The Journal of Experimental Biology 213, 3980-3987

doi:10.1242/jeb.042903

# Chemo-accumulation without changes in membrane potential in the microstome form of the ciliate *Tetrahymena vorax*

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Accepted 3 September 2010

## SUMMARY

**The swimming behaviour of ciliates is mainly determined by membrane potential and transmembrane fluxes. In a chemical gradient, swimming ciliates may approach or move away from the source. Based on experiments on *Paramecium*, it is generally assumed that chemical attractants and repellents affect the swimming behaviour of ciliates by specific changes in the membrane potential. We have examined whether there is a causal relationship between membrane potential and chemo-accumulation in the microstome form of the polymorphic ciliate *Tetrahymena vorax*. Effects of chemo-attractants on the membrane potential of *Tetrahymena* have not been previously reported. Microstome *T. vorax* cells aggregated close to a point source of L-cysteine and the complex meat hydrolysate proteose peptone. Chemo-accumulated cells displayed a significantly higher turning frequency than control cells at a similar cell density. A concentration of 20 mmol $l^{-1}$ L-cysteine did not evoke any detectable change in the membrane potential whereas 1% proteose peptone depolarised the cells by ~12 mV. This is contrary to the current model, which predicts agents that induce a moderate depolarisation to be repellents. A solution of 1% proteose peptone contains 21 mmol$^{-1}$ $Na^+$. A solution of 21 mmol$^{-1}$ NaCl without organic compounds also caused ~12 mV depolarisation but had no aggregating effect on the cells. Collectively, the electrophysiological and behavioural data indicate that chemo-accumulation in the microstome form of *T. vorax* is not governed obligatorily by the membrane potential. We thus suggest that the simple membrane potential model for chemokinesis in *Paramecium* may not be valid for *T. vorax*.**

Key words: *Tetrahymena vorax*, membrane potential, chemo-accumulation, chemo-attraction, swimming behaviour, ciliate, L-cysteine, proteose peptone.

## INTRODUCTION

One of the fundamental processes in life is the ability to sense and recognise chemicals in the environment. Unicellular organisms such as ciliates may respond to different chemicals in the aqueous environment by altering their swimming behaviour (Jennings, 1906). In a chemical gradient, responding ciliates may thus approach or move away from the source. Attractants are often food or mating signals whereas repellents, for instance, may be agents released by lysed cells (Almagor et al., 1981; Levandowsky et al., 1984; Hennessey et al., 1995). The chemosensory responses are guided by complex alterations in ciliary motility, which may lead to either chemotaxis or chemokinesis. The former is characterised by straight, vectorial swimming up (positive chemotaxis) or down (negative chemotaxis) a chemical gradient (Hellung-Larsen et al., 1990; Bell et al., 2007; Nam et al., 2009). By contrast, chemokinesis is more random in nature and involves changes in turning frequency (klinokinesis) and/or swimming speed (orthokinesis) (Fraenkel and Gunn, 1961; Van Houten, 1978; Almagor et al., 1981; Van Houten et al., 1982; Leick and Hellung-Larsen, 1992). The end result of both positive chemotaxis and positive chemokinesis is aggregation of cells close to the source of a chemo-attractant, i.e. chemo-accumulation. Thus, chemo-accumulation is the result of a chemokinetic or chemotactic response and does not in itself provide information about the mechanisms resulting in the aggregation of cells.

Changes in the ciliary motility of ciliates are mainly determined by membrane potential and transmembrane ion fluxes (Naitoh and Eckert, 1969; Eckert, 1972; Eckert and Naitoh, 1972; Naitoh, 1974; Onimaru et al., 1980). According to a model proposed by Van Houten, chemokinesis in *Paramecium* is exclusively guided by changes in the membrane potential (Van Houten, 1979). Chemicals causing moderate depolarisation disperse the cells by increasing the turning frequency. By contrast, chemicals causing strong hyperpolarisation disperse the cells by increasing the swimming speed. Chemo-attractants, such as acetate and folate, moderately hyperpolarise the membrane of *Paramecium* and induce aggregation of cells by reducing the turning frequency and slightly increasing the swimming speed. However, some chemicals, such as $Ba^{2+}$, induce a strong depolarisation, resulting in aggregation by increasing the turning frequency and decreasing the swimming speed. This model for integration of sensory information in *Paramecium* has been proposed as universal for all ciliates and is thus presumably applicable to *Tetrahymena* (Hennessey and Kuruvilla, 1999).

In *Tetrahymena*, chemo-repellents such as lysozymes, GTP and ATP induce membrane depolarisation followed by reversal of the ciliary beat, which elicits a turn of the ciliate (Kuruvilla and Hennessey, 1998; Kim et al., 1999). *Tetrahymena* cells are attracted to various substances, such as amino acids, amines and peptides (Levandowsky et al., 1984; Hellung-Larsen et al., 1986). However, effects of chemo-attractants on the membrane potential in *Tetrahymena* have not been previously reported.

In the present study, we examined a possible correlation between membrane potential and chemo-accumulation in *Tetrahymena vorax*. This species is polymorphous, with a microstome form

predominating in standard culture conditions. The microstome cells take up bacteria and dissolved nutrients by unspecific pinocytosis (Nilsson, 1979; Grønlien et al., 2002). When exposed to potential prey organisms, i.e. other species of relatively small ciliates, the microstome form transforms to a carnivorous macrostome form (Williams, 1961; Buhse, 1966). Because of the great differences in nutrient preference and selectivity of food internalisation (Grønlien et al., 2002), we limited our initial study to the microstome form. A similar study of the macrostome form will be presented later.

Contrary to our expectations, the behavioural responses to the tested chemo-attractants in microstome *T. vorax* were not governed by changes of the membrane potential. Our data thus indicate that the simple membrane potential model for chemokinesis in *Paramecium* may not have general validity for ciliates.

## MATERIALS AND METHODS

### Cells

We used the polymorphous *Tetrahymena vorax* Kidder 1941, strain $V_2$, in which a microstome form predominates in standard culture conditions. The ciliates were kept in culture at 19°C in standard axenic culture medium (Plesner et al., 1964), which is essentially a balanced salt solution with nutrients in the form of 2 g $l^{-1}$ yeast extract (Sigma Chemical Co., St Louis, MO, USA) and 20 g $l^{-1}$ proteose peptone (protein hydrolysate from meat; Sigma Chemical Co.). The experiments were performed on cells in the mid-logarithmic phase of exponential growth. The cell density was ~$5\times10^5$ cells $ml^{-1}$. The size of the elongated cells was ~60×20 μm.

### Experimental solutions

The registration solution contained (final concentrations) 1 mmol $l^{-1}$ NaCl, 1 mmol $l^{-1}$ KCl, 1 mmol $l^{-1}$ $CaCl_2$ and 1 mmol $l^{-1}$ Tris/HCl (pH 7.2). Test solutions were prepared by dissolving the potential attractants in the registration solution. The test solutions contained 0.1–50 mmol $l^{-1}$ cysteine, 10 g $l^{-1}$ proteose peptone, 20 mmol $l^{-1}$ mannitol and 21 mmol $l^{-1}$ NaCl. In addition, the effect of lowering the pH in the registration solution was tested. All chemicals were obtained from Sigma Chemical Co. To analyse the concentration of inorganic ions in proteose peptone, standard equipment for atomic absorption spectroscopy was used.

### Experimental procedures

Prior to the experiments, cells in standard culture medium (2 ml) were gently centrifuged at ~10 ***g*** for 30 s to form a loose pellet. The cells were then suspended in the registration solution and washed twice by repeating the same procedure. The cells were finally suspended in 2 ml of the registration solution. Prior to the recordings, the cells were kept in the registration solution for 1 h. The experiments were performed at room temperature (21–24°C).

#### Recording of chemo-accumulation

The effect of a potential chemo-attractant was assessed by recording the time-dependent accumulation of cells close to a point source of the test solution. A total of 60 washed cells were selected, using a compound microscope and a manually operated micropipette, and placed in a circular cell chamber with a volume of 230 μl and a surface area of 95 $mm^2$. The chamber contained 200 μl registration solution and was topped with paraffin oil to avoid evaporation of water. The experiments were performed on the stage of an inverted microscope fitted with a video camera connected to a video-recorder. The behaviour of the cells was recorded at 25 frames $s^{-1}$.

A glass micropipette with a tip diameter of ~25 μm was filled with the test solution, fixed in a pipette holder and inserted into the cell chamber using a micromanipulator. The tip of the pipette was positioned centrally in the chamber, ~50 μm from the bottom. The level of solution within the pipette was kept 5–10 mm above the level that would result from capillary suction, thus ensuring a constant leakage of solution out of the pipette. When the pipette contained attracting test solutions, the number of cells close to the tip of the leakage pipette increased during the first 10–15 min towards a maximum, stable cell density (Fig. 1). In the area surrounding the pipette tip, the concentration of the attractant was of course considerably less than in the test solution and declined with distance to the pipette. During a period of 15 min, ~8 μl test solution leaked into the cell chamber.

In order to quantify the chemo-accumulating potency of the test solutions, the number of cells within a radius of 0.95 mm from the tip of the leakage pipette was counted every 10 s during the 14th minute after placing the pipette in the cell chamber. The mean number of cells present within the observation area during this 1-min period was recorded as the percentage of the total number of cells in the chamber and was used as a relative measure of chemo-accumulation. Leakage of pure registration solution was used as a control. The observation area constituted ~3% of the surface area of the cell chamber. The experiment was repeated three times for each test solution.

#### Recording of swimming behaviour

In the present study, we measured changes in the swimming pattern, i.e. swimming speed and frequency of turns, by manual tracking of cells displayed on a screen at a magnification of ×126. A change in direction of more than 90 deg was defined as a turn. A directional change of a multiple of this angle was still categorized as one turn if the turn was smooth and uninterrupted. The swimming paths between turns were relatively straight and the speed between turns was approximately constant. Thus, the cells resumed full speed immediately after turning, with periods of acceleration too short to be resolved by our video system. This is probably due to the high surface:volume ratio of ciliates, which is likely to cause the force required to overcome friction during swimming to be much higher than the force needed to accelerate the cell after a turn. The duration of tracks between turns was at least 0.5 s, and the horizontal position of a cell was determined with an accuracy of ~6 μm. Thus, the resolution of the measurements of average swimming speed was better than 12 μm $s^{-1}$.

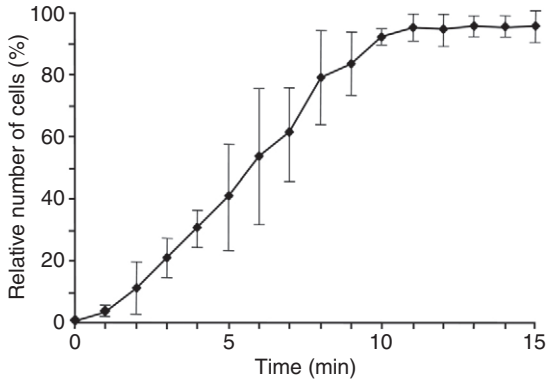


Fig. 1. Aggregation of microstome *Tetrahymena vorax* cells close to a point source of L-cysteine as a function of time. The ordinate shows the relative number of cells within the observation area (3% of the chamber surface area) surrounding the tip of a leakage pipette containing 20 mmol $l^{-1}$ L-cysteine in registration solution. Data are means ± s.d.

In order to determine the sustained swimming behaviour of chemo-accumulated cells, recordings from the 14th minute after start of the experiments were replayed frame by frame. Individual cells were tracked for a period of 1–5 s, during which the frequency of turns and the swimming speed between turns were determined. If a cell collided with an obstacle or another cell, only data prior to the collision were included. For each test solution, three experiments were performed, and 10 of the aggregated cells were tracked in each experiment. As noted, the number of cells in the chamber was 60 and, for the applied chemo-attractants, nearly all of the cells were accumulated in the observation area after 14 min of exposure. The cells to be tracked were chosen from a still picture prior to tracking, attempting to achieve an even spatial distribution of the selected cells. In the control recordings of dispersed cells prior to exposure to the attractant, all the cells in the observation area were tracked.

In order to determine if the cell density *per se* affected swimming behaviour, recordings of unexposed cells were also performed with ~5000 cells in the test chamber, resulting in 80–100 cells within the observation area.

### Electrophysiological recordings

Current clamp recordings were performed at room temperature on the stage of an inverted microscope. The microelectrode and micropipettes used to immobilise cells and to eject test solution were positioned with micromanipulators. During the recordings, the cells were kept stationary by a microsuction pipette with a tip diameter of 10–15 µm. The suction pipette was connected to ambient, subatmospheric or superatmospheric pressure *via* a solenoid valve (Jonsson and Sand, 1987). A selected cell was sucked onto the tip of the pipette by activating the solenoid valve, using a manual trigger. The subatmospheric catching pressure was between –1.0 and –1.5 kPa whereas the holding pressure during the recordings was reduced to –0.5 kPa. The cells are coated with a gel-like material, which tended to clog the pipette. Between each catch, the pipette was therefore cleaned by a short flush of medium through the tip by connecting the pipette to a superatmospheric pressure of ~5 kPa.

The electrical membrane properties were studied using conventional microelectrodes and standard equipment for current clamp recordings. The electrodes were filled with 4 mol $l^{-1}$ potassium acetate adjusted to pH 7.2 with acetic acid, and the electrode resistance was 40–80 MΩ. Electrode penetration of the cell was facilitated by passing a short pulse of high-frequency oscillating current through the electrode. The analogue signals from the current clamp amplifier were fed into an A/D converter (Axon Digidata 1320A) connected to a PC. The PClamp (Axon Instruments, Union City, CA, USA) software package was used for data collection and storage, to generate command signals for current injection and for data processing and analysis.

Test solutions were ejected onto the immobilised cells through a glass micropipette with tip diameter of ~10 µm. The tip of the pipette was positioned ~75 µm from the cell. The cell was flushed with test solution by engaging a solenoid valve, which connected the pipette to a superatmospheric pressure of 1–2 kPa. In control experiments using this procedure, ejection of pure registration solution did not elicit any evident membrane responses.

### Statistics

The significance of the results was tested using a Student's *t*-test ($P<0.05$). Values are presented as means ± s.d.

## RESULTS

### L-Cysteine induces chemo-accumulation in the microstome form of *T. vorax*

The chemo-accumulation of the microstome form of *T. vorax* caused by different test solutions was studied using the technique described in the Materials and methods. Amino acids are known to induce chemo-attraction in *Tetrahymena* (Hellung-Larsen et al., 1986; Levandowsky et al., 1984). Several amino acids were initially tested, and L-cysteine, which was among the most potent attractants, was selected for more detailed experiments.

The microstome form of *T. vorax* was attracted to L-cysteine within the concentration range of 0.1–50 mmol $l^{-1}$. The concentration that induced the most pronounced chemo-accumulation was 20 mmol $l^{-1}$ L-cysteine. Fig. 1 shows the time-dependent aggregation of cells close to the tip of a leakage pipette containing normal registration solution supplemented with 20 mmol $l^{-1}$ L-cysteine. The number of cells in the observation area increased towards a stable, maximum level during the first 10 min of the exposure. Fig. 2B shows the accumulation of ciliates in the observation area 15 min after positioning the pipette in the chamber. In the three parallel tests of 20 mmol $l^{-1}$ L-cysteine, the mean number of cells present within the observation area (3% of the chamber surface area) during the 14th minute of the test period was 95±2% ($N$=3) of the total number of cells in the chamber (60 cells). The corresponding number in control experiments, using leakage pipettes containing only normal registration solution, was 0.7±0.2% ($N$=3). Fig. 2A displays the observation area in one of the control experiments 15 min after positioning of the leakage pipette.

### Proteose peptone induces chemo-accumulation

Proteose peptone is an ingredient in the culture medium commonly used for *Tetrahymena* (Plesner et al., 1964) and is often used as a

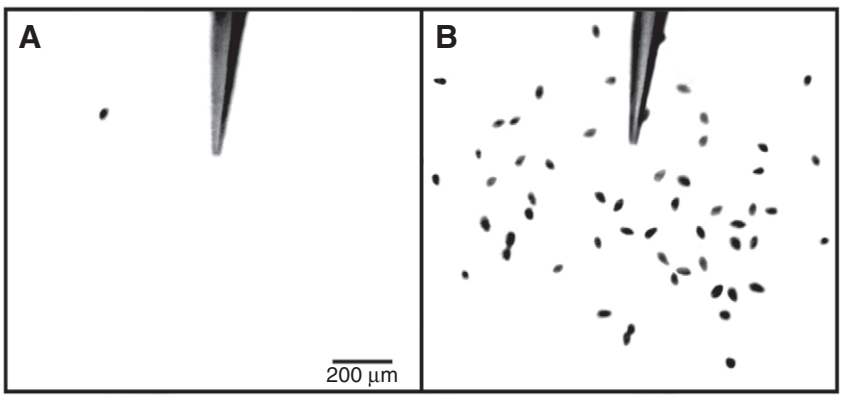


Fig. 2. Accumulation of microstome *Tetrahymena vorax* cells to a point source of L-cysteine. The panels display the observation area surrounding the tip of a leakage pipette containing the test solution. The area covered 3% of the chamber, which contained 60 cells. The photos were taken 15 min after start of the test period. (A) No accumulation of cells occurred close to a leakage pipette containing only registration solution. (B) The ciliates were clearly attracted to a leakage pipette containing 20 mmol $l^{-1}$ L-cysteine in registration solution.

standard attractant in studies concerning behavioural responses in ciliates (Hellung-Larsen et al., 1986; Koppelhus et al., 1994). Therefore, we also examined the accumulation of microstome *T. vorax* cells in registration solution containing 1% proteose peptone. In these experiments, the mean number of cells present within the observation area (3% of the chamber surface area) during the 14th minute of the test period was 97±3% ($N$=3) of the total number of cells in the chamber. The corresponding number in control experiments, using leakage pipettes containing only normal registration solution, was 0.7±0.2% ($N$=3). This result demonstrates that proteose peptone is also a potent attractant for the microstome form of *T. vorax*.

### Moderate changes in osmolarity or pH do not affect chemo-accumulation

Addition of the tested chemo-attractants to the registration solution resulted in increased osmolarity of the solution. In order to test if the increased osmolarity is an attractant in itself, registration solution supplemented with 20 mmol $l^{-1}$ mannitol was used as a test solution. Mannitol is an inert substance that does not interfere with membrane receptors, nor is it transported across the membrane. Only 1.4±0.8% ($N$=3) of the cells were located in the observation area during the 14th minute of exposure to the test solution containing 20 mmol $l^{-1}$ mannitol. We thus conclude that the increased osmolarity was not responsible for the observed accumulation close to the point source of proteose peptone or L-cysteine.

Both proteose peptone and L-cysteine were dissolved in normal registration solution with low buffering capacity, resulting in reduced pH in parallel with increasing concentrations of the solutes. The pH of the test solution with 1% proteose peptone was 7.1, i.e. only slightly different from the pH 7.2 of the registration solution. However, the pH of the test solution with 20 mmol $l^{-1}$ L-cysteine was 6.2. The dissolved L-cysteine constitutes a buffer with high capacity, and a substantial addition of base would have been required to shift the pH to 7.2. Therefore, in order to avoid adding yet another factor to the test solution, we instead tested whether a reduction in pH from 7.2 to 6.2 may cause attraction. In these experiments, the leakage pipette contained registration solution with pH adjusted to 6.2, and only 0.7±0.1% ($N$=3) of the cells were located in the observation area during the 14th minute of the exposure. It is therefore evident that a reduced pH from 7.2 to 6.2 does not in itself induce chemo-accumulation.

### Chemo-accumulation increases turning frequency without affecting swimming speed

In order to elucidate the swimming behaviour of cells accumulated in areas with high concentrations of an attractant, we studied the frequency of turns and the swimming speed between successive turns. The estimates of the degree of chemo-accumulation were based on recordings during the 14th minute after the start of exposure to the attractant, and measurements of sustained changes in behaviour were restricted to the same time period. However, as no accumulation of cells was observed in the control solution, a longer observation period was required to obtain control data from a sufficient number of cells.

Fig. 3 presents the results of the analysis of turning frequency (Fig. 3A) and swimming speed (Fig. 3B) for control cells and for ciliates attracted to 20 mmol $l^{-1}$ L-cysteine and 1% proteose peptone. The swimming speed of control cells was 0.21±0.09 mm $s^{-1}$ ($N$=30). The swimming speeds of cells attracted to L-cysteine (0.18±0.09 mm $s^{-1}$, $P$=0.20, $N$=30) or proteose peptone (0.22±0.08 mm $s^{-1}$, $P$=0.33, $N$=30) were not significantly different from the control speed.

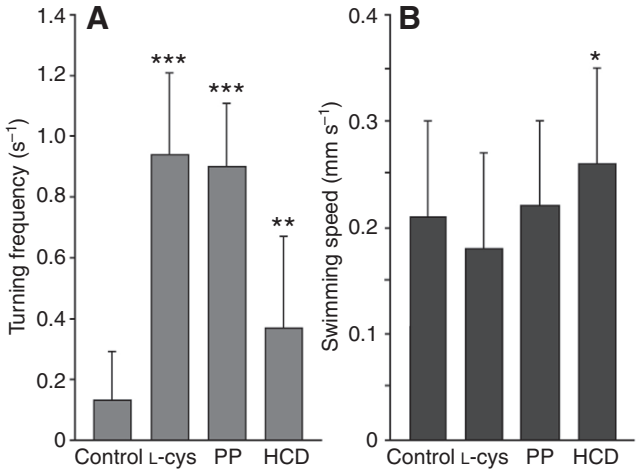


Fig. 3. Swimming behaviour of chemo-accumulated *Tetrahymena vorax* cells. Turning frequency (A) and swimming speed between turns (B) were recorded in cells aggregated close to the tip of a leakage pipette containing 20 mmol $l^{-1}$ L-cysteine (L-cys) or 1% proteose peptone (PP) during the 14th min of exposure. Control values were obtained in tests with pure registration solution in the pipette (Control). The chamber contained 60 cells in the chemo-accumulation experiments. The swimming behaviour of unexposed cells was also recorded at high cell density (HCD), i.e. with 5000 cells in the chamber. Asterisks indicate significant differences compared to control values: *, $P$<0.05; **, $P$<0.001; ***, $P$<0.0001.

The control cells displayed a turning frequency of 0.13±0.16 $s^{-1}$ ($N$=30). During the 14th minute after the start of exposure to L-cysteine and proteose peptone, the turning frequencies were 0.94±0.27 $s^{-1}$ ($N$=30) and 0.90±0.31 $s^{-1}$ ($N$=30), respectively (Fig. 3). Thus, in contrast to the stability of the swimming speed, the frequency of turns was significantly increased in chemo-accumulated cells compared to control cells ($P$<0.0001 for both attractants).

In *Paramecium*, it has recently been observed that cells may swim smoothly up gradients of attractants whereas the turning frequency increases when the cells leave an area with higher concentrations of an attractant (Bell et al., 2007). To elucidate whether the observed increase in turning frequency is a response to leaving an attractive area, we compared the turning frequency of cells in a central and a peripheral region relative to the tip of the pipette in the L-cysteine experiments described above. The central region was defined as the area between 0.08 and 0.24 mm from the tip of the pipette, whereas the part of the observation area extending from 0.8 to 0.95 mm from the tip of the pipette was defined as peripheral. In the central area during the 14th min of exposure, the turning frequency was 0.91±0.34 $s^{-1}$ ($N$=30) whereas the turning frequency in the peripheral area was 0.76±0.31 $s^{-1}$ ($N$=30). These values are not significantly different ($P$=0.25).

The observed changes in swimming behaviour could be a mechanism that is at least partially responsible for the aggregation of cells, or they could be caused by the aggregation. To clarify this, the swimming behaviour of cells were recorded in experiments in which the number of cells in the test chamber was increased from 60 to 5000, resulting in 80–100 cells within the observation area. The reason for the relatively low number of cells in the observation area (3% of the chamber) was due to the tendency of freely swimming cells to concentrate along the edge of the chamber. At the high cell density, the average swimming speed was 0.26±0.09 mm $s^{-1}$ ($N$=30), which is a moderate, significant increase


Fig. 4. Lack of membrane response of a microstome *Tetrahymena vorax* cell stimulated by L-cysteine. The arrows indicate the period of ejection of registration solution with 20 mmol $l^{-1}$ L-cysteine onto the recorded cell, and the trace indicates the membrane potential. A microsuction pipette kept the cell stationary during the recording. The cell had a resting membrane potential of –33 mV, and the membrane potential was unchanged during the stimulation.

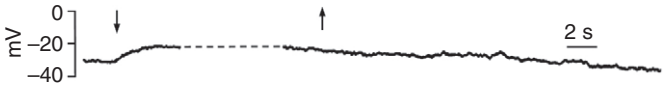


Fig. 6. Membrane response of a microstome *Tetrahymena vorax* cell during prolonged stimulation by proteose peptone. The arrows indicate the period of ejection of registration solution with 1% proteose peptone onto the recorded cell, and the trace indicates the membrane potential. A microsuction pipette kept the cell stationary during the recording. The cell had a resting membrane potential of –29 mV. The stimulation evoked a depolarisation of 9 mV. The membrane was depolarised as long as the cell was stimulated by proteose peptone.

($P$=0.04). The turning frequency was nearly tripled at the high density to 0.37±0.30 $s^{-1}$ ($N$=30), which is a highly significant elevation ($P$<0.001).

Thus, at a similar cell density, cells attracted to L-cysteine and proteose peptone displayed a turning frequency about 2.5 times higher than that of unexposed cells. This difference is highly significant ($P$<0.0001) and indicates that increased turning frequency comprises at least part of the behavioural response to these chemo-attractants. Still, the data demonstrate that our assay is less suited to the detailed study of the mechanisms resulting in the chemo-accumulation of cells because the accumulation in itself influences the swimming behaviour. However, the assay is a simple and efficient method for determining whether a compound is a chemo-attractant.

### The chemo-attractant L-cysteine does not alter the membrane potential

L-Cysteine is a strong attractant for *T. vorax* and, according to van Houten's model (van Houten, 1979), we expected that stimulation with this amino acid would cause a moderate hyperpolarisation of the cells. The membrane response to the potent chemo-attractant L-cysteine was tested in eight microstome *T. vorax* cells, and Fig. 4 presents a typical recording from one of the experiments testing this assumption. To our surprise, in no case did ejection of registration solution with 20 mmol $l^{-1}$ L-cysteine onto the cell evoke any detectable change in the membrane potential. Because the aggregation of cells close to a point source of L-cysteine steadily increased to a stable level after ~10 min, we also tested the membrane response to continuous exposure to 20 mmol $l^{-1}$ L-cysteine for 10 min in five cells. However, even in these cases we could not detect any consistent change in membrane potential.

### The chemo-attractant proteose peptone depolarises the membrane

The microstome form of *T. vorax* was also attracted to proteose peptone. Surprisingly, the cells depolarised moderately in response to proteose peptone. Fig. 5 shows one of these recordings. All 41 cells included in this series of experiments displayed a similar response, and ejection of registration solution with 1% proteose peptone onto the cells induced a depolarisation of 11.9±3.8 mV from a resting membrane potential between –30 and –40 mV. The depolarisation was completely reversible. The membrane remained depolarised during prolonged exposure to proteose peptone for up to 10 min ($N$=3; Fig. 6). This result is in contrast to van Houten's model, which predicts that moderate membrane depolarisation should induce dispersal of the cells (van Houten, 1979).

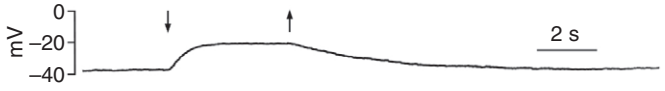


Fig. 5. Membrane response of a microstome *Tetrahymena vorax* cell stimulated by proteose peptone. The arrows indicate the period of ejection of registration solution with 1% proteose peptone onto the recorded cell, and the trace indicates the membrane potential. A microsuction pipette kept the cell stationary during the recording. The cell had a resting membrane potential of –38 mV. The stimulation evoked a depolarisation of 16 mV.

To explore whether the depolarisation induced by proteose peptone was caused by a change in membrane conductance, the membrane resistance was monitored by injecting hyperpolarising current pulses of 200–250 ms duration at a frequency of ~1 Hz during the recording. The *Tetrahymena* cell membrane displays both outward and inward rectification (Onimaru et al., 1980); therefore, the membrane resistance was estimated from the peak value of the potential deflections cased by the constant current pulses. High-resistance microelectrodes are notorious for tending to change their resistance during recording, thus causing erroneous estimates of the input resistance of the cell. Therefore, if the post-stimulus input resistance did not settle at a level deviating less than 5% from the pre-stimulus resistance, the recording was discarded.

Fig. 7A presents a representative recording from one of the cells in this series of experiments. The cell had a resting potential of approximately –30 mV prior to stimulation and was depolarised by ~12 mV during the exposure to proteose peptone. The depolarisation was accompanied by an approximately 30% reduction in membrane resistance. Among the 30 cells tested in this manner, the membrane resistance was reduced by more than 5% during the stimulation in 26 cells. The mean membrane resistance was 427±125 MΩ ($N$=30) before exposure and dropped to 346±113 MΩ during the stimulation. This difference is significant ($P$<0.05).

However, the membrane of *T. vorax* showed pronounced outward rectification upon depolarisation to values above approximately –30 mV (H.K.G., A. K. Jansen, G. E. Bruskeland and O.S., unpublished data). Therefore, to clarify whether the depolarisation induced by proteose peptone was initiated by changes in membrane conductance, the membrane was hyperpolarised by DC current injection during the recording. The complete depolarising response to proteose peptone was then below the potential range in which outward rectification normally occurs. Fig. 7B presents a recording from the same cell that is presented in Fig. 7A, but the cell was hyperpolarised to –55 mV before this recording commenced. Inward rectification at even more hyperpolarised levels is evident from the shape of the hyperpolarising potential deflections induced by the injected current pulses. Exposure to proteose peptone depolarised the cell to the same extent as at the shallower membrane potential, but the peak amplitudes of the current-induced potential deflections and, hence, the membrane resistance were unchanged during the depolarising response. In all of the seven cells tested in this manner,

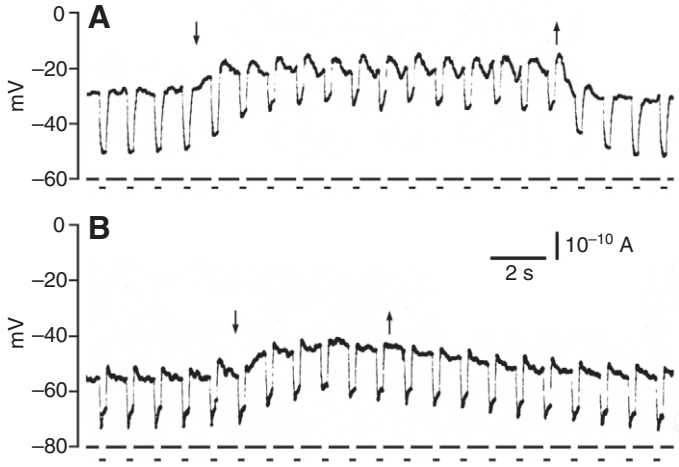


Fig. 7. Membrane resistance of microstome *Tetrahymena vorax* cells stimulated by proteose peptone. The arrows indicate the period of ejection of registration solution with 1% proteose peptone onto the recorded cell, and the upper traces indicate the membrane potential. The lower trace in each panel reflects hyperpolarising current pulses injected during the recording in order to monitor the membrane resistance. A microsuction pipette kept the cell stationary during the recording. (A) Recording from a cell without DC current injection. The membrane resistance of the cell was markedly reduced during the depolarisation induced by proteose peptone. (B) The same cell was hyperpolarised by continuous DC current injection to approximately –55 mV from a resting potential of –30 mV. The membrane resistance of the cell did not change during the depolarisation induced by proteose peptone.

the deviations of the membrane resistance during stimulation was less than 5% of the pre-stimulatory resistance, showing that the depolarisation induced by proteose peptone cannot be explained by a change in the ion permeability of the membrane.

### Elevated concentration of NaCl depolarises the membrane

The data presented above suggest that exposure to proteose peptone might have altered the local ionic environment of the cell. Therefore, the content of inorganic cations in proteose peptone was examined by atomic absorption spectroscopy. The analysis revealed that a 1% solution of this complex meat hydrolysate contains 21 mmol $l^{-1}$ $Na^+$, 2.9 mmol $l^{-1}$ $K^+$ and 1.4 mmol $l^{-1}$ $Ca^{2+}$.

The concentration of $Na^+$ in the applied proteose peptone solution was thus rather high and indicated that the elevated $Na^+$ concentration, rather than the organic components in proteose peptone, might be responsible for the electrophysiological response. Cells were therefore exposed to a test solution consisting of registration solution in which the $Na^+$ concentration was increased to 21 mmol $l^{-1}$ by adding NaCl. Fig. 8 presents a representative recording from one of the eight cells stimulated by this solution, which was completely devoid of added organic components. The electrophysiological response to the exposure was a depolarisation with a mean value of 11.6±2.2 mV ($N$=8), which is not significantly different from the response elicited by 1% proteose peptone in registration solution. The membrane remained depolarised during prolonged exposure to 21 mmol $l^{-1}$ NaCl. These results indicate that the observed depolarisation caused by 1% proteose peptone was simply due to increased extracellular $Na^+$ concentration and not to effects of organic molecules on ion channels in the membrane.

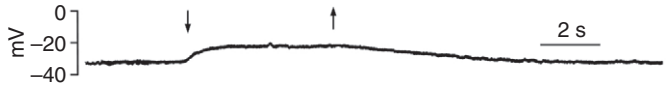


Fig. 8. Membrane response of a microstome *Tetrahymena vorax* cell stimulated by an elevated concentration of $Na^+$. The arrows indicate the period of ejection of registration solution with 21 mmol $l^{-1}$ $Na^+$ onto the recorded cell, and the trace presents the membrane potential. A microsuction pipette kept the cell stationary during the recording. The cell had a resting membrane potential of –33 mV, and the stimulation depolarised the cell by 10 mV.

### $Na^+$-enriched solution is not a chemo-attractant

Contrary to the depolarising proteose peptone solution, the $Na^+$-enriched registration solution was not an attractant for *T. vorax*. In three parallel behavioural experiments testing possible chemo-accumulation as previously described, cells exposed to the modified registration solution containing 21 mmol $l^{-1}$ $Na^+$ did not show any sign of accumulation in the observation area. According to previous studies, compounds inducing depolarisation in *Tetrahymena* are expected to be repellents (Kuruvilla and Hennessey, 1998). However, further investigation is needed to determine whether the response to elevated $Na^+$ concentration is neutral or leads to repulsion.

Collectively, the presented electrophysiological and behavioural data indicate that the chemokinetic behaviour in the microstome form of *T. vorax* is not determined by the membrane potential alone.

## DISCUSSION

In agreement with earlier studies on *Tetrahymena* (Levandowsky et al., 1984; Hellung-Larsen et al., 1986; Leick and Hellung-Larsen, 1992; Koppelhus et al., 1994), we have shown that the microstome form of *T. vorax* is highly attracted to L-cysteine and the complex meat hydrolysate proteose peptone. The swimming behaviour of aggregated cells was studied in the 14th minute after exposure to the attractants and may reveal how cells continue to be aggregated in an attractive environment. For both L-cysteine and proteose peptone, we observed a significant increase in the turning frequency whereas the swimming speed between turns was the same as in control cells (Fig. 3). However, our behavioural assay, which records aggregation close to a point source of the attractant, measures only the result of positive chemokinetic behaviour and is unsuitable for revealing mechanisms that cause chemokinetic approach towards an attractant. Furthermore, aggregation *per se* also increased the turning frequency, although less than in chemo-attracted ciliates at the same cell density, which indicates that the extent to which the observed behaviour of the chemo-accumulated cells reflects mechanisms that keep the cells aggregated is also uncertain.

Nonetheless, our assay is a simple and efficient method for revealing if a compound is an attractant or not, and to quantify its chemo-attractive potency. The assay is thus well suited for pursuing the main agenda of the present project, i.e. to elucidate if there is an obligatory relationship between changes in membrane potential and chemo-accumulation in the microstome form of *T. vorax*.

The observed stability of swimming speed is in accordance with previous reports on chemo-attractive responses in *T. vorax* stimulated by lysine and glycine (Brown and Kerkut, 1981), and in *Tetrahymena pyriformis* stimulated by N-methyl-D-aspartate (NMDA) (Nam et al., 2009). However, it has been reported that L-cysteine elicits an initial increase in swimming speed in *Tetrahymena thermophila* but the swimming speed returned to the control level

during sustained exposure (Levandowsky et al., 1984). In *T. pyriformis*, the swimming speed was higher in cells stimulated by proteose peptone compared to unstimulated cells even after 12 min exposure (Hellung-Larsen et al., 1986).

Evidently, unchanged swimming speed between turns is not a consistent feature of chemo-attractive responses within the *Tetrahymena* genus. A similar inconsistency seems to be the case regarding turning frequency, as previous studies on *T. thermophila* and *T. pyriformis*, in contrast to our data, have shown that chemo-attraction is accompanied by fewer turns (Levandowsky et al., 1984; Leick and Hellung-Larsen, 1985; Hellung-Larsen et al., 1986). In *Paramecium*, the behavioural response to attractants is characterised by an immediate decrease in the frequency of turns and increased swimming speed. Removal of attractive stimuli usually results in increased turning frequency and decreased swimming speed in *Paramecium* (Van Houten and Van Houten, 1982). Evidently, ciliates employ a variety of behavioural strategies to achieve a positive chemokinetic response.

The direction and frequency of the ciliary beat are partly governed by the membrane potential in *Paramecium* (Eckert, 1972; Eckert and Naitoh, 1972; Machemer, 1974; Naitoh, 1974) and *Tetrahymena* (Onimaru et al., 1980). Membrane hyperpolarisation leads to increased frequency of the ciliary beat and causes a faster forward swimming whereas strong membrane depolarisation results in reversal of the ciliary beat and swimming direction (Machemer, 1974). In *Tetrahymena*, chemo-repellents have been reported to produce membrane depolarisation (Kuruvilla and Hennessey, 1998; Kim et al., 1999), whereas no electrophysiological studies on the effects of chemo-attractants have previously been performed. Based on the available data on chemosensory transduction in *Paramecium* (Van Houten, 1979; Preston and Usherwood, 1988; Hennessey et al., 1995; Kim et al., 1997; Van Houten et al., 2000; Bell et al., 2007), the current hypothesis for the relationship between membrane potential and positive chemokinesis in *Tetrahymena* is that most chemo-attractants, such as amino acids, cause moderate hyperpolarisation of the membrane, faster forward swimming and decreased turning frequency (Hennessey and Kuruvilla, 1999). To our surprise, microstome *T. vorax* cells displayed no observable change in membrane potential in response to L-cysteine (Fig. 4).

In Van Houten's model for the correlation between membrane potential and chemokinetic behaviour in *Paramecium* (Van Houten, 1979), compounds inducing very strong depolarisation may also cause chemo-attraction by increasing the turning frequency and decreasing the swimming speed (Van Houten, 1979). Stimulation by 1% proteose peptone depolarised the *T. vorax* membrane by ~12 mV (Fig. 5) and the membrane remained depolarised during prolonged exposure to proteose peptone for at least 10 min (Fig. 6). However, this depolarisation is moderate according to Van Houten's model, which predicts chemo-repulsion in such cases (Van Houten, 1979). Later studies on *Tetrahymena* have also shown that various compounds inducing depolarisations of a similar magnitude are repellents (Kuruvilla and Hennessey, 1998; Kim et al., 1999).

Atomic absorption spectroscopy revealed that a 1% solution of proteose peptone contains 21 mmol $l^{-1}$ $Na^+$, and our electrophysiological tests showed that the high $Na^+$ content of proteose peptone is probably responsible for most or all of its depolarising effect. A test solution that contained 21 mmol $l^{-1}$ NaCl and no organic compounds also depolarised the *T. vorax* membrane by ~12 mV (Fig. 8) but did not induce aggregation of cells. It is well known that *Paramecium* is depolarised by elevation of the extracellular concentrations of $Na^+$ and $K^+$ (Naitoh, 1982; Oami and Takahashi, 2004). We have not explored whether a test solution with 21 mmol $l^{-1}$ NaCl and no organic compounds may lead to cell dispersal. However, aggregating cells stimulated by 1% proteose peptone stayed aggregated and continued to turn approximately every second with a constant speed between turns. A possible chemo-repulsive response to elevated $Na^+$ concentration was thus completely overshadowed by the chemo-attractive components in proteose peptone. This observation is in agreement with a previous study on *Paramecium tetraurelia*, which showed continued chemo-attraction to acetate in a depolarising solution containing 30 mmol $l^{-1}$ KCl (Preston and Hammond, 1998).

Change in ciliary direction is dependent on the concentration of $Ca^{2+}$ in both *Paramecium* (Naitoh and Kaneko, 1972) and *Tetrahymena* (Goodenough, 1983; Grønlien et al., 2001). The attractant NMDA increased the cytosolic $Ca^{2+}$ level ($[Ca^{2+}]_i$) in *T. pyriformis* (Nam et al., 2009) and it is probable that the intracellular transduction mechanism for L-cysteine and proteose peptone involves $Ca^{2+}$. Increased $[Ca^{2+}]_i$ can be due to $Ca^{2+}$ influx through ion channels or release from intracellular stores. $Ca^{2+}$-dependent mechanisms may also be modulated by altering the $Ca^{2+}$ sensitivity of the system without changing the $Ca^{2+}$ concentration. L-Cysteine did not depolarise the membrane, and a possibly elevated $[Ca^{2+}]_i$ can thus not be due to $Ca^{2+}$ influx through voltage-gated ion channels. Furthermore, neither L-cysteine nor the attracting components in proteose peptone altered membrane ion conductances, although the attractants indeed influenced ciliary motility. We therefore suggest that changes in membrane ion conductances are not necessary for changes in swimming behaviour, implying a more complex signal transduction pathway. This topic should be explored in future studies.

We conclude that, in the microstome form of *T. vorax*, there is no unambiguous correlation between changes in membrane potential and the kinetic responses leading to chemo-accumulation. The membrane potential model for chemokinesis in *Paramecium* (Van Houten, 1979) may thus not have general validity for ciliates.

## ACKNOWLEDGEMENTS

We thank the two anonymous reviewers for their numerous suggestions for improvements of the manuscript. This work was supported by The Research Council of Norway (RCN) grant 15927.

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