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# SENSORY MECHANISMS IN *PARAMECIUM*

## I. TWO COMPONENTS OF THE ELECTRIC RESPONSE TO MECHANICAL STIMULATION OF THE ANTERIOR SURFACE

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### INTRODUCTION

Electrophysiological studies have provided evidence that locomotor activity of ciliates is controlled by the electrical properties of the cell membrane (Kinosita, 1954; Kinosita *et al.* 1964; Eckert & Naitoh, 1970). Depolarization causes reversal of the direction in which the cilia beat, and hence a reversal of locomotion in the free-swimming organism. Hyperpolarization causes an acceleration of forward-swimming, primarily due to an increase in the frequency of ciliary beating.

In a preliminary report it was shown that *Paramecium* gives transient electrical responses to small deformations of the cell membrane. Mechanical stimulation of the surface of the anterior end elicits a depolarization, while similar stimulation of the caudal (posterior) surface elicits a hyperpolarization (Naitoh & Eckert, 1969*a*, *b*). Thus, the membrane transduces stimuli arising in the environment into the potential changes which adaptively modify the locomotor behaviour of the ciliate. Depolarization in response to mechanical deformation of the membrane appears to be one of the early events leading to the 'avoiding reaction' (Jennings, 1906) which occurs when the ciliate collides with an obstacle (Naitoh & Eckert, 1969*a*; Eckert, 1972*a*, *b*).

The results presented here suggest that the surface membrane of the anterior end is functionally differentiated so that mechanical stimulation increases its permeability to permit the inward flow of an ionic current. This receptor current depolarizes the entire surface membrane by electrotonic spread, and thereby evokes the regenerative calcium response (Naitoh, Eckert & Friedman, 1972) of the surface membrane.

Two papers will follow dealing with the ionic mechanisms of this receptor potential (Friedman & Eckert, 1972) and the hyperpolarizing receptor potential produced by stimulation of the caudal surface of *Paramecium* (Naitoh & Eckert, 1972*a*).

### METHODS

Specimens of *P. caudatum*, obtained from General Biological Supply, were cultured, harvested, washed, equilibrated, isolated and secured for recording as described elsewhere (Naitoh & Eckert, 1972*b*). Methods of intracellular recording and injection of current were identical in all essentials with those previously described (Naitoh & Eckert, 1968, 1972*b*; Eckert & Naitoh, 1970). Specimens were bathed in

solutions of analytical grade $CaCl_2$ and KCl made up in twice glass-distilled water. Drugs and chloride salts of cations were added or substituted as noted. In all cases the solutions were buffered to pH 7·2 with 1 mM tris-HCl. Recordings in newly changed solutions were obtained after an equilibration period of 1 min or more.

A fine glass stylus with a 10–25 $\mu$m swelling at its tip was driven by the displacement of a piezoelectric phonograph cartridge. The excursion of the stylus and duration of its displacement were controlled by voltage pulses applied to the phonocartridge. Stylus movement was approximately perpendicular to the surface of the specimen, and resulted in a dimpling of the cell surface of up to 5 $\mu$m. The inertia of the stylus and the damping properties of the aqueous medium caused the stylus movement to be slower than the applied voltage pulse; however, the precise time course of stylus movement is not critical for the present study. Mechanical stimuli were applied at intervals of at least 10 sec. except where paired stimuli were used to test refractoriness.

The location of the recording electrode within the cell with respect to the origin of the receptor current is without consequence to the various parameters (amplitude, time course, polarity) of the receptor potential since the cytoplasmic compartment of *Paramecium* is essentially iso-potential for both transient and d.c. potentials (Eckert & Naitoh, 1970). The recording electrode was inserted about midway between the ends of the specimen so as to minimize relative movement between the electrode and the membrane during mechanical stimulation at the ends of the cell.

In all cases, other than those indicated, mechanical stimuli were adjusted to sufficient magnitude to evoke uniformly maximal responses. This avoided variations in relative position of stylus and cell surface. Excursion of the stylus tip did not exceed 20 $\mu$m. Movements of the cell required frequent repositioning of the stylus to keep its position with respect to the cell relatively constant. Experiments were performed at 17–19 °C.

## RESULTS

### *Location of mechanically sensitive regions*

Exploration of the cell surface with mechanical stimuli showed that the anterior and posterior ends of the cell are far more sensitive than the surface of the mid-region. The amplitude of the hyperpolarizing posterior receptor potential (Naitoh & Eckert, 1969*a*) evoked by a given stylus displacement decreased progressively from the caudal end toward the mid-region of the cell. Likewise, depolarizations produced by stimulation rapidly fell off in amplitude as the stimulus was applied further from the anterior end. Stimuli of just sufficient amplitude to elicit maximal responses at the anterior and caudal ends of the cell elicited no signals detectable at normal display sensitivities when applied to the mid-region. Failure to record potentials from stimulation of the mid-region cannot result from algebraic summation of anterior and caudal responses, since the sum of two wave forms of different shape and time course would not be zero.

The correlation of depolarization with stimulation of the anterior surface and hyperpolarization with stimulation of the caudal surface was absolutely consistent, regardless of the orientation of the cell or arrangement of electrodes. This rules out the possibility that the potentials recorded were artifacts induced by movements

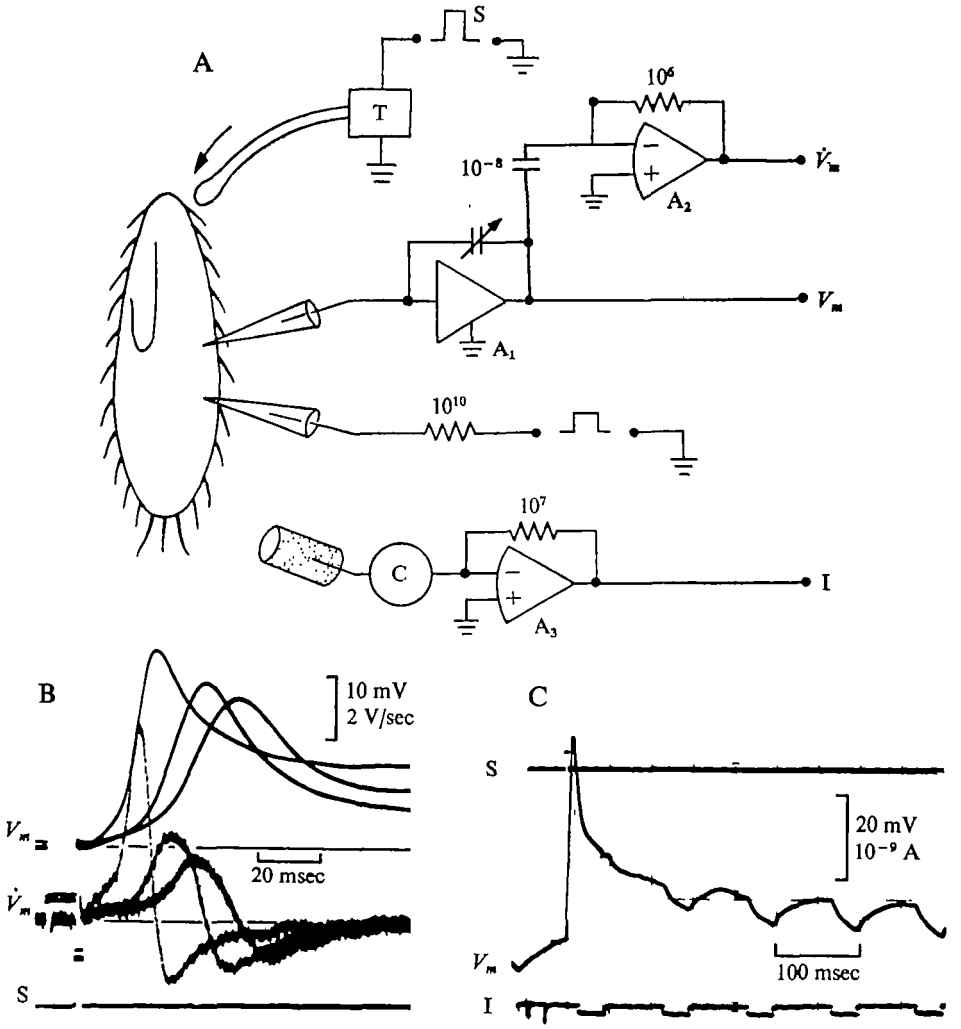


Fig. 1. (A) Arrangement for stimulation and recording. Intracellular current and voltage electrodes were inserted in the mid-region of the specimen half way between anterior and caudal extremes. The anterior end was given a mechanical stimulus with microstylus when voltage pulse (S) was applied to piezoelectric transducer (T). C, calibrator; $A_1$, head stage; $A_2$, $A_3$, operational amplifiers. (B) Graded voltage response to mechanical stimulation of cell anterior. Three sweeps at three intensities of stimulation (stylus excursions). Lowest trace shows voltage applied to transducer, upper trace shows membrane potential, and middle trace shows the time derivative of the voltage signal. (C) Conductance change during response. Hyperpolarizing current pulses (downward deflexion on lowest trace) injected with intracellular current electrode produce RC shifts in membrane potential. Shift is reduced during repolarizing phase of response to mechanical stimulus (upper trace) applied to anterior surface.

imposed on the recording electrode. The results described below were obtained with the stimulus probe located within the anterior 15% of the cell length.

### *General description of anterior response*

Mechanical stimulation of the anterior end produces a depolarization graded in amplitude with the intensity of stimulation (Fig. 1 B). This will be termed the 'anterior

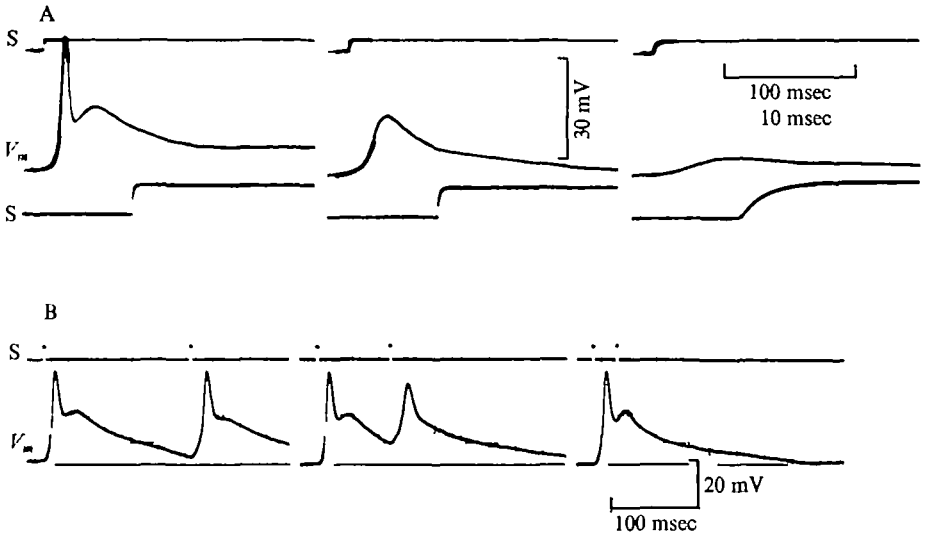


Fig. 2. (A) Rate sensitivity of response. Upper trace: voltage steps of increasing rise time (left to right) applied to piezoelectric transducer. Middle trace: membrane potential produced by mechanical stimuli to anterior surface. Lower trace: expanded sweep of segment intensified on upper traces to show rise time of voltage to transducer. Extent of excursion of microstylus identical in all three cases. (B) Refractoriness. Upper trace: voltage pulses applied to piezoelectric mechanotransducer. Lower trace: potentials recorded with intracellular electrode. As interval between first and second stimulus is reduced the spike component is reduced and finally lost.

response'. Increasing intensities of stimulation produce increasing amplitudes of response up to a saturation level, beyond which no further increase in overshoot occurs. A more quantitative correlation of electrical response with stimulus intensity is not feasible with the present technique of stimulation because of the difficulty of applying uniform stimuli to the surface when the ciliate shifts its position slightly between stimuli.

Inward (hyperpolarizing) current pulses applied to the cell produce smaller RC potential shifts during the depolarization phase of the response than at resting potential (Fig. 1 C). The reduction is more than can be ascribed to the depolarizing activation seen in the steady-state current–voltage curves (Naitoh & Eckert, 1968). This indicates a drop in membrane resistance during the response, and a recovery of the resting resistance during the repolarization.

The effectiveness of stylus displacement in evoking a response depends on the rate of displacement of the stylus (Fig. 2 A) as well as on the extent of displacement (Fig. 1 B). In Fig. 2 A the final displacement of the probe was kept constant while the time constant of the exponential rise in voltage applied to the piezoelectric transducer was increased. This produced a corresponding drop in the rate of displacement of the probe as it tapped the surface of the ciliate. As the *rate* of displacement was reduced the depolarization produced by the stimulus became smaller. Both the rate and degree of deformation should increase with the velocity of a ciliate colliding with an object.

Two stimuli separated by 150 msec or more both evoke similar electrical responses. When the interval is reduced below this duration (Fig. 2 B), the second response is progressively suppressed, and it is essentially absent when the interval is less than 50 msec.

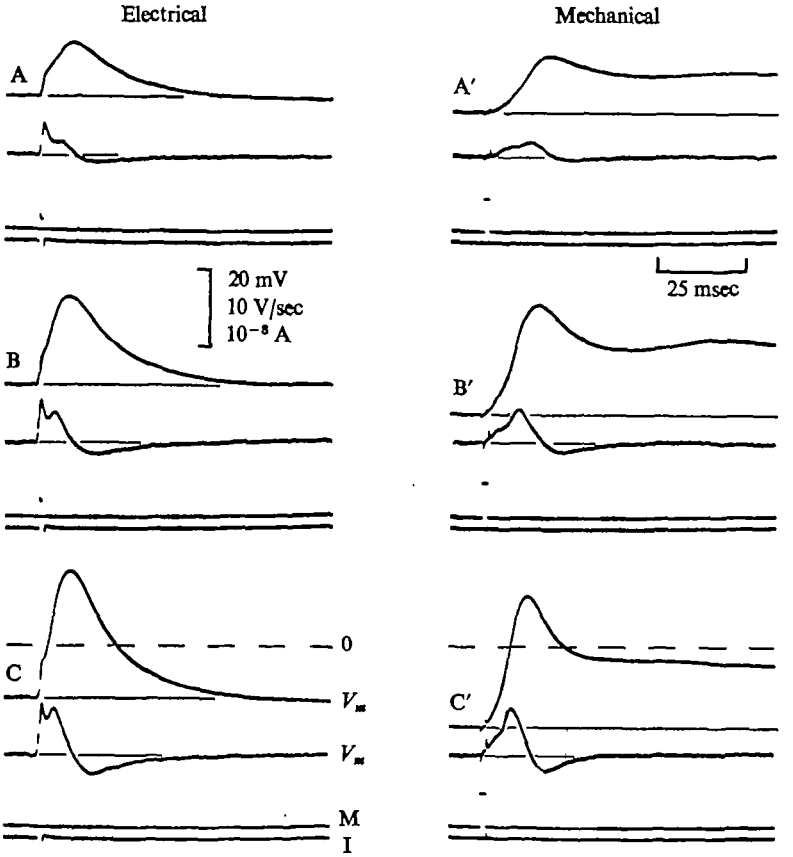


Fig. 3. Depolarizing responses evoked by injection of 2 msec current pulse with intracellular electrode (left), and mechanical stimulation of anterior surface (right). Stimulus intensity increased from A to C and from A′ to C′. M, mechanical stimulus; I, injected current.

### *Two components of the response*

The depolarizing response to increasing intensities of mechanical stimulation of the anterior surface is seen in Fig. 1B. The earliest response to mechanical stimulation is a relatively slow depolarization. The early component is followed by a delayed faster component so that an inflexion appears on the upstroke of the depolarization. Both the early and the later components are graded in rate of rise with the intensity of the stimulus. As the mechanical stimulus is made less effective by lowering the stylus velocity (Fig. 2A), the fast spike-like component disappears, leaving a slow wave which may tentatively be considered the continuation, in the absence of the fast component, of the early slow part of the upstroke.

The faster, spike-like component of the response closely resembles the graded regenerative calcium response of *Paramecium* generated by inward calcium current in response to a depolarization (Naitoh *et al.* 1972). This is evident when responses to electrical and mechanical stimuli are compared in the same cell (Fig. 3). Although there are differences in the time course of the slow components, the spike-like components of responses to mechanical and electrical stimulation are similar in shape and

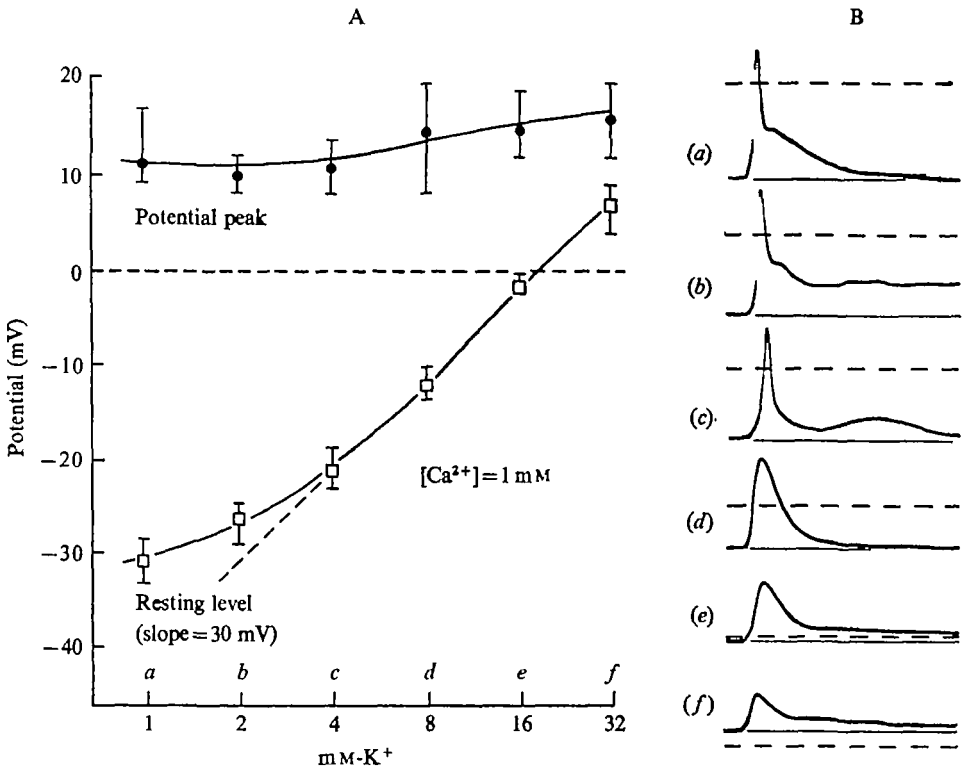


Fig. 4. Response to mechanical stimulation of anterior surface as function of $[K]_o$. $[Ca]_o$ held constant throughout at 1 mM. (A) Resting potential shows slope of 30 mV per tenfold increase in $[K]_o$, peak of response to mechanical stimulation shows slope of < 10 mV. (B) representative electrical records at concentrations (*a–f*) in A. Dashed lines give reference (zero) potential for recordings.

rate of rise. It is reasonable to conclude that the slow depolarization is a receptor potential evoked directly by the mechanical stimulation, and that this serves to evoke the regenerative calcium response.

### *Ionic mechanism of fast component*

If the fast component of the mechanically evoked response is identical to the calcium response evoked by direct electrical stimulation (Fig. 3, A′–C′) it should exhibit similar ionic requirements. This was tested as shown in Figs. 4 and 5. The overshoot exhibited little or no sensitivity to altered $[K]_o$ (Fig. 4). In contrast, the peak of the spike-like component showed an increment of about +20 mV per tenfold increase in $[Ca]_o$ (Fig. 5). Sensitivity to $[Ca]_o$ somewhat below that of an ideal calcium electrode and insensitivity to $[K]_o$ are characteristic of the regenerative calcium response evoked by direct electrical stimulation with injected depolarizing current (Naitoh *et al.* 1972). It is also noteworthy that the changes in amplitude and shape of the spike-like component with altered $[K]_o$ and $[Ca]_o$ parallel those of the electrically evoked responses (figs. 7, 8 and 10 in Naitoh *et al.* 1972).

The overshoot of the response to mechanical stimulation showed little or no sensitivity to $[Na]_o$, $[Mg]_o$, or $[Mn]_o$ (Fig. 6), again in agreement with the electrically evoked

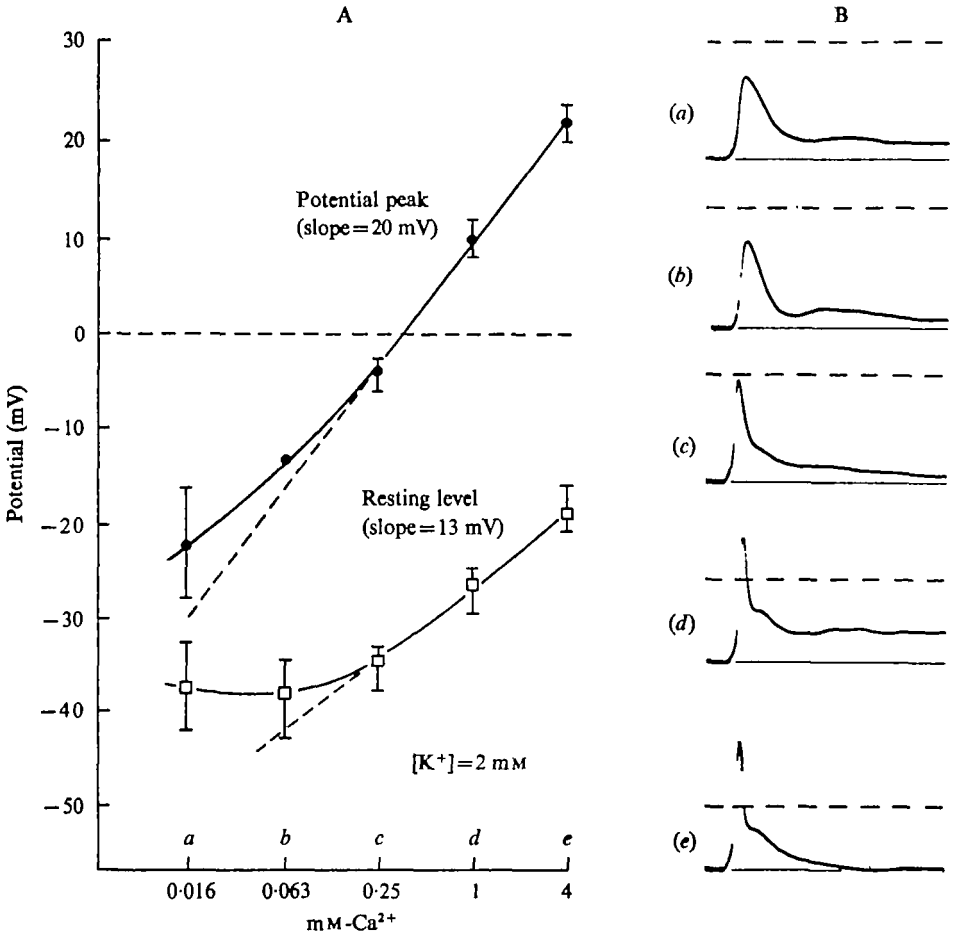


Fig. 5. Response to mechanical stimulation of anterior surface as function of $[Ca]_0$. $[K]_0$ held constant throughout at 2 mM. (A) Resting potential shows slope of 13 mV per tenfold increase in $[Ca]_0$, peak of response to stimulation shows slope of 20 mV. (B) Representative records to concentrations (*a*–*e*) in A. Dashed lines give reference (zero) potential for each recording.

calcium response. The similarity of action of TEA in both cases is especially striking (Naitoh & Eckert, 1972*a*, figs. 8, 9; Naitoh *et al.* 1972, figs. 10 and 11), with TEA causing a slowing of the repolarization, and a small increase in overshoot.

Steady depolarization inactivates the regenerative calcium response evoked by a given intensity of stimulating current (Naitoh *et al.* 1972). Inactivation is also evident in the mechanically evoked response (Fig. 7). The regenerative component is progressively reduced in rate of rise and amplitude when the membrane is depolarized with injected d.c. current. The slow component of the response is also depressed by steady depolarization. This is attributable at least in part to the large increase in membrane conductance (rectification) which accompanies depolarization in excess of 20 mV (Naitoh & Eckert, 1968). Rectification is evident in the disproportionate amount of current required to produce the final increment of depolarization at the far right in Fig. 7. The large conductance at this potential may be short-circuiting the receptor current.

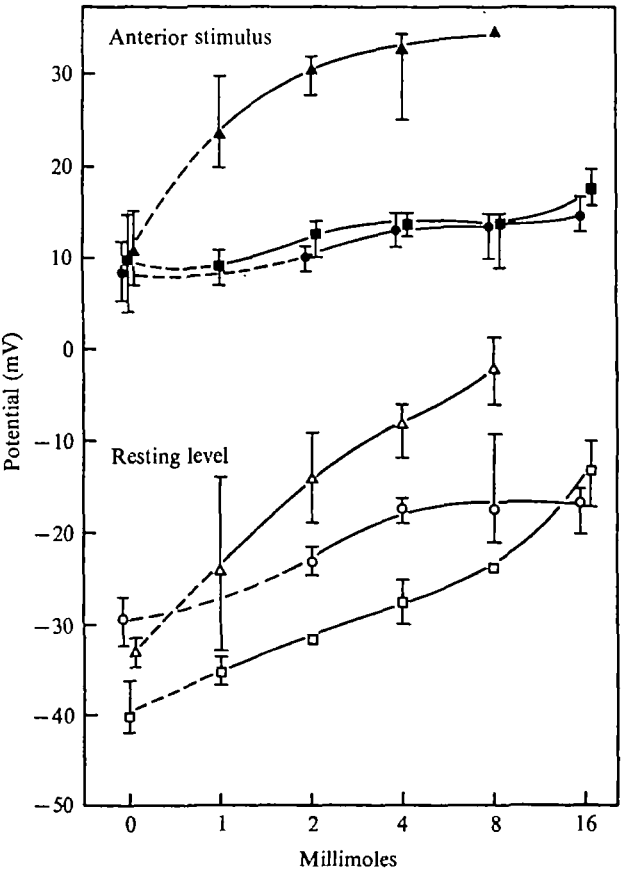


Fig. 6. Effect of $MnCl_2$, $MgCl_2$ and NaCl on overshoot and resting potential. $CaCl_2$ and KCl each 1 mM throughout, while one of the other salts was added in the concentration shown on abscissa. ▲, $Mn^{2+}$; ●, $Mg^{2+}$; ■, $Na^+$.

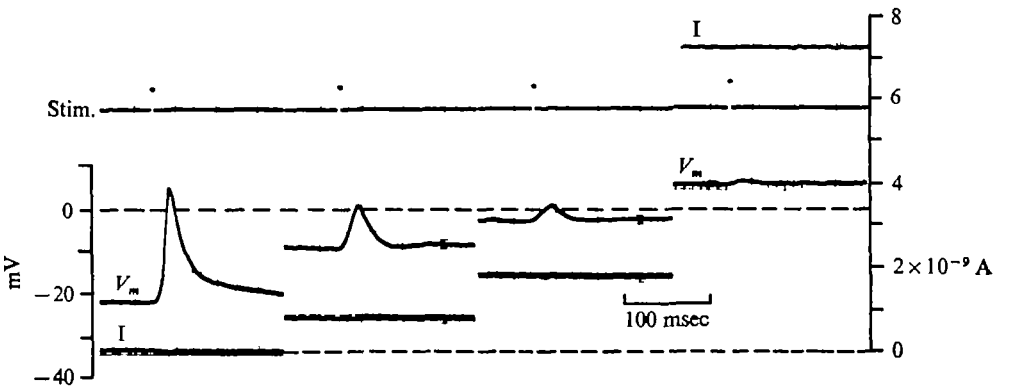


Fig. 7. Response to mechanical stimulation during d.c. depolarizing shifts of the membrane potential. Stim., mechanical stimulus; $V_m$, membrane potential (left-hand scale); I, injected current (right-hand scale). Mechanical stimulus evokes progressively smaller depolarizing response as cell interior is made progressively more positive. Membrane resistance drops markedly beyond depolarization of about 20 mV.

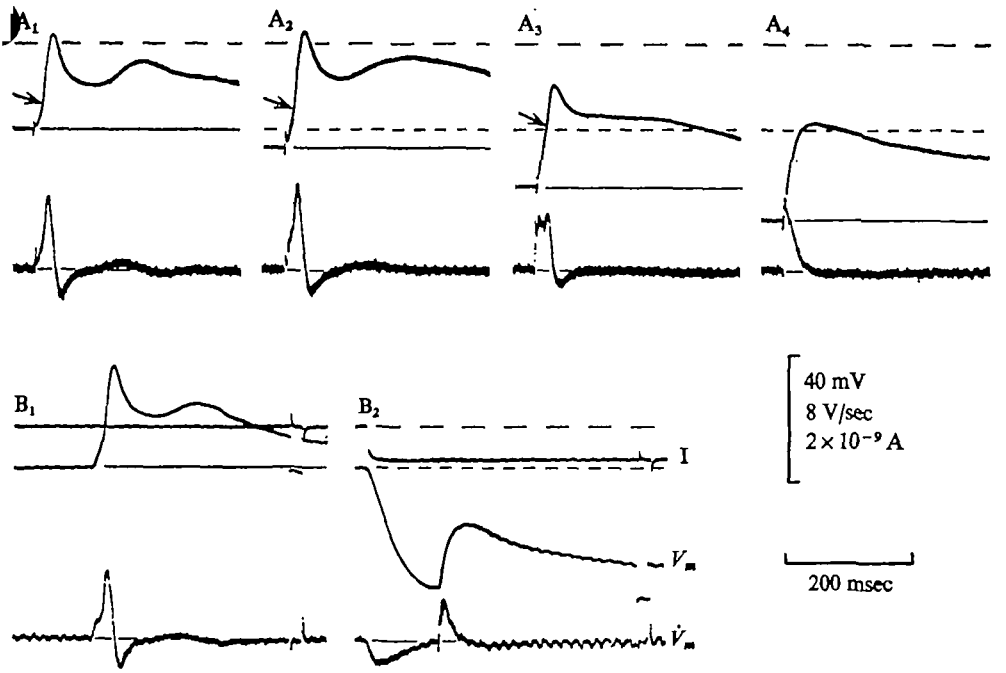


Fig. 8. Suppression of regenerative calcium response by hyperpolarization. (A) Progressive hyperpolarization ($A_1$–$A_4$) with inward d.c. current gradually eliminated the regenerative component leaving the receptor potential unobscured. Dashed lines give zero potential and resting potential. (B) Similar experiment showing the effect of a strong hyperpolarizing pulse beginning prior to the mechanical stimulus ($B_2$). The zero current level is also the zero potential level.

### *The anterior receptor potential*

Various attempts were made to block the regenerative calcium response so that the receptor potential could be examined without eliciting the spike-like component. Tetrodotoxin, procaine, tetracaine, $Mg^{2+}$, $Mn^{2+}$ and $Co^{2+}$ are all ineffective (Naitoh *et al.* 1972). Lanthanum chloride also fails to block the calcium response in *Paramecium* (unpublished observations). It has been reported that increased temperature reduces calcium responses in crustacean muscle (Fatt & Katz, 1953), but this, too, is ineffective in abolishing the regenerative calcium response in *Paramecium*. A method which proved successful was hyperpolarization.

Progressive hyperpolarization of the membrane with inward current produces a progressive suppression of the regenerative component (Fig. 8). With sufficient hyperpolarization only the slow component of the response remains (Fig. 8$A_4$ and $B_2$). The regenerative component returns as an addition to the slow component when the membrane is allowed to return to the resting potential. The slow component, which has no inflexion on the upstroke, is interpreted as the pure receptor potential produced by receptor current entering the cell across the mechanically stimulated membrane. While quantitative comparisons of response amplitude are difficult with the present stimulating technique, it is evident that the initial rate of rise and the amplitude of the receptor component increase with hyperpolarization. This is to be expected (Ginsborg, 1967) if the receptor potential results from an increased conductance of the cell membrane and has an equilibrium potential such that hyperpolarization increases

the driving force on the current carrying ion(s). It is furthermore evident in Fig. 8, $A_1$ that the inflexion (arrows) leading into the regenerative component occurs at an approximately constant value of membrane potential. In $A_4$ the receptor potential barely reaches this potential level.

## DISCUSSION

The results show that the depolarization evoked by mechanical stimulation of the anterior surface of *Paramecium* consists of two different and separable electric components. The direct response to the stimulus is a slow, graded receptor potential which gives rise to a faster, spike-like component. The spike-like component appears to be identical with the regenerative calcium response produced by direct electrical stimulation (Eckert & Naitoh, 1969; Naitoh *et al.* 1972). This conclusion is based on the following similarities between the calcium response and the spike-like component evoked by mechanical stimulation: (1) The spike-like responses to mechanical and electrical stimulation have similar shapes and amplitudes and both arise with inflexions on the upstroke of slower depolarizations (Fig. 3). (2) Both show similar periods of refractoriness (Fig. 2B; fig. 5 in Naitoh *et al.* 1972). (3) Steady depolarization by passage of outward current reduces the overshoot in both cases, presumably due to calcium inactivation and/or increased leakage conductance (Fig. 7 in this paper and fig. 6 in Naitoh *et al.* 1972). (4) $[Ca]_0$ and $[K]_0$ affect overshoot, amplitude and wave shape identically in both cases (Figs. 4 and 5 in this paper; figs. 7 and 8 in Naitoh *et al.* 1972). (5) The relation of amplitude and shape to the ratio $[K]/[Ca]^{\frac{1}{2}}$ is similar in both cases. (6) TEA causes prolongation of repolarizing phases in both cases (fig. 8 in Naitoh & Eckert, 1972; fig. 12 in Naitoh *et al.* 1972). (7) TEA, $K^+$, $Na^+$, $Mg^{2+}$ and $Mn^{2+}$ produce negligible or similar small increments in overshoot in both. (8) Both are suppressed by hyperpolarization (Fig. 8).

It can be inferred, then, that a mechanical stimulus to the anterior surface of the cell leads to an inward current (receptor current) which spreads electrotonically (Eckert & Naitoh, 1970) to produce a depolarizing receptor potential. The electrotonic depolarization then activates the electrically sensitive calcium conductance, and the regenerative 'calcium response' is produced over the entire cell membrane.

The regenerative inward calcium current responsible for the calcium response activates the reversal of ciliary beating and causes the cell to swim in reverse (Eckert, 1972*a*, *b*). Thus, the calcium influx which produces the regenerative component of the potential transient is the event which couples the locomotor response with the local electrical response of the mechanically sensitive receptor membrane at the anterior end of the ciliate. The calcium response, instead of being an all-or-none action potential, is graded with the intensity of the stimulus and the receptor current (Figs. 1B, 3). It can be assumed that this results from gradations in depolarizing stimuli. This is interesting and significant for at least two reasons. First, the cable properties of the ciliate (Eckert & Naitoh, 1970), which permit efficient electrotonic spread of membrane potentials with little decrement, render an all-or-none propagated action potential unnecessary for communication between the receptor surface and the population of effector organelles. Secondly, differences in stimulus strength are reflected in the intensity of calcium influx, and so the locomotor response of the ciliary apparatus is appropriate in intensity to the strength of the stimulus.

In contrast to the metazoan nervous sytem, which is organized largely for the alternation of analogue (receptor and synaptic potentials) and digital (spikes) signals, the far simpler electrophysiological organization of *Paramecium* utilizes only continuously graded transforms in the chain from stimulus to behavioural response.

SUMMARY

1. Specimens of *Paramecium caudatum* were stimulated mechanically with a piezoelectrically driven microstylus. Membrane potentials were monitored and intracellular polarizing currents were passed with glass microelectrodes.

2. Mechanical stimulation of the anterior 15% of the cell's length produced depolarizing transients in membrane potential. This 'anterior response' was amplitude-graded up to a maximum with stimuli of increasing intensities, and was accompanied by a drop in membrane resistance.

3. The anterior response consists of two components, a receptor potential in direct response to stimulus transduction, and a secondarily evoked regenerative component. Both are graded. With hyperpolarization of the cell membrane the regenerative component was suppressed and the receptor potential alone was seen. With hyperpolarization the size of the receptor potential was increased.

4. The regenerative component is identical with the 'calcium response' which is elicited by direct stimulation with injected depolarizing current. This conclusion is supported by similar sensitivity of the overshoot to extracellular concentrations of calcium, similar refractory periods, similar inactivation by depolarization and suppression by hyperpolarization, and similar prolongation of the time course by TEA.

5. It is concluded that local inflow of receptor current through the stimulated membrane of the anterior end depolarizes the cell membrane by electrotonic spread, activating the electrically excited calcium conductance of the membrane, and thereby eliciting the regenerative calcium response.

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