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SHORT COMMUNICATION

ELECTRICALLY INDUCED MOTILE RESPONSES IN FREE-SWIMMING *PARAMECIUM*

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The ciliate *Paramecium* exhibits characteristic motile behaviour. When it is not feeding it normally swims in straight lines. On striking an obstacle or sensing some change in its environment it reverses, reorientates, and sets off in a new direction. The avoidance response is graded, ranging from a slight readjustment in swimming direction to a vigorous reversal for several body lengths before forward swimming is resumed (Jennings, 1906). The underlying ionic events have become clearer in recent years. If the front of the cell is stimulated the excitable membrane there becomes depolarized and calcium ions enter the cell causing the cilia to beat backwards; when the internal calcium concentration drops, due to active extrusion of ions, the cilia resume their forward beating in a staged recovery causing the cell to change direction before moving forward again. In contrast, mechanical stimulation of the rear of the cell causes hyperpolarization of the membrane and results in more rapid forward swimming (Eckert & Brehm, 1979). Although the membrane is clearly different at the front and rear of the cell, little is known about the nature and electrical characteristics of this asymmetry since microelectrode measurements give only average data for the whole cell.

We report here that short electric field pulses produce characteristic motile responses in free-swimming *Paramecium*. The observations are interpreted in terms of an electrophysiological model which gives a direct measure of the front–rear membrane asymmetry. We suggest that such electric field experiments, which have the advantage of being non-invasive, may yield new information about the ciliary membrane.

Specimens of *Paramecium caudatum*, reared in hay infusion, were washed in a solution containing 1 mmol dm^{-3} $CaCl_2$, 2 mmol dm^{-3} KCl and 1 mmol dm^{-3} Tris buffer adjusted to pH 7·0–7·2. All experiments were performed in this solution. Organisms moving in a horizontal Perspex trough (60 mm long, 10 mm wide and 5 mm deep) were photographed from above with a Vinten Mark 3 scientific camera running at 8 frames s^{-1} and coupled to a low-power Zeiss microscope. Horizontal illumination was provided by a projector lamp and condenser lens.

Electric field pulses (from a 400 V power supply controlled by a Bradley type 233

Key words: *Paramecium*, ciliary reversal, electric field, ion gates.

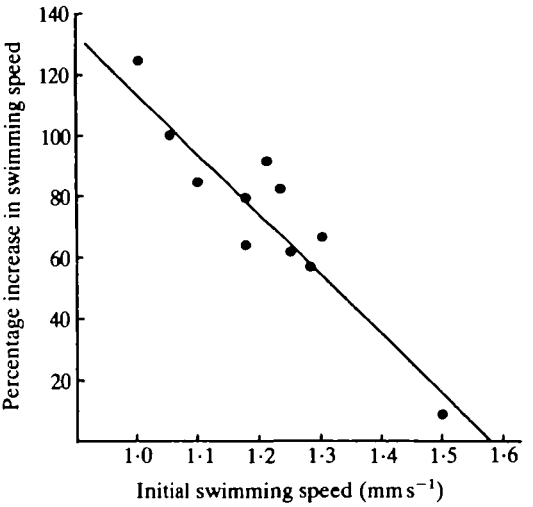

Fig. 1. The percentage increase in forward swimming speed compared with initial speed for cathodal cells, following a pulse of 30 V cm^{-1} and 0·8 ms duration.

pulse generator) were applied between two parallel stainless-steel electrodes placed at the ends of the trough. The shape of the trough constrained most organisms to move within 30° of the chamber axis with roughly equal numbers moving towards each electrode before the pulse was applied. The electric field in the solution during the pulse was calculated from the measured electrical resistivity of the medium (typically $12 \pm 1\,\Omega$m). Film runs were analysed by projecting frame sequences onto a digitizing tablet linked to a microcomputer. Successive positions of cells were tracked manually using a cross-hair cursor, and the computer calculated the average swimming velocities and orientations with respect to the electric field direction both before and after the pulse. No cell was ever subjected to more than one pulse, so obviating the possibility that cells could be adapting to successive stimuli.

Our most striking observation was that *Paramecium* moving towards what subsequently became the cathode when the pulse was applied (and which we call cathodal cells) never reversed. Instead their forward swimming speed increased by an amount governed by both their pre-pulse velocity and the strength of the stimulus. The faster the cell was moving before the pulse the smaller the resulting percentage increase in swimming velocity. This is shown in Fig. 1 for a pulse of 30 V cm^{-1} and 0·8 ms duration, cells being monitored for about 1 s after the pulse. The increase in speed was immediate (i.e. within one ciné frame, or 130 ms) and returned to normal only slowly (after several seconds).

The percentage increase in swimming speed was found to be linearly related to the initial speed for all the pulse combinations investigated. Table 1 gives the slope of the best-fit line and the intercept, U_0, on the speed axis for various pulses. U_0 appears to be independent of pulse strength and duration (being about

Table 1. *Cathodal cell responses to electric field pulses*

Electric field ($V\,cm^{-1}$)	Pulse length (ms)					
	0·2	0·4	0·6	0·8	1·0	1·2
Slopes of regression lines (% $mm^{-1}\,s$)						
15	+3	−2	−48	−30	−69	−88
20	−5	−75	−130	−86	−81	−99
30	−54	−132	−134	−197	−79	−103
36	−95	−90	−82	−108	−114	−136
42	−122	−108	−92	−72	−154	−83
48	−87	−109	−100	−75	−140	−105
57	−60	−123	−141	−71	−75	−91
Intercept U_0 ($mm\,s^{-1}$)						
15	2·9	2·6	2·1	2·6	1·7	1·9
20	2·5	2·0	1·7	2·1	2·0	1·9
30	1·9	1·7	1·7	1·6	1·9	1·8
36	2·1	2·0	2·1	2·0	1·9	1·8
42	1·9	2·0	2·0	2·2	1·8	2·0
48	2·0	1·9	2·0	2·2	1·7	1·8
57	2·1	1·7	1·7	2·4	2·4	2·0

The table gives the slopes and intercepts U_0 of the regression lines (such as that shown in Fig. 1) for cathodal cells and various pulse combinations.

1·8–2·1 $mm\,s^{-1}$), and the effect of increasing the stimulus is to vary the (negative) slope of the regression line from zero up to a maximum of about 150 % $mm^{-1}\,s$. Stronger pulses than those shown in Table 1 (using larger electric fields or longer durations) could not be obtained because of joule heating in the medium and the onset of electro-osmotic flow in the trough. No systematic changes in swimming direction could be found following the pulse.

The response of anodal cells to electric pulses was more complex. Some organisms reacted to weaker pulses as before, accelerating in the forward direction. Again the percentage increase in swimming speed depended linearly on the pre-pulse swimming speed, and the overall results were not significantly different from those in Table 1 for cathodal cells. The remainder reversed (i.e. stopped, then actively swam backwards), turned towards the cathode and resumed normal forward swimming at an increased rate. The fraction (F) of cells that reversed in a given population first rose and then fell with increasing stimulus strength, and for each pulse length there was a particular field strength which optimized F (Table 2).

We have carried out a preliminary analysis of these results using the quantitative physiological model proposed by Hook & Hildebrand (1979, 1980). This model assumes that the cell has a number of ion gates for K^+ and Ca^{2+}, the parameters of which have been inferred principally from intracellular electrode experiments.

Table 2. *Anodal cell responses to electric field pulses*

Electric field ($V cm^{-1}$)	Pulse length (ms)					
	0·2	0·4	0·6	0·8	1·0	1·2
Fraction (F) of anodal cells reversing						
15	0	0	0	0	0·1	0·3
20	0	0	0·3	1·0	0·8	0·9
30	0	0·3	0·8	0·9	0·9	0·4
36	0·3	0·8	0·8	0·8	0·9	0
42	0·6	0·8	0·3	0·1	0·1	0
48	0·3	0·2	0	0·2	0	0
57	0·4	0·1	0	0	0	0
Mean reversing times (s)						
15	0	0	0	0	0·5 ± 0·4	1·3 ± 0·8
20	0	0	0·8 ± 0·8	1·4 ± 1·1	1·6 ± 0·7	1·7 ± 0·9
30	0	1·2 ± 0·8	1·1 ± 0·5	2·1 ± 0·6	1·3 ± 0·8	1·0 ± 0·6
36	0·6 ± 0·6	1·6 ± 0·9	1·1 ± 0·5	0·8 ± 0·6	1·1 ± 0·9	0
42	1·3 ± 0·8	1·4 ± 0·5	1·6 ± 1·0	0·4 ± 0·4	1·1 ± 0·6	0
48	1·4 ± 0·8	1·6 ± 0·9	0	1·1 ± 0·7	0	0
57	0·9 ± 0·7	0·6 ± 0·4	0	0	0	0

Values for mean reversing time are means ± S.E. ($N = 8–17$).

Cilia are assumed to reverse their direction of beat when the ciliary Ca^{2+} concentration exceeds some threshold value. In our experiments the effect of the electric field is to depolarize whichever half of the cell faces the cathode and to hyperpolarize the other half (Offen & Roberts, 1973). In our initial analysis the cell is divided into two halves, one of which is uniformly depolarized whereas the other is correspondingly hyperpolarized. The model then predicts the changes in membrane potential and internal calcium concentration following the imposition of the pulse.

No difference is predicted between cathodal and anodal cell responses if both segments of the model are given the same membrane parameters. An asymmetry can readily be incorporated (without changing the voltage-clamp characteristics of the model) by assuming that more Ca^{2+} channels and fewer K^+ channels are present in the front half. Such an asymmetry has already been posulated by Ogura & Machemer (1980).

If the channel asymmetry is chosen appropriately (with about 75 % of the Ca^{2+} and 25 % of the K^+ channels in the front segment and the remainder in the rear) the two-segment model makes two clear predictions. First, a pulse will never elicit a full-scale depolarization response (reversal) in cathodal cells whereas it will in anodal cells if the pulse is sufficiently intense. Second, if a cell does not reverse following the pulse the intraciliary calcium level will always decrease below the resting value whereas it will rise dramatically when the cell does reverse. The

model can account for the general features of the electric field response if it is assumed that decreased intraciliary calcium concentration is correlated with more rapid swimming; some observations on extracted cells support this view (Naitoh & Kaneko, 1972).

Hook & Hildebrand's model contains a parameter that is determined by the degree of adaptation of the cell to previous stimuli of whatever origin. The longer the time elapsed since the last reversal the greater the intraciliary Ca^{2+} level and the greater the probability of reversal following a pulse. Any given population of cells shows a range of degrees of adaptation and this explains both why the percentage increase in swimming speed depends on the pre-pulse speed and why some cells reverse following a pulse whereas others do not. The model predicts the form of Fig. 1 if Naitoh & Kaneko's data linking swimming speed and Ca^{2+} level are used. Interestingly, the suggestion of Nakaoka *et al.* (1983) that a decrease in Ca^{2+} level produces a decrease in swimming velocity is not borne out by our results, at least under these experimental conditions.

In summary, the simple two-segment model is able to account for the general features of the experimental results, although the numerical predictions are not always accurate. A multi-segment model is being developed which will allow a more realistic simulation of the membrane potential perturbation to be obtained, and will yield a more accurate assessment of the gate asymmetry. Combined with modern video imaging techniques, the electric pulse method should permit the rapid analysis of a wide range of experiments. Two areas of immediate interest are multiple pulse experiments (giving data on membrane gate time constants) and the investigation of pharmacological effects of drugs on the ciliary cell membrane.

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