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# CIRCADIAN CHANGE OF PHOTOSENSITIVITY IN *PARAMECIUM BURSARIA*

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## Summary

Circadian changes in behaviour and electrical properties of the membrane were investigated in *Paramecium bursaria*. Both photoaccumulation and swimming velocity were at a higher level during the day than at night. During the day, swimming velocity decreased as light intensity was increased and the cells almost stopped at the intensity at which they showed the maximum degree of photoaccumulation. When the intensity was increased further, the avoiding reaction was frequently observed. Step increases in the light intensity depolarized the membrane potential. The depolarization was larger in the night cells than in the day cells. The resting potential measured in the dark was about 4 mV more positive in the night cells than in the day cells. Membrane conductance was larger in the day than at night, which can explain the changes in the resting potential and also the photosensitive behaviour.

## Introduction

*Paramecium bursaria* is photosensitive and shows phototaxis by accumulating in a lighted area (Engelmann, 1882; Jennings, 1906). Swimming velocity and/or the beating frequency of cilia is a function of membrane potential (Machemer, 1974), and it has recently been shown that a step increase in light intensity induces depolarization of membrane potential (Nakaoka *et al.* 1987), as a result of which the swimming velocity of cells in the lighted area decreases or the cells stop swimming (Matsuoka & Nakaoka, 1988).

Circadian rhythms have been found in populations of unicellular organisms (Mergenhagen, 1980; Pittendrigh, 1981; Edmunds, 1983; Hasegawa & Tanakadate, 1984; Johnson & Hastings, 1986; Schweiger & Hartwig, 1986). *P. bursaria* shows circadian rhythmicity in its mating reaction (Miwa *et al.* 1987) and photoaccumulation (Miwa *et al.* 1986), both of which show higher levels in the daytime than at night. The present study investigates the relationship between

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circadian changes in behaviour of *P. bursaria* and the electrical properties of the cells.

## Materials and methods

### *Cells*

*Chlorella*-containing *Paramecium bursaria* (stock OK-1, supplied by Dr I. Miwa of Ibaraki University) were cultured in a hay infusion inoculated with *Klebsiella pneumoniae*. The culture was maintained under a fixed illumination cycle of 12 h: 12 h L: D (a fluorescent light of about $10^3$ lx) at 25 °C. Cells in the stationary phase were collected by low-speed centrifugation and suspended in a control solution of the following composition (mmol $l^{-1}$): $CaCl_2$, 0·25; $MgCl_2$, 0·5; KCl, 2; Tris–HCl, 2; pH 7·2 for 1–10 days under the illumination cycle or under continuous illumination. Cells collected 3–5 h after the illumination period and those collected 3–5 h after the dark period were used as typical 'day' cells and 'night' cells, respectively.

All the following measurements were made in the control solution.

### *Swimming behaviour*

A 0·8 mm thick glass microscopy slide, with a hole 15 mm in diameter through it, was fixed on another slide. The *Paramecium* cell suspension was placed in the hole of the upper slide and sealed with a cover-glass coated with Vaseline. This preparation was then placed on a microscope stage and subjected to the illumination cycle described above. Swimming behaviour was recorded with a video camera (Panasonic, WV-1550) mounted on a microscope. To measure photoaccumulation, an interference filter of 550 nm (the cells were sensitive to the light which passed through this filter) was placed in front of a microscope lamp with a variable-voltage d.c. supply and the image of the condenser diaphragm was focused on an area of 0·7 mm diameter on the bottom of the cell suspension. To measure swimming velocity, the diaphragm was opened to obtain uniform illumination. The light for video recording was a lamp with a 750 nm interference filter (the cells were insensitive to the light which passed through this filter).

### *Intracellular recording*

Methods for intracellular recording were similar to those described in a previous report (Matsuoka & Nakaoka, 1988). The electrodes were filled with 0·1 mol $l^{-1}$ KCl and their resistances were 100–150 MΩ. A sample of cells under the illumination cycle was taken, and these cells were deciliated by washing in 5 % ethanol (Nakaoka *et al.* 1987) and resuspended in the control solution. Membrane potential was measured within an hour after deciliation. The resting potential, which was obtained from the potential difference after nulling the electrode, was measured under a microscope light with a 700 nm long-pass filter. Membrane conductance was measured by injecting a constant current (−0·3 to 0·3 nA) through a resistor of 10 GΩ for 0·5 s.

### *Light stimuli*

The light source for photostimulation was a 50 W halogen lamp with a variable-voltage d.c. supply. A 550 nm interference filter was placed in front of the lamp. Pulses of light were obtained by using a camera shutter between the filter and microscope stage. A photo-cell was placed between the shutter and the microscope stage to record the light pulse. Light intensity was measured with a calibrated silicon photodiode.

## Results

### *Photoaccumulation*

*Paramecium* cells accumulated on a spot continuously illuminated at 550 nm.

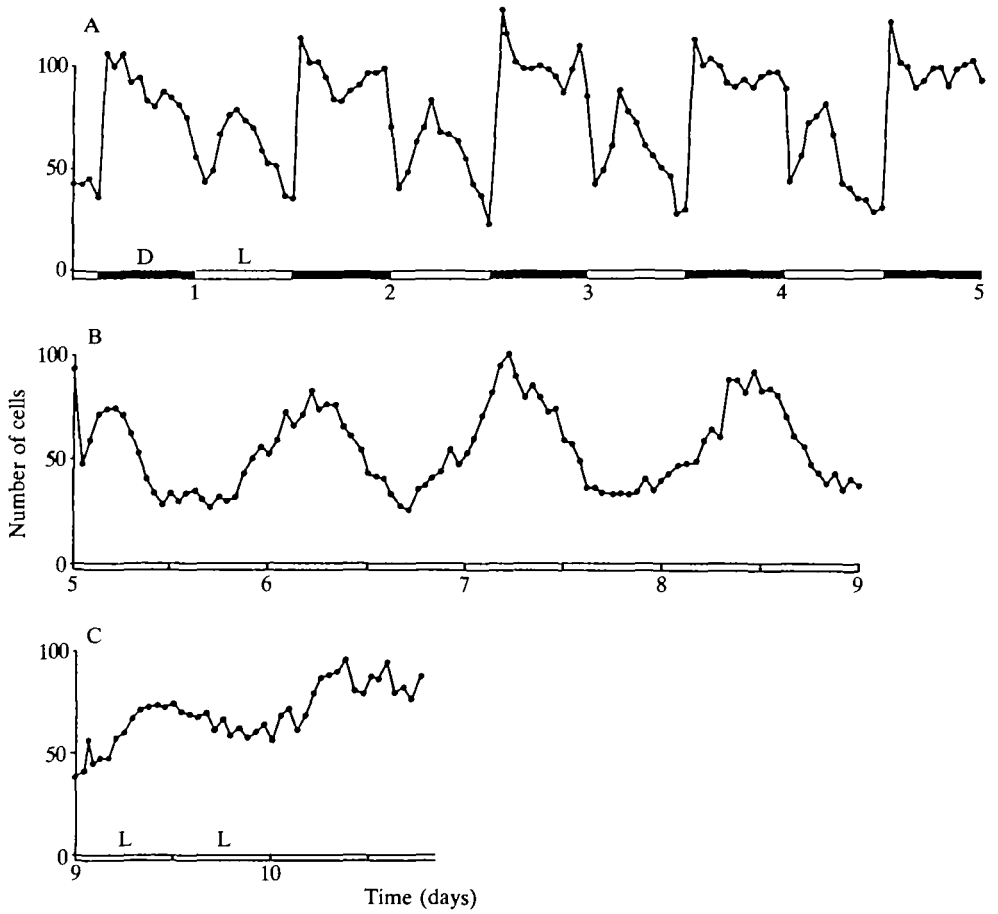


Fig. 1. Circadian change in the number of cells accumulating at a spot continuously illuminated at 550 nm. (A) The first 5 days were under 12 h: 12 h, L: D conditions (DL). (B,C) The cells were kept under constant illumination (LL). C is the continuation of B.

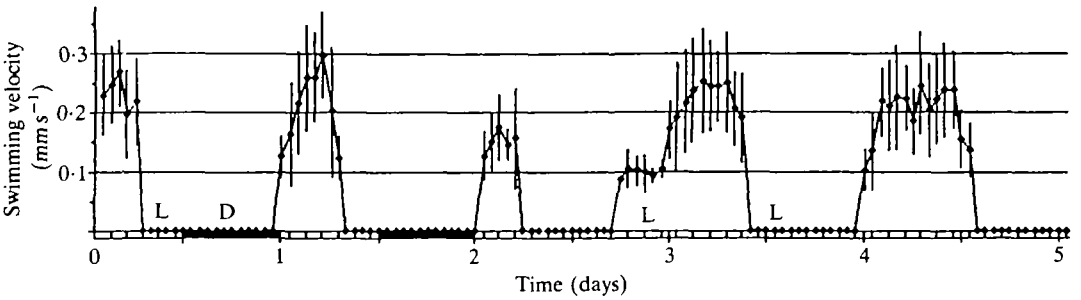


Fig. 2. Circadian change of swimming velocity. The first 2 days were under 12 h: 12 h L: D conditions (LD), then the cells were kept under constant illumination (LL). The swimming velocities of 10–40 cells were averaged. Vertical lines show ± S.D.

With ambient illumination under a 12 h: 12 h L: D cycle, the number of accumulated cells showed rhythmic changes synchronized with the illumination cycle (Fig. 1A). During the light period, the accumulation was at a maximum after 4–5 h. At the beginning of the dark period, the accumulation increased suddenly and became larger than that during the light period. The accumulation decreased rapidly before the end of the dark period.

Under continuous illumination, the cells showed a circadian change in photoaccumulation (Fig. 1B,C): the accumulation during the period corresponding to daytime was larger than that during the period corresponding to night-time. The rhythm almost disappeared after 5 days of continuous illumination.

### *Swimming velocity*

Under the illumination cycle, the cells showed active swimming during the first half of the light period, but during the second half the cells almost stopped swimming and stayed at the bottom of the chamber (Fig. 2). Microscopic observation showed that the ciliary beating of these slowly moving cells was very slow and the cilia of the posterior half had almost stopped beating.

A circadian change in swimming velocity was also found under continuous illumination. In this case, the duration of active swimming was longer than that under the dark–light cycle.

### *Dependence of swimming velocity on light intensity*

The swimming velocity of the cells changed according to the light intensity (Fig. 3). The day cells showed a decrease in swimming velocity as light intensity was gradually increased and stopped moving at an intensity of about 1 mW $cm^{-1}$: the intensity at which the cells showed marked photoaccumulation (Matsuoka & Nakaoka, 1988). Further increase in light intensity induced swimming in which the avoiding reaction occurred frequently. Night cells showed no swimming at intensities below about 1 mW $cm^{-1}$ and showed similar behaviour to day cells at higher intensities (Fig. 3).

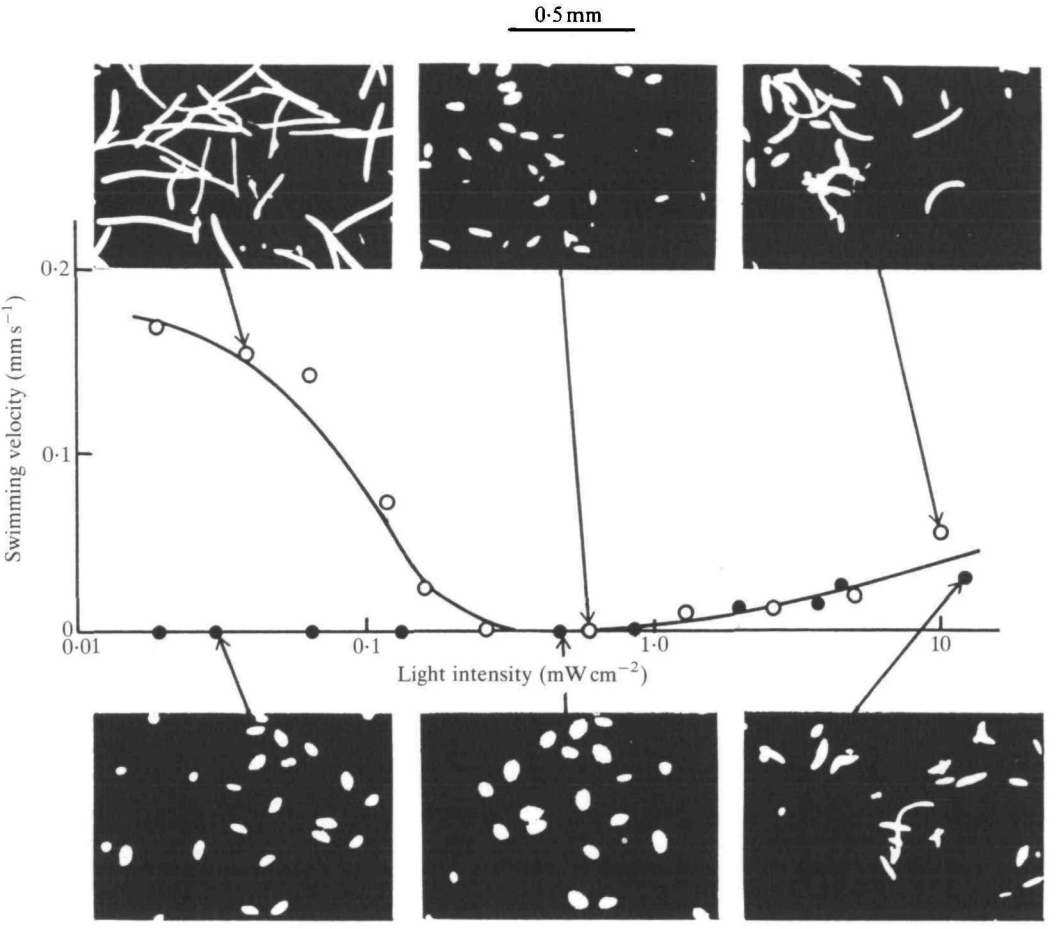


Fig. 3. Swimming velocity at various light intensities. 2–3 min after the illumination with 550 nm light, swimming velocities of day (○) and night (●) cells were measured. Velocities are the mean of 30 cells. Upper and lower photographs show swimming tracks for the points indicated on the curve. The tracks were 2 s exposures.

### *Depolarization induced by photostimulation*

Photostimulation induces depolarization of the membrane potential of *Paramecium* (Nakaoka *et al.* 1987; Matsuoka & Nakaoka, 1988), and this was recorded in both day and night cells (Fig. 4A). All recordings showed that a step increase in light intensity induced a steady depolarization of membrane potential and a step down caused recovery of the potential. The amplitude of the steady depolarization increased with increasing light intensity (Fig. 4B). The light intensity capable of inducing depolarization was lower for night cells than for day cells.

### *Resting potential*

Membrane potential was measured at various times during the illumination cycle (Fig. 5). Although there were large fluctuations in the measurements, the

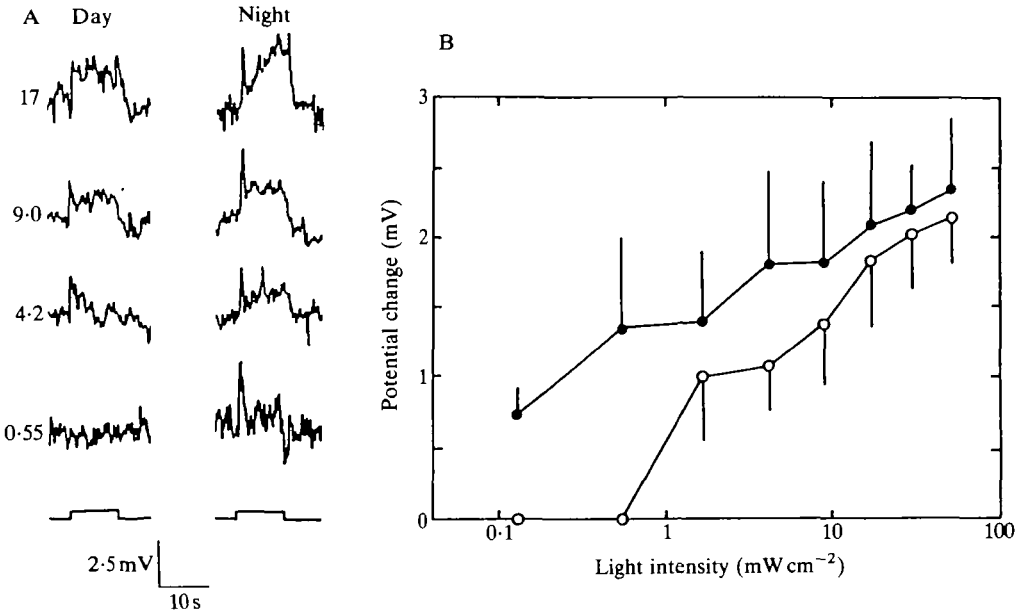


Fig. 4. Potential change induced by photostimulation. (A) Potential records of day and night cells. The time course of photostimulation is shown below the traces. Figures on the left indicate the intensity of light for stimulation in mW cm$^{-2}$. (B) Relationship between light intensity and amplitude of steady depolarization. Measurements from three day cells (○) and four night cells (●) were averaged. Vertical lines indicate S.D.

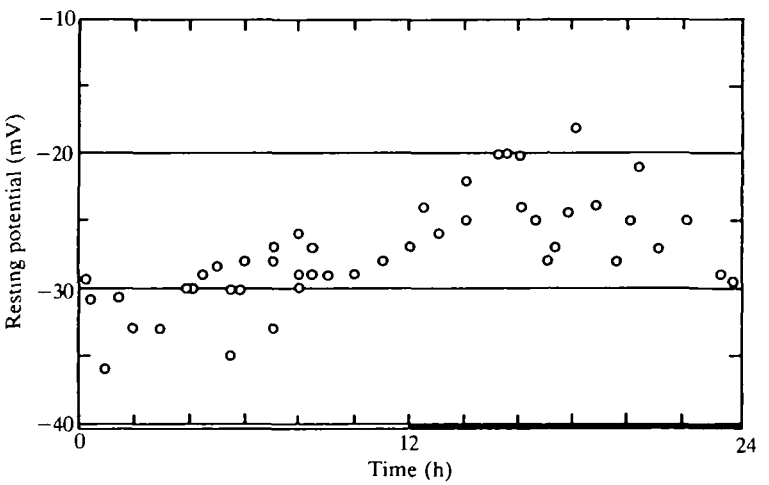


Fig. 5. Resting potentials at various times of day. The measurements were made within 1 h after the cells had been removed from the incubator where they had been subjected to a 12 h:12 h L:D cycle. Each point shows the measurement for a single specimen.

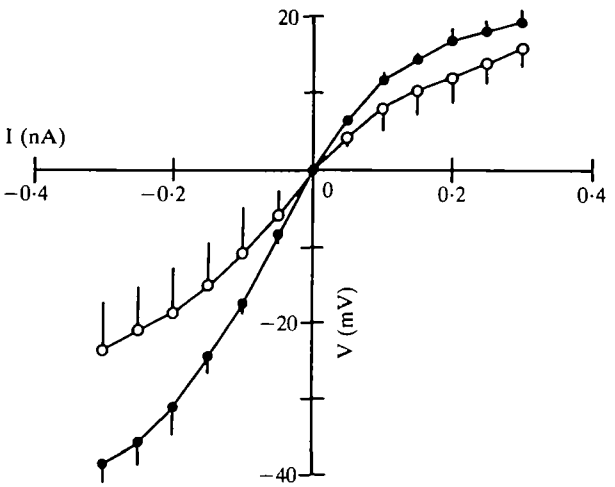


Fig. 6. I–V relationships of day and night cells. Current pulses of 0·5 s were injected into day (○) and night (●) cells and potential shifts from resting level were recorded. Each point is the mean of four cells. Vertical lines indicate S.D.

potential during the light period (mean −29 mV, S.D. 3·5 mV, $N = 26$) was more negative than that during the dark period (−25 mV, S.D. 3·4 mV, $N = 22$).

### *Membrane conductance*

Membrane conductance was measured by injection of current pulses. The I–V relationship (Fig. 6) of the night cells had a steeper slope at around the resting potential than that of the day cells. The mean membrane resistance was 105 MΩ for day cells and 145 MΩ for night cells.

## Discussion

Clear periodic changes in the degree of photoaccumulation and swimming velocity of *Paramecium bursaria* were observed (Figs 1, 2). In the day, when the degree of photoaccumulation in a light spot is large, the swimming velocity of the cells outside the light spot is high, whereas that of the cells within the light spot is very low. Such a difference in swimming velocity is expressed in the relationship between light intensity and swimming velocity (Fig. 3). Swimming velocity of the day cells decreases as light intensity is gradually increased and almost stops at the light intensity at which it has previously been observed that the cells show maximum photoaccumulation (Matsuoka & Nakaoka, 1988). As night cells stop swimming at these light intensities, the degree of photoaccumulation becomes smaller. Apparently, the difference in the swimming velocity between dark and light conditions causes the cells to accumulate in a light spot, as discussed in the previous paper (Matsuoka & Nakaoka, 1988). The periodic change in the swimming velocity in this study corresponds to that of *P. multimicronucleatum* (Hasegawa & Tanakadate, 1984, 1987).

The potential change induced by light stimulation is larger in night cells than in day cells (Fig. 4). Nakaoka *et al.* (1987) have reported that the depolarization induced by light stimulation accompanies an increase in the membrane conductance for $Ca^{2+}$. When the membrane conductance of the whole cell increases, the amplitude of the depolarization elicited by light stimulation will be smaller. Indeed, the membrane conductance of the day cells is larger than that of the night cells (Fig. 6).

Resting potentials during the light period are more negative than those during the dark period (Fig. 5). Machemer (1974) has shown that depolarization by a few millivolts decreases the beating frequency of cilia, so the present results are reasonably related to the change in resting potential. The membrane conductance of *Paramecium* is considered to consist mostly of a $K^+$ conductance (Naitoh & Eckert, 1980), and an increase in the $K^+$ conductance changes the membrane potential to a negative level. It is therefore most probable that rhythmic changes in $K^+$ conductance mediate the observed changes in resting potential.

In addition to the periodic change in membrane conductance, the membrane conductance for $Ca^{2+}$ changes rapidly following photostimulation (Nakaoka *et al.* 1987). This dual change in the membrane conductances causes the changes in membrane potential and, presumably, modulates the photosensitive behaviour.

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