Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 6476–6480, September 1987
Cell Biology

A calcium regenerative potential controlling ciliary reversal is propagated along the length of ctenophore comb plates

(ctenophore cilia/extraciliary recording/inward Ca^{2+} current)

ANTHONY G. MOSS* AND SIDNEY L. TAMM†

Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543

Communicated by Lynn Margulis, May 28, 1987

ABSTRACT **We have used the giant ciliary comb plates of ctenophores to record electrical activity directly from cilia. A compound action potential was recorded extracellularly over most of the length of the comb plate cilia in response to electrical stimulation of the ectodermal nerve net. The ciliary action potential was correlated with intracellularly recorded action potentials, selectively blocked by Ca^{2+}-channel antagonists, and correlated with ciliary reorientation and reversed beating. Dual-electrode recording from different sites on the same comb plate showed that, unlike protistan cilia, the ≈1-mm-long cilia of comb plates are not isopotential. Rather, action potentials are generated 150–200 μm from the base and propagate to the tip of the cilia. These results indicate that voltage-dependent channels that mediate increases in intraciliary Ca^{2+} concentration are distributed over most of the length of the cilia. Consequently, the Ca^{2+}-sensitive machinery controlling ciliary motor responses is also likely to be located along the length of the axoneme.**

Modifications of ciliary and flagellar activity in response to stimuli are controlled by the membrane potential in a variety of protozoan and metazoan cells (1–5). In ciliate protozoa, the best-studied example, depolarizing stimuli activate voltage-sensitive Ca^{2+} channels located exclusively in the ciliary membrane (6–9) leading to Ca^{2+} influx into the cilia and to a graded Ca^{2+}-dependent regenerative response recorded from the cell body (1, 2, 4, 5). The increase in Ca^{2+} concentration triggers a reorientation of the power stroke (ciliary reversal) (10).

The small size of most cilia has prevented direct determination of the distribution of Ca^{2+} channels along the length of the ciliary membrane. Results of previous studies are consistent with either a uniform or an uneven distribution of ciliary Ca^{2+} channels (7, 8, 11).

To the best of our knowledge, the site(s) or mechanism(s) of action of Ca^{2+} within the axoneme also is not known. Calmodulin, a putative ciliary Ca^{2+} sensor (12–15), has been detected along the entire length of cilia (16, 17). In contrast, local application of Ca^{2+} to reactivated cilia and flagella indicate that the Ca^{2+}-sensitive machinery controlling various motor responses is localized to a specific region of the axoneme (18–20).

In this report we take advantage of the large size of ctenophore comb plate cilia to record electrical activity directly from motile cilia. We show that a graded Ca^{2+}-dependent regenerative potential is initiated near the base of the comb plate and propagates to the tip of the cilia. The regenerative response is correlated with reorientation and reversed beating of the comb plate. The Ca^{2+} conductance, and hence the Ca^{2+}-sensitive machinery controlling ciliary motor responses, is, therefore, likely to be distributed along the ciliary length.

MATERIALS AND METHODS

Pleurobrachia pileus was collected locally. Split comb-row preparations for electrical recording were made as described (21) (Fig. 1).

Intracellular microelectrodes (50–80 MΩ, filled with 4 M potassium acetate) were inserted into comb-plate cells (polster cells) from the cut side of the comb plate to avoid mechanical artifacts. Signals were recorded with an intracellular amplifier [WPI Instruments (Waltham, MA), model M701] and stored on an instrumentation recorder [Vetter Instruments (Rebersburg, PA), model D].

Extracellular glass recording electrodes (inside tip diameter, 10–15 μm) were attached to a comb plate by gentle suction, thus splitting the plate longitudinally into a narrow sliver attached to the electrode, and a much wider part which was free to beat (Fig. 1*B*). The seal resistance of the recording pipet, determined by measuring the voltage drop across an electrode attached to the comb plate, was $2.25 \pm 0.25 \times 10^5$ Ω ($n = 11$), regardless of the site of attachment along the ciliary bundle.

Compound extraciliary signals were recorded with ac preamplifiers [Grass Instruments (Quincy, MA), model P15] at a gain of 100 or 1000. Polarity was arranged so that upward or downward deflections of the oscilloscope trace represented net positive or negative charge at the electrode tip, respectively. Two extracellular electrodes were attached at different sites along the same ciliary bundle to measure conduction speed of the electrical signals (see Fig. 4).

Electrical and motor responses of comb plates were elicited by stimulation of the adjoining ectodermal nerve net with trains of bipolar pulses (5 Hz, 5–40 V, 5 msec) delivered by a suction electrode attached to the body surface (Fig. 1*B*) (21).

Ciliary motor responses were imaged with a high-speed video camera and combined by way of a video switcher with a video image of the oscilloscope trace (21). Electromotor correlates were analyzed by still-field video playback (21).

RESULTS

Ctenophores, or comb jellies, swim by means of eight meridional rows of paddle-like ciliary comb plates (Fig. 1*A*) (22). Each comb plate consists of hundreds of thousands of single cilia, ≈1 mm long, which beat together as a unit due to mechanical coupling (23). The cilia are borne on a ridge of thousands of elongated epithelial cells, called polster cells, which are probably electrically coupled (24), and receive

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

*Present address: Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, MA 01545.
†To whom reprint requests should be addressed.

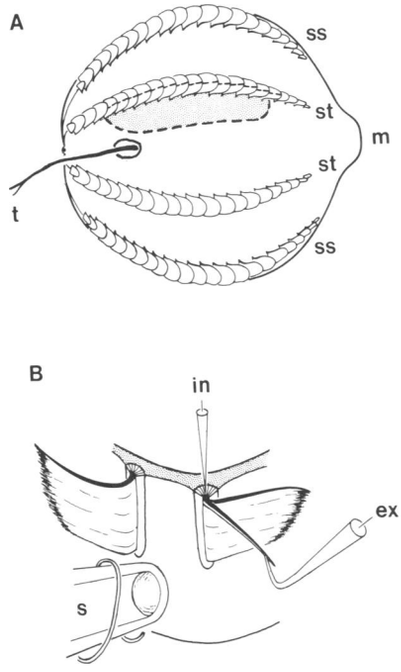

FIG. 1. (*A*) Side view of *Pleurobrachia* showing subsagittal (ss) and subtentacular (st) rows of comb plates. A subtentacular row, sliced longitudinally along its midline to expose the comb-plate cells, is removed together with the adjoining midsubtentacular body surface (dashed line) to make a split-row preparation. t, Tentacle; m, mouth. (*B*) Split-row preparation pinned to a Sylgard wedge in the recording chamber. An intracellular recording microelectrode (in) impales a comb-plate cell from the cut edge of the polster ridge. An extracellular recording pipet (ex) is attached to the distal end of a narrow sliver of the comb plate, allowing the rest of the plate to beat. Electrical and motor responses of the comb plate were elicited by trains of bipolar pulses delivered by a stimulation electrode (s) attached to the adjoining body surface.

synchronous and nearly identical synaptic input from an ectodermal nerve net (21, 25).

Ciliary motor responses, such as reorientation of nonbeating comb plates ("laydown") and reversal of effective stroke direction (26), are correlated with volleys of graded action potentials recorded intracellularly from the polster cells (21). Intracellular recordings from microsurgically deciliated polster cells showed that this regenerative response was lost (27), indicating that excitability resides in the ciliary membrane.

To map electrical activity over the length of the cilia, we recorded *en passant* with extracellular micropipets from various sites along a sliver of a comb plate during pulse-train stimulation of the ectoderm (Fig. 2). From the electrode tip diameter and comb-plate anatomy, we estimate that the signals represent the summed electrical responses of at most 100–200 cilia arising from three or four polster cells.

When attached directly to the polster cells (Fig. 2*a*) or to the proximal 100–200 μm of the cilia (Fig. 2 *b* and *c*), the pipet recorded positive, essentially monophasic potentials that initially grew in amplitude with each successive stimulus in the pulse train (Fig. 2 *b, Upper,* and *f, Lower*; see Fig. 4*B*), similar to intracellularly recorded synaptic potentials (21). The extrinsic monophasic signals from the proximal ciliary region were greater in amplitude and shorter in duration than those recorded from the cell bodies and usually displayed a clear but small negative-going component (Fig. 2 *a–c*, and see Fig. 4), arising from settling of the ac amplifier.

Recordings at more distal sites on the comb plates showed more complex waveforms. When the positive monophasic reached a threshold value, a shoulder or second positive peak appeared, followed immediately by a negative wave (Fig. 2 *d–f*). In some cases the first two positive components merged and summed, resulting in a very large biphasic waveform with amplitudes of >10 mV (data not shown).

To unambiguously identify the components of the extrinsic ciliary signal, we simultaneously recorded intracellularly from a polster cell of the ciliary sliver to which the external recording electrode was attached (Fig. 3). Positive monophasic responses from the cilia were correlated with excitatory postsynaptic potentials recorded in the polster cell soma. The size of the extrinsic monophasic potential varied directly with the magnitude of the intracellular synaptic potential, which could be +20 mV above the resting potential.

Complex extrinsic waveforms were correlated with intracellularly recorded action potentials (Fig. 3). Such wave-

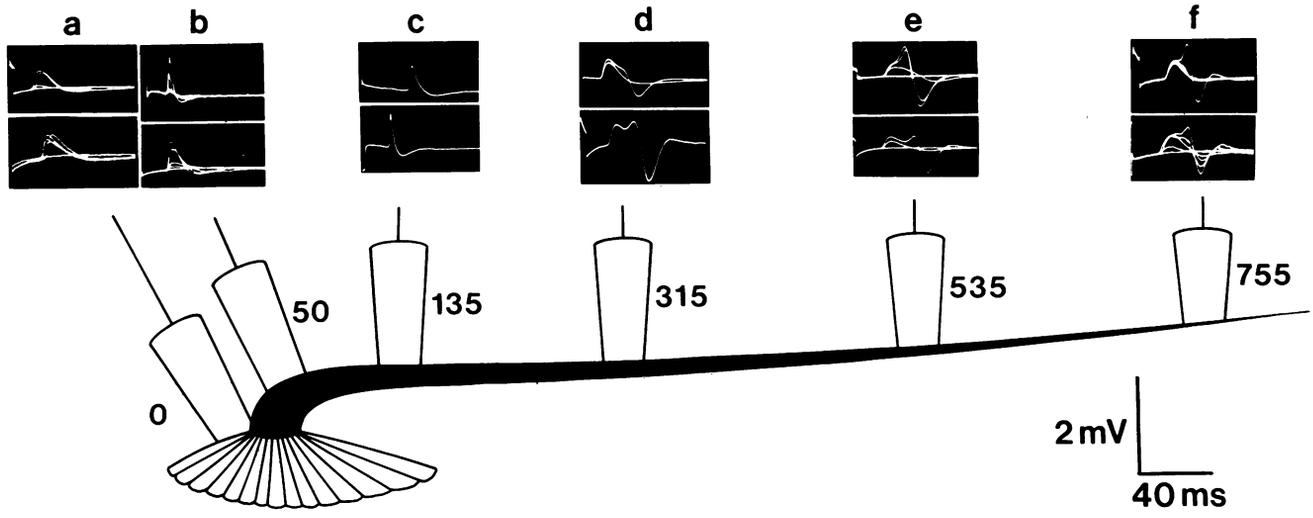

FIG. 2. Extracellular electrical activity recorded at different sites along a comb plate of *Pleurobrachia* during pulse-train stimulation of the ectoderm (compiled from single and dual recordings from >100 plates). Two typical records are presented for each position of the recording electrode, shown in absolute distances (in μm) from the base of an idealized 800-μm plate. Actual total lengths of the comb plates (in μm) in upper and lower records at each site were as follows: *a*, 510 and 930; *b*, 769 and 660; *c*, 730 and 800; *d*, 320 and 440; *e*, 510 and 710; *f*, 760 and 880. One to six electrical impulses are shown in each record. Upward or downward deflections of the oscilloscope trace represent a net positive or negative charge at the electrode tip, respectively. If attached to the polster-cell ridge or to the basal region of the plate (*a* or *b*), the electrode recorded mainly positive monophasic responses, interpreted as synaptic potentials. More distal recordings (*d–f*) displayed, in addition to the monophasic potential, a large negative component representing an inward current.

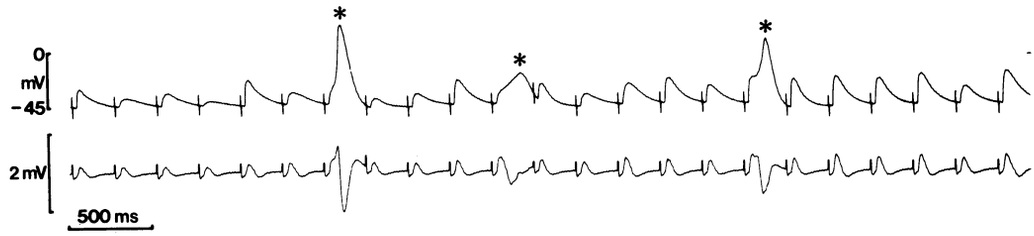

FIG. 3. Simultaneous intracellular (*Upper*) and extracellular (*Lower*) recordings from the same comb plate during pulse-train stimulation of the ectoderm (4-Hz, 5-ms, 20-V bipolar pulses). The intracellular electrode was located in a polster-cell body; the extracellular electrode was attached to the distal end of a ciliary bundle that included the cilia of the impaled cell (see Fig. 1*B*). Three intracellularly recorded regenerative responses of different amplitudes are correlated with extrinsic negative waves (net inward currents) of corresponding magnitudes (asterisks). Variations in amplitude of synaptic potentials are reflected in corresponding changes in the size of the extracellularly recorded monophasic response. Chamber temperature, 8°C. Resting potential prior to stimulation, −55 mV.

forms were typically composed of an initial positive monophasic component, a second positive wave, and a final negative wave. The size of the extrinsic negative component varied directly with the amplitude of the graded regenerative potential recorded intracellularly and occurred only when the positive monophasic signal exceeded threshold. Complex extrinsic waveforms typically had a total duration of 50–100 msec, similar to that of intracellularly recorded action potentials (Figs. 2 *d–f* and 3; ref. 21).

We, therefore, interpret the extracellularly recorded monophasic or first positive potential to be a compound depolarizing postsynaptic potential that originates in the cell bodies and invades the cilia, resulting in an effective outward current leakage across the ciliary membrane. The increase in size of the monophasic response early in the stimulus train is likely the result of recruitment or facilitation of synaptic activity in the polster ridge, producing correspondingly larger ciliary depolarizations (cf. ref. 21).

We interpret the second positive component of the extrinsic waveform as outward current due to depolarizing local circuit activity ahead of the inward current of an action potential. The negative-going component of the external signal is interpreted as that inward current, since it is correlated in time and amplitude with the intracellularly recorded regenerative response.

The time course of electrical activity over the length of the cilia was determined by attaching two recording electrodes at different locations along the same ciliary bundle (Fig. 4). Electrical events did not occur instantaneously over the cilia but showed proximal-to-distal delays or propagation times (28), characteristic for each component of the waveform.

The conduction velocity of the action potential was 26.8 ± 3.8 mm/s (n = 16 plates), as determined by measuring the delay between arrival of the peak negative potential at the two recording electrodes. For these measurements the proximal electrode was located at least 100 μm from the base and displayed a clear downward inflection (inward current). The trough of the negative potential was used because it was readily identifiable and is known to travel at virtually the same velocity as other points in a propagated wave. [The propagation rate for all points of both a linear (passively conducted) and nonlinear (action potential) waveform approaches $2\lambda/R_mC_m$ for (infinite) cylinders (28), where R_m is membrane resistance and C_m is membrane capacitance. Comb-plate cilia roughly approximate such cylinders.]

The distally recorded synaptic potential lasted longer and rose and fell more slowly than the proximally recorded synaptic potential (Fig. 4). However, the amplitude of the synaptic potential was smaller distally in only about half of the cases (24 out of 44 cases); larger distal signals were often

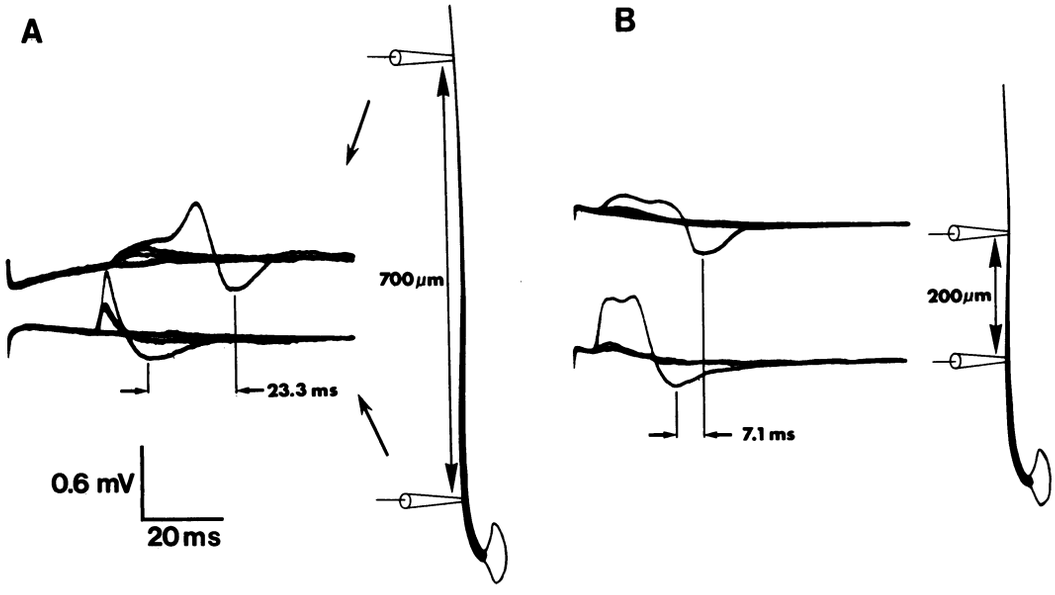

FIG. 4. Conduction velocity of ciliary action potentials. Two extracellular recording electrodes were attached at different sites along the same ciliary bundle (shown in profile). Propagation speeds were determined using the time delay between the lowest point of the negative potential wave (inward action current) at the two sites (indicated by vertical lines below paired records). Electrical activity was evoked by pulse-train stimulation of the ectoderm. Although there are differences between the waveforms of any pair, the components of the proximal and distal signals can still be identified. Conduction velocities of action potentials: 30 mm/s (*A*), 28 mm/s (*B*).

recorded (15 out of 44 cases). The reason for this unusual result is not known (see *Discussion*).

Intracellularly recorded regenerative potentials of polster cells are accompanied by curvature of nonbeating plates in the direction of the reversed effective stroke (reorientation or laydown response; Fig. 5*B* and ref. 26), followed by beating in the reversed direction at high frequency (21). To establish the motor correlates of the extrinsic ciliary signals, recordings from the immobilized sliver of a plate were compared with the accompanying motor activity of the remaining part of the same plate that was free to beat [normally, all the cilia within an intact plate beat synchronously in the same direction (23)].

Reversed beating or reorientation of the free part of a plate was always preceded or accompanied by multiphasic potentials with large negative components recorded from the electrode-attached sliver (n = 15 plates). In contrast, 89% of the cases in which normal beating occurred on the free part of the plate were accompanied by monophasic (synaptic) potentials (n = 18 plates) recorded on the sliver.

More directly, attachment of the recording pipet approximately halfway along the sliver allowed the distal region of the sliver to move, enabling simultaneous recording of electrical and motor activity from the same group of cilia. In such cases, the free end of the sliver did not beat, but bent in the direction of the reversed power stroke (as in the reorientation response) immediately following action potentials (95% of the cases, n = 25) (Fig. 5*A*). Monophasic positive potentials that occurred prior to the action potentials were not associated with changes in curvature of the sliver tip. Similarly, relaxation of the bend was accompanied by monophasic potentials or no signals (n = 12). These results confirm that the negative component of the extrinsic waveform is specifically associated with ciliary reorientation and reversal.

Both reversal of beat direction of comb plates and the intracellularly recorded action potential of polster cells have been shown to be calcium dependent (21, 29, 30). Local application of Co^{2+} (n = 12)-, Cd^{2+} (n = 12)-, or Ca^{2+} (n = 5)-free artificial sea water to a comb plate during pulse-train stimulation completely eliminated the negative-going component of the propagated, extrinsic potential (Fig. 6) without abolishing the synaptic response, although the latter was often reduced. The negative wave usually returned several minutes after termination of Co^{2+} or Cd^{2+} perfusion, although it was often smaller and occurred less frequently in response to pulse-train stimulation. Incomplete recovery of the regenerative potential may be a result of incomplete washout of the test solution or a deleterious effect of the test solution on the ciliary membrane. Co^{2+} or Cd^{2+} also blocked curvature of the tip of the sliver. Therefore, the negative wave of the extracellular signal probably arises from an inward calcium current that triggers comb-plate reorientation and reversed beating. These results further support our hypothesis that the regenerative response is conducted along the length of the cilia.

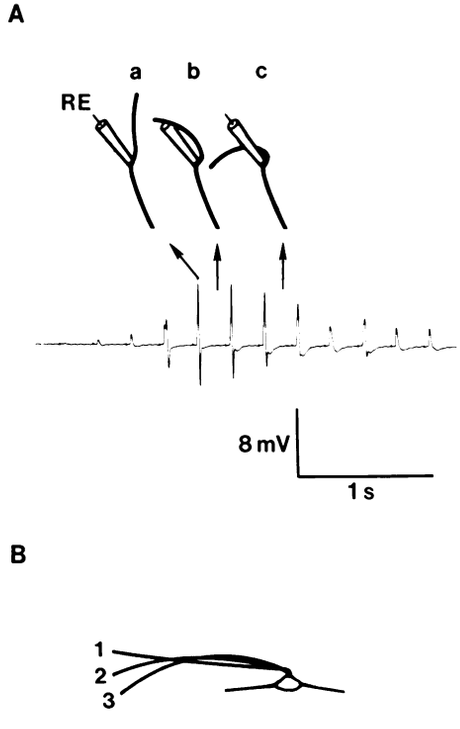

FIG. 5. Electromotor coupling in an electrode-attached ciliary sliver with a free tip. Reorientation of the ciliary tip distal to the recording electrode (RE) (*Upper*, video tracings show profile views of the distal two-thirds of the sliver) and accompanying electrical activity (*Lower*) are shown during pulse-train stimulation of the body surface (4 Hz, 5 ms, 7 V). Initial monophasic synaptic potentials do not elicit curvature of the tip. After the first two action potentials, the tip bent rapidly to the left (tracing b) in the direction of the reversed effective stroke (reorientation response, see *B*). The curvature of the tip became maximal after two more spikes (tracing c), and slowly relaxed over several seconds in the absence of action potentials (data not shown). Time between ciliary tracings a to b, 0.7 s; tracings b to c, 0.33 s. (*B*) Reorientation response of an intact comb plate without attached electrodes. Time interval between successive positions was 0.1 s.

DISCUSSION

The diverse behavioral responses of cilia and flagella are controlled by membrane-regulated Ca^{2+} fluxes (1–5). Depolarizing stimuli activate voltage-sensitive Ca^{2+} channels located exclusively in the ciliary membrane (6–9), leading to influx of Ca^{2+} ions that triggers modification of ciliary activity (10).

Heretofore, the distribution of Ca^{2+} channels along the ciliary membrane has not been determined by electrophysiological methods due to the small diameter (≈0.25 μm) and short length (10–30 μm) of most cilia. Studies on the return of the action potential during ciliary regeneration in *Paramecium* are consistent with either a uniform or an uneven

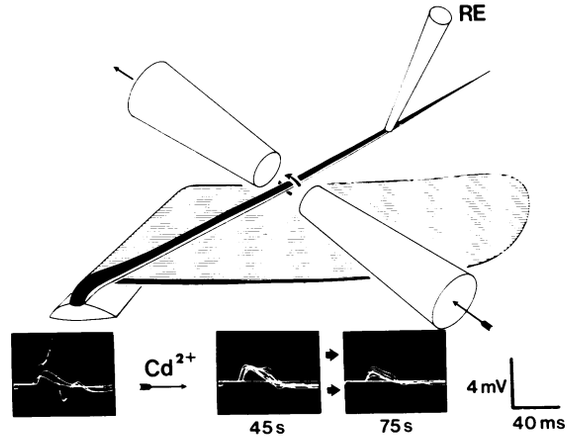

FIG. 6. Block of ciliary action potentials by local application of Ca^{2+} antagonists. Preparation was bathed in normal artificial sea water (35). (*Upper*) Two opposing pipets connected to a push–pull syringe perfusion system were positioned on either side of a comb-plate sliver to which a recording pipet (RE) was attached distally. Before perfusion, a typical triphasic waveform was recorded (*Lower left*). Local perfusion of artificial sea water containing 1 mM Cd^{2+} selectively eliminated the negative-going component of the action potential without significantly reducing the first positive wave (synaptic potential) (*Lower right*, shown 45 s and 75 s after the start of a 30-s pulse perfusion of 1 mM Cd^{2+} in artificial sea water).

distribution of Ca^{2+} channels over the ciliary membrane (7, 8). The asynchrony of ciliary regeneration makes these experiments difficult to interpret. The discovery of two subpopulations of ciliary membrane vesicles, only one of which has Ca^{2+} channels, supports a nonuniform distribution of channels (11).

In this report we take advantage of the great length (≈1 mm) and compound nature (>10^5 cilia) of ctenophore comb plates to record electrical activity directly from motile cilia. We attached suction electrodes to slivers of comb-plate cilia and recorded large compound extraciliary potentials. These signals represent the summed electrical activity of several hundred uniformly oriented cilia, or "fibers," with identical diameter, electrical characteristics, and electrical (synaptic) input; the extrinsic waveform may, therefore, be treated as though recorded from a single fiber.

A negative extrinsic potential arising from a net inward current was recorded over most of the length of comb-plate cilia. This signal is thought to represent a Ca^{2+}-dependent regenerative depolarization responsible for ciliary motor responses, since it is specifically correlated with intracellularly recorded action potentials, it is sensitive to Ca^{2+}-channel blockers, and it is correlated with ciliary reorientation and reversed beating.

These findings strongly suggest that the voltage-dependent calcium conductance is distributed over most of the length of ctenophore comb-plate cilia. Failure to detect an inward current near the base of the comb plate may mean that fewer voltage-dependent channels exist in the proximal region of the ciliary membrane. Alternatively, the apparent absence of an action potential at proximal recording sites may reflect masking of nonregenerative inward Ca^{2+} current by the large synaptic potential invading this region of the cilia, and/or shunting of inward current by the adjacent cell bodies that are coupled by gap junctions (24). Since the extrinsic potentials arise from the net inward or outward current through the membranes of a population of cilia, specific contributions to the waveform cannot be unequivocally related to specific conductances at this time.

It is not clear that the positive monophasic signal represents simply a synaptic potential passively spread along the cilia. Since it rises at different times proximally and distally (Fig. 4) and often does not show decrement with distance from the base, the synaptic potential may be augmented by an additional unknown conductance.

The length constant for a cilium of "infinite" length has been calculated to be as high as one to several millimeters (4, 31), indicating that the short (10–30 μm) cilia of protozoa are essentially isopotential along their length and with the cell body (7, 32). Our dual electrode recordings show that comb-plate cilia are not isopotential, but propagate potentials from base to tip of the 1-mm cilia. Variations in the tightness of the electrode–ciliary seal preclude estimation of the length constant. Since the duration of the net inward current is ≈20 msec and the conduction velocity of the action potential is ≈27 mm/s, the region of inward current occupies ≈0.4 mm, or about half the length of the comb plate. Therefore, a long region of the axoneme probably experiences simultaneous influx of Ca^{2+} during the action potential.

The Ca^{2+}-binding regulatory protein, calmodulin, is present in cilia and flagella and is considered a likely candidate for the axonemal Ca^{2+} sensor (12–15). Fluorescence and immunoelectron microscopy have shown that calmodulin is localized along the entire length of the axoneme (16, 17).

In contrast, local application of Ca^{2+} to different regions of detergent-permeabilized cilia and flagella indicates that the Ca^{2+}-dependent mechanisms controlling motor responses are localized to specific regions of the axoneme (18–20). In particular, the basal region of *Paramecium* cilia is most sensitive to Ca^{2+} in inducing ciliary reorientation, a response analogous to ciliary reversal (18).

Studies on Ca^{2+} entry through voltage-gated Ca^{2+} channels in neurons show that cytoplasmic Ca^{2+} buffering and/or binding restricts the region of elevated free Ca^{2+} to microscopic "domains" centered upon the open channels (33, 34). Ca^{2+}-binding by the axonemal target molecule should also retard diffusion of Ca^{2+} away from the sites of entry, resulting in highly localized Ca^{2+} signals. For effective coupling between Ca^{2+} entry and ciliary motor responses, the axonemal Ca^{2+} sensor should be located close to the Ca^{2+} channels.

Therefore, our finding that Ca^{2+} influx occurs over most of the length of comb-plate cilia implies that the Ca^{2+}-sensitive machinery controlling beat direction is also distributed along the length of the axoneme.

The giant cilia of ctenophore comb plates thus offer distinct advantages for further studies on the excitable membrane of motile cilia and on the site and mechanism of action of Ca^{2+} within the axoneme.

We thank A. Fein, P. Brehm, and K. Dunlap for critical reading of the manuscript; R. Payne and A. Stuart for helpful discussions; and A. Shipley, R. Stephens, and J. Whittaker for loans of equipment. This research was supported by National Institutes of Health Grants GM 27903-07 to S.L.T. and a Lerner–Gray award and Award 1F31MH09240-02 from the National Institute of Mental Health to A.G.M.

1. Eckert, R. (1972) *Science* **176,** 473–481.
2. Naitoh, Y. (1982) in *Electrical Conduction and Behaviour in "Simple" Invertebrates*, ed. Shelton, G. A. B. (Oxford Univ. Press), pp. 1–48.
3. Saimi, Y., Murakami, A. & Takahashi, K. (1983) *Comp. Biochem. Physiol. A.* **74,** 507–511.
4. Machemer, H. (1986) *Progr. Zool.* **33,** 205–250.
5. Kung, C. & Saimi, Y. (1982) *Annu. Rev. Physiol.* **44,** 519–534.
6. Ogura, A. & Takahashi, K. (1976) *Nature (London)* **264,** 170–172.
7. Dunlap, K. (1977) *J. Physiol. (London)* **271,** 119–133.
8. Machemer, H. & Ogura, A. (1979) *J. Physiol. (London)* **296,** 49–60.
9. Thiele, J. & Schultz, J. E. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3688–3691.
10. Naitoh, Y. & Kaneko, H. (1972) *Science* **176,** 523–524.
11. Thiele, J., Klumpp, S., Schultz, J. E. & Bardele, C. F. (1982) *Eur. J. Cell Biol.* **28,** 3–11.
12. Gitelman, S. E. & Witman, G. B. (1980) *J. Cell Biol.* **98,** 764–770.
13. Satir, B. H., Garofalo, R. S., Gilligan, D. M. & Maihle, N. J. (1980) *Ann. N.Y. Acad. Sci.* **356,** 83–91.
14. Stommel, E. W., Stephens, R. E., Masure, H. R. & Head, J. F. (1982) *J. Cell Biol.* **92,** 622–628.
15. Otter, T., Satir, B. & Satir, P. (1984) *Cell Motil.* **4,** 249–267.
16. Maihle, N. J., Dedman, J. R., Means, A. R., Chafouleas, J. G. & Satir, B. H. (1981) *J. Cell Biol.* **89,** 695–699.
17. Ohnishi, K., Suzuki, Y. & Watanabe, Y. (1982) *Exp. Cell Res.* **137,** 217–227.
18. Hamasaki, T. & Naitoh, Y. (1985) *Proc. Jpn. Acad.* **61B,** 140–143.
19. Okuno, M. (1986) *Dev. Growth Diff. (Suppl.)* **28,** 82 (abstr.).
20. Katada, J., Shingyoji, C. & Takahashi, K. (1986) *Cell Struct. Funct.* **11,** 510 (abstr.).
21. Moss, A. G. & Tamm, S. L. (1986) *J. Comp. Physiol.* **158A,** 311–330.
22. Tamm, S. L. (1982) in *Electrical Conduction and Behaviour in "Simple" Invertebrates*, ed. Shelton, G. A. B. (Oxford Univ. Press), pp. 266–358.
23. Tamm, S. L. (1984) *J. Exp. Biol.* **113,** 401–408.
24. Satterlie, R. A. & Case, J. F. (1978) *Cell Tissue Res.* **193,** 87–91.
25. Horridge, G. A. & Mackay, B. (1964) *Q. J. Microsc. Sci.* **105,** 163–174.
26. Tamm, S. L. & Moss, A. G. (1985) *J. Exp. Biol.* **114,** 443–461.
27. Moss, A. G. & Tamm, S. L. (1985) *J. Cell Biol.* **101,** 270 (abstr.).
28. Jack, J. J. B., Noble, D. & Tsien, R. W. (1975) *Electric Current Flow in Excitable Cells* (Oxford Univ. Press), pp. 60–66.
29. Moss, A. G. & Tamm, S. L. (1984) *J. Cell Biol.* **99,** 186 (abstr.).
30. Nakamura, S. & Tamm, S. L. (1985) *J. Cell Biol.* **100,** 1447–1454.
31. Dietmer, J. W. (1986) *J. Physiol. (London)* **380,** 551–574.
32. Dietmer, J. (1984) *J. Physiol. (London)* **355,** 137–159.
33. Chad, J. E. & Eckert, R. (1984) *Biophys. J.* **45,** 993–999.
34. Simon, S. M. & Llinas, R. R. (1985) *Biophys. J.* **48,** 485–498.
35. Cavenaugh, G. (1956) *Formulae and Methods VI of the Marine Biological Laboratory Chemical Room* (Marine Biological Laboratory, Woods Hole, MA).