Light-Induced Electrical Potential Changes and Motility in Desmids

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Abstract. Motility of the desmid *Cosmarium cucumis* depends on light: switching the light on induces a large fraction of previously immotile cells to start moving, and switching it off causes many motile cells to stop. Turning light on or off causes light-induced electrical potential changes which can be measured with internal microelectrodes. The electrical gradient within the cell is not correlated with the light gradient. Consequently, the cell cannot obtain information concerning the spatial distribution of the incident light, e.g. for phototactic orientation. However, light-induced potential changes could serve as signals for photokinesis, since switching the light on causes a transient increase and switching the light off a transient decrease in the electrical potential of the front half as compared to the rear half or the extracellular space.

Key words: *Cosmarium* — Electrical potential changes — *Micrasterias* — Motility — Photomovement

Introduction

Most desmids move with a gliding mechanism as a result of the extrusion of slime from apical pores in the cell wall (Kol 1927; Jarosch 1962; Kiermayer and Staehelin 1972). Movement is controlled by light: desmids show all three basic photomovement responses found in microorganisms (Diehn et al. 1977). First, there is a pronounced positive photokinesis, i.e. a dependence of the movement speed and the percentage of motile cells in a population on light intensity (Neuscheler 1967a, b; Häder and Wenderoth 1977). Second, many desmids show positive phototaxis, an oriented movement toward the light source (Bendix 1960a, b). Phototactic orientation is the result of active steering. The meandering path in darkness becomes straighter in light which also increases the net speed of movement (Stahl 1878; Hygen 1938). The third photomovement reaction, the photophobic response, is induced by sudden changes — both step-up and step-down — in light intensity (Häder and Wenderoth 1977). Upon perceiving

a stimulus, a cell can either swing sidewards or reverse its direction of movement. In the latter case, the cell stops producing slime from the rear end and starts excreting a new slime stream from the former front pores; thus, the cell reverses its polarity.

The action spectra of all the three photomovement responses coincide with the absorption spectrum of the cells in vivo. This indicates that the photosynthetic pigments — chlorophylls and accessory pigments — are involved in photoperception (Wenderoth and Häder 1979). This is strengthened by the finding that photokinesis and, partially the photophobic response, are impaired by inhibitors of the photosynthetic electron transport chain (Häder 1981). Phototaxis, however, seems to be independent of the photosynthetic machinery since it is not affected by inhibitors of the electron transport nor by uncouplers of photophosphorylation (Häder 1982a).

The next step in the reaction chain is the sensory transduction following the primary perception of the light stimulus. Some microorganisms have been shown to utilize light-induced electrical potential changes which simultaneously serve as an effective means of amplification (Schmidt and Eckert 1976; Häder 1982b). Many photosynthetic organisms produce light-induced potential changes which depend directly upon the photosynthetic apparatus (Sybesma et al. 1981; Remish et al. 1981). The present paper describes changes in the electrical potential and motility induced by changes in the illuminance of the cells.

Materials and Methods

Most experiments were carried out with the unicellular desmid, *Cosmarium cucumis* strain 612—10, obtained from the culture collection in Göttingen (Schlösser 1982), and some control experiments with *Micrasterias denticulata*, isolated as described recently (Häder and Wenderoth 1977). The organisms were cultured in 200 ml Erlenmeyer flasks in MSX medium (Waris 1953) at 16 °C under a light-dark regime (16 h 500 lx from fluorescent lamps and 8 h dark).

The cells were harvested by centrifugation ($1100 \times g$ for 5 min), treated in a homogenizer (20 s Bühler homogenizer at 12,000 rpm) to free the organisms from the slime mass they had produced during growth, and finally washed and suspended in fresh medium.

Movement was followed under an infrared monitoring beam with an infrared-sensitive video camera (National WV 1350E) mounted on top of an inverted microscope (Zeiss ICM 405) and recorded on a 1/2 inch time lapse video recorder (National NV 8030E). The actinic light was produced by a quartz halogen slide projector (Leitz, Prado) connected to a timer which allowed light-dark cycles of 30 min each. The light-induced behaviour was followed on a video monitor (Sony, PVM 201CE). The proportions of cells in which a stop or start of movement was induced by a change in illumination were determined and compared to the controls. Control values were obtained by calculating those fractions of the population which started or stopped moving at an arbitrarily chosen point of time in the absence of a stimulus (constant light or dark). In order to obtain statistically relevant data, a total number of more than 5000 individuals were analyzed.

Light-induced electrical potential changes were recorded using microelectrodes with tip diameters of about 1 μm filled with 0.2 mol.l^{-1} KCl. The monitoring electrode was placed at various sites of the

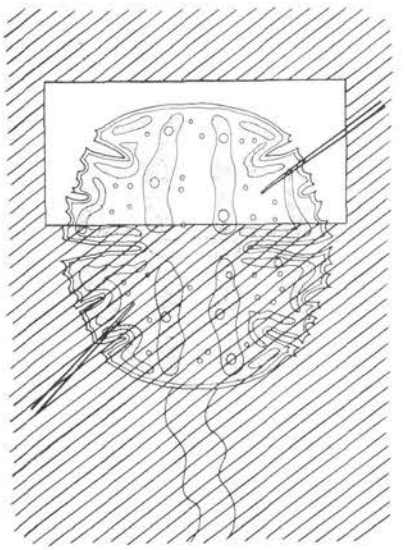

Fig. 1. A cell impaled with a measurement electrode in the front half and a reference electrode in the rear half. The cell was stimulated with a rectangular light field on the front half.

cell and the reference electrode either inside or outside the cell. Fig. 1 shows a typical result of several hundred cells tested. The electrodes were connected to an amplifier (BAK Standard Wide Band Electrometer ELS A-4) by a FET preamplifier to guarantee high input impedance. The amplifier output was monitored on a storage oscilloscope (Tektronix Model 314) and recorded on videotape for later analysis. The actinic light was switched on and off mechanically (by means of a shutter) rather than electrically to avoid electrical noise which could interfere with the measurement. The onset of the actinic light was recorded on the second channel of the oscilloscope using the photovoltage of a Si-glass diode inserted in the light beam.

Results and Discussion

The cells do not glide continuously but rather rest for certain periods of time after moving for a while. When the actinic light was switched on after a dark period of 30 min about 24 % of all cells in the population showed a response within 60 s. In this fraction of responding cells, the stimulus induced motility in 71 % of previously resting cells while 29 % of previously motile cells stopped moving (Fig. 2). When the light was switched off after 30 min of light about 23 % of the cells responded. Out of these responding cells about 39 % of resting cells started moving while 61 % motile cells stopped moving. In both the light and dark controls about 7 % of all cells changed their state of motility at an arbitrarily chosen time point. This indicated that turning the actinic light on (not constant light!) preferentially induced motility while switching it off (not constant darkness!) caused cells to stop

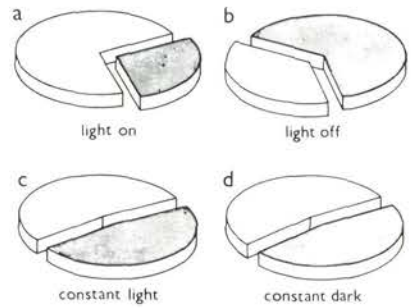

Fig. 2. Proportions of responding cells which stopped moving (hatched sectors) or started moving (open sectors) when actinic light (1000 lx white light) was switched on (*a*) or off (*b*) and under constant light (*c*) or darkness (*d*) at an arbitrarily chosen point of time.

Table 1. χ^2-test ($K = 2$, $P < 0.01$) for the number of responding cells (N_R) out of the total number of cells observed (N_T)

Conditions	χ^2-value	N_R	N_T
Light on	38.72	230	940
Light off	39.48	229	778
Constant light	2.87	114	1603
Constant dark	2.70	118	1752

moving. The statistical significance for both responses has been proven using the χ^2 test (Table 1).

$$\chi^2 = \sum_{N=1}^{K} \frac{(V_o - V_e)^2}{V_e}$$

assuming a value of K = 2 for the two groups of starting and stopping organisms. V_o stands for the observed and V_e for the expected number of responding organisms.

Are the induction or cessation of movement related to intracellular electrical potential changes? When a microelectrode was placed in the front half and the reference electrode in the rear half of the cell, switching the light off caused a transient depolarization of about 17 mV and switching it on again resulted in a transient hyperpolarization of about 19 mV (Fig. 3). The electrical signals were identical regardless of whether the electrodes were inserted in the left or the right half of the cell. Similar responses were also found when a lateral half of a cell was irradiated or when the reference electrode was outside the cell. This suggested that the organism cannot derive the direction of the light from light-induced electrical

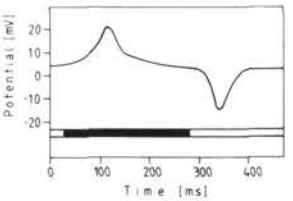

Fig. 3. Electrical potential changes (copied from a video recording of the oscilloscope trace) measured between two electrodes inserted in the front and rear halves of the cell induced by switching the light (1000 lx) off or on (light = white bar, darkness = black bar).

signals. Thus, phototactic orientation seemed to be determined independently of electrical potential changes. However, the electrical signal was correlated with motility: switching the light on caused a transient rise in the electrical potential and induced movement. Switching the light off induced a transient potential in the opposite direction. This signal could be utilized for the photokinetic induction of movement.

Due to the geometry and the cellular peculiarities of the organism, impalement with two microelectrodes was not always successful. Furthermore, light-induced potential changes could only be measured in about the same percentage of cells as that found to respond to a change in illuminance. This also indicated that there was a correlation between motility and light-induced potential changes.

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