

Passive Electrical Properties of *Paramecium* and Problems of Ciliary Coordination

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ABSTRACT Potential recordings made simultaneously from opposite ends of the cell indicate that the cytoplasmic compartment of *P. caudatum* is nearly isopotential. Measured decrements of the spread of steady-state potentials are in essential agreement with calculated decrements for a short cable model of similar dimensions and electrical constants. Action potentials and passively conducted pulses spread at rates of over 100 μm per msec. In contrast, metachronal waves of ciliary beat progress over the cell with velocities below 1 μm per msec. Thus, electrical activity conducted by the plasma membrane cannot account for the metachronism of ciliary beat. The electrical properties of *Paramecium* are responsible, however, for coordinating the reorientation of cilia (either beating or paralyzed by NiCl_2) which occurs over the entire cell in response to current passed across the plasma membrane. In response to a depolarization the cilia assume an anteriorly directed orientation ("ciliary reversal" for backward locomotion). The cilia over the anterior half of the organism reverse more strongly and with shorter latency than the cilia of the posterior half. This was true regardless of the location of the polarizing electrode. Since the membrane potential was shown to be essentially uniform between both ends of the cell, the cilia of the anterior and posterior must possess different sensitivities to membrane potential.

INTRODUCTION

In 1920 Taylor reported microsurgical experiments from which he concluded that a fibrillar "neuromotor" system originating in a central "motorium" of the ciliate *Euplotes* was the anatomical basis for coordination between reorientation (reversal) of widely separated ciliary membranelles and cirri. This led to recurring speculation regarding the signaling capability of intracellular fibrillar systems (Sleigh, 1962; Bullock and Horridge, 1965; Ochs, 1965; Kitching, 1961). Recent experiments (Okajima and Kinosita, 1966; Naitoh and Eckert, 1969 *a*) have cast serious doubt on Taylor's interpretation, and together with other studies (Kinosita, 1954; Kinosita et al., 1964 *b*; Naitoh, 1958, 1966;

Naitoh and Eckert, 1969 *b*) have implicated electrical activity of the plasma membrane in the control of ciliary orientation in ciliates. This should not be confused with the suggestion (Grebecki, 1965) that the metachronal waves of ciliary beat¹ are propagated by impulses passing along the cell membrane.

There are two familiar concepts for the transmission of electrical signals by biological membranes, which are therefore relevant to problems of ciliary coordination. (*a*) The resistive and capacitive properties of a cell permit membrane currents to spread passively from one part of a cell to another (Hodgkin and Rushton, 1946). If the membrane resistance is sufficiently high compared with the longitudinal intracellular and extracellular current pathway the cytoplasmic compartment approaches an isopotential state. That is to say, any potential change at any point between cell interior and exterior will develop to the same degree across all other portions of the plasma membrane with minimal amplitude decrement and time lag. If, on the other hand, the internal resistance of a membrane-limited compartment or the extracellular resistance encountered by the current is sufficiently high as compared with the membrane resistance, that compartment will deviate significantly from the isopotential condition and an electrotonic potential will exhibit a corresponding decay with distance from its point of origin. (*b*) Voltage-sensitive regenerative conductance changes give rise to an all-or-none action potential (Hodgkin, 1937; Hodgkin and Huxley, 1952) which is propagated with a velocity determined in part by the passive electrical ("cable") properties of the cell.

In this paper we examine (in the context of the passive electrical properties of *Paramecium*) the spread of signals which participate in the coordination of ciliary activity. A potential change generated by current introduced into the cell at one end spreads passively over the cell membrane with a small (< 10%) amplitude decrement, and with a velocity of more than 100 μm per msec. While this is not consistent with the velocity of ciliary metachronism, it is consistent with changes in the direction of ciliary beat which occur in response to depolarization. Finally, the data reveal that the cilia of anterior and posterior portions of *Paramecium* exhibit different sensitivities to membrane potential.

MATERIALS AND METHODS

Specimens of *P. caudatum* were held immobilized in the test solution so that one polarizing electrode and two recording electrodes could be introduced. The current-passing electrode and one recording electrode (proximal) were inserted near one end of the organism, and the second recording electrode (distal) was inserted near the other end.

¹ Cilia normally beat with a small phase difference with respect to their immediate neighbors along an axis which can be either in the plane of the power stroke or at some angle, up to 90°, to that plane. The result is a series of "metachronal" waves of ciliary beat which in different materials progress with or against the power stroke or at an angle to it. The direction of progression shifts in accordance with changes in the direction of the power stroke. Metachronism is described in more detail by Sleight (1962).

The current electrode and proximal recording electrode were inserted near the anterior end in about half the experiments and near the posterior end in the remaining experiments.

Pulses of constant current were supplied to the polarizing electrode from a pulse generator through a resistor of 10^{10} ohms. The bath was held at virtual ground and current pulses were monitored by means of an operational amplifier (George A. Philbrick Researches, 1966). The bath was connected to the summing junction of the amplifier through a 5×10^8 ohm KCl-agar lead. Essentials of the setup are described in further detail elsewhere (Naitoh and Eckert, 1968 *a*) and are illustrated in Fig. 2.

Recordings were made with 10–30 megohm 3 M KCl capillary glass electrodes of conventional tip dimensions connected to neutralized capacity amplifiers. Potential signals were electronically differentiated by means of operational amplifiers equipped with passive networks of 20 msec time constant (Fig. 2). Polarizing currents, intracellular potentials, and their derivatives were displayed with an oscilloscope and photographed.

Special care was exercised to employ equal gain and capacity neutralization in the two recording channels so as to avoid artifacts in amplitude and time course comparisons. This was done in two stages throughout experiments after the electrodes were in their recording position. First, gain and neutralization were adjusted to equalize the recorded wave shape and amplitude of square calibration pulses applied between the bath and virtual ground. Second, the capacity neutralization of one channel was further adjusted so that the derivative recordings of the square pulse recorded by the two channels were of precisely equal amplitude.

Except where otherwise noted, the extracellular medium consisted of 1 mM CaCl_2 plus 2 mM KCl in double glass-distilled water. All-or-none action potentials (Naitoh and Eckert, 1968 *b*) were obtained by exposing the specimen to a solution of 1 mM CaCl_2 plus 2 mM BaCl_2 . Solutions were uniformly at pH 7.2 with Tris-HCl.

Illumination for photomicrography was provided by a substage electronic flash synchronized with the pulse generator which supplied the stimulus current pulse. In some experiments the specimen was exposed to 1 mM NiCl_2 until the cilia were paralyzed (Kúznicki, 1963). They were then returned to the Ni-free experimental medium. Although paralyzed, the cilia retain their ability to orient in response to stimuli (Naitoh, 1966; Naitoh and Eckert, 1969 *b*).

Specimens of *P. caudatum* used for these experiments were obtained from Turtox Biological Supply Inc (Chicago, Ill.) and were cultured in a hay infusion at 22°C. Experiments were performed at 16°–19°C.

RESULTS

Membrane Constants

Surface areas of specimens were calculated by the method of Fortner (1925), who approximated the shape of *Paramecium* by assuming the cell body to be equal to the sum of two half-ellipsoids of revolutions. The length, L , of the specimen is multiplied by both the diameter, d , at the widest part of the cell, and the factor 2.3:

$$A = 2.3 dL. \quad (1)$$

This formula does not take into account either the increase in membrane surface area due to the prominent polygonal sculpturing of the surface (Pitelka 1963, Fig. 14), or the surface area contributed by the cilia. It will be termed "uncorrected surface area."

The plasma membrane of *Paramecium* is continuous over each cilium, forming a sheath of nearly cylindrical form $0.2 \mu\text{m}$ in diameter and about $11 \mu\text{m}$ long (Sleigh, 1962). Thus each cilium has a surface area of about $7 \mu\text{m}^2$. The cilia of *P. caudatum* number about 38 per $100 \mu\text{m}^2$ of uncorrected surface area (Wichterman, 1953), which adds $266 \mu\text{m}^2$ or 266% to the uncorrected surface

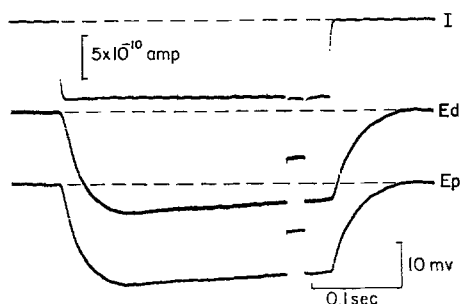


FIGURE 1. Electrotonic potentials in response to 300 msec hyperpolarizing current pulse delivered with microelectrode near one end of cell. Trace *I*, current monitor; trace *Ep*, signal from recording electrode (proximal) adjacent to current-passing electrode; trace *Ed*, signal from recording electrode (distal) inserted near other end of cell. Two recording electrodes were $125 \mu\text{m}$ apart in a specimen measuring $210 \mu\text{m}$ overall. Calibration pulse toward the ends of the potential wave forms was used to normalize the gain of both channels, and to detect any changes in relative recording sensitivity. The corrected deflection in trace *Ed* was 100% of that in trace *Ep*.

area. Since the cable properties of individual cilia are unknown it is difficult to calculate the contribution of the ciliary component of the cell membrane to the passive electrical properties of the cell.

Input resistance of the cell (resistance encountered by a current passed between two electrodes inside and outside the cell, respectively) was determined in a medium of 1 mM CaCl_2 plus 1 mM KCl by the measurement of steady-state transmembrane potentials elicited in response to 300-msec pulses of hyperpolarizing current (Fig. 1). Uncorrected values of membrane resistance, R_m , based on uncorrected surface area calculated as shown above, range from 2.2 to $6.7 \times 10^8 \text{ ohms} \cdot \text{cm}^2$ for hyperpolarizations of 15 – 24 mv (Table I). Resistance drops somewhat when the cell is exposed to higher concentrations of cations (Naitoh and Eckert, 1968 *a*). Time constants (τ = time to 63% of steady-state potential) of the electrotonic wave shapes were measured from photographs, and ranged from 20 to 27 msec, yielding uncorrected values for membrane capacitance of 3.9 – $9.3 \mu\text{F}/\text{cm}^2$. The true

value of membrane capacitance must be significantly lower than these figures when proper allowance is made for the area of membrane over the cilia. Likewise, the true membrane resistance must be considerably higher than the uncorrected value calculated above.

Cable Properties

In considering the cable properties of *Paramecium* it is expedient to treat the slipper-shaped cell as a cylinder in which the plasma membrane forms the

TABLE I

PASSIVE ELECTRICAL PROPERTIES OF *P. caudatum*

A 300 msec inward current pulse was applied with an intracellular electrode located near one end of the specimen. Electrotonic potentials were recorded with two other electrodes, one of which was inserted near the polarizing electrode while the other was inserted near the opposite end of the specimen. E_p is the potential recorded with the electrode inserted near the current electrode. E_p/I is the input resistance of the cell. Uncorrected values for membrane resistance and capacitance are termed such because the cable properties of the cilia are not known.

A	B	C	D	E	F	G	H	I	J
Specimen No.	Length L	Maximum width d	Location of polarizing electrode	Distance between recording electrodes	Decrement calculated for entire length, L D_c	Decrement measured between electrodes D_m	$\frac{E_p}{I}$	Uncorrected membrane resistance	Uncorrected membrane capacitance
	10^{-4} cm	10^{-4} cm		10^{-4} cm			$10^6 \Omega$	$k\Omega \cdot \text{cm}^2$	$\mu\text{F}/\text{cm}^2$
1	180	35	p*	75	0.01	0.03	22	3.1	7.8
2	210	45	p	121	0.01	0.01	24	5.1	3.9
3	175	30	p	132	0.02	0.05	19	2.2	9.3
4	200	40	a†	52	0.01	0.00	15	2.7	8.3
5	215	43	a	109	0.01	0.03	18	3.7	5.8
6	285	52	a	190	0.01	0.02	20	6.7	4.2

* Posterior half of organism.

† Anterior half of organism.

circumference, and in which the ends of the cylinder are sealed with infinite resistances. The model cylinder is given a length equal to that of the organism. In this form the cell can be treated as a "short cable" as in case 3 of Weidmann (1952). The space constant, λ , can be calculated from the uncorrected specific resistance, R_m , of the plasma membrane and the resistivity of the cytoplasm by application of the standard cable equation (Hodgkin and Rushton, 1946):

$$\lambda = \sqrt{r_m / (r_i + r_o)} \quad (2)$$

in which r_m is membrane resistance of one unit length of cable (ohm·cm), r_i is

resistance of the core (cytoplasm)² of one unit length (ohm/cm), and r_o is the effective external longitudinal resistance of one unit length (ohms/cm) encountered by the electrotonic current.³ Thus, for a specimen 40 μm in diameter with a membrane resistance of 2700 ohm·cm², r_m is 2.2×10^9 ohms· μm , r_i is 1200 ohms/ μm , and the calculated space constant, assuming an infinite cable and negligible external resistance, is 1350 μm . The decay of an electrotonic potential can be calculated for the short cable model of over-all length, L , by the relationship (Weidmann, 1952)

$$E = E_0 \frac{\text{Cosh} [(L - x)/\lambda]}{\text{Cosh} [L/\lambda]}, \quad (3)$$

in which E is the electrotonic potential recorded at any distance x from the origin of the potential at one end of the sealed cable. At that end $x = 0$, and the potential is E_0 . If E is measured at the opposite end of the cable ($x = L$), the equation reduces to:

$$E = \frac{E_0}{\text{Cosh} [L/\lambda]}. \quad (4)$$

The calculated decrement of a steady-state electrotonic potential over the length of the cell is defined as:

$$D_e = 1 - E/E_0. \quad (5)$$

Table I lists dimensions and membrane constants of six specimens as well as the calculated decrement (column F) in electrotonic spread from end to end of each specimen. The calculated decrement in all six specimens was a drop of the order of 1% from one end of the cell to the other end.

It was not practical to test these calculations directly by the insertion of electrodes at the extreme ends of specimens; however, experiments were performed with one recording electrode in the anterior half, and one in the posterior half of the organism, separated by distances of about half the length of the organism. Steady-state electrotonic potentials in response to 300-msec current pulses introduced near the proximal electrode were recorded with both electrodes to determine the degree of signal decrement with distance

² The specific conductance of the cytoplasm of *Paramecium* was measured directly by Gelfan (1926-27) between two intracellular KCl-agar-filled capillary electrodes. His published value of 0.0068 mho per cm, used in our calculations, is consistent with unpublished determinations of electrolyte osmolarity in *Tetrahymena* made by Dr. P. B. Dunham of Syracuse University.

³ The effective longitudinal resistance to electrotonic current of the extracellular medium must be appreciable in a solution of the present ionic strength and undoubtedly higher than the internal resistance encountered by electrotonic current. However, it will be assumed for these calculations to approach zero, because no present method allows for its simple calculation. As a result the calculated length constant will err on the high side.

(Table I, column G). The measured decrement, $D_m (= 1 - E_d/E_p)$, in which E_d and E_p are the steady-state potentials recorded, respectively, with distal and proximal electrodes), ranges up to 0.05 for the distances involved. These values are higher than the values of decrement (column F) calculated for the full length of each organism.

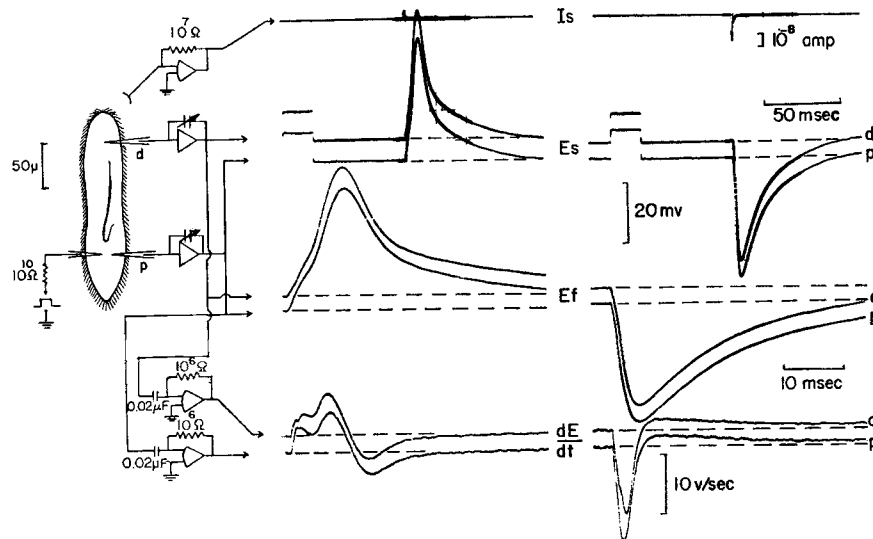


FIGURE 2. Depolarizing and hyperpolarizing electrotonic potentials evoked by 1 msec current pulses. Diagram, specimen showing insertion of proximal (*p*) and distal (*d*) recording electrodes and current electrode. Operational amplifier in current-monitoring configuration is shown at top, and two operational amplifiers in differentiator configurations at bottom. Output signals of the five amplifiers are shown at right for depolarizing current pulse (left column) and hyperpolarizing pulse (right column). Upper two sets of traces are shown at slow (*s*) sweep velocity. Trace *I* shows stimulating current; trace *Es* shows proximal, *p*, and distal, *d*, voltage records. The lower two sets of sweeps are at four times faster (*f*) velocity. Signal originating from the proximal electrode is displayed on lower trace of each pair. Discontinuity in the rising phase of the depolarizing potential represents transition from a passive RC response to the regenerative depolarization of the graded calcium spike (Eckert and Naitoh, 1969). The square calibration pulses on the slow potential (*Es*) traces are 10 mv, 20 msec.

Sources of possible disagreement between calculated and measured values of decrement are obvious. The cell is not a perfect cylinder with a diameter equal to the maximum width of the cell; nor are its ends sealed by infinite resistances. Equations 3 and 4 assume one-dimensional current flow, and hence are most valid for cylindrical elements in which the diameter is far less than the length of the cylinder. Finally, and perhaps most important as a source of error is the simplifying assumption that the resistance encountered by the extracellular current is negligible. The values calculated for steady-state cable loss must, for this reason alone, be lower than the true decrement.

Fig. 2 illustrates the potential responses of the cell to injected current pulses of short duration. During a 1.0 msec pulse the membrane potential recorded with two electrodes near opposite ends of the cell is rapidly driven to a level which it passively descends at the end of the pulse. Comparison reveals little difference in amplitude or time course between the recordings taken from a point near the current electrode and from the distal electrode inserted near the opposite end of the cell, 125 μm distant. The experiment was repeated on six specimens with consistent results.

A short depolarizing current pulse resulted in two stages of potential drop,

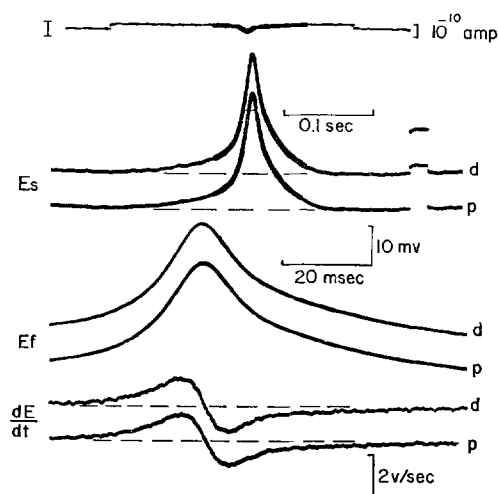


FIGURE 3. Barium action potential recorded simultaneously from two locations in a specimen. Trace *I*, current monitor; traces *Es*, slow sweep recordings from proximal (*p*) and distal (*d*) electrodes; traces *Ef*, same at five times greater sweep velocity; lowest set, time derivatives of potentials displayed in traces *Ef*. Action potential was elicited by a near rheobase current delivered with polarizing electrode inserted near one end of cell adjacent to proximal recording electrode. Distal recording electrode was inserted toward the opposite end of the cell, 82 μm away. Cell had an over-all length of 197 μm .

the first a passive response to current, and the second corresponding to the graded regenerative depolarization noted previously (Eckert and Naitoh, 1969). This is seen to better advantage in the differentiated signals recorded in the lowest set of traces in Fig. 2. The derivative recording typically showed an extra notch on the rising phase of the potential recorded with the more distant electrode. Responses to short pulses of hyperpolarizing current were similar with the exception that regenerative responses were absent.

The barium action potential (Kinosita et al., 1964 *a*; Naitoh and Eckert, 1968 *b*) was initiated with low level current pulses so that the action potential would originate in that part of the plasma membrane, if any, with the lowest

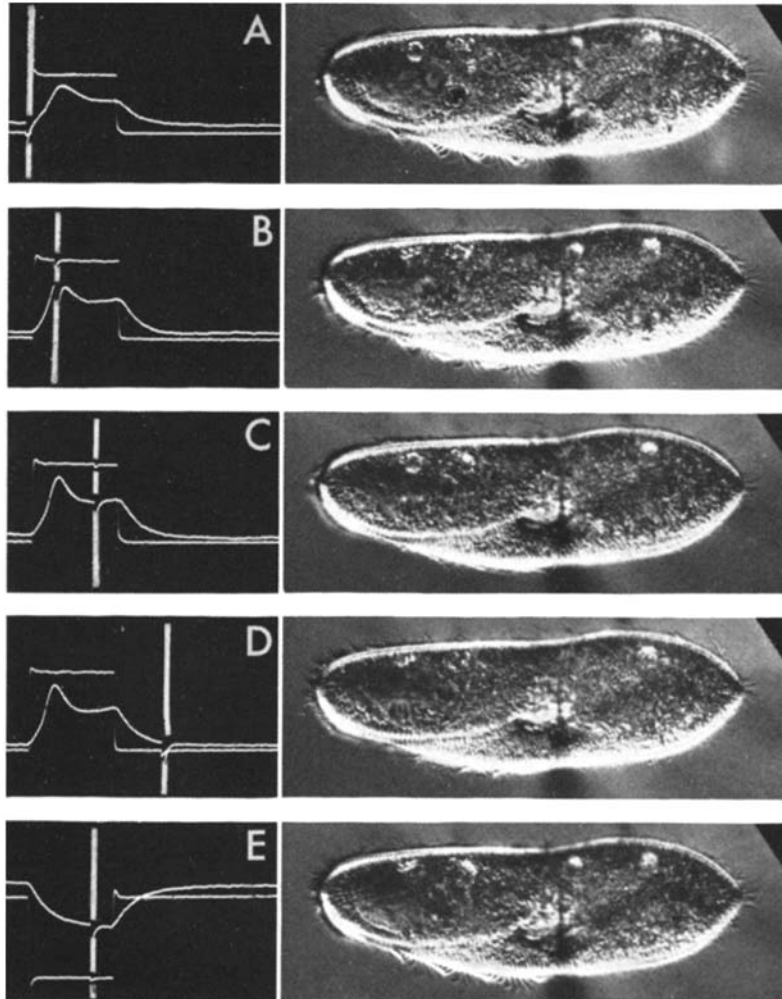


FIGURE 4. Responses of beating cilia to changes in membrane potential. Left-hand column of oscilloscope records shows potential changes recorded (upper trace) in response to intracellular injection of constant current pulses (lower trace). Vertical bar coincides with capacitive artifacts which signal discharge of strobe unit which provided illumination for each corresponding photomicrograph at right. Duration of each current pulse was 60 msec. Potential change from resting level to peak of action potential in B-D was 22 mv.

firing level. The current was injected near one end of the organism. Fig. 3, which is representative of all such experiments done on 10 organisms, shows that the action potential is recorded synchronously near both ends of the cell with no measurable delay.

Ciliary Reorientation Correlated with Depolarization

The temporal and spatial relationships between uniform changes in membrane potential and ciliary activity were examined by photographing specimens at different times after the onset of applied current pulses. In the experiment shown in Fig. 4 the actively beating cilia were "stopped" photographically by discharge of an electronic flash. The points in time at which the photomicrographs were taken can be detected in each corresponding record at the left by capacitive artifacts in the current (lower) and potential (upper) traces. The normal pattern of metachronal beat was seen prior to the depolarization induced by applied transmembrane current (Fig. 4 A). 25 msec after the peak of the depolarization a change in ciliary orientation had taken place so that a metachronal pattern was no longer apparent in the plane of the photograph (Fig. 4 C). Under visual observation this appeared to be associated with an anteriorly directed orientation (reversal) of the beating cilia.

At the peak of the depolarization (Fig. 4 B) the metachronal pattern was photographed in an intermediate condition. 35 msec after the end of the current pulse (Fig. 4 D) the cilia were again in an intermediate state leading toward recovery of the normal pattern of ciliary beat. Hyperpolarization had no dramatic effect on the appearance of the cilia as photographed in Fig. 4 E. However, as reported earlier (Kinosita et al., 1964; Naitoh and Eckert, 1969 *a*), hyperpolarization was associated with an increase in the frequency of beat.

Still photographs taken of beating cilia are difficult to interpret in the context of ciliary orientation (direction of beat) because of the three-dimensional complexity of the beat (Párducz, 1954). Interpretation of ciliary orientation was facilitated by repeating the previous experiment with Ni-paralyzed specimens (Fig. 5). The static orientation of Ni-paralyzed cilia is believed to be determined by the same mechanism which controls the direction of the effective stroke in nonparalyzed beating cilia (Naitoh, 1966).

The "resting" orientation of the paralyzed cilia prior to application of current (Fig. 5 A) was toward the posterior end of the cell. This position corresponds to forward locomotion (Naitoh, 1966). Concomitant with depolarization and regenerative reversal of the membrane potential (Eckert and Naitoh, 1969) the cilia swung forward to assume an anteriorly directed orientation (Fig. 5 B-D). This corresponds to the anteriorly directed power stroke characteristic of the "reversal" of nonparalyzed beating cilia (Naitoh, 1966). Maximum reversal of the paralyzed cilia was photographed about 40 msec after onset of depolarizing current and about 20 msec after the peak of the action potential (Fig. 5 E). This is in general agreement with Kinosita et al. (1965) who found that the latency from the onset of the spontaneous barium action potential (Naitoh and Eckert, 1968 *b*) and the maximum reversal of beating cilia ranged from 22 to 36 msec. The subsequent return of

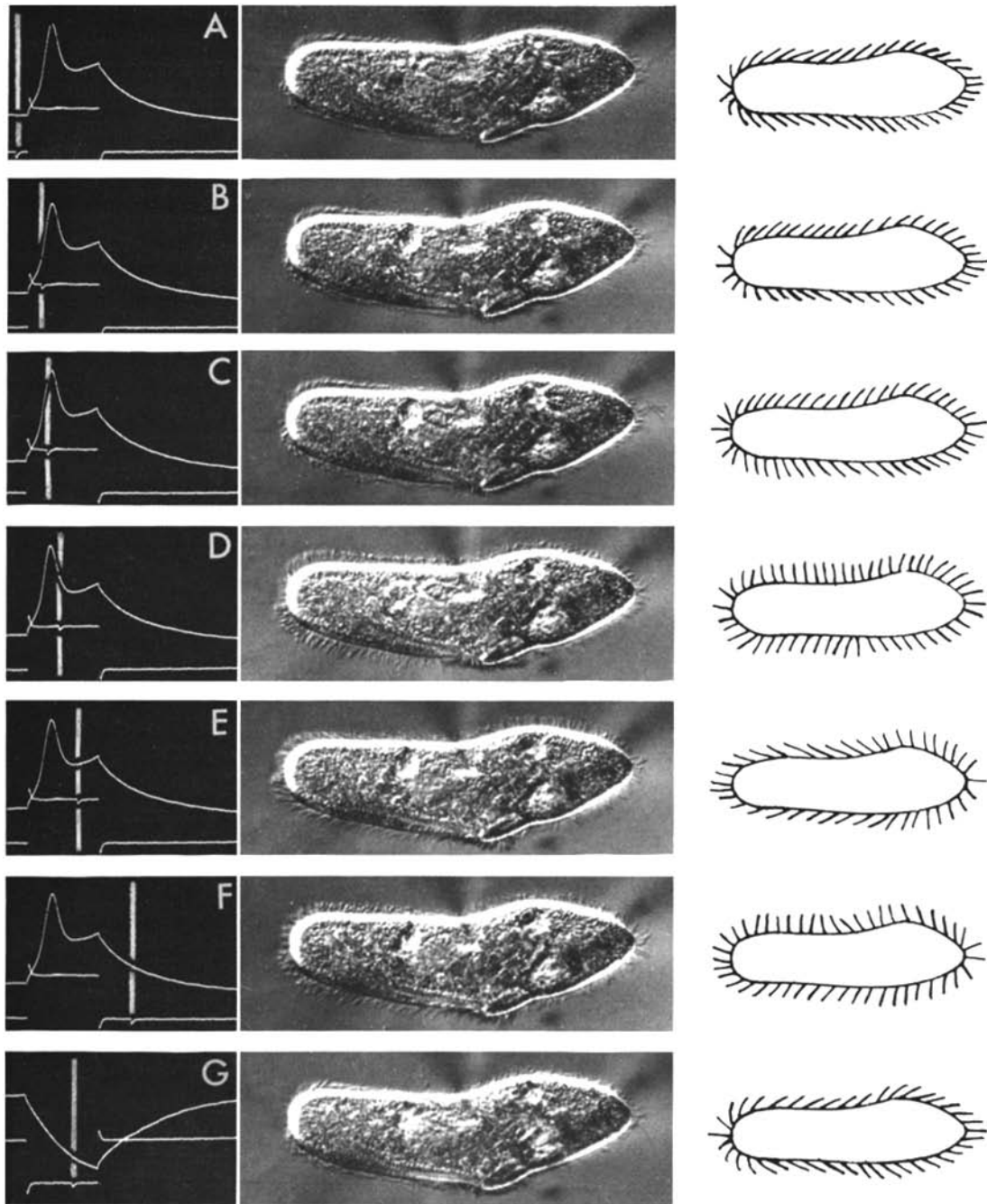


FIGURE 5. Orientation responses of Ni-paralyzed cilia to changes in membrane potential. This experiment was identical to that shown in Fig. 4 except that Ni treatment eliminated beating movements of cilia, and therefore permitted clearer visualization of ciliary orientation. Schematic drawings at right give our interpretation of corresponding photographs. Duration of each current pulse was 60 msec. Potential change from resting level to peak of action potential was 39 mv.

the cilia to their resting orientation was well under way 30 msec after the end of the current pulse (Fig. 5 F). Hyperpolarization of the membrane potential caused no perceptible deviation in this specimen from the ciliary orientation seen at the prevailing resting potential (Fig. 5 G). However, in most specimens tested hyperpolarization induced a perceptible accentuation of the posteriorly directed resting orientation of the cilia.

The cilia of the anterior part of the cell responded to depolarization with a stronger reorientation (reversal), and did so with a shorter latency than the cilia of the posterior half of the cell. The peristomal cilia, which perhaps are the most sensitive (Grebecki, 1965), could not be clearly seen in our photomicrographs. The current-passing electrode was inserted near the middle of the cell in the experiment of Fig. 5. This, together with the cable properties described above, rules out any likelihood that the cilia over the anterior half responded more strongly because of regional differences in the membrane potential. A more pronounced reorientation of cilia over the anterior half was found consistently in all experiments regardless of the intracellular location of the current electrode.

DISCUSSION

Ciliary movement in *Paramecium* and other ciliates exhibits two levels of coordination: (a) beating movements of adjacent cilia are phase-related so that metachronal waves sweep across the cell with velocities ranging from 0.1 to 0.7 $\mu\text{m}/\text{msec}$ (Sleigh, 1962); (b) the direction of the effective stroke (and the direction of metachronal wave progression, which is closely related) can change and thereby alter the direction of net force applied to the medium. Maximal reorientation of the cilia results in about a 180° shift in the direction of the power stroke, and is termed ciliary reversal (Jennings, 1899; Gray, 1928). Worley (1934) showed that the two levels of coordinated movement differed in their sensitivities to narcosis, and therefore concluded that they operate by separate mechanisms. More recent work (Naitoh, 1966, 1969), utilizing Ni-paralyzed and glycerinated specimens of *Paramecium*, has provided additional evidence for the independence of ciliary orientation (direction of effective stroke) from the beating movements of the cilia.

Coordination of Metachronism

Debate surrounding the basis for coordination of metachrony is summarized elsewhere (Sleigh, 1962, 1967; Pitelka and Child, 1964; Kinoshita and Murakami, 1967; Jahn and Bovee, 1967; Machemer, 1969). "Mechanical" theories generally hold that metachronal progression is due to both the mutual entrainment of the neighboring cilia by virtue of least mutual interference, and the apparent mechanical sensitivity of cilia (Machin, 1963; Murakami, 1963; Cleveland and Grimstone, 1964; Brokaw, 1966; Thurm, 1968). In

contrast, "neuroid" theories propose conducted regulatory impulses as the basis for interciliary coordination (Sleigh, 1962).

Taylor's (1920) neuromotor fibrils have recently been shown to be unimportant in the coordination of ciliary reversal (Okajima and Kinoshita, 1966; Naitoh and Eckert, 1969 *b*). The only form of rapid nondecremental signal conduction for which there is evidence in biological systems is the action potential of electrically excitable membranes (Hodgkin, 1958). Grebecki (1965) proposed that impulses conducted along a gradient of excitability underlie metachronal coordination in ciliates. Phase coordination of ciliary beat (metachronism) would require that the metachronal wave front propagate with a velocity identical to the velocity of the regulating signal. In an essentially isopotential cell like *Paramecium* a potential change across one portion of the plasma membrane spreads very rapidly over the entire cell membrane. For this reason signals (either action potentials or electrotonic potentials) in *Paramecium* spread at rates well above 100 μm per msec (Figs. 2 and 3). In contrast, metachronal waves progress at rates of 0.1–0.7 μm per msec (Sleigh, 1962). We can safely conclude that the phasing of ciliary beat cannot have its origin in electrical signals carried by the plasma membrane. Indeed, the literature contains no evidence that the cycle of ciliary movement is phase-related to potential changes of any kind in protozoa. This contrasts with reported changes in membrane potential correlated with movements of the comb-plate cilia of ctenophores (Horridge, 1965), and with minute potential fluctuations reportedly correlated with ciliary movement in lamellibranch gills (Satir, 1961). Mechanical artifacts have not yet been ruled out in these two cases.

The cortex in *Paramecium* and many other ciliates contains a membrane-limited reticulum of interconnected alveoli (Allen and Eckert, 1969). It is possible that the cortical reticulum generates and conducts action potentials of the conventional type intracellularly at a velocity consistent with the velocity of the metachronal wave. However, data supporting the mechanical basis for metachronism (see above) cast doubt on the necessity for conducted signals in the phasing of ciliary beat.

Coordination of Ciliary Reversal

The passive electrical properties of the plasma membrane permit the rapid spread of changes in membrane potential which coordinate changes in orientation of the cilia in *Paramecium*. Depolarization has been known for some time to cause a reorientation (reversal) of the cilia (Kinoshita, 1954; Kinoshita et al., 1964 *a*; Naitoh, 1958, 1966; Naitoh and Eckert, 1969 *a, b*). Our results have shown that the potential change is essentially uniform over the entire cell and spreads with negligible latency. For example, receptor potentials arising from local receptor currents in response to deformation of the

cell membrane (Naitoh and Eckert, 1969 *a, b*) spread electrotonically over the entire cell membrane and can thereby effect a change in the orientation of the entire complement of cilia. This is of major importance to the normal locomotor behavior of the ciliate. The control of ciliary orientation (Fig. 5) by rapid electrotonic spread of membrane potentials explains the findings of Worley (1934) on *Paramecium*, *Stentor*, and *Spirostomum*, of Okajima (1953) on *Opalina*, and also the findings of Okajima and Kinoshita (1966) on *Euplotes* (see also Naitoh and Eckert, 1969 *b*) showing that the reversal of the effective stroke occurs on both sides of an incision made in any direction or in any part of the cell. It is also consistent with the observation of Párducz (1956) that the initial manifestation of a strong reversal of the ciliary beat in *Paramecium* is a single synchronous beat of all the cilia in the reversed direction before the new pattern of reversed metachronal wave progression sets in.

The locomotor behavior of ciliates frequently depends on interciliary coordination more complex than simple synchronous changes in orientation or metachronism (Jennings, 1906; Jensen, 1959; Grebecki, 1965; Machemer, 1965). Such semiindependence of cilia may be explained in large part by the observation in Fig. 5 that the cilia over different portions of the cell surface respond with differing degrees of reversal and with differing latencies to changes in membrane potential which are shown to be spatially uniform over the entire cell. We believe this results from differences in the sensitivity and/or threshold of the mechanical effector mechanisms responsible for the reversal response of the respective cilia to given levels of membrane potential.

Machemer (1969) has proposed an anterior-posterior gradient and a cyto-stomal gradient of an unspecified nature to explain patterns of metachronal propagation. His hypothesis includes the reversal of the anterior-posterior gradient during ciliary reversal, and therefore differs conceptually from the regional differences in sensitivity to a uniform membrane potential which are shown here. In our view changes in the direction of metachronism are a secondary result of changes in the direction of the effective stroke. Jensen's (1959) proposal of three pacemaker sites (anterior, posterior, and cytopharyngeal) is also inconsistent with our findings. The anterior and posterior ends *do* exert different influences on ciliary orientation, but by virtue of their different receptor properties (Naitoh and Eckert, 1969 *a*) rather than because of any inherent local pacemaker activities. Furthermore, because of the passive electrical properties of the cell shown above, the cilia cannot tell at which end of the cell a change in membrane potential was initiated; they can only detect changes in membrane potential.

The anterior-posterior difference in ciliary response shown in Fig. 5 is in general agreement with Grebecki's (1965) hypothesis that the reversal response of the cilia shows a stomatocaudal gradient of sensitivity. However, contrary to his suggestion that it is a gradient of membrane excitability which underlies

the regional differences in ciliary responses, our findings indicate a gradient in the mechanical responsiveness of the cilia to a spatially uniform membrane potential.

The difference in behavior of anterior and posterior cilia in response to uniform depolarization of the plasma membrane has, for the sake of simplicity, been tentatively interpreted as the result of differences in sensitivity of the respective cilia to membrane *potential*. An alternative possibility is that the cilia respond instead to membrane *current* and that there are regional differences in membrane current due to an unequal distribution over the cell of membrane resistance. Naitoh (1958) found systematic regional differences in membrane resistance in *Opalina*. Low membrane resistance was correlated in that species with a greater mechanical response to depolarization of the membrane.

In summary, naturally occurring changes in membrane potential (Kinosita et al., 1964 *a*; Naitoh and Eckert, 1969 *a, b*; Eckert and Naitoh, 1969), conducted passively over the cell with great rapidity and with negligible decrement, control the orientation of all the cilia. This, together with differences in the sensitivities of the cilia of different regions of the cell to a spatially uniform membrane potential, provides a basis for the control of locomotor behavior in ciliates.

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