J. Physiol. (1984), **355**, *pp.* 137–159
With 12 text figures
Printed in Great Britain

EVIDENCE FOR TWO VOLTAGE-DEPENDENT CALCIUM CURRENTS IN THE MEMBRANE OF THE CILIATE *STYLONYCHIA*

BY JOACHIM W. DEITMER

From the Abteilung für Biologie, Ruhr-Universität, D-4630, Bochum, F.R.G.

(*Received 22 February 1984*)

SUMMARY

1. Action potentials and voltage-dependent membrane currents have been investigated in the fresh-water hypotrich ciliate *Stylonychia mytilus*, using two intracellular micro-electrodes.

2. The inward current–voltage (I_{in}–V) relationship has two maxima, the first around −45 mV, and the second around −17 mV (resting and holding membrane potential being −50 mV). The shape of the I_{in}–V relationship is virtually unaltered in the presence of the K-channel blockers tetraethylammonium, 4-aminopyridine or internal Cs.

3. The inward currents exhibit a differential sensitivity to both external Co and Cd; the inward current activated at potentials $\geqslant$ −40 mV is more sensitive to these divalent cations than the inward current activated at around −45 mV. This suggests the presence of two different types of Ca inward currents.

4. Both types of inward currents are present when Ca is replaced by Ba (or Sr). The small inward current recorded between −48 and −40 mV relaxes similarly in Ca and in Ba solutions. The larger inward current, recorded at −30 or −20 mV, relaxes rapidly in Ca solution but only slowly and incompletely in Ba solution.

5. A two-pulse protocol revealed that for both types of inward currents inactivation may depend partially upon the influx and/or intracellular accumulation of the charge-carrying divalent cation. There appears to be a significant difference in the degree of inactivation of the two types of inward currents, however, when Ba is the charge carrier.

6. When the cell spontaneously released, or was induced to release its membranellar band (row of compound cilia), the second, 'all-or-none' component of the action potential, and the maximum of the I_{in}–V relationship at −45 mV disappeared. The first, graded peak of the action potential and the larger maximum of the I_{in}–V relationship remained essentially unaltered. The smaller Ca current and the action potential shoulder also disappeared when the anterior half of the cell (with most of the membranellar band) was severed, but not when the posterior half was cut off. When recording from a membranellar band vesicle both types of inward currents were present.

7. The results suggest that the two components of the action potential may correspond to the two types of Ca currents. These Ca currents are separable by their localization in the membrane. The smaller Ca current appears to be restricted to the

membranellar band. It is possible that the presence of the two voltage-dependent Ca currents in the membrane of *Stylonychia* may be functionally related to the difference between the beating behaviour of the membranellar band cilia and that of the rest of the compound cilia.

INTRODUCTION

Since the first intracellular potential measurement in the ciliated protozoa *Paramecium* (Kamada, 1934) the relationship between electrical excitation, control of ciliary activity and cellular behaviour has been the subject of a variety of investigations (for references see Machemer & de Peyer, 1977; Kung & Saimi, 1982). The membrane excitability depends upon a voltage-sensitive Ca inward current, which gives rise to a graded Ca action potential. In addition to this voltage-dependent membrane process, Ca ions are also involved in controlling frequency and reversal of ciliary beating (Eckert, Naitoh & Machemer, 1976). Removal of the cilia results in a reversible loss of the electrically excitable response and the voltage-dependent Ca inward current (Ogura & Takahashi, 1976; Dunlap, 1977; Machemer & Ogura, 1979). The deciliation studies suggest that the voltage-dependent Ca channels are restricted to the ciliary membrane, and underline the direct connexion between voltage-dependent Ca inward current and ciliary reversal (Eckert, 1972).

In contrast to the holotrich ciliate *Paramecium*, the hypotrich ciliate *Stylonychia* generates a *two-peak* action potential (de Peyer & Machemer, 1977). These two components are represented by a first, large, graded peak, and a second, small, 'all-or-none' peak. Mechanical stimulation to the cell anterior evokes a depolarizing mechanoreceptor potential, which gives rise to a similar two-peak action potential (de Peyer & Machemer, 1978; de Peyer & Deitmer, 1980). Both components of the action potential depend upon the external Ca concentration, and they are abolished when Ca is replaced by an impermeable ion, e.g. Mg or Mn. Both Sr and Ba can replace Ca as charge carriers during the action potential (de Peyer & Deitmer, 1980). Addition of external Na, Rb or Cs, or changing the external K or Cl concentration, do not alter this basic pattern of the composite action potential.

The aim of the present study was to analyse the composite electrical excitability under voltage-clamp conditions with respect to the different types of ciliary organelles in *Stylonychia*. The results suggest that the membrane contains two types of voltage-dependent Ca-current activation processes, one of which appears to be restricted to the (ciliary) membrane of the membranellar band. Preliminary reports of some of these results have been given previously in abstract form (Deitmer, 1982, 1983*a*).

METHODS

The ciliate *Stylonychia mytilus* syngen I (Hypotricha) was cloned and cultured in Pringsheim solution at 18 °C. The cells were fed with the phytomonad alga *Chlorogonium elongatum* every 3 days. In a series of cell cultures 0·35 mM-CsCl was added to the Pringsheim culturing solution. This was an attempt to induce some accumulation of Cs in the cytoplasm of the cells to reduce K outward currents. The population growth of these cell cultures, however, decreased considerably, and the behaviour of most cells changed in the first few days in the Cs-containing Pringsheim solution. Typically, the reversal of ciliary beating and thus backward swimming were prolonged, indicating

an increased action potential duration. This suggests that Cs was indeed taken up by the cells and the K permeability reduced. Hypotrich ciliates are regarded as unicellular organisms which are highly advanced in evolutionary terms: they have a more differentiated morphology, and the ciliary system is more elaborate than in holotrich ciliates, such as *Paramecium*. Cilia of hypotrichs are usually grouped into clusters of five to eighty, forming rod-like cirri or velum-shaped membranelles. This differentiation, and the specific location of membranelles and cirri, provide a highly developed locomotor system, enabling these cells not only to swim forward and backward (like *Paramecium*), but also to walk and rest on smooth surfaces (Verworn, 1889; Machemer, 1965).

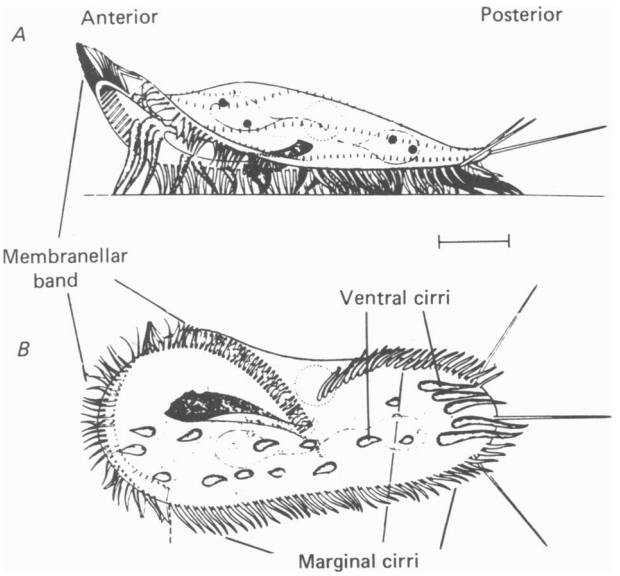

Fig. 1. Drawing of the hypotrich ciliate *Stylonychia mytilus* (after Machemer): *A*, lateral view; *B*, ventral view. The dotted line on the lower left (*B*) indicates the extension of the frontal membranellar band and the beginning of the marginal cirri. The membranelles surround the anterior of the cell and extend to the oral groove; the marginal cirri surround the posterior of the cell. For further details see Methods. Scale bar = 50 μm.

Fig. 1 *A* and *B* shows a lateral and a ventral view of *Stylonychia*. The cell is 200–250 μm in length, 40–50 μm in width, and 15–20 μm in dorsoventral extension. The organization of the membranellar band, which surrounds the anterior pole of the cell and extends toward the oral groove, suggests that its function might be to provide a feeding water current (Machemer, 1966). Recent high-speed cinematography of cirri and membranelles of *Stylonychia* under membrane voltage clamp revealed that these two types of organelles display different motor responses to the same voltage stimuli (Deitmer, Machemer & Martinac, 1983, 1984).

For experimentation, cells were washed and equilibrated in the normal experimental solution containing 1 mM-$CaCl_2$, 1 mM-KCl and 1 mM-Tris HCl or 1 mM-HEPES (*N*-2-hydroxyethyl piperazine-*N'*-ethanesulphonic acid) to give a pH of 7·4 ($\pm$0·1). In some test solutions Ca was partially or completely replaced by equivalent amounts of Co, Ba or Mn, or Cd (10^{-6}–10^{-4} M) was added to the normal solution. In other experiments, tetraethylammonium (TEA, up to 5 mM) and/or 4-aminopyridine (4-AP, up to 1 mM) were added. The experiments were performed with the experimental chamber held at a temperature of 17–18 °C by means of a constant perfusion of a microscope cooling table.

Cell fragmentation. Some experiments used cells which had released their membranellar band either spontaneously, or after some mechanical irritation (e.g. by strongly poking the anterior part of a cell with a glass micropipette). Sometimes the membranellar band was destroyed during this

procedure, but often it formed a sickle-shaped membrane vesicle with beating membranelles. This vesicle contained 50–80 % of the membranelles, depending on how many adoral membranelles were attached. It was 30–40 μm long and approximately 10 μm in diameter. It moved in rotations like on a spiral ('tumbling'). The rest of the cell, having lost its membranellar band, displayed an almost normal locomotion, and eventually regenerated the membranellar band (Wallengren, 1901; Dembowska, 1938).

In other experiments, the cell was cut in two parts. When the cell was cut transversely into two parts (see upper diagrams of Fig. 10*A* and *B*), both fragments often remained intact and functional for several hours or longer. For the present study usually thirds or halves of a cell were destroyed by large current injection and/or capacity over-compensation of the intracellular voltage electrode. The remaining part was left to seal in, and then penetrated by two micro-electrodes. The increased input resistance usually indicated a good healing of the cell membrane.

Electrical recording. The cells were penetrated by two micro-electrodes (borosilicate capillaries with filament, outer diameter 1 mm), one electrode being filled with 1 M-KCl for membrane potential recording, the other one filled with 2 M-K citrate (in some experiments 3 M-CsCl) for current injection. A third micro-electrode, filled with 1 M-KCl, was placed outside the cell for differential measuring of the membrane potential. The resistance of the micro-electrodes was 20–60 MΩ in normal bathing solution. Further details have been described previously (de Peyer & Machemer, 1977; de Peyer & Deitmer, 1980; Deitmer, 1981).

The membrane resting potential and the membrane input resistance were always measured at the beginning of each experiment in normal solution. The input resistance was measured by injecting a negative constant-current pulse of small amplitude (10^{-10} A). Only cells having a resistance of 50 MΩ or more were taken for further experimentation. Transfer to a solution different from the normal solution was done by perfusing the experimental chamber (volume *ca.* 1 ml), exchanging at least 10 times the volume of the chamber.

The voltage clamp was performed by means of a feed-back system using a high-gain differential amplifier (AD 171 K). The membrane current was monitored by a current–voltage converter connected to the bath via a 1 M-KCl–agar bridge, and displayed on an oscilloscope. The voltage was usually clamped to a stable steady state within 200–500 μs after a 10 mV step. The time-to-peak of the early inward current was approximately 1·5–4·5 ms. Thus, the clamp was sufficiently fast to record the peak inward current free of capacitive transients.

RESULTS

Recordings of action potentials, elicited by intracellular current injection and mechanical stimulation of the cell anterior are shown in Fig. 2. Both means of stimulation lead to the generation of the two-peak action potential. Injection of different current intensities (Fig. 2*A*) revealed the graded nature of the first, large action potential peak, and the near 'all-or-none' nature of the second peak.

In voltage-clamp experiments the cell membrane was held at its resting potential of −50 mV (±2 mV), and was de- and hyperpolarized in steps of various amplitudes and of 30–80 ms duration (Fig. 3*A*). Depolarizations of only 2–3 mV in amplitude were sufficient to elicit a small transient inward current (0·5–2 nA) which rapidly increased with depolarizations up to +6 mV (−44 mV) to about 5 nA. The transient inward current grew only slightly larger upon +10 mV depolarization, i.e. at −40 mV, but further depolarization evoked steeply increasing inward currents.

Depolarizing pulses to −30 mV elicited an inward current which increased with further depolarizations and reached its maximum between −20 and −15 mV. The maximum inward current amounted to 24 ±2·8 nA (±S.D., n = 12). For depolarizations beyond −15 mV the inward current decreased.

A maintained outward current appeared with depolarizations larger than −30 mV,

and increased with voltage. At a membrane potential of −15 mV the steady-state outward current usually amounted to less than 10 nA, and at −10 mV the outward current was between 20 and 30 nA.

Fig. 3*B* shows typical current–voltage relationships. There is a first maximum of the inward current at a membrane potential between −45 and −43 mV, and another

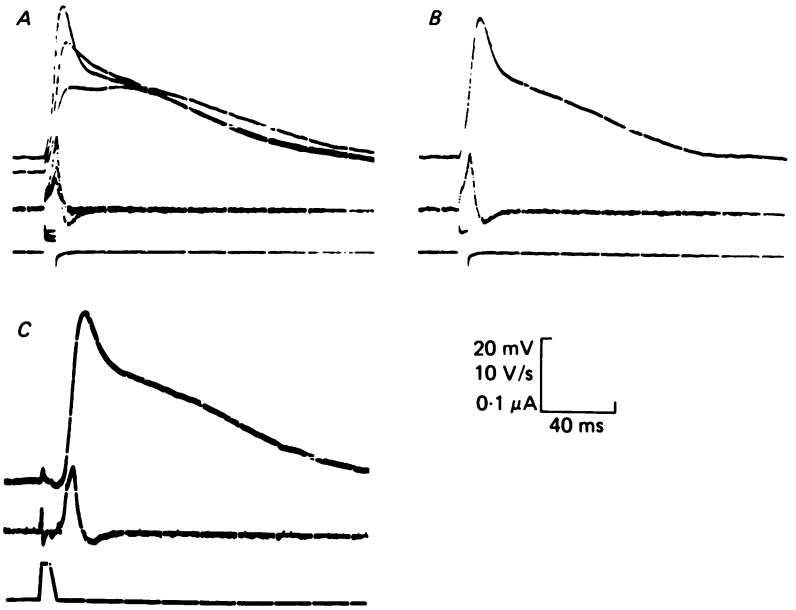

Fig. 2. Two-peak action potentials (upper traces) elicited by intracellular current injection (*A* and *B*, lower traces) or by mechanical stimulation of the cell anterior (*C*, lower trace indicates voltage ramp pulse to piezo-crystal driving a micropipette as mechanical stimulator), and the first derivative of the potential changes (dV/dt, middle traces). In *A* three different current intensities evoke active responses demonstrating the graded nature of the first peak and the near 'all-or-none' characteristics of the second component of the action potential.

maximum near −17 mV. The inset of Fig. 3*B* shows part of the inward current–voltage (I_{in}–V) relationship up to −30 mV on an enlarged scale to show the maximum near −44 mV more clearly.

The steady-state I_{in}–V relationship revealed a strong outward-going rectification upon depolarization beyond −20 mV, and a moderate inward-going rectification upon hyperpolarization of the membrane (Fig. 3*B*). Both types of membrane rectifications decreased considerably when Ca was replaced by Mg (see also de Peyer & Deitmer, 1980).

A fast outward current (Hagiwara & Saito, 1959; Neher, 1971; Connor & Stevens, 1971) was sometimes evident at a membrane potential of between −35 and −20 mV (see trace of Fig. 4 and Fig. 6).

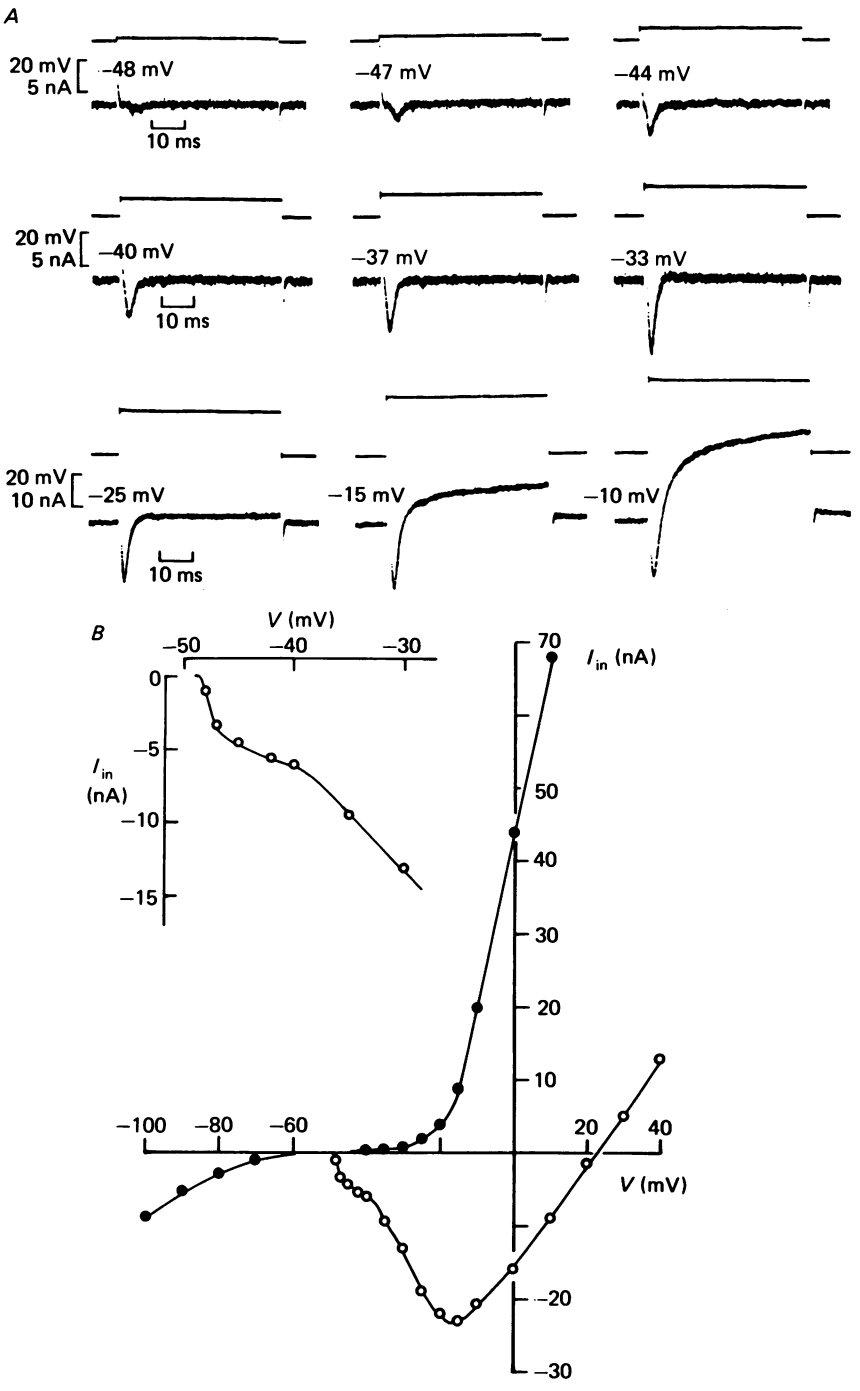

Fig. 3. *A*, membrane currents (lower traces) recorded following 50 ms depolarizing voltage-clamp pulses (upper traces); the figures between the traces indicate the membrane potential during the step (holding membrane potential: −50 mV). Note the appearance of an early, transient, inward current at very small depolarizations (at −48 and −47 mV). A net steady-state outward current appears at a membrane potential of −25 mV and

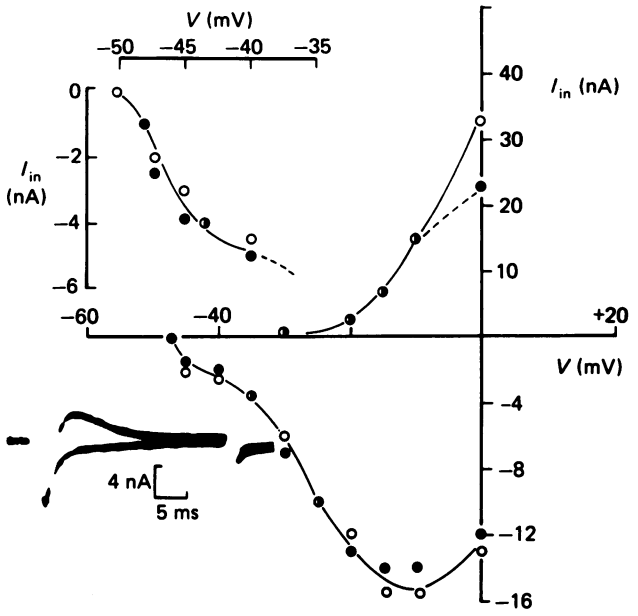

Fig. 4. I_{in}–V relationships of one cell in normal solution (○) and after addition of 3 mM-TEA and 1 mM 4-AP (●). No significant difference in the course of the I_{in}–V relationship in the presence of K-channel blockers is apparent. The traces (lower left) are superimposed recordings of currents following a voltage pulse from −50 to −30 mV before and after addition of TEA and 4-AP, indicating the reduction of a fast outward current, while the peak inward current was virtually unchanged. The inset shows part of I_{in}–V relationships obtained in cells with intracellular Cs, either taken up after addition of Cs to the culturing medium (●) or by insertion of a current electrode filled with 3 M-CsCl (○).

The effect of K-channel blocking agents

In order to reduce the K outward current during the depolarizing voltage steps, drugs known to block K channels were used in some experiments. Fig. 4 shows I–V relationships before and after the addition of 3 mM-TEA and 1 mM-4-AP. The outward rectification was reduced at membrane potentials more positive than −10 mV. The fast, transient, outward current was always reduced in the presence of these drugs (see traces in Fig. 4). The inward currents, however, remained virtually unaltered except for a somewhat slower decay due to the reduction of outward current. The I_{in}–V relationship still showed two distinct maxima.

If Cs was brought into the cells, either by filling the current electrode with CsCl or by adding CsCl to the cell culture medium (see Methods), the first maximum of the I_{in}–V relationship at −44 mV was still prominent (Fig. 4, inset), clearly separated

above. *B*, I_{in}–V relationship of the cell membrane: steady-state currents (●) following de- and hyperpolarizing pulses, and the early, fast, transient current (○) following depolarizing pulses. Note the inflexion in the early I_{in}–V relationship at membrane potentials between −48 and −40 mV. This part of the I_{in}–V curve is enlarged in the inset on the upper left.

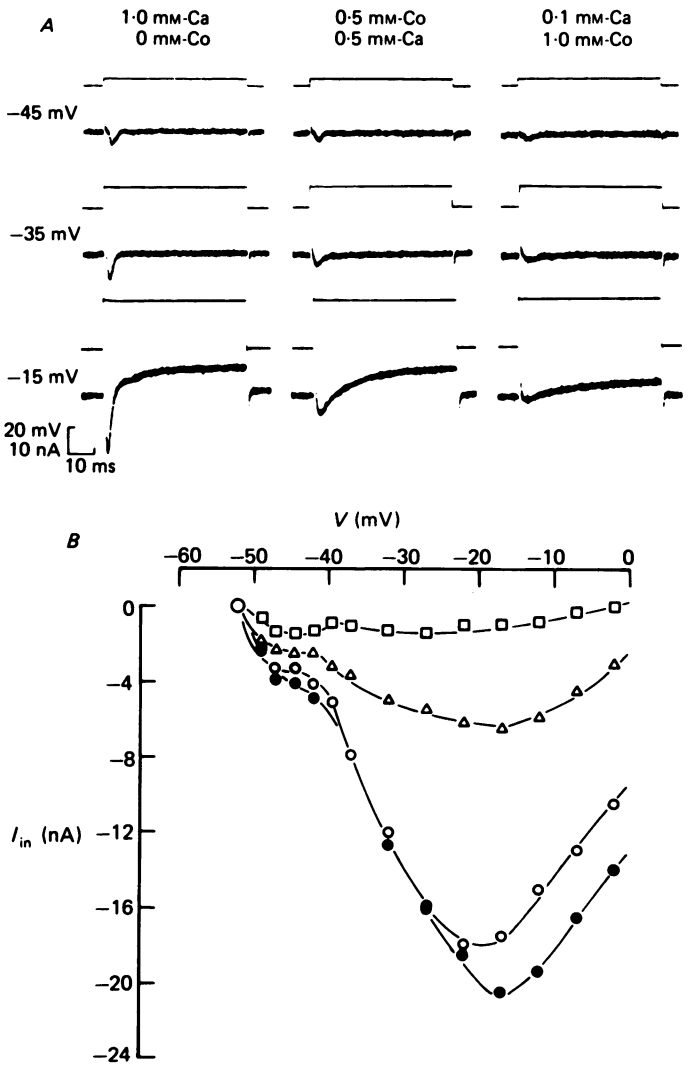

Fig. 5. The effect of Co on the early inward currents: *A*, membrane currents (lower traces) recorded during depolarizing voltage-clamp pulses (upper traces) in normal solution and in solution with reduced external Ca concentrations, where Ca was replaced by Co (0·5 and 1·0 mM). *B*, early I_{in}–V relationships of the same cell as in *A* successively superfused with normal solution (1·0 mM-Ca; ●), 0·5 mM-Ca, 0·5 mM-Co (△), normal solution (○), and 0·1 mM-Ca, 1·0 mM-Co (□). In both Co-containing solutions the distinct inflexion in the early I_{in}–V relationship became even more pronounced than in the control.

by the notch in the I_{in}–V relationship from the second, large maximum at −17 mV (not shown here). Thus, the outward current can be considerably reduced by TEA, 4-AP or internal Cs without affecting the shape of the I_{in}–V relationship. This has also been shown in *Paramecium* (Brehm & Eckert, 1978; Satow & Kung, 1979).

Effect of Co and other divalent cations

When Ca was partially replaced by Co in the external solution, the inward current was substantially reduced. Fig. 5*A* shows voltage-clamp recordings in the presence of 0·5 and 1·0 mM-Co. The inward currents were decreased when compared to those in the absence of Co. It is also conspicuous that the outward current was activated with a slower time course, levelling into a steady state of smaller amplitude in 1·0 mM-Co. This may indicate the influence of Ca inward current on the K outward current suggested previously for this cell (de Peyer & Deitmer, 1980), and other preparations (see e.g. Meech & Standen, 1975; Heyer & Lux, 1976).

Fig. 5*B* shows I_{in}–V relationships of one cell, successively superfused with normal saline, 0·5 mM-Ca + 0·5 mM-Co, normal saline again, and then 0·1 mM-Ca + 1·0 mM-Co. The two maxima of the I_{in}–V relationship were reduced by Co. The voltage dependence of the inward currents remained essentially unchanged by Co. The reduction by Co of the second maximum of the I_{in}–V curve was relatively larger than that of the first. With 0·5 and 1·0 mM-Co, the peak inward current at −45 mV was reduced to 62 and 29 % while the peak current at −17 mV was reduced to 32 and 6 % respectively. The reduction of the external Ca concentration to 0·5 and 0·1 mM (without adding Co) reduced the peak inward currents at all potentials by approximately 10 and 35 %, respectively. These data suggest that the inward current activated at small membrane depolarizations, i.e. near −45 mV, is less sensitive to Co than the inward current activated by larger depolarizations.

In a series of experiments $CdCl_2$ (10^{-6} and 10^{-4} M) was added to the normal solution. Cd also reduced both types of inward currents, but again with different efficacy. The inward current activated by small depolarizations, i.e. at −45 mV, was less sensitive to Cd and was reduced to 50 % by approximately 10^{-4} M-Cd, while the inward current activated by larger depolarizations, i.e. at around −20 mV, was already reduced to 50 % by 10^{-5} M-Cd.

When Ca was completely replaced by Mg, Co or Mn (nominally Ca-free), no inward currents or action potentials could be elicited by membrane depolarizations. Increasing the external Ca concentration, on the contrary, produced larger inward currents. In experiments where Ca was substituted by Ba or Sr, early inward currents and action potentials were still generated upon membrane depolarization. The two maxima of the I_{in}–V relationship were both present in Sr and Ba solutions, indicating that Sr and Ba carry charge in both types of inward currents. This is supported by previous observations in solutions containing various amounts of Ca, Sr and Ba (de Peyer & Deitmer, 1980; Ballanyi & Deitmer, 1984).

Activation and inactivation of the two types of inward currents in Ca and Ba

Depolarizing voltage steps to −45 mV and to −30 mV or −20 mV elicited membrane currents, which contained representative examples for the two types of inward currents. Sample recordings of these membrane currents evoked by 30 ms voltage-clamp pulses to −45 mV and to −30 mV in the normal, Ca-containing solution and in nominally Ca-free Ba solution are shown in Fig. 6. Voltage pulses to −45 mV elicited a transient inward current in both Ca and Ba of 4·5 and 5·0 nA amplitude, respectively. Often the currents in Ba were up to 20 % smaller than those in Ca at this potential step. In the presence of the K-channel blockers TEA and/or

4-AP or internal Cs these currents were often reduced to 3–4 nA, but otherwise unaltered. At this membrane potential there was no indication for a K outward current even after elimination of the inward current. The time-to-peak of the inward currents in both Ca and Ba was 3–4 ms, indicating similar activation kinetics for both divalent cation currents. The mean decay of these transient inward currents often showed two exponential time courses with time constants of 1·3 and 2·3 ms in Ca, and 2 and 3 ms in Ba, respectively.

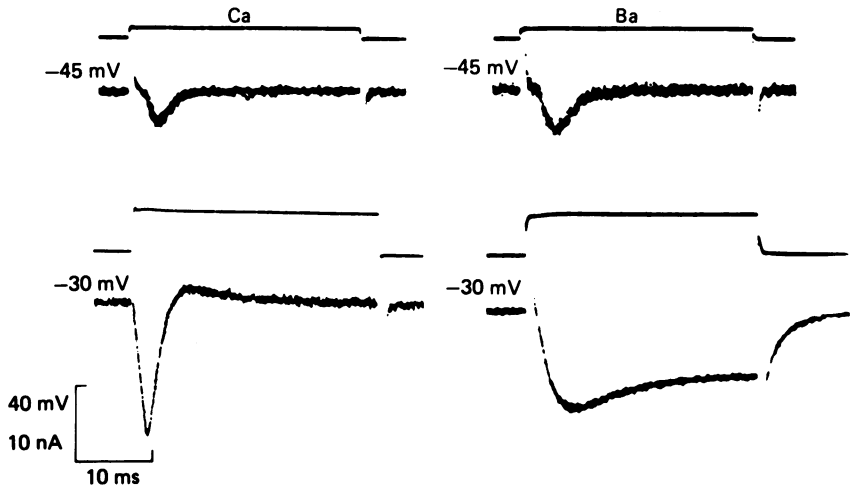

Fig. 6. Membrane currents (lower traces) following voltage-clamp steps to −45 and −30 mV (upper traces) in normal Ca solution and after replacing Ca by Ba. The current traces recorded at −45 mV are two superimposed sweeps.

The voltage pulse to −30 mV elicited a transient inward current of 17·5 nA in Ca and an inward current with a transient peak of 12·5 nA and a steady state of 8·5 nA in Ba. While in Ca this inward current transient rapidly decayed and turned into a small transient outward current, the inward current in Ba was maintained during the length of the pulse duration. The time-to-peak of the Ca inward current at −30 mV was approximately 2 ms, while the Ba inward current reached its maximum within about 6 ms. The mean decay of the transient inward current in Ca displayed a fast time course, presumably affected by some transient outward current. In Ba the inactivation kinetics of this inward current at −30 mV were considerably changed. The inward current decayed to 90% in 5 ms or more and achieved an apparent steady state at 75–90% of the maximum amplitude even with pulses as long as 1 s. There were at least two time courses of inactivation (relaxation) of these currents in Ba: a relatively fast one which occurred within several milliseconds and a slow one which extended over seconds.

It is evident that the apparent relaxation of the net inward current at −45 mV was very similar in Ca and in Ba solutions, while the time course and the degree of the apparent relaxation of the net inward current at −30 mV were distinctly different when Ca or Ba carried the current.

A double-pulse protocol (see Brehm & Eckert, 1978; Tillotson, 1979) was chosen to study inactivation of the two types of inward currents in both Ca and Ba. When two depolarizing pulses with an interval of 30–50 ms, were applied, the amplitude of the pulse II current was dependent on the amplitude of pulse I. In a first series, the test pulse was kept a constant step to −45 mV, while the pre-pulse varied (Fig. 7). The membrane currents shown (Fig. 7*A*) were recorded with pulse I steps to −45, −10 and +70 mV. The pulse II current appeared more reduced with the pre-pulse step to −10 mV than to −45 or +70 mV. The result of several experiments of this kind is plotted in Fig. 7*B*. The peak amplitude of the pulse II current relative to the peak current without a preceding pulse first decreased steeply with increasing voltage of pulse I. In the Ca solution, 50% inactivation of the inward current at −45 mV was achieved with a pulse I step to −40 mV, being maximal with a pulse I step to +10 mV, where inactivation amounted to 65%. With pulse I steps larger than +60 mV, inactivation decreased again, and with a pulse I step to +90 mV it was only 27%. In Ba, a more complex relationship between inactivation and pulse I amplitude appeared. Inactivation amounted to 40% with a pulse I step to −40 mV, and then decreased to about 30% at pulse I steps to 0 and +10 mV absolute membrane potential. Beyond a pulse I step to +30 mV inactivation decreased and was only 15% with a pulse I step to +90 mV. For all pulse I amplitudes the inactivation was less in Ba than in Ca by 10–20%, and for pulse I steps to −10, 0 and +10 mV the difference in inactivation was up to 33%.

A similar pulse protocol was used to study inactivation of the second type of inward current, activated in this experiment at −20 mV in both Ca and Ba (Fig. 8). The membrane currents shown (Fig. 8*A*) were recorded with the first pulse to −40, −20, 0 mV (+10 mV in Ba), +70 and +90 mV. The interval between the two pulses was 30 or 50 ms in Ca and 30 ms in Ba. With rising pulse I amplitudes the peak pulse II inward current in Ca first decreased and then increased again, while in Ba the peak pulse II current remained almost unaffected by pulse I. The graph (Fig. 8*B*) of the relative pulse II current amplitude demonstrates this large difference in the degree of inactivation in Ca and in Ba. Inactivation of 80% (30 ms interval) and 70% (50 ms interval) was obtained with pulse I steps to 0 and +10 mV in Ca. In Ba maximal inactivation was only 13%, obtained with pulse I steps to −15 mV. A similarly large difference in the inactivation of this current carried either by Ca or Ba has also been found with repetitive voltage-clamp steps to −20 mV, when inactivation was frequency dependent in Ca solution but not in Ba solution (Deitmer, 1983*b*).

Excitation without the membranellar band. It has been known since the work of Jennings & Jamieson (1902) that hypotrich ciliates can survive the release of their membranellar band. The release of the membranellar band may occur spontaneously, or can be achieved by an experimental stress such as that associated with electrode penetration or bath solution change. The action potential elicited in a cell devoid of its membranellar band has a distinctly different shape as compared with the action potential of intact cells. Fig. 9*A* shows a typical action potential of such a cell; the second, 'all-or-none' peak is greatly reduced in amplitude or even absent. The first, graded peak of the action potential, however, appears unaffected in amplitude and maximum rate of rise. As shown in Fig. 9*B* under voltage-clamp conditions no inward current was induced by a depolarization to −45 mV, and only a small inward current

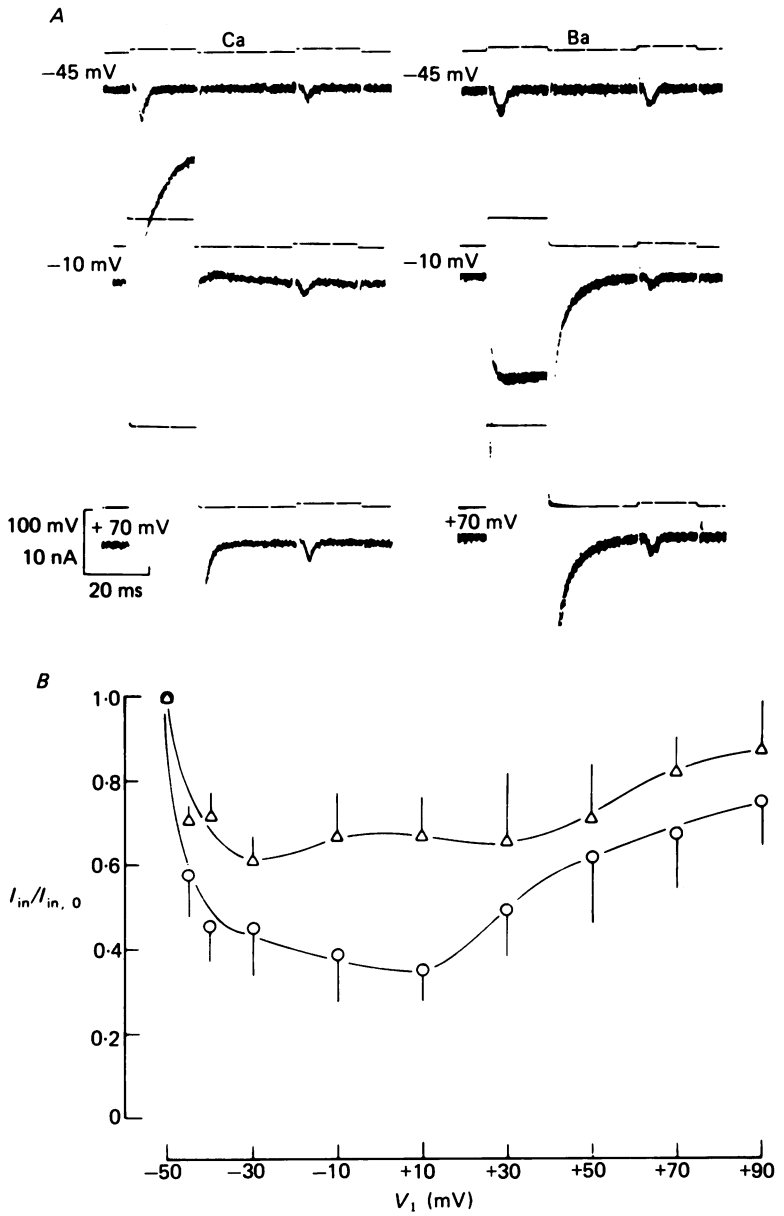

Fig. 7. Inactivation of the inward current in Ca and Ba solutions using a double-pulse protocol with the first, conditioning, pulse to various membrane potentials and the second, test, voltage step to −45 mV (holding potential −50 mV). *A*, membrane currents (lower traces) with the conditioning pre-pulses to −45, −10 and +70 mV (upper traces). *B*, linear plot of the relative amplitude of the inward current, $I_{in}/I_{in,0}$, upon voltage step to −45 mV with (I_{in}) and without ($I_{in,0}$) pre-pulse, *versus* the pre-pulse voltage, V_1, in Ca solution (circles), and in normally Ca-free Ba solution (triangles). Bars represent + or −1 S.D. of the mean ($n = 5$).

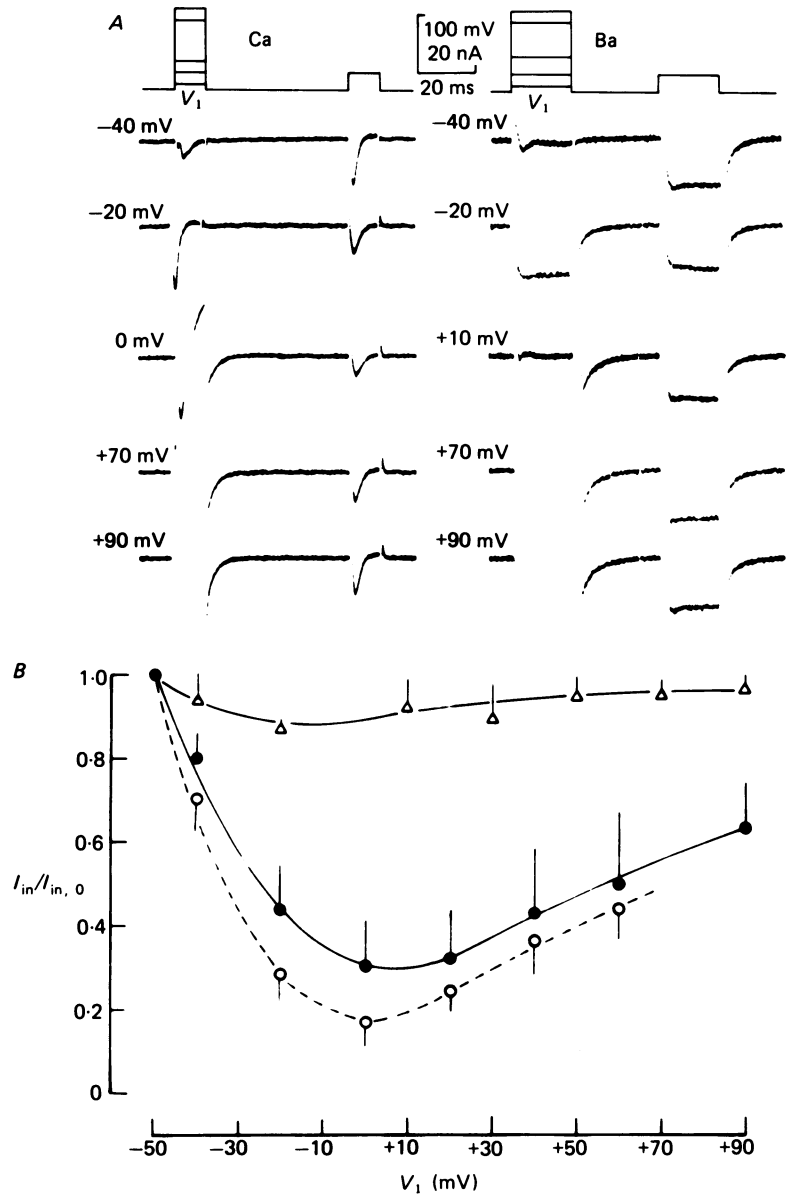

Fig. 8. Inactivation of the inward current in Ca and Ba solutions using a double-pulse protocol with the first, conditioning, pulse to various membrane potentials and the second, test, voltage step to −20 mV (holding potential −50 mV). *A*, membrane currents (lower traces), with the conditioning pre-pulses to −40, −20 mV, 0 mV (+10 mV in Ba), +70, +90 mV (upper traces). *B*, linear plot of the relative amplitude of the inward current, $I_{in}/I_{in,0}$ upon voltage step to −20 mV with (I_{in}) and without ($I_{in,0}$) pre-pulse, *versus* the pre-pulse voltage, V_1, in Ca solution (circles) and in nominally Ca-free Ba solution (triangles). The duration of the pre-pulse was either 20 ms with an interval of 30 ms in Ca and Ba (open symbols), or 10 ms with an interval of 50 ms in Ca solution (filled symbols). Bars represent + or −1 S.D. of the mean (n = 4–6).

was recorded after a step to −40 mV. The I_{in}–V relationship of the same cell is shown in Fig. 9*C*. There was no maximum at around −45 mV as in the intact cell (see Fig. 3*B*). The maximum of the I_{in}–V relationship occurred at a membrane potential of −18 mV, i.e. at the same membrane potential as the second maximum of the I_{in}–V relationship obtained in intact cells. In the experiment shown the total maximum inward current was reduced by approximately 40%, and in other experiments by 30–60%, presumably due to the reduction of the cell size.

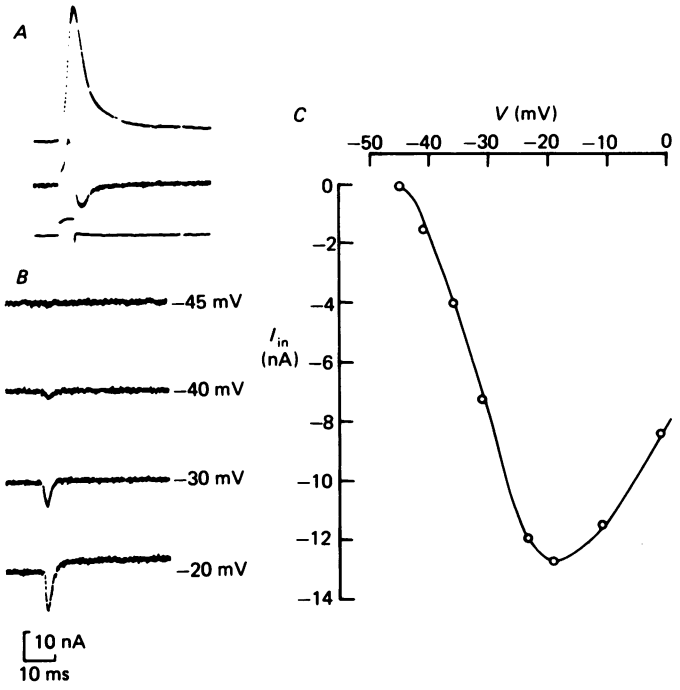

Fig. 9. Data from a cell which had released its membranellar band. *A*, action potential recording (upper trace) and its first derivative (dV/dt, middle trace) elicited by a 10 ms intracelluar current pulse (lower trace). Note the absence of the second component of the action potential. *B*, membrane currents of the same cell recorded at various membrane potentials (indicated by the figures on the right). Note the absence of an inward current at −45 mV. *C*, the inward I_{in}–V relationship of this cell reveals only a single maximum at −18 mV.

Electrophysiology of cell fragments. From the different types of compound cilia forming functionally different motor organelles in this hypotrich ciliate the possibility was tested of a non-homogeneous distribution of Ca channels in the membrane. Therefore the cells were cut transversely, or parts of the cells were destroyed, to obtain cell fragments (as indicated in the Methods, and in the upper diagrams of Fig. 10*A* and *B*). The remaining anterior or posterior cell fragments were penetrated with two micro-electrodes for electrophysiological recording and voltage clamping. When the anterior third or half of a cell was removed, and recordings made from the posterior part, the shape of the action potential changed (Fig. 10*A*) as compared with the normal action potential of intact cells (Fig. 2). The first, graded peak of the action

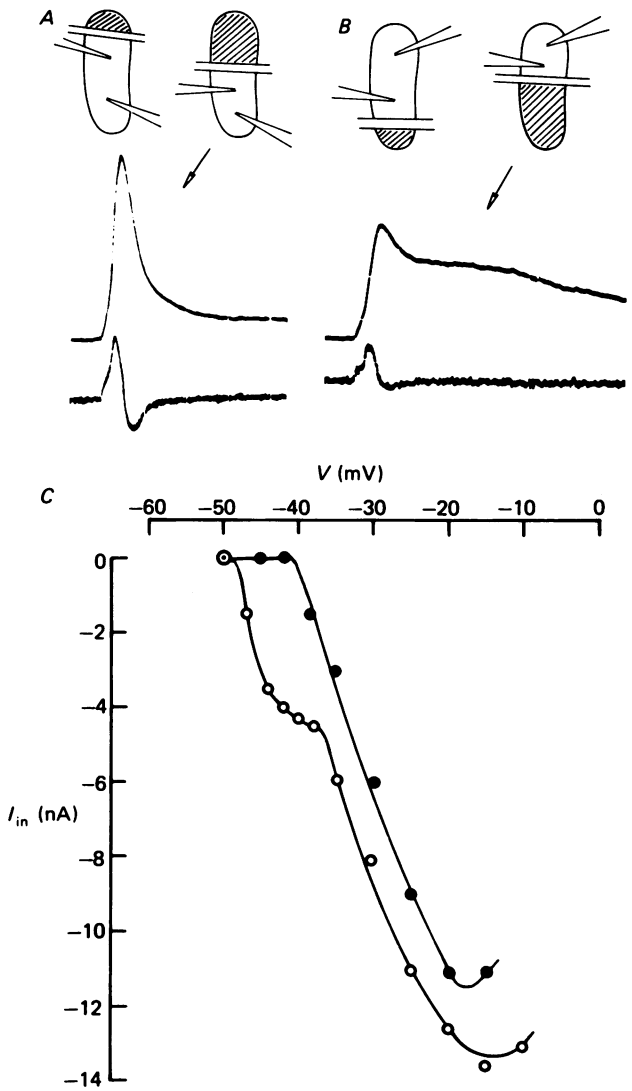

Fig. 10. Schematic drawings of four sample cell fragments (ventral view; *A* and *B*) and approximate positioning of the two intracellular micro-electrodes after severing part of the cell (indicated by the hatched area), and, below, the corresponding sample recordings of electrically evoked action potentials (upper traces) and their first derivative (dV/dt, lower traces). Note the absence of the second component of the action potential after loss of the anterior cell part (*A*). *C*, I_{in}–V relationship of an anterior (open circles) and a posterior (filled circles) cell fragment.

potential remained unaffected (sometimes slightly enlarged), while the second, 'all-or-none' peak disappeared. If, however, the posterior third or half of a cell was removed, and records made from the anterior part, the action potential had still two peaks, a first, graded one, which was often somewhat reduced in amplitude and maximum rate of rise, and a second, 'all-or-none' peak (Fig. 10*B*).

Fig. 10*C* shows typical I_{in}–V relationships of an anterior cell fragment and a

posterior cell fragment, both of which had approximately half the normal cell size. The maximum inward current (at −15 to −18 mV) was therefore reduced to 40–60 % of that typically recorded in intact cells (as shown in Fig. 3*A* and *B*). In the I_{in}–V relationship of the posterior cell fragment no inward current was recorded up to −42 mV, which resulted in the elimination of the maximum at around −44 mV observed in both anterior cell fragments and intact cells. The first maximum of the inward current in the anterior cell fragment amounted to approximately 4 nA at

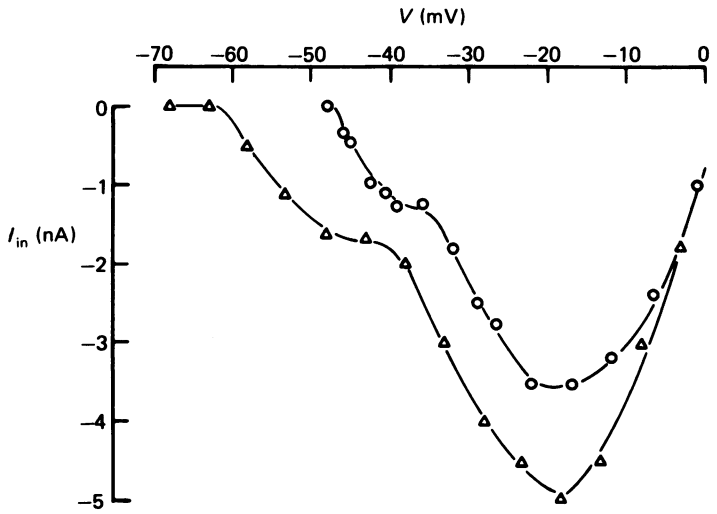

Fig. 11. I_{in}–V relationship with (triangles) and without (circles) hyperpolarizing pre-pulse (50 ms to −70 mV, holding potential −50 mV) obtained from a membranellar band vesicle. Note the presence of two maxima in both curves.

membrane potentials between −44 and −38 mV. The second maximum of the I_{in}–V relationship was at −18 mV for the posterior, and at −15 mV for the anterior cell fragment.

Are both types of Ca currents in the membrane of the membranellar band? The results presented above suggest that the Ca current activated at around −45 mV, i.e. by small depolarizations of the membrane, is associated with the membranellar band. They do not indicate, however, whether the membranellar band contains only this type of inward current or both. In three cases I successfully inserted two separate micro-electrodes into a spontaneously released membranellar band vesicle. Fig. 11 shows two I_{in}–V relationships, obtained with and without a hyperpolarizing pre-pulse (to −70 mV for 50 ms). Both I_{in}–V relationships revealed a first and a second maximum. The amplitude of the first maximum had decreased to about 50 %, and that of the second maximum to less than 20 %. This also indicates some non-homogeneous distribution in the cell membrane of the Ca channels corresponding to the two types of inward currents.

The termination of a hyperpolarizing pulse was observed to be followed by a transient inward current. Fig. 12 shows typical recordings of the membrane current during and after a hyperpolarizing voltage step from −50 to −65 mV (50 ms in

duration). The 'post-hyperpolarization inward current' disappeared, when the membranellar band had been released, or when the anterior end of the cell had been cut off (Fig. 12). When recording from the anterior cell fragment or from a membranellar band vesicle, however, this transient inward current was present. When Ca was replaced by Ba (or Sr), a similar 'post-hyperpolarization inward current' was recorded, which disappeared in Ca-free, Mg- or Mn-containing solution, or when Cd was present. It seems that this 'post-hyperpolarization inward current'

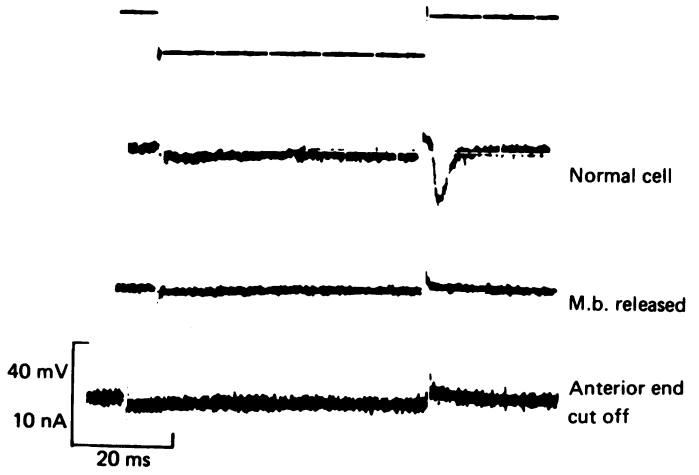

Fig. 12. Membrane currents (lower traces) recorded during and after a 50 ms, −15 mV hyperpolarizing voltage pulse (upper trace) in an intact cell (second trace), in a cell without its membranellar band (M.b.; third trace), and in a posterior cell fragment (fourth trace). The holding potential was −50 mV in each case. Note the absence of the transient inward current following the hyperpolarizing step ('post-hyperpolarization current') in cells without their membranellar band or without their entire anterior part.

is identical to the Ca inward current usually activated at potentials between −48 mV and −40 mV. It may suggest that this type of inward current inactivates only partially, and/or some inactivation of this current is removed by membrane hyperpolarization. Although the exact mechanism concerning the generation of this 'post-hyperpolarization inward current' remains obscure, these results contribute to the evidence for a non-homogeneous distribution of voltage-dependent Ca channels in the membrane of *Stylonychia*.

DISCUSSION

The results suggest that in the membrane of the hypotrich ciliate *Stylonychia*, which generates a two-component action potential, there are two Ca inward currents activated at different membrane potentials. In addition to the classical Ca current (de Peyer & Deitmer, 1980), which displays a voltage characteristic similar to that of other ciliates and nerve and muscle cells (see e.g. Hagiwara & Byerly, 1981), there is a Ca current in *Stylonychia*, which is activated by depolarizations of only a few millivolts from the resting membrane potential. This Ca current appears as a distinct

maximum in the I_{in}–V relationship near −44 mV. It is less sensitive to Ca antagonists, and displays a different inactivation in Ca-free Ba solution as compared to the 'classical Ca current'. The corresponding Ca channels seem to be restricted to the membrane of the anterior part of the cell, presumably to the membranelles.

Action potential and early inward currents. The two components of the action potential and the two inward currents in *Stylonychia* are dependent upon Ca and are unaffected by external Na (de Peyer & Machemer, 1977; de Peyer & Deitmer, 1980). The present experiments were performed in the absence of external Na. Addition of Na to the bathing solution did not alter the action potential or the inward currents. The action potential and both types of inward currents disappeared in the absence of external Ca, and were inhibited by external Cd, Co or Mn. Both currents could be carried by Ba and Sr. It can be concluded therefore that the inward currents induced by membrane depolarization are pure Ca currents, and that the corresponding ion channels are selective for Ca (Sr and Ba). The inward currents in *Stylonychia* do not behave in any way like the slow Na current recently described for *Paramecium* (Saimi & Kung, 1980).

The two components of the action potential seem to be associated with the two types of voltage-dependent inward currents. This is inferred by the concomitant loss of the second action potential component and the first peak of the I_{in}–V relationship near −45 mV after release or removal of the membranellar band. The late occurrence of the second action potential component and the fast inactivation of the inward current activated at around −45 mV appear to contradict this hypothesis. However, if this inward current inactivates only *partially*, it could prolong the action potential and thereby produce the second action potential component. The experiments with hyper- and depolarizing pre-pulses under voltage-clamp conditions tend to support that the inward current activated at −45 mV may not fully inactivate. At a potential around −30 mV, i.e. near the amplitude of the second component of the action potential, a very small underlying inward current would be sufficient to produce this second component due to the absence of any large K outward current at this potential. The 'all-or-none' nature of the second action potential component seems compatible with the steep voltage dependence of the Ca inward current activated between −48 and −42 mV. On the other hand, the graded nature of the first action potential component appears to be related to the inward current which has a peak at −17 mV in the I_{in}–V relationship.

Two types of voltage-dependent Ca inward currents have been described in egg cells of the starfish *Mediaster* (Hagiwara, Ozawa & Sand, 1975) and the tunicate *Halocynthia* (Okamoto, Takahashi & Yoshii, 1976). In these cells one type of Ca inward current flows through a classical Ca channel, and the other either depends on external Na, or flows through membrane channels also permeable to Na ions.

The voltage clamp and the analysis of peak inward currents

A problem of the present experiments might arise from insufficient space voltage-clamp control of the cell membrane. Since the voltage-dependent Ca channels are believed to reside exclusively in the ciliary membrane, as elegantly shown in *Paramecium* (Ogura & Takahashi, 1976; Dunlap, 1977; Machemer & Ogura, 1979), there may be a significant series resistance within the cilia due to their small diameter

(0·25 μm). Thus, local ciliary currents might occur, produced by non-homogeneity of the ciliary membrane potential. In *Stylonychia* the dense packing of cilia in the cirri and membranelles may additionally result in a resistance in series due to small interciliary spaces. If the resistivity of ciliary content (cytoplasm and axoneme) and the interciliary space (external medium) are taken into account to calculate their length constants, the measured amplitude of the early inward current would cause a potential drop along the length of a cilium of at most 1–2 mV. This calculation assumes a similar value for the ciliary membrane and somatic membrane resistances; if the ciliary membrane resistance is larger, as suggested by Machemer & Ogura (1979), the ciliary space clamp would be even more precise. Since an inward current was apparent already with a command depolarization of 2–3 mV, a serious space-clamp artifact seems unlikely. Furthermore, an invasion-like response from an unclamped region was not observed with depolarizations larger than +10 mV, as would be expected to occur from an uncontrolled space clamp. There is no direct experimental evidence yet, however, to rule out the possibility of a space-clamp problem in ciliates. In any case, even if the recorded inward currents near −45 mV were invasions from unclamped membrane regions, they would nevertheless indicate activation of a voltage-dependent Ca response.

Another problem in the present study was the presence of K outward currents, which cannot be eliminated completely in ciliates. A fast K outward current following membrane depolarization might alter the peak and thus the observed potential dependence of the early inward current. The application of K-channel blockers in these experiments suggested, however, that the *peak* inward current was little or not at all affected by K outward currents. The activation of a steady-state current only developed positive to −25 mV, and up to −10 mV this current activated relatively slowly. In *Paramecium* substantial reduction of the K outward current left the peak inward current, and thus the I_{in}–V relationship, unchanged (Brehm, Eckert & Tillotson, 1980). The time-to-peak of the early inward current was also unaltered by even large doses of externally applied or internally injected K-channel blockers. Gradually replacing external Ca by Ba reduced the outward current without greatly altering the general course of the I_{in}–V relationship with its two maxima (Ballanyi & Deitmer, 1984).

Inactivation of the two types of inward currents

The early inward current, activated between −48 and −42 mV, decayed with a similar time course when either Ca or Ba acted as charge carriers. This is in sharp contrast to the inward current evoked at potentials positive to −40 mV, which decayed rapidly in Ca solution, even in the presence of K-channel blockers, but relatively slowly and incompletely in Ba solution. In Ca-free Ba solution large inward currents were maintained over hundreds of milliseconds or even seconds (de Peyer & Deitmer, 1980). This may indicate a reasonably stable voltage clamp even in the long cirral cilia. On the other hand it seems to rule out inactivation of inward current due to extracellular depletion of the charge-carrying ion, as was found for the Ca current in the T-tubules of frog skeletal muscle (Almers, Fink & Palade, 1981). The inactivation of the inward current activated at −30 or −20 mV depended in a U-shaped manner upon the voltage of the conditioning pre-pulse, as in *Paramecium*

(Brehm & Eckert, 1978; Brehm *et al.* 1980), in molluscan nerve cells (Tillotson, 1979; Plant & Standen, 1981; Plant, Standen & Ward, 1983; Chad, Eckert & Ewald, 1984) and in insect muscle fibres (Ashcroft & Stanfield, 1982). As suggested by these authors, inactivation of the Ca inward current may be directly associated with the influx of Ca into the cell and/or accumulation of Ca at the inner membrane side. In most of these preparations inactivation was considerably smaller or even absent when Ca was replaced by Ba. A reduced inactivation has recently been described for Ca currents in the *Paramecium* mutant *dancer*, which was suggested to be due to a structural mutation of the Ca channel or its immediate lipid environment (Hinrichsen & Saimi, 1984).

The inactivation pattern found for the inward current activated at around −45 mV suggests a more complex mechanism. Inactivation still seems to be dependent upon the amplitude of the pre-pulse, but is more prominent at small pre-pulses, i.e. to −45 mV and −40 mV, and less prominent at pre-pulses to −20 mV up to +20 mV as compared to the inactivation of the inward current activated at −20 mV following the same pre-pulses (Figs. 7 and 8). Another difference was that the inward current activated at −45 mV significantly inactivated also in Ca-free Ba solution. A similar inactivation of Ca and Ba inward currents has been described in other preparations, e.g. in the egg cell of the marine polychaete *Neanthes* (Fox, 1981) and in cultured mouse myeloma cells (Fukushima & Hagiwara, 1983). In both examples voltage-dependent inactivation of the Ca current was found. Inactivation of the Ca channel in the tunicate *Halocynthia* was recently suggested to be dependent on Ca inward current in the cleavage-arrested embryo and potential dependent in the egg (Hirano & Takahashi, 1984). At present it cannot be decided whether the inactivation of the inward current activated at −45 mV in *Stylonychia* is also, at least partially, voltage dependent.

Localization of the two types of Ca currents and their functional significance

The two voltage-dependent Ca currents appear to have different localizations in the membrane of *Stylonychia*. Two experimental results suggest a non-homogeneous distribution of Ca channels; (1) the release of the membranellar band selectively abolished the inward current activation between −48 and −42 mV; (2) cutting of the membranellar band or the anterior part of the cell resulted in the selective elimination of this type of inward current. Thus, the inward current activated at −45 mV seems to be restricted to the membrane of the membranellar band, while the other occurs in the (ciliary) membrane of both anterior and posterior cell parts. It cannot be excluded, however, that one type of inward current is only *predominantly*, and the other type only to *a small extent* represented in the membrane of the membranellar band. On the other hand, the separation of both types of Ca currents may be even sharper than the results suggest, since the membranellar band vesicle usually includes a few frontal and/or marginal cirri.

The presence of two types of voltage-dependent Ca currents in the membrane of *Stylonychia* may be functionally significant. The low threshold and apparently steep activation kinetics of the Ca current around −45 mV might be important for the generation of spontaneous action potentials. Each action potential triggers ciliary reversal in ciliates (Eckert, 1972; Machemer & de Peyer, 1977), and thus backward

swimming. In contrast to *Paramecium*, which usually swims only forward (due to the absence of spontaneous action potential generation), *Stylonychia* generates action potentials spontaneously (4–12/min) and reverses ciliary beating following each action potential, thus causing backward jumps (Machemer, 1965, 1970). These spontaneous reversals may optimize food collection by means of an efficient biotope screening.

This would, however, not explain the need for a localization to the two types of voltage-dependent Ca currents. The distribution of the ion channels in the membrane associated with the two types of inward currents may rather be related to the control of ciliary activity in the various types of motor organelles. The activity of the membranelles does not appear to be co-ordinated with the activity of ventral and marginal cirri (Machemer, 1966). High-frequency cinematography of all three groups of ciliary organelles under membrane voltage-clamp control has demonstrated that the beating frequencies of ventral and marginal cirri are similarly tied to membrane potential changes, but are very different from the activity of the membranelles which display little frequency variation with either membrane hyper- or depolarization (Deitmer *et al.* 1983, 1984). Small membrane depolarizations of 2–4 mV, which are sufficient to activate a Ca inward current, have no influence on the marginal or ventral cirri (Machemer & de Peyer, 1982; Deitmer *et al.* 1984). It is therefore tempting to speculate that the inward current activated at around −45 mV and restricted to the membranelles is associated with the control of membranellar activity. The existence of this second type of voltage-dependent Ca current may provide a 'separate gear' for independent motor control of the membranelles.

I would like to thank Drs M. E. Barish, S. Hagiwara, H. Machemer, B. Martinac, W. J. Moody and W. R. Schlue for helpful discussions during this investigation and for comments on an earlier version of the manuscript, Ms Rita Erdmann for expertly carrying out the photographic work and Mrs Gerda Thubeauville for her patience with typing several versions of the manuscript. This investigation was supported by the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 114, TP A5.

REFERENCES

ALMERS, W., FINK, R. & PALADE, P. (1981). Calcium depletion in frog muscle tubules: the decline of calcium current under maintained depolarization. *Journal of Physiology* **312**, 177–207.

ASHCROFT, F. M. & STANFIELD, P. R. (1982). Calcium inactivation in skeletal muscle fibres of the stick insect, *Carausius morosus*. *Journal of Physiology* **330**, 349–372.

BALLANYI, K. & DEITMER, J. W. (1984). Concentration-dependent effects of Ba^{2+} on action potential and membrane currents in the ciliate *Stylonychia*. *Comparative Biochemistry and Physiology* (in the Press).

BREHM, P. & ECKERT, R. (1978). Calcium entry leads to inactivation of calcium channel in *Paramecium*. *Science* **202**, 1203–1206.

BREHM, P., ECKERT, R. & TILLOTSON, D. (1980). Calcium-mediated inactivation of calcium current in *Paramecium*. *Journal of Physiology* **306**, 193–203.

CHAD, J., ECKERT, R. & EWALD, D. (1984). Kinetics of calcium-dependent inactivation of calcium current in voltage-clamped neurones of *Aplysia californica*. *Journal of Physiology* **347**, 279–300.

CONNOR, J. A. & STEVENS, C. F. (1971). Voltage clamp studies of a transient outward membrane current in gastropod neural somata. *Journal of Physiology* **213**, 21–30.

DE PEYER, J. E. & DEITMER, J. W. (1980). Divalent cations as charge carriers during two functionally different membrane currents in the ciliate *Stylonychia*. *Journal of Experimental Biology* **88**, 73–89.

De Peyer, J. E. & Machemer, H. (1977). Membrane excitability in *Stylonychia*: properties of the two-peak regenerative Ca-response. *Journal of Comparative Physiology* **121**, 15–32.

De Peyer, J. E. & Machemer, H. (1978). Hyperpolarizing and depolarizing mechanoreceptor potentials in *Stylonychia*. *Journal of Comparative Physiology* **127**, 255–266.

Deitmer, J. W. (1981). Voltage and time characteristics of the potassium mechanoreceptor current in the ciliate *Stylonychia*. *Journal of Comparative Physiology* **141**, 173–182.

Deitmer, J. W. (1982). Two voltage-dependent Ca^{2+} conductances in the membrane of the ciliate *Stylonychia*. *Pflügers Archiv* **392**, suppl. R 19.

Deitmer, J. W. (1983*a*). Activation and inactivation of two voltage-dependent Ca channels. *Biophysical Journal* **41**, 293a.

Deitmer, J. W. (1983*b*). Ca channels in the membrane of the hypotrich ciliate *Stylonychia*. In *The Physiology of Excitable Cells*, ed. Grinnell, A. D. & Moody, Jr, W. J., pp. 51–63. New York: A. R. Liss, Inc.

Deitmer, J. W., Machemer, H. & Martinac, B. (1983). Simultaneous recording of responses of membranelles and cirri in *Stylonychia* under membrane voltage-clamp. *Journal of Submicroscopic Cytology* **15**, 285–288.

Deitmer, J. W., Machemer, H. & Martinac, B. (1984). Motor control in three types of ciliary organelles in the ciliate *Stylonychia*. *Journal of Comparative Physiology* **154**, 113–120.

Dembowska, W. S. (1938). Körperreorganisation von *Stylonychia mytilus* beim Hungern. *Archiv für Protistenkunde* **91**, 89–105.

Dunlap, K. (1977). Localization of calcium channels in *Paramecium caudatum*. *Journal of Physiology* **271**, 119–133.

Eckert, R. (1972). Bioelectric control of ciliary activity. *Science* **176**, 473–481.

Eckert, R. & Brehm, P. (1979). Ionic mechanisms of excitation in *Paramecium*. *Annual Review of Biophysics and Bioengineering* **8**, 353–383.

Eckert, R., Naitoh, Y. & Machemer, H. (1976). Calcium in the bioelectric and motor functions of *Paramecium*. *Proceedings of the Society of Experimental Biology* **30**, 233–255.

Fox, A. R. (1981). Voltage-dependent inactivation of a calcium channel. *Proceedings of the National Academy of Sciences of the U.S.A.* **78**, 953–956.

Fukushima, Y. & Hagiwara, S. (1983). Voltage-gated Ca^{2+} channel in mouse myeloma cells. *Proceedings of the National Academy of Sciences of the U.S.A.* **80**, 2240–2242.

Hagiwara, S. & Byerly, L. (1981). Calcium channel. *Annual Review of Neuroscience* **4**, 69–125.

Hagiwara, S., Ozawa, S. & Sand, O. (1975). Voltage clamp analysis of two inward current mechanisms in the egg cell membrane of a starfish. *Journal of General Physiology* **65**, 617–644.

Hagiwara, S. & Saito, N. (1959). Voltage–current relations in nerve cell membrane of *Onchidium verruculatum*. *Journal of Physiology* **148**, 161–179.

Heyer, C. B. & Lux, H. D. (1976). Control of the delayed outward potassium currents in bursting pace-maker neurones of the snail, *Helix pomatia*. *Journal of Physiology* **262**, 349–382.

Hinrichsen, R. D. & Saimi, Y. (1984). A mutation that alters properties of the calcium channel in *Paramecium tetraurelia*. *Journal of Physiology* **351**, 397–410.

Hirano, T. & Takahashi, K. (1984). Comparison of properties of calcium channels between the differentiated 1-cell embryo and the egg cell of ascidians. *Journal of Physiology* **347**, 327–344.

Jennings, H. S. & Jamieson, C. (1902). Studies on reactions to stimuli in unicellular organisms. X. The movements and actions of pieces of ciliate infusoria. *Biological Bulletin* **3**, 225–234.

Kamada, T. (1934). Some observations on potential differences across the ectoplasm membrane of *Paramecium*. *Journal of Experimental Biology* **11**, 94–102.

Kung, C. & Saimi, Y. (1982). The physiological basis of taxes in *Paramecium*. *Annual Review of Physiology* **44**, 519–534.

Machemer, H. (1965). Analyse langzeitlicher Bewegungserscheinungen des Ciliaten *Stylonychia mytilus* Ehrenberg. *Archiv für Protistenkunde* **108**, 91–107.

Machemer, H. (1966). Zur Koordination und Wirkungsweise der Membranellen von *Stylonychia mytilus*. *Archiv für Protistenkunde* **109**, 257–277.

Machemer, H. (1970). Korrelation zwischen Membranpotential und Fortbewegung bei *Stylonychia*. *Naturwissenschaften* **57**, 398–399.

Machemer, H. & de Peyer, J. E. (1977). Swimming sensory cells: electrical membrane parameters, receptor properties and motor control in ciliated protozoa. *Verhandlungen der Deutschen Zoologischen Gesellschaft*, pp. 86–110. Stuttgart: F. Fisher Verlag.

MACHEMER, H. & DE PEYER, J. E. (1982). Analysis of ciliary beating frequency under voltage clamp control of the membrane. *Cell Motility* **1**, suppl., 205–210.

MACHEMER, H. & OGURA, A. (1979). Ionic conductances of membranes in ciliated and deciliated *Paramecium*. *Journal of Physiology* **296**, 49–60.

MEECH, R. W. & STANDEN, N. B. (1975). Potassium activation in *Helix aspersa* neurones under voltage clamp: a component mediated by calcium influx. *Journal of Physiology* **249**, 211–239.

NEHER, E. (1971). Two fast transient current components during voltage clamp on snail neurons. *Journal of General Physiology* **58**, 36–53.

OGURA, A. & TAKAHASHI, K. (1976). Artificial deciliation causes loss of calcium-dependent responses in *Paramecium*. *Nature* **264**, 170–172.

OKAMOTO, H., TAKAHASHI, K. & YOSHII, M. (1976). Two components of the calcium current in the egg cell membrane of the tunicate. *Journal of Physiology* **255**, 527–561.

PLANT, T. D. & STANDEN, N. B. (1981). Calcium current inactivation in identified neurones of *Helix aspersa*. *Journal of Physiology* **321**, 273–285.

PLANT, T. D., STANDEN, N. B. & WARD, T. A. (1983). The effects of injection of calcium ions and calcium chelators on calcium channel inactivation in *Helix* neurones. *Journal of Physiology* **334**, 189–212.

SAIMI, H. & KUNG, C. (1980). A Ca-induced Na-current in *Paramecium*. *Journal of Experimental Biology* **88**, 305–325.

SATOW, Y. & KUNG, C. (1979). Voltage sensitive Ca-channels and the transient inward current in *Paramecium tetraurelia*. *Journal of Experimental Biology* **3**, 149–161.

TILLOTSON, D. (1979). Inactivation of Ca conductance dependent on entry of Ca ions in molluscan neurons. *Proceedings of the National Academy of Sciences of the U.S.A.* **76**, 1497–1500.

VERWORN, M. (1889). Psychophysiologische Protistenstudien. *Experimentelle Untersuchungen, Jena*, pp. 1–219.

WALLENGREN, H. (1901). Zur Kenntnis des Neubildungs- und Resorptionsvorganges bei der Teilung der hypotrichen Infusorien. *Zoologische Jahrbücher der Anatomie* **15**, 1–58.