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A NOVEL TYPE OF CILIARY ACTIVITY IN *STYLONYCHIA MYTILUS*: ANALYSIS OF POTENTIAL-COUPLED MOTOR RESPONSES IN THE TRANSVERSE CIRRI

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Summary

The motor responses of the transverse cirri of *Stylonychia mytilus* were investigated by applying high-speed microcinematography and step voltage-clamp. As a response to hyperpolarization, the transverse cirri began to swing posteriorly from an inactive upright posture at rest. The deeply inclined posture was maintained as long as the hyperpolarizing pulse was on. Upon depolarization, the cirri began to swing towards the anterior end of the cell and continued regular cyclic beating, orienting the effective stroke anteriorly. Motor responses of the transverse cirri occurred in quasi-planar motion, allowing analysis of the bend configuration along the full length of the cirri. Beating activity induced during sustained depolarization was virtually stable, with different oscillation profiles at base and tip. Cyclic movement of the distal region was enhanced at large amplitudes of depolarization. Termination of a hyperpolarizing voltage step induced a transient depolarization-type anterior beating, and termination of a depolarizing step induced a transient posteriad inclination of the transverse cirri. In both hyperpolarization- and depolarization-induced motor responses, a shear angle analysis of the initiation of the response indicated that sliding displacement of doublet microtubules was initiated at the base and propagated towards the tip. The discovery in a ciliary organelle of a very distinct response to hyperpolarizing and depolarizing stimulation is highly useful for the analysis of ciliary electromotor coupling. The functions of intraciliary Ca^{2+} in the regulation of the motor responses are discussed.

Introduction

The ciliary motor response in ciliates is closely coupled to membrane potential changes (Machemer, 1986, 1990). In *Paramecium* and *Stylonychia*, it has been established that membrane potential directly controls both the direction and the frequency of beating (Machemer, 1988; Machemer and Deitmer, 1987). Depolar-

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ization of the cell membrane induces a counterclockwise shift of the ciliary beating direction and an increase in frequency of reversed beating (depolarization-induced ciliary activity, DCA). Hyperpolarization of the membrane, in contrast, induces a clockwise shift of beating direction that orients the effective stroke towards the cell posterior and is associated with a rise in beat frequency (hyperpolarization-induced ciliary activity, HCA).

Recently the voltage-coupled motor responses in the frontal cirri of *Stylonychia* have been analyzed using axial view recordings (Sugino and Machemer, 1988, 1990). Quantitative analysis of the variables of three-dimensional ciliary beat cycles revealed that, unlike in *Paramecium*, the beat orientation of the frontal cirri is insensitive to negative voltage steps. In this paper we introduce a new type of motor response discovered in the transverse cirri of *Stylonychia*, which occur on the posterior oral surface. The activity of the transverse cirri is responsible for the characteristic backward jump of hypotrich ciliates. This pronounced motion seems to be coupled to the electrical activity of the membrane. In the transverse cirri of *Stylonychia*, DCA is a cyclic movement with the effective stroke directed anteriorly. HCA, in contrast, is a noncyclic motion; the cirri swing posteriorly and remain inclined during hyperpolarization. Both DCA and HCA of these cirri occur virtually in one plane. These two distinct motor responses, when performed by the same cirrus, reinforce the notion of the existence of two different programs for activating the ciliary axonemes under membrane potential control. Because of the ease of the observation, the transverse cirri may serve as a model for the study of mechanisms linking the bioelectric and motile events in the ciliary machinery.

Previously we demonstrated that Ca^{2+} is a major intraciliary messenger, with levels decreasing during HCA and increasing during DCA (Mogami *et al.* 1990). Furthermore, quantitative approximations indicate that, during HCA, Ca^{2+} is removed from the cilia, depleting bound calcium (Mogami and Machemer, 1991). Our data show that down-and-up regulation of intraciliary Ca^{2+} from a resting level has an interesting spatial correlate in voltage-dependent motor activation of the transverse cirri.

Materials and methods

Stylonychia mytilus (wild type) were cultured in Pringsheim solution and fed with the green phytomonad *Chlorogonium elongatum*. After the logarithmic growth phase, cells starved for 2–5 days were washed and equilibrated for at least 1 h in the experimental solution containing 1 mmol l^{-1} KCl, 1 mmol l^{-1} $CaCl_2$ and 1 mmol l^{-1} Tris–HCl, pH 7.4. All experiments were carried out at a controlled temperature of 17–18 °C.

For conventional voltage-clamp experiments, cells were impaled by two glass microelectrodes, which were aimed at the anterior and posterior macronuclei (one electrode for monitoring voltage filled with 1 mol l^{-1} KCl, 40–60 MΩ, the other for current passing filled with 2 mol l^{-1} potassium citrate, 30–60 MΩ). The tips of the intracellular electrodes were oriented parallel to the longitudinal cell axis. The

electrophysiological methods for mounting and recording from single *Stylonychia* cells have been summarized elsewhere (Machemer and Deitmer, 1987). Cells showing typical resting potentials of −55 to −45 mV (−48.6±2.7 mV, N=41) with input resistances of more than 30 MΩ were used for the experiments.

The transverse cirri of *Stylonychia* are located on the oral ('ventral') surface of the posterior end of the cell (for identification of the ciliary organelles, see Machemer and Deitmer, 1987). To obtain a lateral view of the cirri, cells were rotated, after impalement, around the axis connecting the tips of two electrodes. The reorientation of the cell from a dorso-ventral to a lateral view was guided by two additional microneedles, which also supported the cell during the experiment. Details of the method of cell rotation have been summarized elsewhere (Mogami *et al.* 1991). Usually, three out of five transverse cirri (T3–T5; Machemer and Deitmer, 1987) were observed in one focal plane (see Fig. 1) and selected for the analysis of the movement.

For microcinematographical recordings, a 16 mm high-speed shutter camera (Locam 164 5DC, Red Lake) was connected to a compound microscope equipped with interference contrast optics (Carl Zeiss, objective ×16) and run at 250 frames per second under electrically triggered stroboscopic illumination (Strobex model 136, Chadwick-Helmuth). Recorded images were projected at a magnification of about ×150 and analyzed frame by frame.

For the assessment of cirral movement, we directly measured the angular changes of the cirral shaft. The base angle represented in this paper is the tangential angle of the center line of the cirrus at a fixed distance of 5 μm (about twice the radius of the cirri) from the base. The tip angle is the tangential angle at the most distal section of an image. Shear angle curves were obtained from the measurement of the tangential angles at intervals of 2.5 μm from base to tip of the cirrus. All angles are represented by deviation (positive in the anterior direction) from the normal to the cell surface at the cirral base.

Results

Voltage control of transverse cirri

When the membrane was clamped at the resting potential, the transverse cirri were motionless ('inactive'). The cirri were inclined posteriorly (10–20° from the right angle to the cell surface) with minor bends in the proximal and distal regions (Fig. 1). Two separate responses were induced by hyperpolarizing and depolarizing potential shifts from the resting potential: a posteriad reorientation followed by a maintained, deeply inclined posture as a hyperpolarization-induced ciliary activity (HCA, Fig. 1A), and a cyclic beating towards the anterior cell end as a depolarization-induced ciliary activity (DCA, Fig. 1B). Unlike the three-dimensional beating of most ciliary organelles, including cirri and membranelles in *Stylonychia* (Machemer, 1988; Machemer and Deitmer, 1987), the movements of the transverse cirri were highly polarized in space. Axial views of the response (Fig. 2A,B) demonstrated that the movements, both HCA and DCA, are

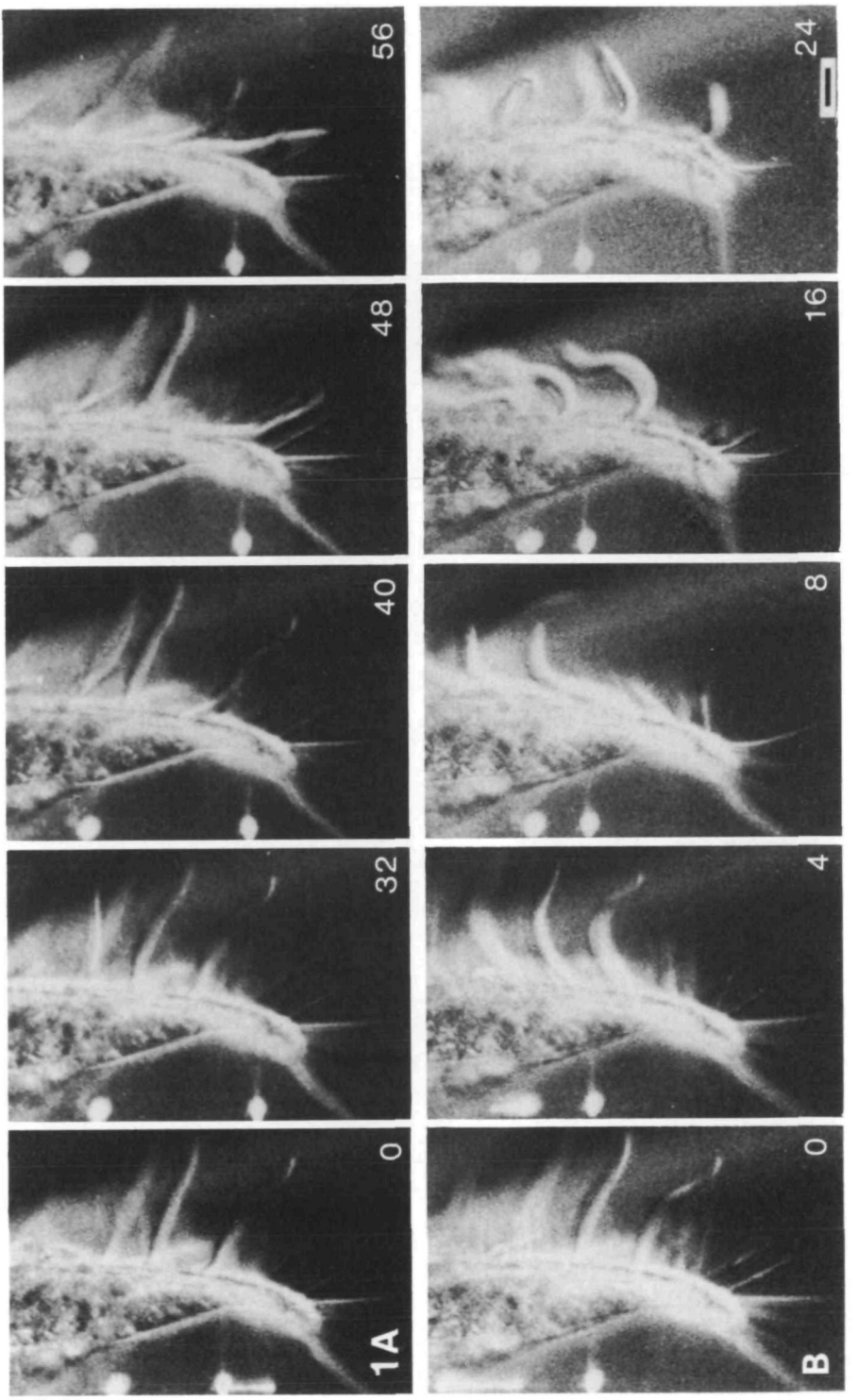
1A
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Fig. 1. Sequential micrographs from high-speed recordings of the voltage-dependent responses of the transverse cirri (T3–T5) of *Stylonychia*. (A) Posteriad single inclination induced by a hyperpolarization of −10 mV. (B) Anteriad repetitive beating induced by a depolarization of 10 mV. The anterior end of the cell is towards the top of the figure. Numbers in each frame represent time (in ms) from the onset of the voltage-clamp pulse. Scale bar, 10 μm.

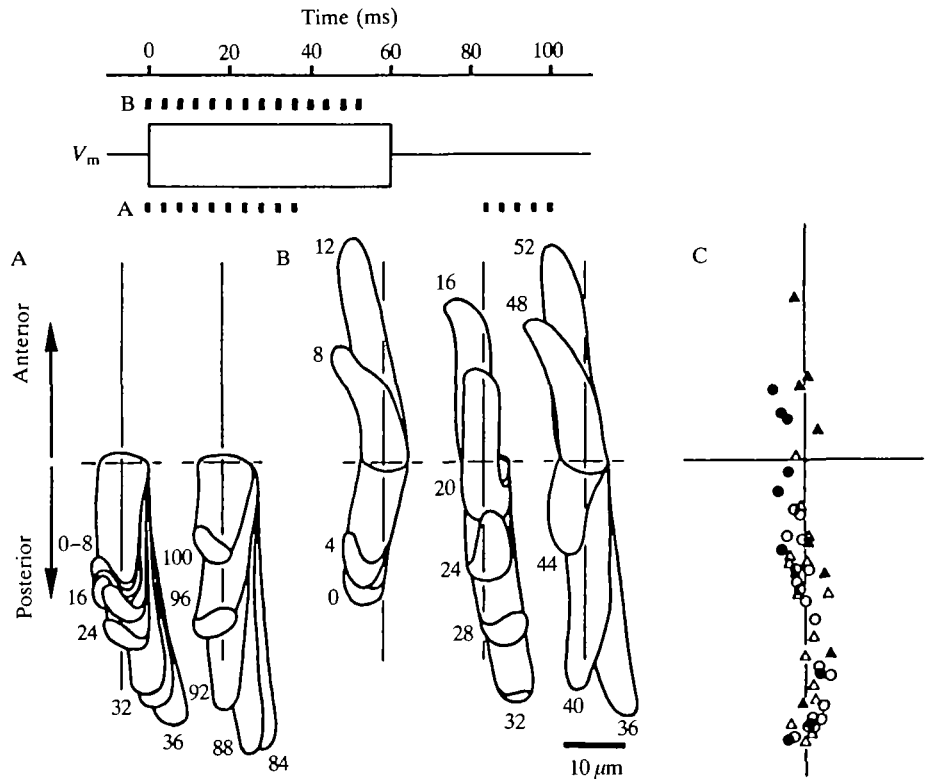

Fig. 2. Axial views of the motor responses of transverse cirri. (A,B) Superimposed tracings (viewed from tip to base; anterior cell end up) of the cirral movement induced by hyperpolarization (−10 mV; A) and depolarization (10 mV: B). Numbers near tracings show the time (in ms) from the onset of the clamp pulse (60 ms) as indicated in the inset. (C) Plot of the position of the tip of the cirrus during the responses induced by hyperpolarizations of −5 (○) and −20 mV (△) and depolarizations of 5 (●) and 20 mV (▲). The data are from a single cirrus at times shown in A and B and projected onto the same focal plane (parallel to the cell surface). Vertical lines indicate the anteroposterior cell axis passing through the base of the cirrus. Inset: time scale, including voltage pulses (V_m) and selected frames during hyperpolarization (A) and depolarization (B).

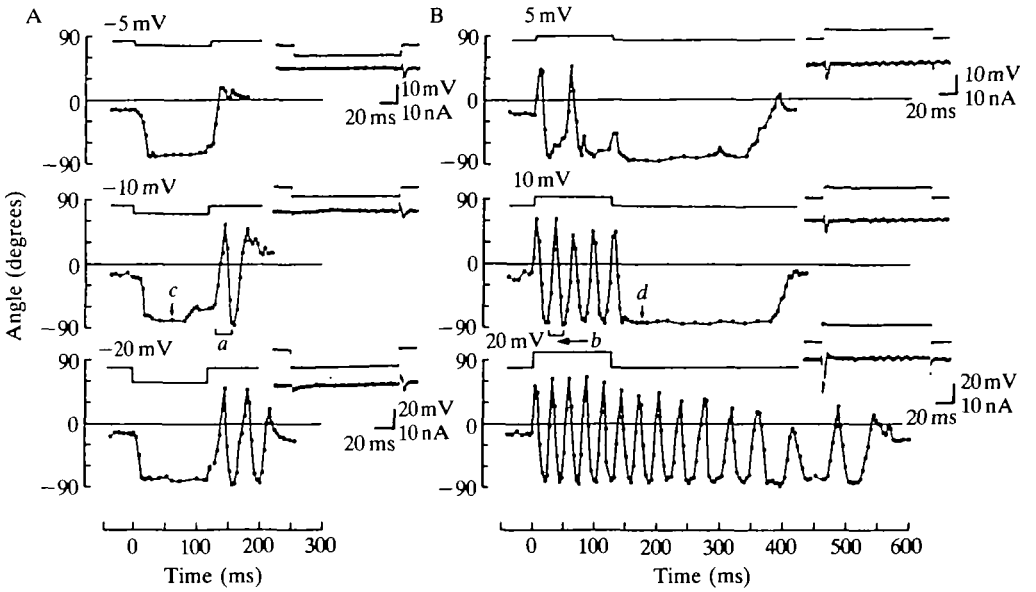

Fig. 3. Motor responses of transverse cirri induced by various amplitudes of hyperpolarizing (A) and depolarizing (B) 120-ms voltage steps. Movements were assessed by the changes in base angle as a function of time. Insets show original oscilloscope records of the voltage stimulus (upper traces) and the membrane current (lower traces). Marked times (*a–d*, middle diagrams) correspond to the bend configuration of the cirral shaft shown in Fig. 9. All data are from the same cirrus.

restricted to nearly a single plane roughly parallel to the cell axis. The plane was reproduced in the responses to different amplitudes of negative- and positive-going shifts of membrane potential (Fig. 2C). The polarized property of the motion allowed us to analyze the movement over the whole length of the cirrus in one focal plane of the microscope.

We have analyzed the voltage- (Fig. 3) and time-dependencies of HCA and DCA (Fig. 4) as represented by the changes in base angle. During HCA, the base angle shows a negative shift corresponding to posterior inclination. The latent period of the inclination response decreased with increasing amplitude of hyperpolarization. A brief (10 ms) pulse did not induce the response (Fig. 4A, top). After inclining posteriorly, the cirri became quiescent in a deeply inclined posture with a steady base angle near −90°, where they formed one sharp bend in the proximal region and two bends of opposite direction in the middle and the distal regions. The inclined posture appeared over a wide range of hyperpolarization (up to −100 mV), and no repetitive beating activity was observed. At suprathreshold hyperpolarization (exceeding 10 mV step), the cirri maintained the inclined posture until the end of the pulse (Fig. 4A) and began to swing back immediately after repolarization. The end of HCA occurred with a delay of about 20 ms, irrespective of the amplitude and the duration of hyperpolarization

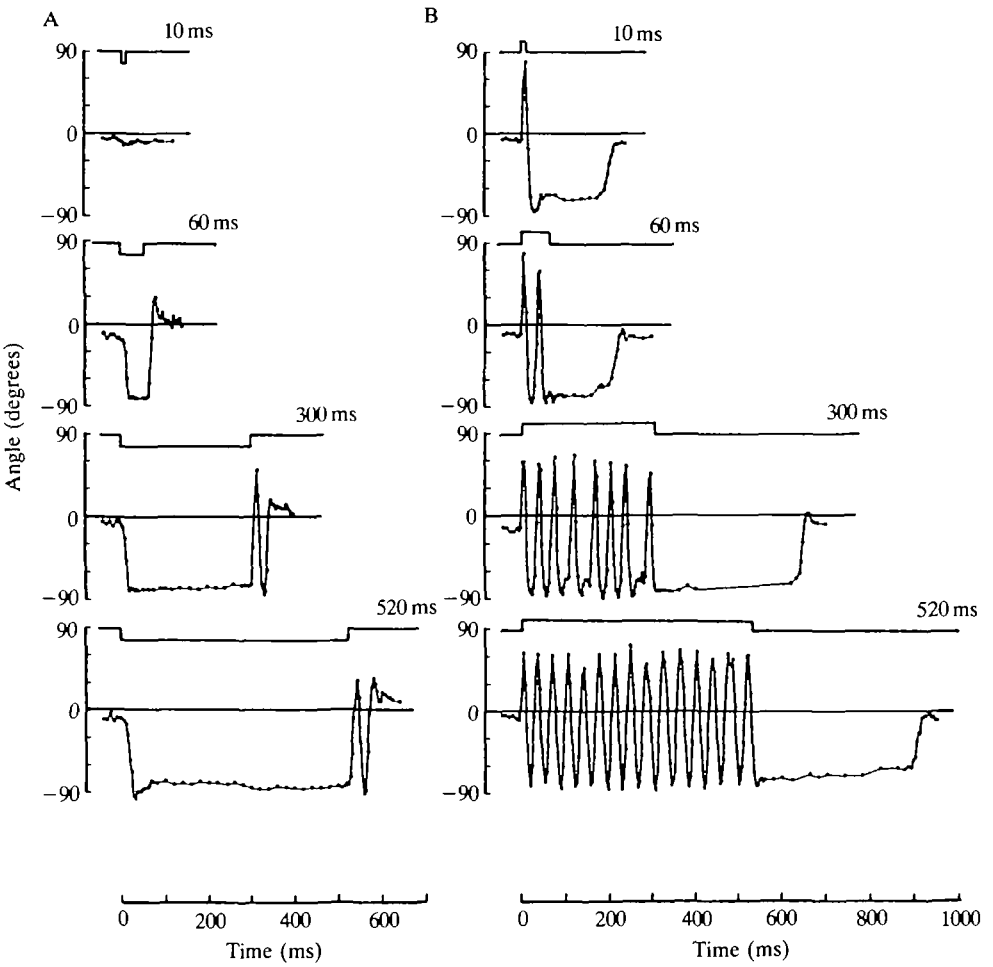

Fig. 4. Effects of voltage-step duration on motor responses of transverse cirri. (A) Hyperpolarization (−10 mV); (B) depolarization (10 mV). Changes in base angle at different pulse durations are shown as a function of time. Data are combined from two cirri (10 and 520 ms from one cirrus; 60 and 300 ms from another).

(Fig. 5A,B). The swing-back went beyond the resting position, inducing a few oscillations. This post-hyperpolarization oscillation was potentiated with increases in both amplitude (Fig. 3A) and duration of the hyperpolarizarion (Fig. 4A).

Positive shifts of the base angle during DCA are more voltage-sensitive and occur within a shorter latency as compared with HCA (Fig. 4B, top). The initial anteriad swing was always followed by cyclic activity, the effective stroke being oriented towards the cell anterior. The frequency and amplitude of DCA rose with increases in the depolarization amplitude (Fig. 3B); the response was saturated by steps greater than 20 mV. The cirri maintained beating activity while the depolarization persisted. In small depolarizing steps (e.g. <10 mV, Fig. 3B, top)

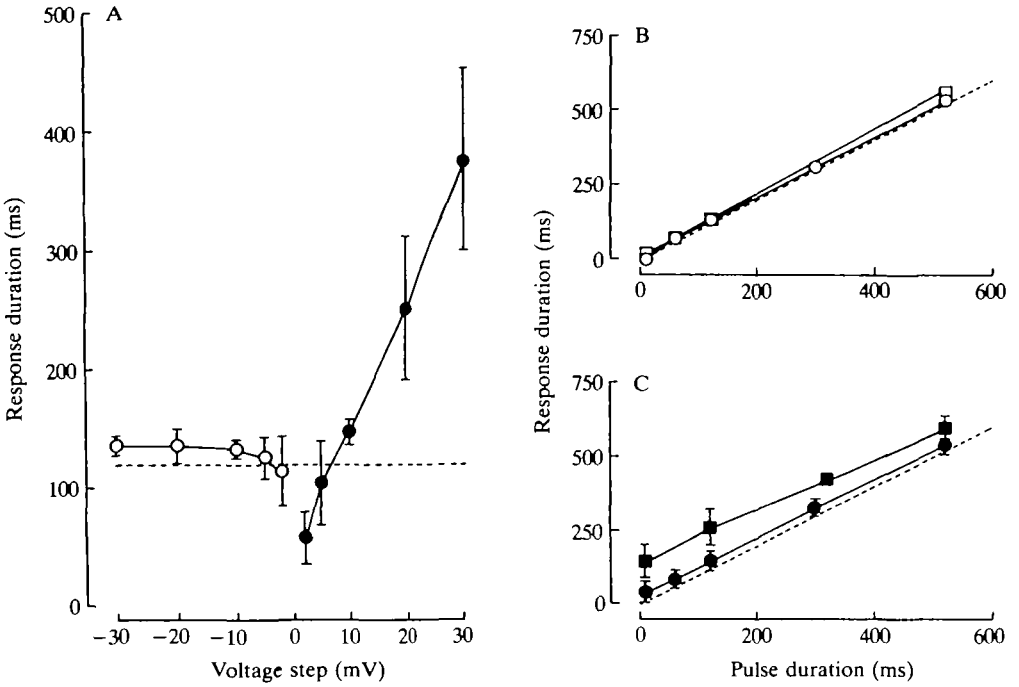

Fig. 5. Voltage- and time-dependency of the response duration. The duration of posteriad inclination (open symbols) and anteriad beating (closed symbols) were measured from the onset of the voltage step, including latency. (A) Response duration plotted against the amplitude of a 120-ms voltage step. (B,C) Response duration plotted against the voltage-step duration (squares, 20 mV; circles, 10 mV). Mean±S.D. from at least seven cirri are shown. Standard deviations in B are within the size of the symbols. Dashed lines indicate the end of the voltage step.

the beating tended to be unstable, with the cirri resting at the end of the recovery stroke. Larger depolarizations (≥10 mV) consistently induced the cirri to beat until the end of the depolarization. In contrast to HCA, DCA lasted beyond the end of the pulse (Fig. 3B, bottom), the response increasing in duration with increase in amplitude of the voltage step (Fig. 5A). The duration of DCA increased in proportion to the stimulus duration (Fig. 5C). Interestingly, DCA induced by moderate (10 mV) depolarization ended in synchrony with the voltage step, irrespective of pulse duration (Figs 4B and 5C).

Anterior beating during DCA did not terminate at the neutral posture. When the membrane potential was repolarized from a depolarization of 10 mV or below, the cirri became transiently quiescent in the deeply inclined posture (Fig. 3B, top and middle). After larger depolarizations, cirri continued beating beyond the end of voltage steps at reduced frequency and amplitude (Fig. 3B, bottom) and eventually became quiescent in the inclined posture. From this posture they later returned to the neutral state.

Initiation of the responses

The latency of the motor response differed along the length of the cirri. In both HCA and DCA, the activity primarily occurred at the base and propagated towards the tip. Fig. 6 shows the shear angle (tangential angle of the cirral shaft) along the length of the cirri. Since the motion of transverse cirri is nearly planar, we take the tangential angle as a direct measure of the sliding displacement of the peripheral doublet microtubules. In HCA, deflection from the neutral position to

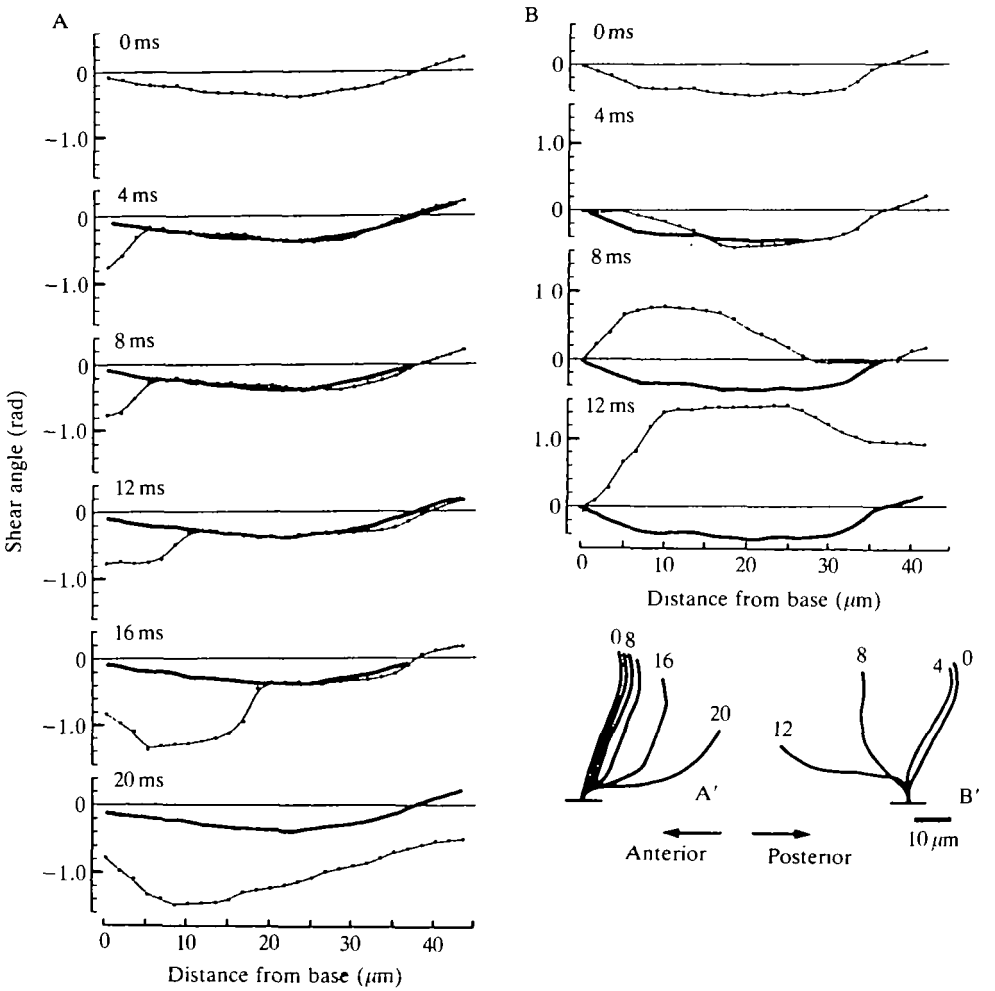

Fig. 6. Shear angles of a transverse cirrus in the first movement following the initiation of a voltage-dependent response (—●—). (A) Hyperpolarizing voltage step (−10 mV); (B) depolarizing voltage step (5 mV). Numbers beside the curves indicate the time (in ms) from the onset of the voltage step (0 ms). (——) The shear curves obtained at 0 ms. Insets (A',B') show superimposed tracings of the cirral shaft in lateral view with bend forms corresponding to the shear angle curves.

the negative (=posterior) side occurred first in the proximal region (Fig. 6A). In DCA, the initial response was a proximal deflection to the positive (=anterior) side (Fig. 6B). The propagation velocity of the sliding activity increased with stimulus amplitude; it was greater during DCA than during HCA.

Since sliding of doublet microtubules occurs in one direction as a result of force generation of dynein arms towards the tip (Sale and Satir, 1977; Mogami and Takahashi, 1983), the initial angular changes in the opposite directions between HCA and DCA indicate that sliding movement was activated on opposite sides of the axoneme; on the left side in HCA and on the right side in DCA, with respect to the cell axis and viewed from tip to base of the cirri.

Steady-state responses

As long as a hyperpolarizing pulse was maintained, the cirri maintained the deeply inclined posture. This posture was occasionally interrupted by a sudden relaxation of the proximal bend, with the initial bend angle at the tip being unchanged (data not shown).

A depolarization above threshold induced regular anterior beating activity as long as the depolarization was maintained (Figs 4B and 7A). The frequency of the beating was almost constant during the pulse (Fig. 7B). Normalization of beating with respect to the beat period shows the basic pattern of the angular changes during the complete cycle. Fig. 7C characterizes the pattern of cyclic movement in proximal and distal regions at different amplitudes of depolarization. The sinusoidal profile of the pattern of the proximal region differs substantially from that of the distal region. With an increase in the pulse amplitude from 10 to 20 mV, the beat frequency increased by 10.0±3.0 % (N=4). Voltage-dependent changes in beating activity are found in the pattern of the distal region, where the beating amplitude extended anteriorly (positive-side deflection) by 20–30° at the higher voltage, whereas the most posterior (negative side) level was unchanged. In contrast, changes in beat amplitude were not observed in the proximal pattern. Bend activation of DCA was spatially more differentiated following small depolarizations; here, angular changes occurred predominantly in the proximal region of the cirrus (Fig. 8).

Off-responses

Recovery from posteriad inclination was frequently followed by oscillatory movements. As shown in Fig. 3A, an approximately direct transition to the neutral posture was observed after a small hyperpolarization. With increases in hyperpolarization amplitude, the recovery included some post-stimulus oscillations (DCA-type off-response). The number of oscillations increased with the stimulus amplitude. Post-hyperpolarization oscillations were also a function of stimulus time: one additional cycle of the off-response was observed when

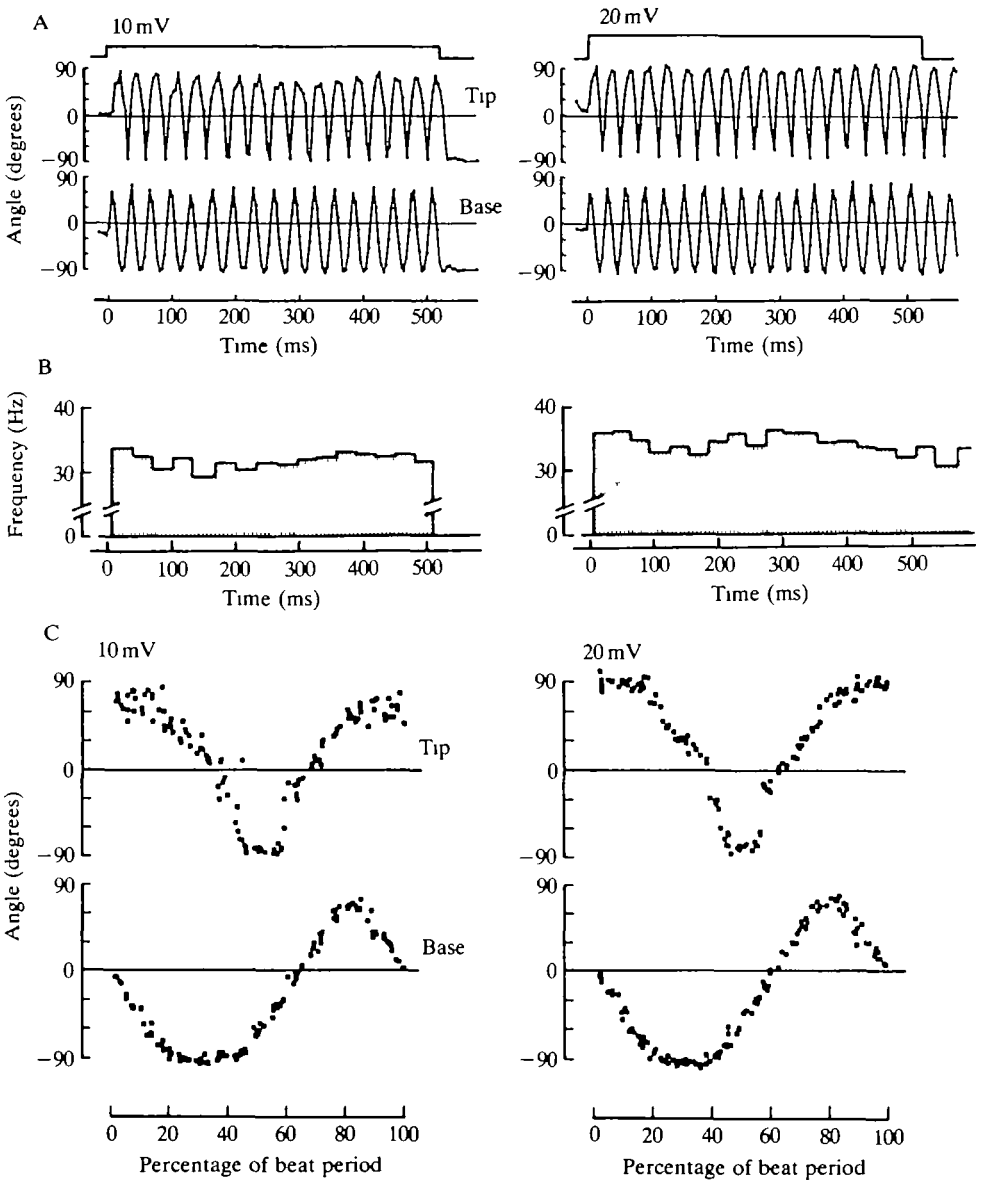

Fig. 7. Cyclic activity of transverse cirri induced by depolarizing voltage steps of two different amplitudes (10 mV, left-hand column; 20 mV, right-hand column). (A) Changes in tip angle (upper) and base angle (lower) as a function of time. (B) Frequency profiles of the beating shown in A. (C) Normalized beat cycles at the tip (upper) and base (lower). For each cycle shown in A, the beat period was measured from the angular changes at the base. Time intervals between passes across 'zero' degrees were obtained by interpolation of the nearest neighbors between which the angle changed sign from negative to positive. The time base for each point in C is given as a percentage of the beat period to the interval obtained.

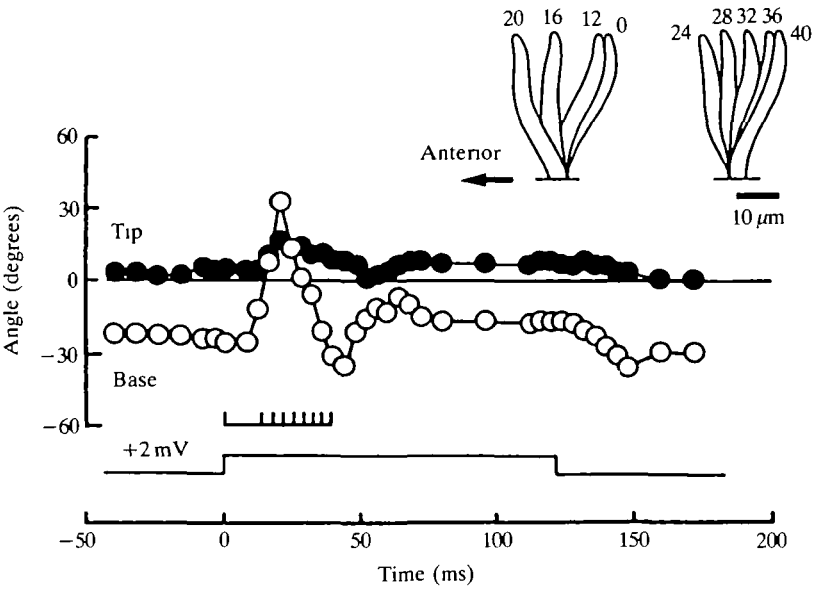

Fig. 8. Threshold activation of a transverse cirrus induced by a small depolarization (2 mV from the resting level of −51 mV). Angles at the base (○) and tip (●) are plotted as a function of time. Inset; superimposed tracings of the cirrus corresponding to the times indicated.

stimulus pulse duration exceeded 60 ms. Further enhancement of the off-response was not noted (Fig. 4A).

Post-hyperpolarization oscillations (Fig. 9A) are similar to those of anterior beating during DCA (Fig. 9B). Superimposed tracings and the corresponding shear curves of post-hyperpolarization beating indicate a sharp bend with its convex side towards the posterior and propagating from base to tip, suggesting a recovery stroke directed posteriorly. Sliding displacement for the anteriad effective stroke was somewhat reduced along the length of the cirrus as compared to normal DCA, while equal displacements occurred for the recovery stroke. The overall spatial similarity between the DCA-type off-response and DCA suggests that termination of hyperpolarization is associated with an influx of Ca^{2+} into the cilia.

Stepping back from depolarization induced the cessation of anteriad beating. Cirri took a posteriorly inclined posture before returning to the neutral orientation (HCA-type off-response, Figs 3B, 4B). The form of termination of the DCA response was voltage- and time-dependent. Fig. 9C,D shows the similarity of the bend configurations of posteriorly inclined postures recorded during hyperpolarization (HCA, Fig. 9C) and after depolarization (HCA-type off-response, Fig. 9D). It is, therefore, likely that the transverse cirri, after termination of DCA, transiently pass through a functional state similar to that induced by hyperpolarization.

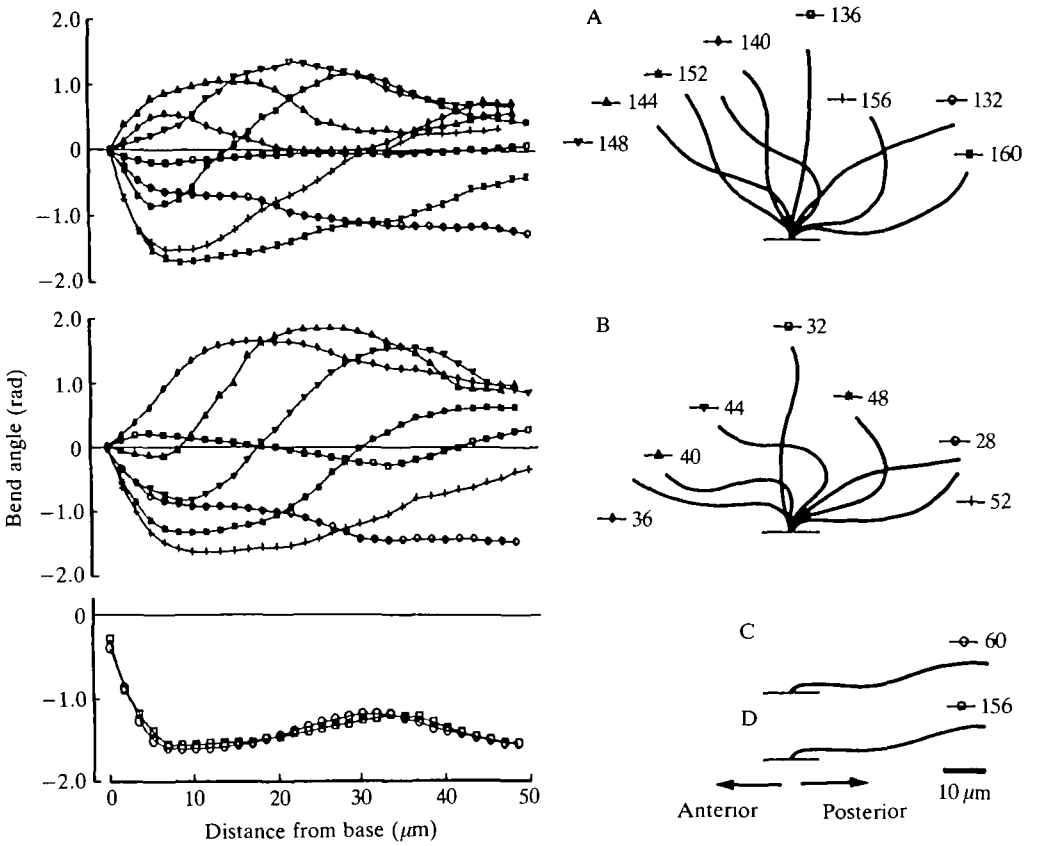

Fig. 9. Spatial analysis of off-responses. (A) Bend configuration of the post-hyperpolarization DCA-type off-response. (B) Anterior beating during depolarization (DCA). (C) Deeply inclined posture during hyperpolarization (HCA). (D) Post-depolarization posture in an HCA-type off-response. Right-hand column, tracings of the cirral shaft, and left-hand column, the corresponding shear angle curves of the same cirrus. A–D are the recordings corresponding to *a–d* in the middle diagram in Fig. 3, with times (in ms) from the onset of the pulse marked near the tracings.

Discussion

General properties of the cirral motor responses

The responses of ciliary organelles in ciliates are characterized by cyclic gyration in a counterclockwise direction viewed from tip to base. Changes in activity, induced by a stimulus, occur by modification of the gyratory profile. Motion of the transverse cirri of *Stylonychia* differs from that of the majority of ciliary organelles in ciliates and even from that of other groups of cirri of the same species. A nearly planar movement of the transverse cirri occurs in two modes: a noncyclic posteriad inclination following membrane hyperpolarization, and a cyclic anteriad beating following depolarization (Fig. 2). This novel finding of distinct responses to hyperpolarizing and depolarizing stimulation is a useful tool in the analysis of

electromotor coupling in ciliates. In the cilia of *Paramecium*, graded transitions exist between these activities (Machemer and Sugino, 1989).

In order to determine the common properties of the voltage-dependent responses of various ciliary organelles, a comparative approach may be informative. We find the following similarities between the electromotor coupling of the transverse cirri and of the marginal and frontal cirri of *Stylonychia* (de Peyer and Machemer, 1982*a*,*b*, 1983; Sugino and Machemer, 1988, 1990; Mogami *et al.* 1991): (1) inactivity of the cirri at the resting potential; (2) directionality of the initial movements, that is, a posteriad swing at the onset of hyperpolarization and an anteriad swing with depolarization; (3) maintenance of HCA and DCA over the entire time of the potential shift.

In *Paramecium*, the cilia are inactive only when the membrane is slightly depolarized (Machemer and Sugino, 1989) so that point 1 applies to depolarization only. The persistence of a motor response during a continued voltage shift (point 3), which is applicable to all ciliates so far investigated, indicates that the intraciliary messenger (and modulators) is 'clamped' to the membrane potential.

A voltage-dependent activation of the cirri from quiescence (points 1 and 2) suggests the existence of differential activation of sliding movements. Regarding unidirectionality of ciliary gyration in ciliates, it follows that, with membrane hyperpolarization, shear forces of the peripheral doublet microtubules are activated in the right half-cylinder of the axoneme (as viewed from tip to base), and with depolarization, in the left half-cylinder (Machemer, 1977). The switching-point hypothesis (Satir, 1985) envisions two molecular switches in the extreme posture of mollusc lateral gill cilia ('hands-up' and 'hands-down') which alternately turn on separate halves of the axoneme. With this perspective, at least one additional switch might be envisioned for the transverse cirri to account for the neutral resting posture. Alternatively, the oscillatory motion of the transverse cirri during DCA suggests a predominantly continuous circumferential transfer of sliding activity between doublet microtubules comparable to conclusions from observations of the three-dimensional movement of the frontal cirri of *Stylonychia* (Sugino and Machemer, 1988, 1990) and from the analysis of planar movement in sea urchin sperm flagella (Baba *et al.* 1990). Different details of the programming of the transfer of the activity in the transverse cirri from those in the other organelles might explain the specialized movement of the transverse cirri. A revised version of the switching-point hypothesis (Satir and Sleigh, 1990) suggests an increase in the number of interdoublet switches, indicating a convergence with an alternative hypothesis ('rotary sliding machine', Machemer, 1977; 'active site rotation model', Baba *et al.* 1990).

We have recently demonstrated that, when applying large positive voltage steps under voltage-clamp in *Stylonychia* (so as to exceed the calcium equilibrium potential; $V_m > E_{Ca}$; calcium driving force outward), an HCA-type activation occurred in the transverse and marginal cirri. With the membrane potential clamped below the calcium equilibrium potential ($V_m < E_{Ca}$; calcium driving force inward), the cirri showed DCA (Mogami *et al.* 1990; Mogami and Machemer,

1991). This supports the hypothesis that Ca^{2+} removal from, and binding to, axonemal sites are signalling steps to induce HCA and DCA, respectively (Mogami and Machemer, 1990). In particular, these data suggest a decrease in $[Ca^{2+}]_i$ from the resting level to initiate the posterior swing and an increase in $[Ca^{2+}]_i$ to initiate the anterior swing of the transverse cirri (point 2).

Spatial properties of the responses

Analysis of the responses of transverse cirri revealed a tipward propagation of shear activity (Fig. 5), in agreement with data from cilia from other sources (cf. Baba and Mogami, 1987). If shear activity were initiated by changes in Ca^{2+} concentration, it would, nevertheless, be unlikely that propagation of the activity was caused by a Ca^{2+} 'diffusion wave'. Voltage-activated Ca^{2+} channels are distributed over almost the entire length of cilia (Moss and Tamm, 1987; Thiele *et al.* 1982), which are good cables for propagation of the voltage signal (see Machemer, 1986). It follows that depolarization induces a nearly homogeneous increase in Ca^{2+} concentration along the ciliary shaft. This evidence suggests that there are gradients of Ca^{2+}-sensitivity along the axoneme. In fact, ionophoretic application of Ca^{2+} to detergent-extracted *Paramecium* cilia indicates a high sensitivity of the basal axoneme for Ca^{2+}-induced ciliary reorientation (Hamasaki and Naitoh, 1985). The data of these authors are also consistent with our observations that, with very small depolarization, shear angles at the base exceeded those at the ciliary tip (Fig. 8); the latter eventually rose with increasing depolarization (Fig. 7C).

Off-responses

The transverse cirri show a peculiar behavior after the membrane has been stepped back to the resting potential: HCA is followed by a DCA-type anteriad beating, and DCA is followed by an HCA-type posteriad inclination (Figs 3 and 4). A post-DCA HCA-type activation of cilia was documented in *Paramecium* following a large action potential (Machemer, 1974) and in the marginal cirri of *Stylonychia* after a step back to the resting potential from depolarization (de Peyer and Machemer, 1983).

Stepping back from hyperpolarization elicits a transient inward Ca^{2+} current ('anode-break excitation') by opening a low-threshold Ca^{2+} channel in *Stylonychia* (Deitmer, 1984). Our observation of a DCA-type off-response following HCA is explained by assuming that low-threshold Ca^{2+} channels exist in the transverse cirri and that the 'anode-break current' raises $[Ca^{2+}]_i$. Conversely, the HCA-type off-response following DCA is explained by the conventional assumption that a raised Ca^{2+} pumping rate (from residual Ca^{2+} influx during sustained depolarization; Brehm *et al.* 1980) extends beyond the rapid closure of the Ca^{2+} channel to decrease $[Ca^{2+}]_i$ below the resting level.

The observed off-responses of the cilia may be explained by time-dependent changes in Ca^{2+}-sensitivity of the motile machinery. If the threshold $[Ca^{2+}]_i$ needed to induce HCA were to rise gradually during sustained depolarization, a

rapid repolarization could induce HCA even at the resting potential. A sustained hyperpolarization may lower the threshold $[Ca^{2+}]_i$ needed to induce DCA. Hamasaki *et al.* (1989) demonstrated a Ca^{2+}- and cyclic-AMP-dependent axonemal polypeptide phosphorylation in *Paramecium*. These phosphorylations and dephosphorylations of axonemal components might be associated with slow regulations of axonemal Ca^{2+} sensitivity.

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