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32. The work of developing the proposal outlined in this article was performed by a series of ad hoc committees chaired by Prof. W. E. Gordon, Rice University. In the reports that constitute (*1*) and (*30*) are listed the names of all who contributed to the studies, which were supported by the National Academy of Sciences and by the National Science Foundation under grant GA-27447. I thank S. A. Bowhill and W. E. Gordon for critical comments on the manuscript.

Bioelectric Control of Ciliary Activity

Locomotion in the ciliated protozoa is regulated by membrane-limited calcium fluxes.

Roger Eckert

Recent findings offer compelling evidence that membrane-regulated changes in the concentration of intracellular calcium ions control ciliary activity and, thereby, the locomotion of ciliated protozoa such as *Paramecium*. The relations between membrane function and ciliary activity appear to be as follows: The intracellular concentration of free calcium ions (Ca^{2+}) is maintained far below the extracellular concentration. An increase in the intracellular Ca^{2+} concentration produces a shift in the direction of ciliary beating along with an increase in frequency. In the absence of stimulation the Ca^{2+} which slowly leaks into the cell is pumped out at a similar rate to maintain a steady state. Depolarization increases the Ca^{2+} conductance of the membrane, permitting a strong influx of extracellular Ca^{2+}. As a consequence, the Ca^{2+} in the cell cortex reaches a concentration sufficient to activate a shift in the direction of the effective stroke as well as an increase in the frequency of beating. This causes the ciliate to swim backward. Thus, the cell membrane in responding to environmental stimuli with altered conductances and potentials controls the ciliary apparatus by regulating the rate at which Ca^{2+} leaks into the cell.

The electrical properties of the ciliate membrane are of interest for several reasons. Besides providing a relatively complete mechanistic picture of integrated locomotor behavior, recent technical advances in this area have opened the way for some novel approaches to the investigation of membrane function. In one such approach, use is made of a mutant of *Paramecium* in which electric properties of the cell membrane have been genetically altered (*1*).

Membrane Potential and Ciliary Activity

A relationship between ciliary activity and electric current is evident in the galvanotaxis of *Paramecium* (*2*). When a current is passed through the medium in which a *Paramecium* is grown, the cilia on the side of the cell facing the anode increase their frequency of beating. The cilia on the cathodal side beat in reverse if the current is strong enough. The result of such differential ciliary activity is that the paramecium swings around so that the anterior end points toward the cathode, and then swims, with servo correction of its orientation, toward the cathode. At currents sufficiently strong to produce reversal over more than half the surface from the fore end back, migration is toward the anode. Jahn (*3*) has suggested that paramecia act as core conductors, since the cell membrane provides a large resistance compared to the cytoplasm and the surrounding volume conductor of pond water. Although Jahn chose to stress electrophoretic migration of Ca^{2+} in the applied field, it now appears to be the local potential difference across the cell membrane produced by the applied current which is the basis of the galvanotaxis. Thus, the membrane on the side facing the anode is somewhat hyperpolarized by inward electrotonic current while the membrane facing the cathode is somewhat depolarized by outward electrotonic current. As noted below, depolarization elicits ciliary reversal and hyperpolarization elicits an increased frequency of beating in the "normal" direction.

The importance of membrane potential in the control of ciliary activity has become evident from the work on *Paramecium* and *Opalina* at Tokyo University (*4*, *5*), and more recently from our laboratory at the University of California, Los Angeles (*6–8*). It has been demonstrated by means of intracellular recording and stimulating techniques that depolarization and hyperpolarization are both accompanied by an increase in the frequency of ciliary beat-

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ing. Depolarization also produces a shift in the plane through which the cilium moves during the effective stroke. The direction of the effective stroke can shift with respect to the direction of the stroke characteristic of normal forward locomotion. Since there is an essentially fixed angular relation between the plane of the effective stroke and the orientation of the metachronal wave front, the latter shifts by about the same angle as the effective stroke (*9*). Ciliary reversal is not an all-or-none shift in the direction of the power stroke, but is, instead, continuously graded through all angles up to a maximum of about 180° (*9*, *10*). The mechanism of ciliary beating and the mechanism of altering the orientation of the effective stroke are both poorly understood, and are not included in this article.

A stylized example of ciliary reversal produced by membrane excitation is shown in Fig. 1. Ciliary beating was eliminated with 1 m*M* nickel chloride (*11*) to simplify documentation of the orientation response of the cilia. In the absence of stimulation, Ni^{2+}-paralyzed cilia pointed toward the rear of the paramecium, approximately as they are positioned at the end of the power stroke. When the cell was stimulated with a pulse of outward current across the membrane, the depolarizing electric response was followed by an anteriorly directed shift in the orientation of the cilia. This reached a maximum about 30 milliseconds after the peak of the response, and then the cilia returned to their resting positions.

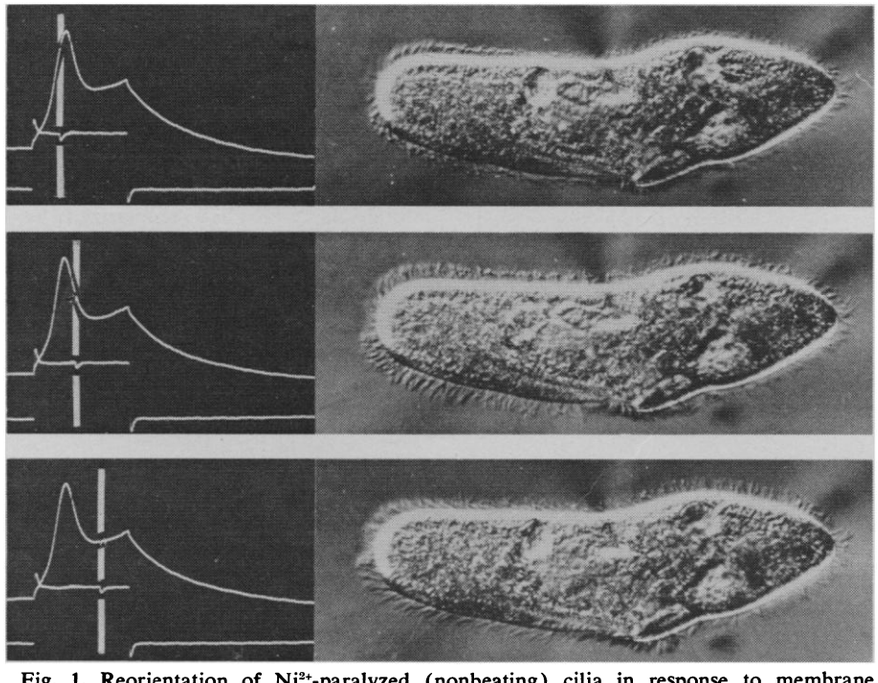

Fig. 1. Reorientation of Ni^{2+}-paralyzed (nonbeating) cilia in response to membrane excitation in *P. caudatum*. Photomicrographs taken by strobe illumination during electrical recording (left) at instant shown by vertical white bar. Upper trace, membrane potential; lower trace, 60-msec stimulating current pulse injected with intracellular current electrode. Fore end of the specimen is at the left. [Permission of Rockefeller University Press (*8*)].

Intracellular Calcium Ions Regulate Cilia

Removal of extracellular Ca^{2+} results in an uncoupling of ciliary reversal from changes in membrane potential. There is reason to believe that the importance of extracellular Ca^{2+} lies in its effect on intracellular Ca^{2+} concentrations (*12*). A direct role of intracellular Ca^{2+} in the control of ciliary reversal is indicated by recent experiments of Naitoh (*14*) in which he utilized as models paramecia extracted with the neutral detergent Triton X-100 after the method of Gibbons *et al.* (*15*). Naitoh found that the extracted paramecium swims forward in a solution of 4 m*M* ATP (adenosine triphosphate), 4 m*M* $MgCl_2$, 50 m*M* KCl, 10 m*M* tris buffer, and Ca^{2+} between 10^{-8} and 10^{-7} mole/liter. The cilia beat with perfectly coordinated metachrony even though the cell interior is freely exchangeable with the exterior. When the concentration of Ca^{2+} is raised progressively from 10^{-8} to 10^{-5} mole/liter there is a gradual shift in the plane of the effective stroke. At about 10^{-6} mole/liter the effective stroke is directly lateral to the long axis of the cell, causing the extracted paramecium to spin about in one place without progress forward or backward. As the concentration is increased further, the direction of the effective beat shifts more toward the anterior, so that the extracted cell swims backward.

The experiments with Triton-extracted paramecia extend earlier work in which nonbeating cilia in models of *Paramecium* extracted in glycerine were shown to swing into an anteriorly directed orientation when the concentration of Ca^{2+} in the reactivation medium was raised in the presence of ATP (*16*). The Ca^{2+}-evoked shift in orientation of the nonbeating cilia characteristic of the glycerinated model is reminiscent of the anteriorly directed shift exhibited by nonbeating cilia of living specimens paralyzed with 1 m*M* $NiCl_2$ when the membrane is depolarized (Fig. 1).

Membrane Potential and Intracellular Calcium Ions

It is noted above that appropriate stimuli produce anteriorly directed reorientation of static cilia, and reversed beating in active cilia. In the extracted models of *Paramecium* these effects (forward shift of static cilia and reversal of beating cilia) are produced by raising the concentration of Ca^{2+}, while in the living cell they are produced by membrane depolarization. This suggests that in the living ciliate depolarization may produce ciliary reversal by increasing the concentration of intracellular Ca^{2+}. If the calcium sensitivity of the mechanism producing reversal is the same in the extracted models as in the living cell, the free Ca^{2+} of the forward-swimming living cell must be in the range of 10^{-7} mole/liter or below. In crustacean muscle and squid axon, for comparison, the concentration of intracellular Ca^{2+} is well below 10^{-6} mole/liter (*16a*). For *Paramecium* the extracellular concentration of Ca^{2+} generally ranges between 10^{-4} and 10^{-2} mole/liter.

The net rate at which Ca^{2+} leaks into the cell is defined as the calcium current (*17*),

$$I_{Ca} = g_{Ca} (V_m - E_{Ca}) \quad (1)$$

where I_{Ca} is the calcium current, proportional to conductance of the membrane to Ca^{2+}, g_{Ca}, and the electromotive force (EMF) acting on Ca^{2+}, which is the difference between the membrane potential, V_m, and the equilibrium potential for Ca^{2+}, E_{Ca}. If the intracellular concentration of Ca^{2+} is 10^{-5} times the external concentration, E_{Ca} should be in the range of +150 mv. Since the resting potential of *Paramecium* ranges from −20 to −40 mv (*18*) the EMF acting on Ca^{2+} is in the neighborhood of 180 mv. In the resting state Ca^{2+} must be extruded from the cell at a rate equal to the rate at which it leaks into the cell, so as to keep the net flux of Ca^{2+} at zero. Any increase in the Ca^{2+} conductance, g_{Ca}, should increase the Ca^{2+} influx as described by Eq. 1. This occurs when the membrane of *Paramecium* is given a depolarization stimulus (*19*). The influx of Ca^{2+} can be inferred from the data of Fig. 2 in the light of the ionic hypothesis of Hodgkin, Huxley, and Katz (*20*). The membrane responds to a depolarizing stimulus with a graded regenerative depolarization (Fig. 2, A–C) in which the peak potential (overshoot) is related to the extracellular Ca^{2+} concentration (Fig. 2E). As the concentration of extracellular Ca^{2+} is raised the overshoot is increased. This effect is most pronounced for external concentrations of calcium, barium, and strontium ions (*21*) and is interpreted as the result of an inward Ca^{2+} current which flows as a consequence of a transient increase in membrane conductance to Ca^{2+} (Ba^{2+}, Sr^{2+}). The inward Ca^{2+} current is analogous to the inward Na^+ current in neuronal or muscle membranes. Although it is a regenerative current, it does not produce an all-or-none action potential. Instead, the "calcium response," which resembles the calcium response of crustacean muscle (*22*) and nerve terminals (*23*), is graded with the degree of electrotonic depolarization. Figure 3A shows the relations presumed to exist between changes in membrane potential in response to stimuli (abcissa) and transient increases in Ca^{2+} conductance. The consequent transient Ca^{2+} currents produced by Δg_{Ca} are shown in Fig. 3B. These relations resemble those for Na^+ conductance and Na^+ current in squid axons (*20*).

Those portions of the surface membrane of *Paramecium* covering the cilia constitute about two-thirds of the total surface membrane area (*8*). If it is assumed that these portions of the surface membrane participate in the calcium response, one can estimate the minimum number of calcium ions carrying charge into each cilium through the surface membrane to produce a calcium response of a given amplitude. From this one can calculate (*24*) the approximate increment in free calcium concentration produced in each cilium by such a calcium response. Assuming a specific membrane capacitance of 10^{-6} farad/cm^2, the capacitance of the membrane covering one cilium will be 6×10^{-14} farad. To produce a depolarization of 1 mv across this capacitance requires 6×10^{-17} coulomb or 3×10^{-22} mole of Ca^{2+}. Dispersed in free solution in the volume of a cilium, this amount of additional Ca^{2+} will produce in the cilium an increment in the Ca^{2+} concentration of 10^{-6} mole/liter (*24*). This calculation neglects the percentage of nondiffusible space within the cilium, and the short-circuiting effect of a potassium efflux during the calcium response. Correction for these unknowns would increase the value calculated for the increment in calcium concentration.

In summary, depolarization causes a transient increase in the conductance of the membrane to Ca^{2+}. Since Ca^{2+} has a large electrochemical gradient ($V_m - E_{Ca}$) directed from the cell exterior to cell interior, a net influx of Ca^{2+} occurs, and produces a regenerative depolarizing calcium response even though the stimulating current (receptor current or microelectrode current) crosses the membrane in the opposite (outward) direction. Because of the large ratio of surface to volume of each cilium, the Ca^{2+} crossing the membrane of each cilium during a calcium response is sufficient to raise the intraciliary Ca^{2+} concentration by increments of at least 10^{-6} mole/liter.

Membrane-Regulated Calcium Controls Reversal

The central idea of this article is that the calcium current (Ca^{2+} influx) produced by an increase in Ca^{2+} conductance of the surface membrane results in ciliary reversal. It is assumed that following a depolarizing stimulus the concentration of Ca^{2+} within the cilia rises as the inward calcium current increases.

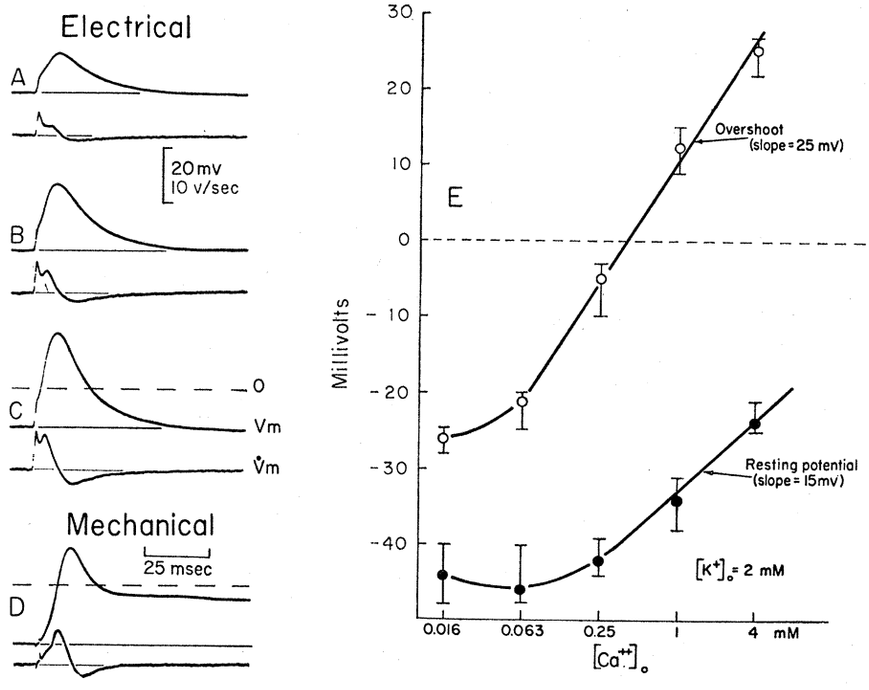

Fig. 2. The calcium response in *P. caudatum*. (A–C) Regenerative excitation (calcium responses) graded in amplitude (V_m) and rate of rise ($\dot{V}_m$) with increasing intensity of 2-msec current pulse delivered with intracellular electrode. (D) Electrical response to mechanical stimulation of anterior surface. Results in A through D were recorded from the same cell in 1 m*M* $CaCl_2$ and 2 m*M* KCl. (E) Resting potential and peak amplitude (overshoot) of maximal regenerative responses to electrical stimulation plotted against extracellular Ca^{2+} concentration. The concentration of KCl was held constant at 2m*M* throughout. [Modified from (*6*) and (*19*)]

The proposed sequence of events leading from a depolarizing stimulus to ciliary reversal is outlined in Fig. 4. In the resting state there is a steady influx of Ca^{2+} because of its very steep electrochemical gradient. The influx is balanced in the resting state by an equivalent efflux driven by active transport. An increase in Ca^{2+} conductance, g_{Ca}, produced by depolarization permits an increase in Ca^{2+} influx according to Eq. 1; this will cause influx to exceed Ca^{2+} efflux. The inward Ca^{2+} current continues until g_{Ca} returns to normal or until the rate of extrusion by active transport of Ca^{2+} increases to compensate for the increased influx. The result is a temporary increase in the intracellular concentration of Ca^{2+} which activates the mechanism for reversed beating of the cilia.

The hypothesis that ciliary reversal is coupled to membrane potential by a membrane-limited influx of Ca^{2+} receives support from the following observations.

1) Reversal occurs in extracted models of *Paramecium* when the concentration of Ca^{2+} is raised to 10^{-6} mole/liter or higher. It is probable that in the living cell similar increases in intracellular Ca^{2+} concentration also produce reversal. As shown above, an increment of 10^{-6} mole of Ca^{2+} per liter or more can be produced in each cilium of a living paramecium as the result of a brief increase in Ca^{2+} conductance (calcium response) of the surface membrane in response to a depolarizing stimulus.

2) Extracellular Ca^{2+} is essential for reversal in response to both electrical and ionic stimuli applied to the living cell (*4, 13, 25, 26*). Since the influx of Ca^{2+} depends on the Ca^{2+} concentration gradient ($V_m - E_{Ca}$), there will be essentially no net influx of Ca^{2+} if the external concentration is lowered so that the electrochemical gradient for Ca^{2+} approaches zero (Eq. 1).

3) Stimuli which depolarize the membrane also evoke reversal of ciliary beat. These include outward current injected into the cell with a microelectrode (*27*) or depolarizing receptor current evoked by mechanical stimulation (*6, 7*); or depolarization of the cell by an increase in extracellular cations (*4, 18, 25, 28*); and spontaneous depolarization (*4, 29*). Further evidence implicating membrane-limited Ca^{2+} influx in the coupling of ciliary reversal to membrane depolarization comes from high speed cinematography of ciliary activity in *Paramecium* carried out during electrophysiological stimulation and recording (*30*). Reversal of ciliary beating was correlated with the occurrence of a calcium response, and never occurred in response to depolarizing stimuli which produced potential changes that were purely electrotonic. The period of reversed beating grew in duration as stronger stimuli evoked calcium responses of greater amplitude and rate of rise.

4) It can be predicted from Eq. 1 that I_{Ca} will reverse sign (calcium will flow out across the membrane against its concentration gradient) if V_m is made more positive than E_{Ca}. When this occurs depolarization should not produce reversal (Fig. 3, B and C). This, indeed, is the case. Naitoh (*27*) found that low and moderate outward currents produced reversal in *Opalina*, but that at very high intensities of outward current (strong intracellular positivity) the normal stroke continued without reversal during the passage of current. Instead, reversal occurred at the "break" of the high intensity current pulse. Epstein and Machemer (unpublished) have filmed the suppression of ciliary reversal in *Euplotes* and *Paramecium* during strong intracellular positive pulses (above +70 mv), and have confirmed that reversal occurs at the termination of the pulse. Calcium influx is presumably delayed during such strong positive pulses until the term ($V_m - E_{Ca}$) grows larger as the potential drops exponentially away from E_{Ca} following the end of the current pulse. The analogous behavior occurs in the Ca^{2+}-dependent release of the transmitter substance from the presynaptic terminal of the giant synapse in squid (*31*).

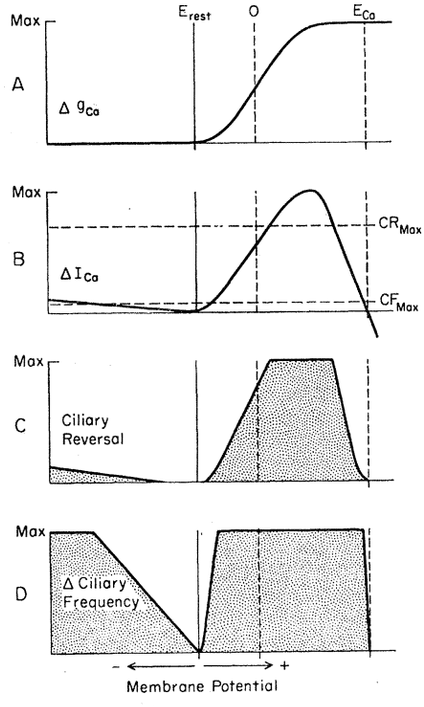

Fig. 3. Proposed relations between membrane potential, calcium conductance, calcium current, ciliary reversal, and ciliary frequency. (A) Peak transient increases in calcium conductance, Δg_{Ca}, produced by sudden changes in V_m from resting potential (E_{rest}) to various hyperpolarized (toward left) or depolarized (toward right) membrane potentials. Sigmoid curve is characteristic of electrically excitable membranes (*20*). (B) Corresponding transient change in inward calcium current, ΔI_{Ca}, based on Eq. 1. When ΔI_{Ca} is above the level labeled CF_{max}, ciliary frequency is maximal, and when ΔI_{Ca} is above the level labeled CR_{max}, ciliary reversal is maximal. (C) Reversed beating increases with the intracellular concentration of Ca^{2+}, which is closely related to ΔI_{Ca} of plot B. (D) Frequency of beating more sensitive to I_{Ca} than is reversal. Plots are hypothetical and are not intended to depict precise quantitative relations.

5) One can also predict from Eq. 1 that if g_{Ca} shows no large decrease with progressive hyperpolarization (increased intracellular negativity) I_{Ca} will increase linearly with the membrane potential because the electrochemical potential acting on Ca^{2+} increases as the cell interior is made more negative (Fig. 3, B and C). Thus, ciliary reversal should occur with sufficiently strong hyperpolarization. Naitoh (*27*) found this to be the case in *Opalina*. With small and moderate hyperpolarizing currents, beating frequency increased, and with stronger hyperpolarizing currents the cilia reversed their direction of beating.

6) The calcium current hypothesis (Fig. 4) predicts that failure of the membrane to undergo an increase in calcium conductance in response to a depolarizing stimulus will uncouple the response of the ciliary apparatus from the membrane potential, since depolarization under those circumstances will not produce an increased Ca^{2+} influx. Mutants of *P. aurelia* with modified locomotor behavior have been produced and isolated by Kung (*32*). One of these, named "Pawn," fails to reverse ciliary beating when stimulated with increased potassium or calcium chloride. Electrophysiological examination (*1*) showed that depolarizing current injected into Pawn produces only a passive (electrotonic) drop in mem-

brane potential with no sign of a regenerative calcium response (that is, no strong Ca^{2+} influx). Failure of depolarization to produce a calcium response in Pawn, and the concomitant failure of this mutant to show ciliary reversal upon depolarization are strong evidence for the causal relation between calcium influx and ciliary reversal.

7) The calcium current hypothesis requires that Ca^{2+} be removed from the cell or sequestered inside the cell so as to lower the intracellular Ca^{2+} and terminate ciliary reversal after a transient net Ca^{2+} influx (Fig. 4). Since the rate of active removal (metabolically energized pump) should be slowed down by lowered temperature, the hypothesis predicts that reversal in response to a given stimulus will last longer at reduced temperatures. This is, in fact, the case; the duration of reversed swimming induced by depolarization with increased cation concentration varies inversely with temperature with a Q_{10} of about 2.0 (*33*).

Thus, the calcium current hypothesis states that reversal of ciliary beating occurs when Ca^{2+} influx produces a sufficient increase in the intraciliary concentration of Ca^{2+}. This normally occurs when depolarization causes an increase in Ca^{2+} conductance, and Ca^{2+}, driven by its electrochemical potential, enters the cilia at an accelerated rate. There is growing evidence that membrane-limited Ca^{2+} fluxes regulate contraction in smooth (*34*) and certain cardiac (*35*) muscle. Calcium influx is also implicated in the release of neurotransmitter substances from nerve terminals (*23*). In all these instances depolarization increases the Ca^{2+} conductance of the surface membrane. Extracellular Ca^{2+} is also required for release of certain hormones in response to depolarization of the cell membrane (*36*). In skeletal muscle most of the intracellular Ca^{2+} is released from an extensive sarcoplasmic reticulum (*37*). It has been postulated that a similar release of Ca^{2+} may take place from the cortical reticulum of ciliates during excitation to supplement Ca^{2+} influx through the surface membrane (*38*).

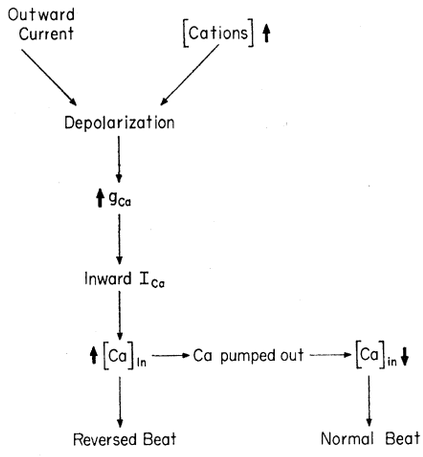

Fig. 4. Sequence of steps leading from stimulation to reversal of ciliary beating. Any depolarizing stimulus, such as an electrotonic outward current, results in a transient increased conductance to Ca^{2+}. According to Eq. 1, this results in an influx of Ca^{2+} (I_{Ca}). As a consequence the concentration of Ca^{2+} in cilia and cortex ($[Ca]_{in}$) rises, activating the mechanism for reversal. Subsequent removal of Ca^{2+} by active processes restores the normal beating orientation of the cilia.

Ion Antagonism

When ciliary reversal is induced by transferring paramecia from an "equilibration medium" to a "stimulation medium" of different ionic strength (for example, increased KCl or decreased $CaCl_2$) the duration of reversed swimming varies complexly with the relative concentrations of the monovalent cation (usually K^+) and the Ca^{2+} in the equilibration and stimulation solutions (*25, 28*). As first noted by Jahn (*39*), the duration of reversal is most closely related to the ratio $[X^+]/[Ca^{2+}]^{1/2}$ of the stimulation medium (where $[X^+]$ is the monovalent cation concentration). This is reminiscent of the antagonistic effects of extracellular Na^+ and Ca^{2+} in amphibian cardiac muscle (*40*). Interpretations of these experiments must take into account that the relative amount of extracellular Ca^{2+} affects membrane resistance and excitability (*18*), and that increasing the concentration of cations depolarizes the membrane (*4, 18, 41*). Other uncertainties include the extent and time course of changes in membrane potential and membrane conductance produced by stimulation with salt solutions. In spite of these complexities, such experiments have led to several related theories of reversal based on the premise that Ca^{2+} is bound to anionic sites on or near the cell membrane, and that reversal of ciliary beating is a consequence of its removal or displacement from those sites (*25, 39, 42*). These theories all fail to explain how depolarization by outward current produces ciliary reversal, and are limited to considerations of ciliary reversal by manipulation of the chemical environment.

Naitoh (*25*) has presented the most exhaustive and quantitative study of cation antagonism in the ciliates. In this article I have interpreted his findings in terms of the calcium current hypothesis instead of the cation exchange hypothesis (*39*). Several reasonable assumptions enter into this new interpretation. First, I have assumed that there is a transient increase in calcium conductance, g_{Ca}, when the membrane is depolarized by increased cation concentrations, just as there is when the membrane is depolarized by an outward current delivered with a microelectrode (Fig. 2). It is well established that an increase in cation concentration produces depolarization (*4, 18, 41*). Second, I have assumed that the rise in total membrane conductance observed at increased values of the ratio $[K^+]/[Ca^{2+}]^{1/2}$ (*18, 43*) results largely or entirely from an increase in g_K. (A simultaneous increase in g_{Ca} would serve only to reinforce the argument which follows.) Finally, the simplifying assumption is made that the permeability to anions such as Cl^- is much lower than the permeability of the membrane to cations. This agrees with the available data (*18, 19, 43, 44*).

It is important first to note that when the concentration of extracellular K^+ remains constant an increase in extracellular Ca^{2+} concentration decreases the membrane conductance (*18, 43*).

When the membrane potential is in a steady state (that is, $dV_m/dt = 0$) the net current through the membrane must be zero, and therefore the rate of charge influx must equal the rate of charge efflux. This means that charge carried by Ca^{2+} entering the cell through the increased Ca^{2+} conductance subsequent to a depolarizing shift in membrane potential cannot appreciably exceed the charge simultaneously carried out of the cell. Since K^+ has the proper electrochemical gradient to carry charge out of the cell (*6*) and is the most concentrated inorganic cation in the cell (*44*), the rate of charge efflux is limited largely by the K^+ conductance. Because Ca^{2+} influx cannot exceed an equivalent charge efflux during the poststimulatory steady state, any factor (such as an increase in the extracellular concentration of Ca^{2+} relative to the concentration of K^+) which decreases K^+ conductance can indirectly reduce Ca^{2+} influx by reducing K^+ efflux. Thus, increasing extracellular Ca^{2+} at constant extracellular

K^+ concentrations has at least two effects: (i) the term $(V_m - E_{Ca})$ in Eq. 1 becomes larger, and thus affects I_{Ca} positively; (ii) K^+ conductance drops, and thus reduces I_{Ca} indirectly by limiting K^+ efflux. As a result, inward Ca^{2+} current (and thus the intensity and duration of ciliary reversal, or both) can either increase or decrease when the concentration of extracellular Ca^{2+} or K^+ is changed, depending on which effect predominates (*45*).

Relative concentrations of K^+ and Ca^{2+} in the equilibration medium prior to stimulation with elevated KCl also affect the duration of reversal (*25*). This is to be expected, since external cation concentrations affect internal concentration during equilibration (see *44*).

In summary, the antagonistic effects of Ca^{2+} and K^+ can result from simultaneous opposing effects of increased extracellular Ca^{2+} concentrations on the influx of Ca^{2+}.

Membrane-Regulated Calcium Controls Frequency

Depolarization and hyperpolarization both evoke an increase in beating frequency. It is significant that very low (about 10^{-9} mole/liter) concentrations of extracellular Ca^{2+} lead to a reversible loss of motility in *Paramecium* (*46*). The ciliated cells of the amphibian oviduct also cease beating when the extracellular concentration of Ca^{2+} is reduced with EGTA [ethyleneglycol-bis-(aminoethylether)tetraacetic acid], and resume beating when Ca^{2+} is replaced (*47*). Thus, ciliary activity in living cells requires Ca^{2+}. However, ciliary activity in ciliate (*14*, *15*) and epithelial cells (*48*) extracted with Triton X-100 requires Mg^{2+} instead of Ca^{2+} for reactivation with adenosine triphosphate (ATP). In these preparations the frequency is entirely independent of the Ca^{2+} concentration, suggesting that the calcium requirement for ciliary beating in the living cell is not specific for the ciliary apparatus. Calcium may, for example, be required for certain reactions along the metabolic pathway leading to the final energy donor for ciliary beating. This is suggested by the observation that cilia of living cells inactivated by removal of Ca^{2+} are reactivated by addition of Ca^{2+}-free ATP (*48*). Recent experiments on ciliated epithelium indicate that the activation of ciliary activity produced by a mechanical stimulus results from a transient influx of Ca^{2+} through a transient increase in Ca^{2+} conductance (*49*).

It will be assumed tentatively that increased frequency of beating in ciliates occurs in response to an increase in the intracellular concentration of Ca^{2+}, and that the ciliary frequency reaches a maximum (CF_{max}) at a concentration of intracellular Ca^{2+} below that required to produce full ciliary reversal (CR_{max}) (Fig. 3D). Both hyperpolarization and depolarization can cause an increase in Ca^{2+} influx (Fig. 3B) and therefore an increase in ciliary frequency (Fig. 3D). However, hyperpolarization is less effective than depolarization for producing reversal because of the relatively shallow slope of the current-voltage curve with hyperpolarization (Fig. 3B). In this way a small or moderate hyperpolarization may produce increased frequency of beating without ciliary reversal.

The involvement of Ca^{2+} in the regulation of beating frequency is not firmly established in the ciliates, but rests largely on inferences. It is therefore important that the relations depicted in Fig. 3 be taken as a working hypothesis for further experimentation on the regulation of beating frequency.

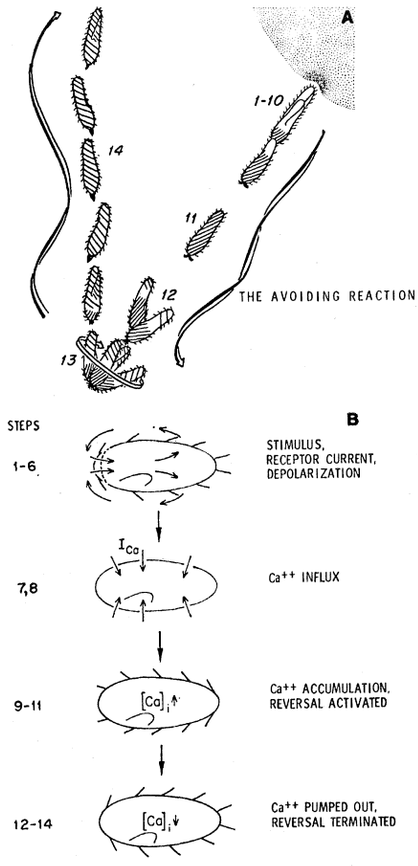

Fig. 5. Physiological steps in the avoiding reaction. (A) Retreat from stimulus, and resumption of forward locomotion. (B) The sequence of steps corresponding to the numbers in diagram A. Step 1, stretch of anterior membrane upon collision with obstacle; step 2, local increase in membrane conductance; and step 3, inward receptor current through stimulated membrane. Step 4, electrotonic spread of receptor current produces step 5, outward current through rest of membrane (arrows show current flow). Step 6, depolarization of cell membrane (receptor potential) produces step 7, increase in calcium conductance. Step 8, inward Ca^{2+} current; step 9, rise in intracellular Ca^{2+} concentration; step 10, cilia reverse beat; and step 11, cell swims backward. Step 12, Ca^{2+} pumped out; step 13, intracellular concentration of Ca^{2+} drops, cilia resume normal orientation; and step 14, cell swims forward. [Diagram A adapted from Grell (*61*) after Párducz (*10*)]

Control of Locomotion

The most common locomotor response seen in the ciliates is the "avoiding reaction" first described by Jennings (*50*). It consists of a temporary reversal in the direction of the ciliary power stroke, propelling the ciliate backward (Fig. 5A). The reaction is evoked by mechanical stimulation of the anterior end (*6*, *7*, *50*), ultraviolet irradiation of the anterior end (*51*, *52*), and a variety of chemical stimuli (*50*, *53*). The avoiding reaction comes to an end when the cilia resume the normally directed power stroke for locomotion in the forward direction. Stimulation of the posterior end mechanically (*50*, *54*) or by local ultraviolet irradiation (*51*, *52*) causes the ciliate to accelerate forward locomotion by increased frequency of ciliary beating.

Recent studies of the local mechanosensitivity of the surface of *Paramecium* (*6*, *7*) indicate the method by which local stimuli evoke such adaptive behavioral responses. A small tap delivered to the anterior surface of the cell with a microstylus produces a depolarization, whereas a similar stimulus applied to the posterior surface produces hyperpolarization (Fig. 6A). Both are graded according to the intensity of the stimulus. The response to stimulation of the cell anterior varies with the extracellular Ca^{2+} concentration, while the response to stimulation of the posterior varies with extracellular K^+ (Fig. 6C). This is interpreted in the light of the ionic hypothesis (*20*) to

result from an increased Ca^{2+} conductance produced by mechanical stimulation of the membrane at the anterior end, and an increased K^+ conductance produced by stimulation of the membrane at the posterior end. Receptor currents which arise locally in response to mechanical stimulation spread electrotonically to change the membrane potential over the entire cell membrane (*8*). Electrotonic depolarization caused by the receptor current flowing into the cell through the stimulated membrane of the anterior end evokes the regenerative Ca^{2+} response shown in Fig. 2, A–D. The regenerative Ca^{2+} response can be suppressed by hyperpolarizing the membrane to demonstrate the pure receptor potential elicited by a mechanical stimulus applied to the anterior (Fig. 6B). Thus, the change in potential produced by a stimulus applied to the anterior end of a paramecium consists of two components, a graded receptor potential produced by the influx of positive charge through the stimulated membrane, and a subsequent graded regenerative Ca^{2+} response due to an electrically excited increase in Ca^{2+} conductance distributed over the cell membrane.

Stimulation of the posterior end produces an outward K^+ current through the stimulated membrane. The hyperpolarization produced by this receptor current spreads electrotonically to the entire surface membrane. In this case there is no secondary regenerative response. The hyperpolarization simply increases the electrochemical force acting on Ca^{2+} (Eq. 1) and thereby may increase Ca^{2+} influx.

Thus, the membrane potential, which at rest lies between E_{Ca} and E_K (*18*), can undergo hyper- and depolarizing shifts as the result of changes in g_{Ca} and g_K (Fig. 7A), and these in turn regulate calcium movements across the surface membrane. It is the transducer properties of topographically segregated membrane components that determine whether a stimulus restricted to one part of the cell will cause hyperpolarization and accelerated forward locomotion, or depolarization and backward locomotion (Fig. 7B). Adaptive control of locomotor behavior is therefore based on anatomical as well as biophysical factors.

The steps leading from a stimulus applied to the anterior end to the completed avoiding reaction are summarized in Fig. 5B. Depolarization produced by a stimulus (for example, collision of the anterior end with an obstacle, entry into a solution of higher salt concentration) spreads electrotonically over the cell membrane (*6*, *8*). This leads to a general increase in Ca^{2+} conductance. As a result, extracellular Ca^{2+}, driven by its electrochemical gradient (Eq. 1) enters the cell through the increased conductance of the cell membrane. The resulting increase in Ca^{2+} concentration within the cell cortex and cilia induces reversal of the power stroke and an increase in frequency of beating. The ciliate thereby switches from forward to backward locomotion. As the depolarization subsides and the calcium conductance of the membrane returns to normal, the intracellular concentration of Ca^{2+} is lowered again by extrusion of Ca^{2+} out of the cell through the surface membrane or is temporarily sequestered by mitochondria or cell reticulum. The cilia then resume normal forward swimming movements, bringing the avoiding reaction to an end.

Calcium that may enter with the receptor current at the stimulated anterior end is unlikely to produce an appreciable increase in Ca^{2+} concentration throughout the entire cell cortex. Increased concentrations of Ca^{2+} in the cilia therefore depend on the excitation being distributed over the membrane, allowing Ca^{2+} influx over the entire cell surface. In this way the individual cilia are all under the local control of a distributed membrane-

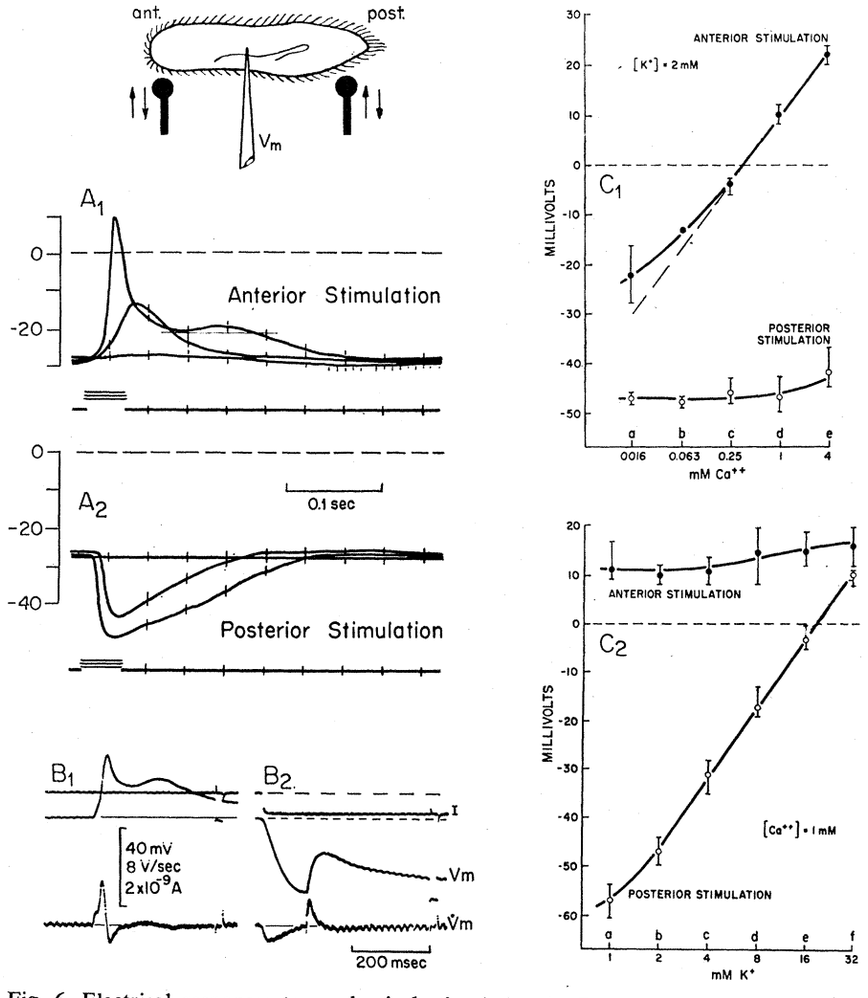

Fig. 6. Electrical responses to mechanical stimulation in *P. caudatum*. Stimuli applied with electrically driven microstylus to anterior (A_1) and posterior (A_2) surfaces. Lower traces in A_1 and A_2 show pulses energizing the microstylus. (B_1) Response to mechanical stimulation of cell anterior; (B_2) mechanical stimulus applied to same cell during hyperpolarizing current. The regenerative calcium response was thereby suppressed, leaving unobscured anterior receptor potential (*I*, current trace; V_m, membrane potential; $\dot{V}_m$, first time derivative of V_m). Curves in C_1 show effects of extracellular calcium concentrations on peak values of potentials evoked by anterior and posterior mechanical stimulation. Curves in C_2 show effects of extracellular potassium concentrations. [Modified from (*6*)]

regulated Ca^{2+} flux. The regenerative calcium response, thus, has the dual functions of: (i) amplifying the electrotonic depolarization produced by receptor current through a voltage-sensitive increase in Ca^{2+} conductance and (ii) increasing the intracellular Ca^{2+} concentration rapidly and simultaneously throughout the cell cortex for the activation of ciliary reversal.

Control of the finer aspects of locomotor behavior of ciliates depends, at least in part, on topographical differences in membrane properties, and perhaps to some extent on sensitivities of the individual cilia to the activating agent, Ca^{2+}. Thus, the cilia distributed over the surface of the cell do not all respond equally to a uniformly distributed membrane potential. This was found in experiments with *Opalina* in which reversal of the cilia of the anterior right side of the ventral surface were found to have the highest sensitivity for outward current (*27, 55*). As the intensity of outward current was increased, reversal spread over the rest of the cell while the response at the anterior right side became more intense. This distribution of responsiveness was correlated with topographical differences in membrane resistance. The surface regions giving the strongest ciliary reversal to a given membrane depolarization had the lowest membrane resistances. In more recent experiments, in which paramecia paralyzed with $NiCl_2$ were used, ciliary reversal was stronger and had a shorter latency at the anterior end of the organism (Fig. 1) even though the membrane potential was essentially uniform over the entire surface (*8*). This could result either from topographical differences in the amount of Ca^{2+} which flows in through the membrane in response to depolarization, or from differences in the sensitivity of individual cilia to free intracellular calcium.

It has been proposed that the metachronal waves of in-phase activity which sweep over the population of cilia are coordinated by signals propagated over the cell surface (*10, 56*) from pacemaker regions (*57*). The problem of metachronal coordination is reviewed elsewhere (*47, 58*), but it should be noted here that the evidence rules out any one-to-one relation between membrane signals and the cycle of ciliary movement. Recordings made from ciliates (*8*) and epithelial cells (*47, 59*) show no fluctuations in potential correlated with metachronal waves (*60*). Paramecia and ciliated epithelium extracted with a lipid-solubilizing detergent, Triton X-100, continue to show well-coordinated metachronism when ciliary activity is reactivated by addition of ATP and Mg^{2+} (*14, 48*). Thus, the beating cycle itself, and the coordination between neighboring cilia, do not require electrical integrity of the membrane. Direct bioelectric regulation of ciliary activity is limited to the modulation of beating frequency and regulation of the three-dimensional orientation of the ciliary movements.

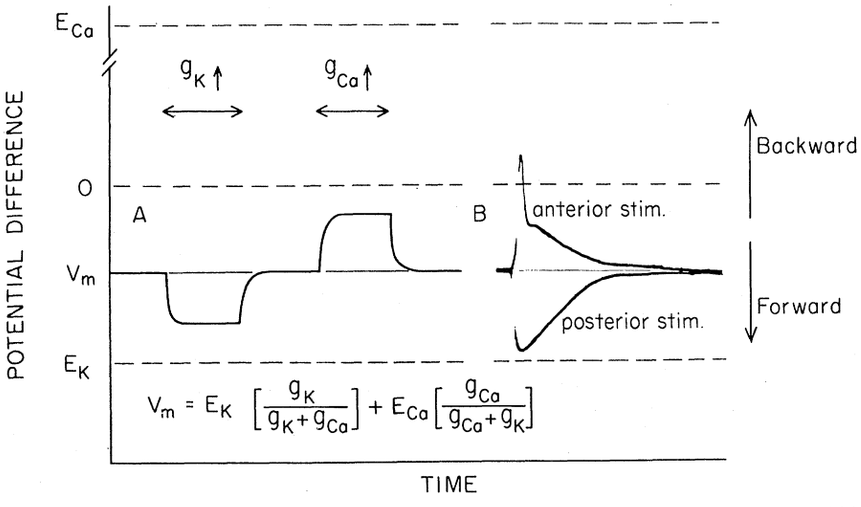

Fig. 7. Membrane potential regulated by calcium and potassium conductances. (A) Example of potential changes produced by changes in g_K and g_{Ca}. E_{Ca} and E_K are drawn in positions relative to zero. (B) Actual recordings showing potential shifts produced by mechanical stimulation of the anterior and posterior ends of *P. caudatum*. Depolarization leads to backward locomotion, hyperpolarization to accelerated forward locomotion.

Conclusions

I have proposed that the direction and frequency of beating of cilia is regulated by the intracellular concentration of free calcium ions in the vicinity of the ciliary apparatus, and that the intracellular accumulation of Ca^{2+} is regulated by the local and distributed electrical responses of the cell membrane to environmental stimuli. Thus, Ca^{2+} appears to act as the agent which couples the mechanical responses of the active cilia to the electrical responses of the cell membrane. Each of the steps between stimulus and ciliary reversal is graded according to the intensity of the previous step so that the locomotor reaction varies in intensity with the strength of the stimulus intercepted by the membrane. While the metazoan nervous system converts analog signals (graded receptor potentials) to digital signals (all-or-none action potentials), and these back into graded analog signals (synaptic potentials), the bioelectric organization of the ciliate is based entirely on analog-to-analog transforms. Nonetheless, the cellular and subcellular mechanisms of ciliates (for example, sensory transduction, electrotonic spread, electric excitation) are similar to those of excitable cells in the Metazoa. It is interesting for evolutionary and physiological considerations that the same general mechanism of membrane-regulated calcium fluxes used in the control of a variety of cellular activities in the Metazoa had already evolved for regulation of locomotor functions in the ciliated Protozoa.

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an intracellular compound, Ca-X; upon lowering the intracellular calcium concentration, X would be liberated and would activate the reversal mechanism. We now know that it is important to consider the effects of intracellular Ca^{2+} on the properties of the cell membrane, for intracellular calcium concentrations determine electrical threshold in excitable membrar ➡ [I. Tasaki, A. Watanabe, K. Takahashi, *Proc. Nat. Acad. Sci. U.S.* **48**, 1177 (19(➡ S. Hagiwara and K. Naka, *J. Gen. Physiol.* **48**, 141 (1964)]. Reduction of intracellular Ca^{2+} converts the graded electrical excitability of crustacean muscle membrane into all-or-none action potentials in which extracellular Ca^{2+} carries the inward current (see S. Hagiwara and K. Naka, above). Thus, in Kamada's experiment the reduction of the intracellular calcium concentration may have induced reversal because of its effect on membrane current or membrane potential (see text). Ueda (*13*) noted that injection of Ca^{2+}-precipitating agents into *Opalina* causes a reduction in resting potential. Calcium ions injected into the ciliate failed to evoke reversal (see T. Kamada, above), perhaps because the injected solution is encapsulated as a vacuole before the Ca^{2+} can diffuse to the periphery.

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62. I am grateful to my collaborators Drs. Y. Naitoh, A. Murakami, K. Friedman, M. Epstein, and H. Machemer for stimulating and informative discussions and arguments, and for permission to cite unpublished data. The work reported from our laboratory together with the preparation of the manuscript was supported by grants from the National Science Foundation and the U.S. Public Health Service.