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A NOVEL TYPE OF MECHANORECEPTION BY THE FLAGELLA OF *CHLAMYDOMONAS*

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Summary

A novel type of mechanosensory mechanism is found in *Chlamydomonas reinhardtii*. When a cell is captured with a suction pipette and a negative pressure is applied, the cell produces repetitive Ca^{2+} impulses at a frequency of 0.5–1.0 Hz. The impulse frequency increases with the applied pressure. The impulses are produced when the flagella are sucked into the pipette but not when the cell body is sucked in leaving the flagella outside the pipette. Cells with short flagella produce impulses of small amplitude. Thus, the site where the cell senses mechanical stimuli and generates the impulse current must be localized at the flagella. The amplitude, shape and ion selectivity of the pressure-induced impulses are distinct from the all-or-none flagellar current that is evoked by photostimulation. The impulses are possibly produced by a combination of currents passing through mechanosensitive channels and Ca^{2+} channels. This response probably functions to modulate flagellar beating and thereby to regulate the behaviour of the cell.

Key words: mechanoreceptor current, mechanosensitive channel, flagellar motility, photoshock response, *Chlamydomonas*.

Introduction

A number of protists have been found to respond to mechanical stimuli with a variety of behavioural responses through a change in membrane potential (Naitoh and Eckert, 1969; Eckert, 1972; de Payer and Machemer, 1978; Wood, 1970). *Paramecium caudatum* produces a depolarizing receptor potential upon mechanical stimulation at the anterior surface and a hyperpolarizing receptor potential upon stimulation at the posterior surface. The depolarizing receptor potential elicits a Ca^{2+} action potential and an increase in intracellular Ca^{2+} concentration, resulting in a reversal of the direction of the ciliary beat (Naitoh and Eckert, 1969; Naitoh and Kaneko, 1972). The hyperpolarizing receptor potential, in contrast, is caused by an opening of K^+ channels and results in an increase in the forward swimming speed.

The biflagellated protist *Chlamydomonas* has systems in which the membrane potential or the intracellular Ca^{2+} concentration controls the behaviour of the organism. Upon stimulation by strong illumination, a *Chlamydomonas* cell changes its flagellar beat pattern and displays a phobic response. This is mediated by an influx of Ca^{2+} (Schmidt and Eckert, 1976; Harz and Hegemann, 1991; Sineshchekov, 1991). An increase in intracellular Ca^{2+} concentration also appears to be essential for another kind of photoresponse, phototaxis (for a review, see Witman, 1993). It is not known, however, whether this organism has any mechanoreception system. Here, I show that it responds to mechanical stimulation applied with a suction pipette. Interestingly, the cell responds to the stimulus with repetitive impulse currents with a variable frequency between 0.5 and 1.0 Hz that increases with increasing pressure. Thus, *Chlamydomonas* appears to have an ingenious mechanoreception system in which stimulus intensity is converted to impulse frequency.

Materials and methods

Cell culture

A cell-wall-deficient *Chlamydomonas reinhardtii* mutant, *cw2*, was used. This mutant enables one to record the membrane currents with a suction electrode with a loose seal (Harz and Hegemann, 1991). Vegetative cells were grown in liquid culture (Gorman and Levine, 1965) under 12 h:12 h light:dark illumination. A double mutant with defects in the cell wall and flagellar inner dynein arm (*pf23cw2*) was constructed using standard methods (see Harris, 1989) and used in experiments with short-flagellated cells. This mutant is non-motile.

Electrophysiological methods

A suction pipette was used to record the membrane current as well as to stimulate the cell with hydrostatic pressure. Electrophysiological experiments were carried out as reported by Yoshimura (1994) with minor modifications. A cell was caught on a suction pipette under observation with an inverted microscope (Nikon MD, Tokyo, Japan). The cell was illuminated with a 500 W xenon arc lamp (Ushio XLD-500D,

Tokyo, Japan) through a red filter (>600 nm, R-60, Kenko, Tokyo, Japan) and neutral density filters. The positions of the flagella were recorded with a CCD camera (Hamamatsu Photonics C2400, Hamamatsu, Japan) and then determined on a video monitor (final magnification: ×5300). The response did not depend on illumination; the same response was observed in the absence of illumination. When the photoresponse was examined, the red filter was replaced with a 500 nm interference filter (half bandwidth 10 nm, Melles Griot, Tokyo, Japan) and a mechanical shutter. The light intensity of the photostimulation was $20\,W\,m^{-2}$.

Suction pipettes with a resistance of 100–200 MΩ were used. The current flowing through the suction pipette was amplified with a patch-clamp amplifier, filtered through a 3 kHz low-pass Bessel filter (EPC7, List-Medical, Darmstadt, Germany), and recorded with a DAT recorder (59ES, Sony, Tokyo, Japan) modified so as to be capable of recording direct current. The potential of the pipette was held at that of the bath solution. The stored data were digitized at 10 kHz with an A/D converter (ADX-98H, Canopus, Kobe, Japan) fitted to a personal computer (PC9801 VX, NEC, Tokyo, Japan).

Mechanical stimulation

To stimulate the cell mechanically, negative pressure was applied through a suction pipette. The pressure was switched between atmospheric pressure and the test pressure using a three-way stopcock (Sigurdson *et al.* 1987). Because the cells responded to a small pressure, the test pressure was monitored with a water manometer instead of a mercury manometer, which has been used in previous studies (Guharay and Sachs, 1984; Martinac *et al.* 1987) (1 cmH_2O=98.1 Pa). For estimation of the pressure at the position of the pipette, the suction pipette and its holder were replaced with a pressure transducer (PDL-40GB, Kyowa, Tokyo, Japan). After the three-way stopcock had been turned, the alteration in the pressure was completed within 0.2 s. Its steady-state value was about 68 % of the pressure at the manometer. The pressure shown in the text is the pressure at the manometer. No pressure was applied to hold the cell onto the pipette because most cells, once caught, remained attached to the pipette without applied pressure.

Solutions

The experimental solution consisted of 1 $mmol\,l^{-1}$ KCl, 0.3 $mmol\,l^{-1}$ $CaCl_2$, 0.2 $mmol\,l^{-1}$ EGTA and 5 $mmol\,l^{-1}$ Hepes. pH was adjusted to 7.1 with *N*-methylglucamine. This solution contains 0.10 $mmol\,l^{-1}$ free Ca^{2+} (calculated after Goldstein, 1979). The effects of verapamil (Sigma), BAY K 8644 (Research Biochemicals; Natick, USA) and ω-conotoxin GVIA (Peptide Institute; Osaka, Japan) were examined either by continuously recording from a single cell while perfusing with the test solution or by recording from several cells after suspending them in the test solution. In the latter case, cells were washed at least three times with the test solution and the response of more than 10 cells was examined.

The results are based on observations on 597 cells. All experiments were carried out at 23–26 °C.

Results

Production of an impulse in response to suction

When a *Chlamydomonas* cell was caught with its flagella inside a suction pipette (Fig. 1) and a negative pressure was applied through the pipette, the cell produced repetitive currents with a delay of 5–12 s after the onset of suction (Fig. 2A). The impulses, measured with the suction pipette, were due to currents flowing out of the pipette. The amplitudes of the impulses were small initially but increased to more than 5 pA over several seconds (Fig. 2A,B). The impulses with small amplitudes observed initially appeared to consist of many spiky currents, whereas the impulses recorded during the steady state were smoother. The amplitude of the current varied even at steady state. This is, however, not due to any instability in the recording conditions since the charge carried by each impulse was found to be almost constant. For instance, the charges of the last four impulses shown in Fig. 2B were 231, 240, 239 and 230 fC. The impulses appeared at almost constant intervals during the application of suction pressure (Fig. 2C). After the release of suction, the impulses continued at increasing intervals; the impulses disappeared abruptly when the interval rose to 2–3 s (Fig. 2A,C).

An increase in applied pressure increased the frequency of the impulses but not their amplitude (Fig. 3A,B). The average frequency at 20 cmH_2O was almost twice as high as that at 5 cmH_2O. The frequencies at 5, 10 and 15 cmH_2O are significantly different from each other (P<0.01 by two-tailed t-test). This indicates that the intensity of the mechanical stimulus is translated into impulse frequency. The threshold of the response seemed to be between 5 and 10 cmH_2O since most cells responded to a pressure of 10 cmH_2O but not to a pressure of 5 cmH_2O.

The latency of the response varied greatly from cell to cell. However, it did not depend on the size of the applied pressure (Fig. 3C). When suction pressure was applied for a longer period, the impulses disappeared during suction. This adaptation process also differed significantly in duration from

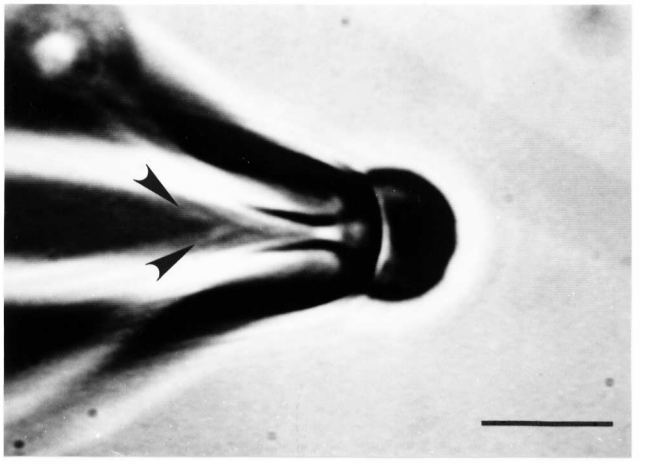

Fig. 1. A *Chlamydomonas* cell caught on a suction pipette with its two flagella (arrowheads) inside the pipette. Scale bar, 10 μm.

cell to cell (range 17–300 s; median 61 s, *N*=30). The variation in these processes might reflect some cellular conditions that could not be controlled in this experiment.

Site of mechanoreception

The repetitive impulses occurred only when the flagellum was sucked into the pipette. The impulses were generated when either one flagellum or two flagella were trapped. 86 % (*N*=66) of cells produced impulses when the flagellum (or flagella) was sucked into the pipette and 0 % (*N*=70) when the cell body was sucked into the pipette leaving the flagellum outside. In the

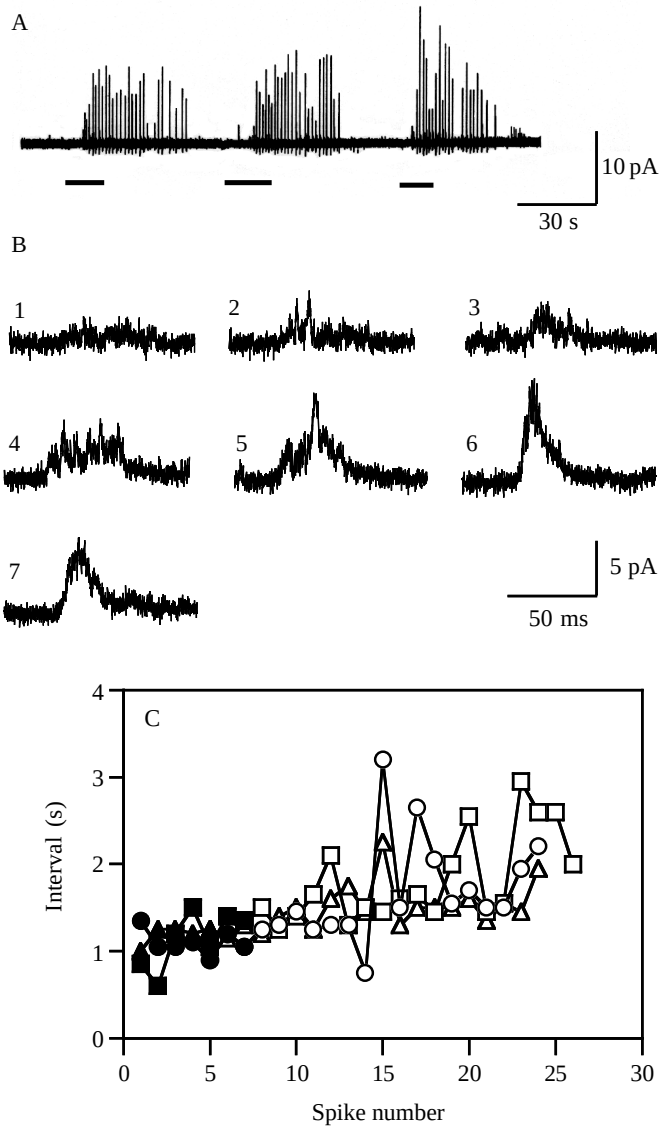

Fig. 2. Response of *Chlamydomonas* to negative pressure applied with a suction pipette. (A) When pressure was applied (bar below the trace), the cell produced a train of impulses. This response was repeated three times. The current was recorded with the suction pipette. The direction of the current was outwards from the pipette. (B) Seven consecutive individual impulses recorded after the application of suction pressure. The shape of the impulse changed with the growth of current. (C) The interval between impulses during the response to mechanical stimulation. The interval between impulses was almost constant during the application of pressure (filled symbols) but increased after the release of pressure (open symbols). The frequencies of the three sets of impulses shown in A are plotted.

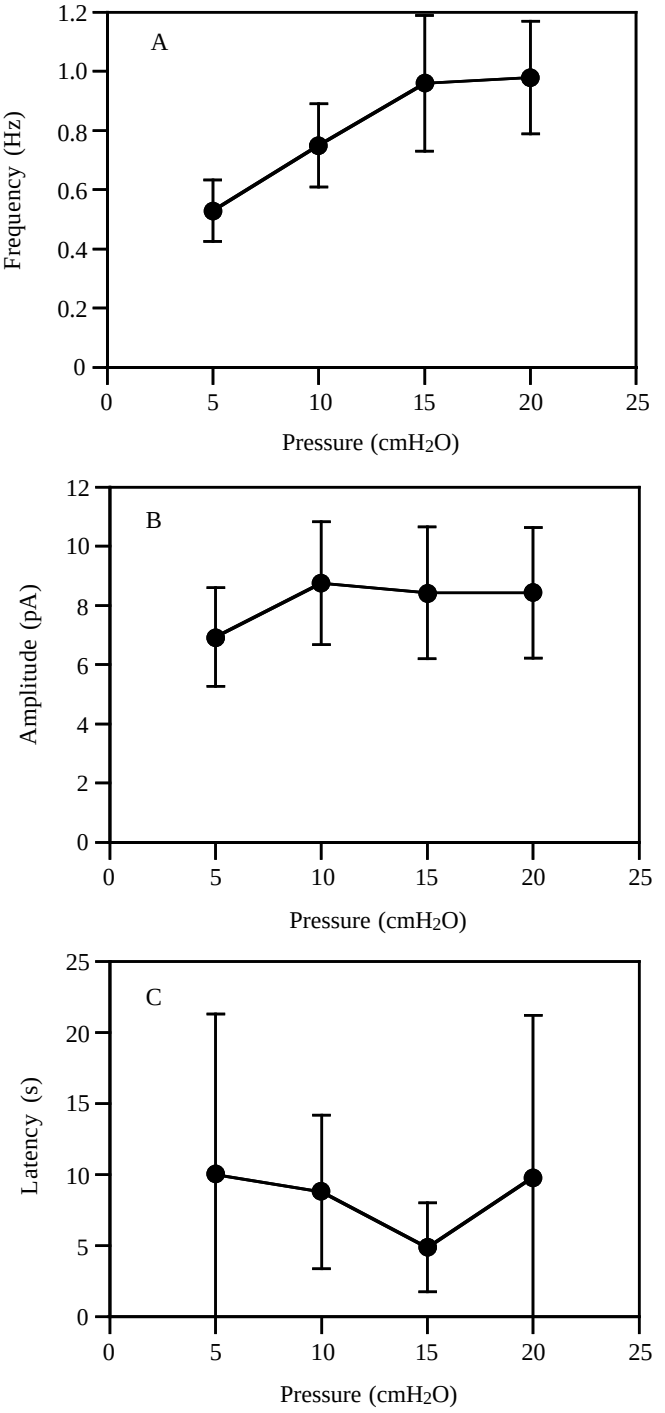

Fig. 3. (A) Relationship between the negative pressure and the frequency of impulses. The frequency of impulses during the application of pressure increased with the magnitude of the applied pressure. (B) The amplitude of impulses did not change with the pressure. (C) The latency of the beginning of the impulses after the onset of stimulation did not change with the pressure. Means and standard deviations are shown. *N*=10 (5 cmH_2O), *N*=13 (10 cmH_2O), *N*=15 (15 cmH_2O) and *N*=12 (20 cmH_2O). 1 cmH_2O=98.1 Pa.

Table 1. *Impulse amplitudes of cells with various flagellar lengths*

Cell type	Impulse amplitude (pA)	Flagellar length (μm)
Control (*cw2*)	11.9±3.6	11.5±1.7
Short flagellar cell (*cw2*)	5.4±1.2	5.2±1.3
Short flagellar cell (*pf23cw2*)	5.1±2.0	3.5±1.2

Values represent mean ± S.D. in 20 cells.

following experiments, cells with their both flagella inside the pipette were used unless otherwise indicated.

To obtain information about the location of the channels along the flagella, two types of short-flagellated cells were examined: a *cw2* cell in the process of regenerating its flagella after deflagellation, and a mutant *pf23cw2* that has defects in the inner dynein arm and lacks motility. The flagella of these cells were about half the normal length. In both types of cell, the amplitudes of the impulses were halved compared with those of the control (Table 1). The impulse amplitude seemed to be proportional to the flagellar length, although extensive analyses have not been performed because these cells rarely produced regular impulses.

When both flagella were sucked into the pipette, two peaks of current were often observed. In such cases, the peak height of the impulse depended on exactly how the two currents overlapped. However, the total charge that moved was almost constant, as stated above. The two peaks may represent the currents in the two separate flagella, since the number of peaks was never greater than two.

Effects of extracellular ions and channel inhibitors

The pressure-induced impulse disappeared when Ca^{2+} was omitted from the medium (Table 2). Therefore, the impulse is probably produced by an inward Ca^{2+} current. Another divalent cation, Ba^{2+}, is known to substitute well for Ca^{2+} as an ion carrier of most Ca^{2+} channels but not as an intracellular messenger. When Ca^{2+} was replaced with Ba^{2+}, no impulse was observed upon suction. Instead, small current fluctuations appeared (Fig. 4). This suggests that Ba^{2+} flows through the channels but cannot generate impulses.

To examine the characteristics of the mechanosensitive channel, I tested the effect of Gd^{3+} and amiloride, which preferentially block mechanosensitive channels in *Xenopus* oocytes and developing mouse muscle cells (Yang and Sachs, 1989; Franco and Lansman, 1990; Lane *et al.* 1991). Both completely inhibited the production of impulses (Table 2), indicating that the mechanosensitive channel participates in impulse production.

Ca^{2+} channels might also play a role in evoking impulses. An inhibitor, ω-conotoxin GVIA, which strongly blocks the N-type Ca^{2+} channel (see Hille, 1992), has been reported to inhibit the photo-induced stop response with a K_i of 130 nmol l^{-1} (Hegemann *et al.* 1990). It inhibited the pressure-induced impulses at 0.6 μmol l^{-1} (Table 2). BAY K 8644, an agonist of the L-type Ca^{2+} channel, decreased the amplitude of the impulses (Table 2). Verapamil has been reported to block the photo-induced Ca^{2+} current at 600 μmol l^{-1} (Harz and Hegemann, 1991). The pressure-induced impulses were inhibited by verapamil at 600 μmol l^{-1}. These findings suggest the involvement of a Ca^{2+} channel.

Table 2. *Inhibition of impulses by chemicals*

Treatment	Concentration (μmol l^{-1})	Impulse	Method*
Control		+	S
Ca^{2+}-free		–	P, S
Ba^{2+}	100†	–‡	S
Gd^{3+}	10§	–	P
Amiloride	1000	–	S
ω-Conotoxin	0.6	–	S
BAY K 8644	2	Small	P
Verapamil	600	–	P

+, more than 80 % of cells generated impulses in response to suction; –, no impulses were produced from at least 10 cells.

*Effect was tested by perfusion (P) or suspension (S). Cells with one or two flagella placed inside of the pipette were used.

†In the presence of 300 μmol l^{-1} Ba^{2+} and 200 μmol l^{-1} EGTA.

‡Small flickering current (Fig. 4).

§In the presence of 100 μmol l^{-1} Ca^{2+} and 0 mol l^{-1} EGTA.

Photostimulation of cells producing impulses

The above results indicate that a Ca^{2+} influx at the flagella is responsible for the impulses. In this respect, the impulse resembles the flagellar current evoked during the photoresponse. The flagellar current occurring after the generation of a photoreceptor current has been shown to be an all-or-none Ca^{2+} current, which is believed to be produced by voltage-dependent Ca^{2+} channels (Harz and Hegemann, 1991; Harz *et al.* 1992). Although the ion species and the location where the current occurs are similar, several observations suggest that the pressure-induced impulse differs from the photo-induced flagellar current (see Discussion).

Suspecting that the pressure-induced impulse and the photo-induced flagellar current are produced by different mechanisms, I next investigated the relationship between them. Cells producing pressure-induced impulses were stimulated with light so that the amplitudes of the two types of current could be directly compared and any interference between them could be detected. Photostimulation evoked a photoreceptor

Fig. 4. When Ca^{2+} was replaced with Ba^{2+}, current fluctuations were observed but no impulse was generated upon application of pressure (bar below the trace).

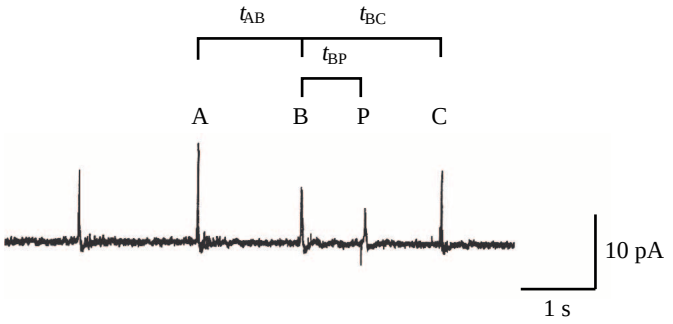

Fig. 5. Photostimulation of cells producing impulses. When a cell producing pressure-induced impulses (A, B and C) was stimulated with light, the cell evoked a photoreceptor current (downward current of P) and a flagellar current (upward current of P). The intervals between the pressure-induced impulses are represented as t_{AB} and t_{BC}. The interval between a photo-induced flagellar current and the preceding pressure-induced current is represented by t_{BP}.

current and a flagellar current (Fig. 5), as previously observed (Harz and Hegemann, 1991; Harz *et al.* 1992). The flagellar current generated by photostimulation was found to be smaller than the pressure-induced impulse (Fig. 5) irrespective of the phase at which the photostimulus was applied after the generation of a pressure-induced impulse (38±20 % on average, Fig. 6A). This suggests that its small amplitude is not due to a relative refractoriness.

Photostimulation delayed the generation of the following pressure-induced impulse ($t_{BC}>t_{AB}$, see Fig. 5). The increase in interval was analysed in order to determine whether the membrane depolarization or the Ca^{2+} influx that are produced by photostimulation affect the periodicity of the pressure-induced impulses. The interval (t_{BC}/t_{AB}) was 1.43±0.44 (Fig. 6B) whereas that measured without photostimulation was 1.05±0.24 (N=40). Statistical analysis indicates that the variances in t_{BC}/t_{AB} increased after photostimulation (P<0.01). This suggests that the delay was not constant but varied with the phase of the photostimulus. Apparently, the later the phase of photostimulation (t_{BP}/t_{AB} in Fig. 5), the later the pressure-induced impulse occurred after the photostimulation (t_{BC}/t_{AB}) (Fig. 6B). The impulse after photostimulation was produced with a delay of less than one cycle (compare the solid line with the broken line in Fig. 6B). The intercept of the regression line does not appear to be unity (P<0.2), suggesting that photostimulation did not completely reset the phase of the impulses, unlike the phase resetting during an extra systole of the heart pacemaker.

Swimming behaviour after mechanical agitation

The Ca^{2+} influx at each impulse may well change the flagellar beating pattern because Ca^{2+} influx occurs at the flagella and because Ca^{2+} is known to regulate flagellar motility (Hyams and Borisy, 1978; Bessen *et al.* 1980; Kamiya and Witman, 1984). However, my experimental conditions did not allow me to observe the flagellar beat pattern directly, because the flagella had to be located inside the suction pipette. As an indirect method of gaining insight into the effect of

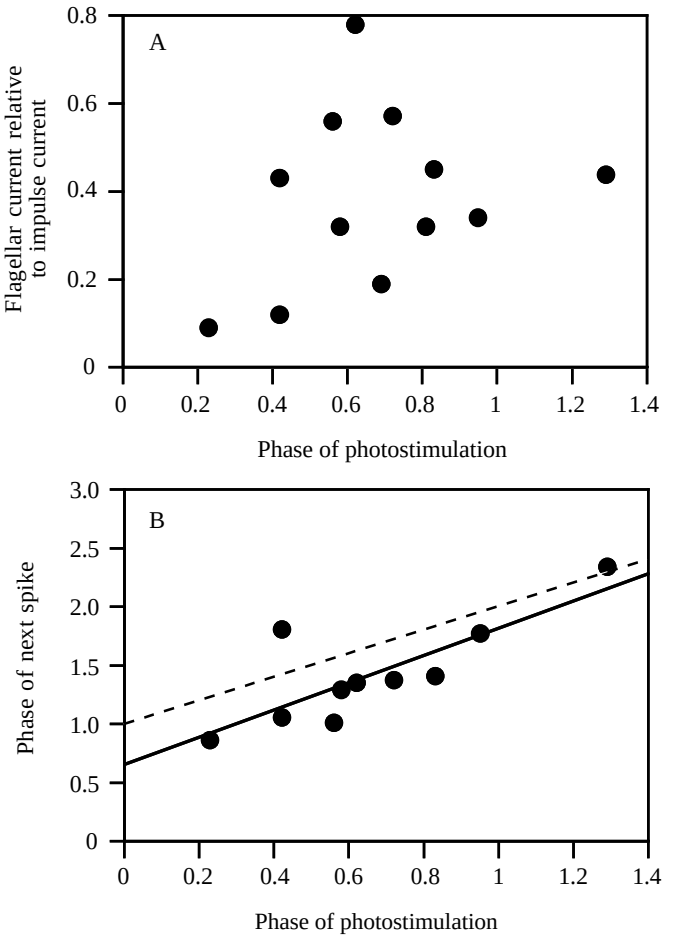

Fig. 6. The relationship between the flagellar current of the photoresponse and the impulses of the mechanoresponse. (A) The photoreceptor currents normalized by the amplitude of the impulse just before photostimulation are plotted against the phase of photostimulation (t_{BP}/t_{AB} in Fig. 5). The photoreceptor current was smaller than the amplitude of pressure-induced impulses irrespective of the phase of photostimulation. (B) The phase of the pressure-induced impulse just after photostimulation (t_{BC}/t_{AB} in Fig. 5) plotted against the phase of photostimulation (t_{BP}/t_{AB} in Fig. 5). The production of the next impulse was delayed after photostimulation. The regression line ($t_{BC}/t_{AB}=1.16t_{BP}/t_{AB}+0.66$; $r^2=0.657$) is shown as a solid line. The broken line represents the theoretical line that would result if the next impulse were delayed for one cycle ($t_{BC}/t_{AB}=t_{BP}/t_{AB}+1$).

mechanical stimulation on the behaviour, I recorded the swimming track immediately after the cells had been agitated by pipetting. In the absence of agitation, cells usually swim straight forward along a helical path (Fig. 7A). However, those cells that were mechanically agitated were observed to change their swimming direction abruptly (Fig. 7B) at a frequency of 0.81±0.30 Hz (12 cells). This erratic movement lasted for several tens of seconds after the agitation. The pressure-induced impulses also continued for several tens of seconds after the end of mechanical stimulation and had a similar frequency (0.6–0.8 Hz, Fig. 2C). This behavioural response disappeared in a Ca^{2+}-free medium (Fig. 7C) and in the

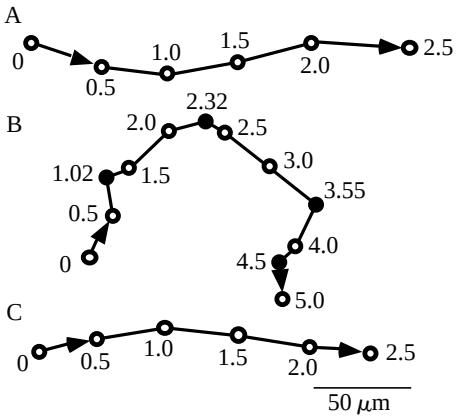

Fig. 7. Traces of swimming cells observed under red light. (A) Cells swam along a straight path in the absence of a stimulus. (B) A cell agitated by pipetting abruptly changed swimming direction (filled circle) at 0.95–1.3 s intervals. (C) Cells did not turn spontaneously but swam straight forwards when extracellular [Ca^{2+}] was depleted. The times (in seconds) are shown beside the position of the cell.

presence of channel blockers (gadolinium, ω-conotoxin GVIA). These observations suggest that the mechano-induced impulse transiently modifies the flagellar beat pattern each time it is generated.

Discussion

Production of repetitive impulses at flagella

The present study has shown that a *Chlamydomonas* cell produces repetitive impulses upon application of a sustained mechanical stimulus with a suction pipette. The impulses do not appear to be an artefact caused by the periodic movement of flagella. Any perturbation of the suction electrode that might be caused by flagellar movement cannot produce any leak current, since the electrode and the bath solution were held at the same potential. In addition, the impulse frequency (0.5–1.0 Hz) is about two orders of magnitude slower than the flagellar beat frequency (about 60 Hz). Lastly, an immotile mutant, *pf23cw2*, also produced repetitive impulses.

This mechanoresponse has the following three novel characteristics: (1) the site for the response is located in the flagella; (2) the impulse frequency increased with the intensity of the stimulus; and (3) the impulse is specific to mechanoreception and is distinct from the flagellar action potential produced by photostimulation.

Chlamydomonas cells produced impulses when their flagella were sucked into the pipette but not when their cell bodies were sucked in. The failure to detect impulses in the latter case indicates that no currents occurred both through the membrane inside the pipette and through that located outside. This is because, in a cell as small as a *Chlamydomonas*, any ionic flow through the membrane outside the suction electrode will also be recorded by the suction pipette. For instance, when the eyespot and flagella are outside the suction pipette, the photoreceptor current and the flagellar current are recorded as currents flowing inwards through the pipette; when the eyespot and flagella are located inside the pipette, they are recorded as outward currents (Harz and Hegemann, 1991; Sineshchekov, 1991; Harz *et al.* 1992). In the present case, the photoreceptor current that occurred outside the pipette during the photoresponse was also recorded as an inward current (Fig. 5). This observation indicates that the flagellum or its base is responsible for both sensing mechanical stimuli and producing impulses.

The small amplitude of impulses in short flagella suggests that the mechanoreceptors and ion channels responsible for the impulses are uniformly distributed along the flagellum. A recent study has shown that the voltage-dependent channels that operate during the photoresponse are also located along the flagellum (Beck and Uhl, 1994). Alternatively, it is possible that the cell senses the stimulus at the flagellar base and that the magnitude of the stress depends on the flagellar length.

Mechanoreception in *Chlamydomonas* thus differs from that in *Paramecium*, which receives stimuli at the soma and produces action potentials at the cilia (Ogura and Takahashi, 1978; Ogura and Machemer, 1980). The mechanosensitive site of *Stylonychia mytilus* and *Stentor coeruleus* has also been shown to be located at the cell membrane of the soma (de Payer and Machemer, 1978; Wood, 1989). The present study, however, does not exclude the possibility that there are some mechanoreceptors on the cell body that are not involved in the production of impulses.

The discovery of repetitive impulse production in *Chlamydomonas* is somewhat surprising because the generation of spikes at frequencies depending on stimulus intensity has not been reported in protists, although it is common in multicellular organisms. In mechanoreception in multicellular organisms, frequency coding is essential because information about the stimulus intensity must be transmitted to the central nervous system by way of nerve fibres. *Chlamydomonas*, with a diameter of less than 10 μm, has no need to transmit such information. Instead, the impulses may directly modulate the behaviour. The finding that the cell displays a turning response after mechanical stimulation, at a frequency similar to that of the pressure-induced impulses, suggests that the mechano-induced impulse transiently modifies the flagellar beat pattern each time it is generated. Another biflagellated alga, *Spermatozopsis similis*, has also been reported to show multiple changes in swimming pattern after mechanical stimulation (Kreimer and Witman, 1994).

Channels that pass the impulse current

The mechanical stimulus induced a current at the flagella of *Chlamydomonas*. It has been shown that a similar flagellar current occurs during the photoresponse (Harz and Hegemann, 1991; Harz *et al.* 1992). This flagellar current is produced only when the preceding photoreceptor current exceeds a certain threshold level. Thus, the flagellar current is produced in an all-or-none manner, probably by the opening of voltage-dependent Ca^{2+} channels. The pressure-induced impulses, however, differ from the photo-induced flagellar current in the

following respects: (1) the amplitude of the pressure-induced impulse is larger than that of the photo-induced current; (2) the pressure-induced impulses were spiky at the beginning of the response, but photo-induced currents were always smooth; (3) the pressure-induced impulses varied in size and shape, whereas the photo-induced current had a constant shape and amplitude; (4) Ca^{2+} can be replaced by Ba^{2+} for the production of the photo-induced current (Harz *et al.* 1992), but not for the production of pressure-induced impulses (Fig. 4). These results therefore indicate that the pressure-induced impulse is produced by channels that are not involved in the photo-induced flagellar current, although some participation of voltage-dependent channels may occur. Because no current as large as the pressure-induced impulse was generated by photo-induced depolarization alone, the channels responsible for impulse generation should be channels that can be opened only in the presence of mechanical stimuli. These are, namely, mechanosensitive channels.

Since the pressure-induced current is larger than the photo-induced flagellar current, the influx of Ca^{2+} during the impulse should depolarize the cell membrane and thereby also open voltage-dependent channels. If so, what is observed during a mechanically induced impulse must be a combination of currents that flow through the two types of channels: a mechanosensitive channel and a voltage-dependent Ca^{2+} channel.

Kreimer and Witman (1994) have reported that a mechanical shock applied to a biflagellated alga (*Spermatozopsis similis*) elicits a striking response that is comparable to the photo-induced response to extremely strong light. They concluded that the mechanical shock response involves a larger Ca^{2+} influx than that in the photoshock response. Although dealing with different organisms, their findings agree well with the finding of my electrophysiological study that the pressure-induced Ca^{2+} current is larger than the photo-induced Ca^{2+} current.

Regulation of the periodicity of impulses

When a cell in the process of generating pressure-induced impulses was stimulated with light, it did not produce as large an impulse as the pressure-induced impulse. As discussed above, it is probable that the depolarization upon photostimulation opens the voltage-dependent Ca^{2+} channels but not the putative mechanosensitive channels. The mechanosensitive channels may remain inactive during the interval between the impulses even if pressure and depolarization are provided; an impulse is generated when the inactivation of the mechanosensitive channels is somehow removed.

What is the basis for this inactivation? The finding that a photoresponse delayed the production of an impulse in response to mechanical stimulation by a variable amount depending on the phase of stimulation indicates that the interval is controlled by a quantitative factor. Thus, the mechanosensitive channels may be inactivated by a factor such as intracellular Ca^{2+} or membrane potential. From the observation of the continuously fluctuating current in the Ba^{2+}-containing solution, I speculate that Ca^{2+} controls the periodicity. The experiment with Ba^{2+} shows that Ba^{2+} can flow through the channels but, unlike Ca^{2+}, its current was not intermittent. This may be due to the absence of inactivation by Ca^{2+}, as in those Ca^{2+} channels that have been reported to be inactivated fully by Ca^{2+} but only slightly by Ba^{2+} (Brehm and Eckert, 1978; Eckert and Tillotson, 1981; Eckert and Chad, 1984). In the present experiment, the intracellular Ca^{2+} concentration may be elevated by the influx of Ca^{2+} and thus inactivate the mechanosensitive channels, while the elevated Ca^{2+} concentration may be lowered by a Ca^{2+}-sequestering system such as a Ca^{2+} pump, by diffusion or by organic anions. When the intracellular Ca^{2+} concentration is reduced below a certain level, the inactivation is removed and the next impulse is produced. Such an interplay between the receptor and the Ca^{2+} pump has been suggested in the agonist-mediated oscillation of intracellular Ca^{2+} concentration in non-excitable cells (Stojilkovic *et al.* 1993).

The present study has demonstrated for the first time the presence of a mechanoresponse in *Chlamydomonas*. *Chlamydomonas*, a popular organism in genetic and molecular biological studies, may be an excellent material for future studies on mechanoreception at the molecular level.

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