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Electric-field effects on gravikinesis in *Paramecium*

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Abstract Equilibrated *Paramecium caudatum* cells exposed to a constant DC gradient reorient with their depolarized anterior ends toward the cathode (galvanotaxis). Voltage gradients were applied to cells swimming either horizontally or vertically. Their velocity and orientation were recorded and compared to unstimulated cells. The DC field increased the horizontal velocity (= reference) up to 175% (galvanokinesis). Swimming velocities saturated after 1 min and were unchanged during the following 4 min. The upward and downward swimming velocities of stimulated cells were below those of horizontal swimmers. The difference in vertical rates (determining gravikinesis) was independent of variations in absolute velocity. Normalization of vertical velocities to horizontal velocities (= 100%) separated DC-field dependent changes from gravity-induced changes in velocities. A weak voltage gradient (0.3 V/cm) was most effective in raising downward gravikinesis up to threefold (−202 μm/s) above the unstimulated reference (−66 μm/s) and to change sign of gravikinesis in upward swimmers (−43 μm/s → + 33 μm/s). We conclude that DC-field stimulation is equivalent to a depolarizing bias on gravikinetic responses of *Paramecium*. The stimulation does not directly interfere with mechanoreception, but modulates somatic Ca^{2+} entry to induce contraction of the cell soma. This presumably affects the gating of gravisensory transduction channels.

Key words Mechanoreception · Gravitransduction · Gravikinesis · DC-field stimulation · *Paramecium*

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Introduction

Locomotion in ciliates is modulated by gravity, which influences the orientation (Verworn 1889) and swimming rate of cells (Machemer et al. 1991, 1993; Ooya et al. 1992; Bräucker et al. 1994). A physiological signaling pathway for gravitaxis is difficult to separate from physical mechanisms of orientation (see Machemer and Bräucker 1992). Gravikinesis, on the other hand, results from changes in ciliary activity, which is under electrophysiological control (Machemer 1986). The present evidence suggests that gravitransduction and the resulting behaviour of *Paramecium* occurs along classical sensorimotor coupling pathways.

The elucidation of gravity-induced responses of single cells is hampered by many problems: (i) Gravity is difficult to manipulate. (ii) The energy of the stimulus generated by terrestrial gravity is small. It depends on the difference in density between a functional "statolith" and the surrounding medium, and on statolith size (Volkmann and Sievers 1992; Machemer 1995). (iii) Individual sedimentation and gravikinetic behaviour of motile cells is subtle, variable, and not easy to document necessitating large numbers of cells to be measured. (iv) Little is known about the signaling pathways between a defined stimulus input and documented gravisensory responses.

Cellular gravisensory transduction poses interesting questions especially with regard to the effective gravitational force. In ciliates, this force (in the order of 10^{-10} N) is likely to interfere with the mechanosensory organization of the somatic cell membrane, which has been extensively studied (see Machemer and Deitmer 1985). A Ca^{2+}-dependent, depolarizing mechanoreceptor conductance prevails at the anterior cell ends, and a K^+-dependent, hyperpolarizing mechanoreceptor conductance prevails at the posterior ends of *Paramecium* and *Stylonychia* (De Peyer and Machemer 1978b; Ogura and Machemer 1980). This bipolarity of

mechanoreception expressed by the organization of the soma membrane leads to two antagonizing and overlapping gradients of sensitivity, the biological function of which was enigmatic so far. A previously published hypothesis relates the topography of mechanosensory organization of *Paramecium* to gravisensory transduction. It predicts a novel type of vector-guided gravikinesis: reduction in downward swimming rates, and increase in upward swimming rates. The hypothesis has been experimentally confirmed (Machemer et al. 1991) including data obtained under weightless conditions (Machemer et al. 1993a) and hypergravity (Bräucker et al. 1994).

Presumably, the graviresponses in ciliates have an electrophysiological basis but direct evidence of the physiological events of transduction is so far missing. The present study uses persistent DC-field stimulation to investigate gravisensory transduction. DC-field stimulation of free swimming cells is equivalent to extracellular voltage clamp. A DC gradient induces *Paramecium* to orient parallel to the field lines with the anterior end pointing to the cathode (see Machemer 1988b). Our data reveal a high sensitivity of gravikinesis to shifts of the steady-state membrane potential and suggest that the membrane potential modulates gravikinesis via a Ca^{2+}-dependent tonus of contraction in the cell cortex.

Parts of this work were presented in preliminary form at the XI. CEBAS Workshop held in September 1995 at Bochum, Germany.

Materials and methods

Cells and solutions

Cultures of *Paramecium caudatum*, line G3, were reared in cerophyl solution (Cerophyl Laboratories, Inc., Kansas City, USA), 0.2% w/v cerophyl powder in bidistilled water, autoclaved, buffered to pH 7.2 by modified Sörensen buffer (sodium salts only), cultured at 22°C in 14 h-10 h light-dark regimen, bacterized with *Enterobacter aerogenes*, and harvested after 3 days in the early stationary phase. The cells were adapted to oxygen at 50 to 60% air saturation in culturing solution at least 16 h in advance of the experiments. They were then collected in experimental solution (in m*M*: $CaCl_2$ 1, KOH 1, $MgSO_4$ 0.1, MOPS 1.5 pH 7.2) using negative gravitactic accumulation, and were transferred to the experimental chamber. Here, the cells were equilibrated for 4 more hours.

Experimental chamber

The acrylic chamber (outer dimensions: 65 × 110 × 15 mm) enclosed a central fluid space of 26 mm × 40 mm × 1.6 mm, equivalent to 1.7 ml, and two peripheral sections filled with 1.5% agar in experimental solution surrounding the platin electrodes for buffering reasons (see Fig. 6 in Machemer et al. 1991). The electrodes ran parallel to each other at a distance of 75 mm. A central field of the chamber (16 × 21 mm) was used for recording cells. A ring of 48 light-emitting diodes generated an 800 lx dark-field illumination of the cells. The emission wavelength (565 nm; halfwidth 28 nm) is near the minimum spectral sensitivity for photoresponses in *Paramecium caudatum* (Iwatsuki and Naitoh 1982).

Voltage gradients and galvanotaxis

Linear DC voltage gradients ranging between 0.2 and 0.9 V/cm were applied during part of the experiments. A gradient was established using a high-voltage constant current source. The current was calibrated so as to generate a predetermined voltage gradient across the resistance of the medium. The resistance of the experimental solution inside a chamber (without cells) was routinely measured before starting, and persistence of the voltage gradient was monitored during the experiment. Many free swimming ciliates including *Paramecium* align parallel to the field lines of a DC voltage gradient, while they actively swim toward the cathode. For a *Paramecium* 250 µm long with an electrically homogeneous membrane, a voltage gradient of 0.2 V/cm corresponds to a maximal cathodal depolarization of 2.5 mV at the anterior cell end and an anodal hyperpolarization of the same size at the posterior cell end. A gradient of 0.9 V/cm elevates local depolarizations and hyperpolarizations to maximally 11 mV. Thus, the applied electric stimuli were within the range of physiological stimulation. A gradient of 0.2 V/cm did not generate sufficient vertical orientation. The behaviour of the cells once exposed to a persistent gradient of 0.9 V/cm did not fully return to normal. Therefore, we restricted evaluations of the data to gradients from 0.3 to 0.8 V/cm.

Experimental protocol

A total of 150 to 500 equilibrated cells was used for each experiment. Swimming paramecia were video taped using a time scheme as shown in Fig. 1. In the absence of a DC field and with the chamber kept in horizontal position, cell motion was continuously recorded for 3 min. After a dark interval of 3 min the voltage gradient was switched on for 5 min. Galvanotactic swimming was recorded, while the polarity of the gradient was reversed every 30 s. Upon reversal of the gradient, *Paramecium* immediately switched the swimming direction performing a U-turn (that is, away from the new anode and heading toward the new cathode). Relaxation to normal behaviour was recorded up to 32 min after turning off the gradient. The chamber was then slowly reoriented to the vertical position. After a 3-min dark period, the DC field was turned on again for 5 min inducing the cells to perform vertical galvanotaxis (Fig. 1A). Changing the polarity of the voltage gradient every 30 s caused *Paramecium* to switch between upward and downward swimming. After turning off the voltage gradient, 2 min of relaxation of *Paramecium* were recorded, while the chamber was kept in the vertical position.

The following control experiments were added to the standard protocol: (i) To test the time dependence of galvanotactic swimming, the experimental scheme was run without changing the horizontal position of the chamber (Fig. 1B). (ii) For determinations of swimming rates and gravikinesis of unstimulated cells (Figs 9, 10, open symbols), we alternated 4 times in sequence horizontal and vertical swimming periods of 2 min and inserted a 2-min dark interval between each recording period. For all experiments and controls, the cells were illuminated during periods of recording only. A particular cell sample was exposed to no more than a single voltage gradient. The sequence of applied gradient strength was chosen stochastically to minimize a possible bias arising from time-dependent fluctuations in culture conditions.

Recording and evaluation

Cell movements were recorded using a CCD-videocamera (Panasonic F10, 25Hz framing rate) equipped with a macro-lens.

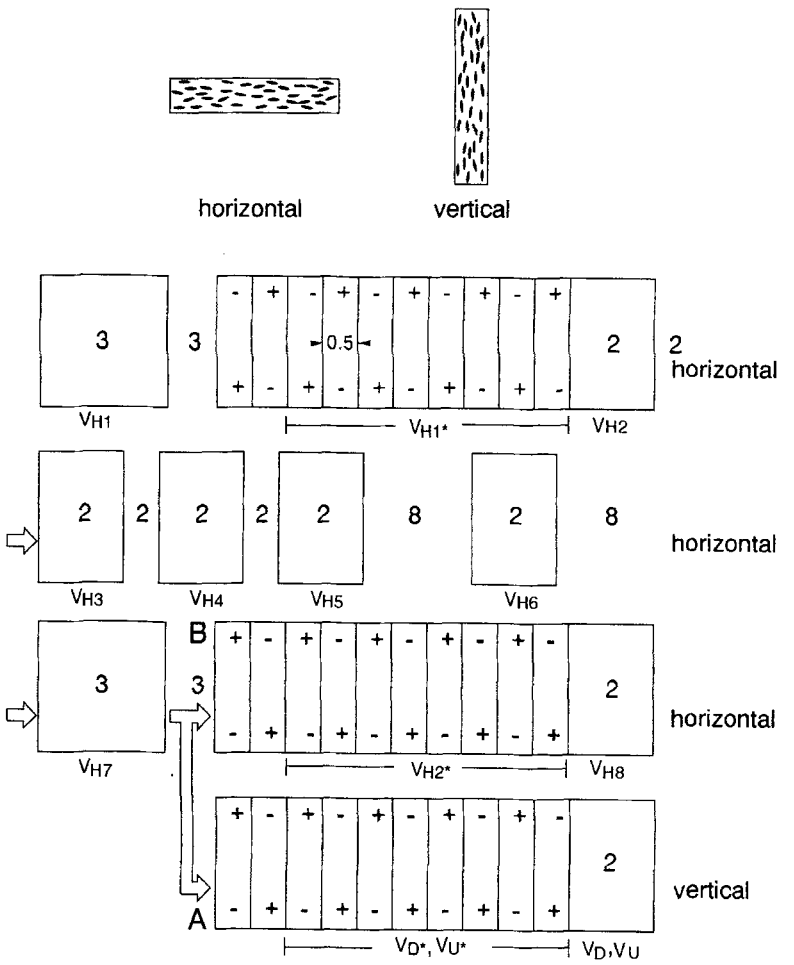


Fig. 1A, B Experimental protocol. The sequence of boxes indicates treatment (±, voltage gradient) and times during and between events (*arrows*; *numbers* = min). Positions of the chamber (illustrated *on top*) are given to the *right*. *Indices below boxes* refer to sequential measurements of swimming velocity (V_H horizontal, V_D downward, V_U upward) with voltage gradient applied (*) or without electric stimulation. Note that the first and second galvanotactic runs were not used for the evaluations. **A** Regular sequence. **B** Alternative horizontal control to check for effects of the DC field on the experimental cells (see Fig. 3)

Camera, chamber, and illumination were mounted on a 90°-tilting device allowing reorientations of the recording unit from horizontal to vertical and vice versa (Machemer et al. 1991). Tilting with a minimum of mechanical disturbance (angular rate approximately 1.5° s) was done by hand using a lever.

From each 3-min recording period 6 time windows of 2.5 s duration were used for evaluations (4 windows from 2-min periods). These records were separated by 20 s-intervals allowing other specimens to enter the video field. Swimming tracks of *Paramecium* were generated from superposition of field sequences (Machemer and Bräucker 1992). Judging from field size and swimming velocity of *Paramecium*, an individual cell was tracked 1.3 times on average (Bräucker et al. 1994). A colour sequence of the track documented the time sequence and direction of locomotion. Tracks of steady locomotion were identified visually. Rare events of unsteady locomotion, such as from ciliary reversals, were disregarded. Velocity and orientation data were calculated and stored automatically.

The sedimentation rate of *Paramecium caudatum* (110 μm/s), was lately determined in our laboratory using $NiCl_2$-immobilized cells (n = 1226) from the same culture line and employing identical culturing methods (Watzke 1995).

Data treatment and statistics

For calculations of swimming velocities and components of gravikinesis, we used tracks inside sectors ± 15° of vertical field lines (see Fig. 3). The velocity class distributions show that a sector of ± 15° is characteristic of a majority of cells even when applying weak voltage gradients. Unstimulated cells, after several hours of adaptation to the experimental conditions, emphasized low velocities compared with stimulated cells (Fig. 2) in agreement with previous data (Machemer et al. 1993b). The left-hand shift of the peak of unstimulated cells was fully reproducible in all experimental groups before and after electric stimulation (Fig. 2). The swimming rates of stimulated cells, on the other hand, were largely Gaussian distributed. The difference in distribution characteristics confirms the need for nonparametric statistics (Sachs 1984) for data comparison. Our data are represented by medians (confidence intervals: 95%). We used the U-test (Mann-Whitney) with error probability of ≤ 5% to test for significance of differences of the median in swimming rates.

Results

Effects of voltage gradients on horizontal and vertical swimming rates

An externally applied voltage gradient changes the swimming rates of ciliated cells in a complex manner because the ciliary motor responses are locally modulated. Depolarization-induced ciliary activity (DCA) near the cathodal (= anterior) cell surface gives

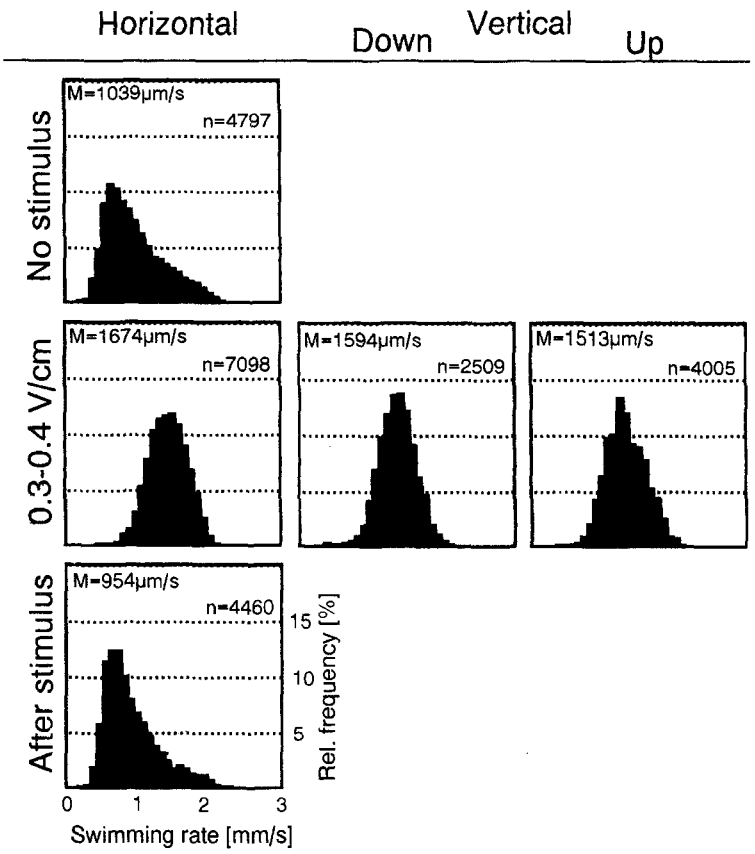


Fig. 2 Comparison of velocity class distributions in weak gradients (see Fig. 1, V_{H1*}, V_{D*}, V_{U*}, before and 32 min after the end of electric stimulation (V_{H1}, V_{H7}). In the *column* of horizontal swimmers, long-term adapted, unstimulated cells showed a reproducible left-hand shift of the distribution peak. Distributions of stimulated cells are approximately Gaussian irrespective of chamber position. Sectors of $\pm$ 15° were applied to stimulated cells, all directions to unstimulated cells

gradually way to hyperpolarization-induced ciliary activity (HCA) near the anodal (= posterior) surface (Machemer 1988b). While weak depolarization induces the cilia to decrease their normal beating rate (or, with raised stimulus strength, increase the frequency of reversed beating), hyperpolarization augments the normal beating rate (see Machemer 1986). Cells head toward the cathode (galvanotaxis) because normally beating cilia outnumber those beating in reverse (Machemer 1988b), and swimming along the field lines minimizes lateral imbalances in ciliary activity (Koehler 1926; Jahn 1961). For a given field strength, the resulting swimming rate is hard to predict: variations in cell size, membrane input resistance and state of adaptation add to antagonizing effects of depolarizing and hyperpolarizing stimulation on ciliary activity (Mogami et al. 1990).

In a *horizontal* chamber, unstimulated cells swim in arbitrary directions, and their median velocities do not show a directional bias (Fig. 3A,B; Machemer et al. 1991). A linear voltage gradient changes the orientation and the swimming velocity of *Paramecium*. Cells head toward the cathode, and the vast majority of specimens swim parallel to the field lines at enhanced velocity (Fig. 3C–F). Orientation improves with the strength of the electric field (Fig. 3E). Gradients between 0.3 and 0.8 V/cm raise the swimming velocity with respect to unstimulated horizontally swimming cells to a range between 130% and 175% with a maximum at 0.4 V/cm (Fig. 4). Galvanokinesis does not rise linearly with the strength of the electric field because both hyperpolarization-induced augmentation of normal ciliary activity and depolarization-induced ciliary depression or even reversal affect the rate of locomotion in a complex manner (Machemer 1988b). Weak voltage gradients (0.3 V/cm, 0.4 V/cm) and strong gradients (0.7 V/cm, 0.8 V/cm) evoked similar values of galvanokinesis.

In the *vertically* oriented chamber, effects of gravity and of the DC field superimpose. Figure 5 (A, C) shows a more precise orientation of upward swimmers as compared to downward swimmers (negative gravitaxis). The downward velocity of stimulated cells (V_{D*}) exceeds their upward velocity (V_{U*}). Increases in field strength have no obvious effects on the vertical swimming velocity (Fig. 5B, D). Comparison with stimulated horizontal swimmers (Fig. 3D, F) shows that upward swimming rates are reduced, whereas the downward swimming rates are not increased under DC-field stimulation.

Gravikinesis affects the difference between observed downward and upward swimming velocities ($V_D - V_U$) (Machemer et al. 1991). How can gravikinesis, under the applied depolarizing or hyperpolarizing stimulation, be isolated from responses unrelated to gravity? Complications arise because the value of $V_D - V_U$ might vary with duration of the stimulus and as a

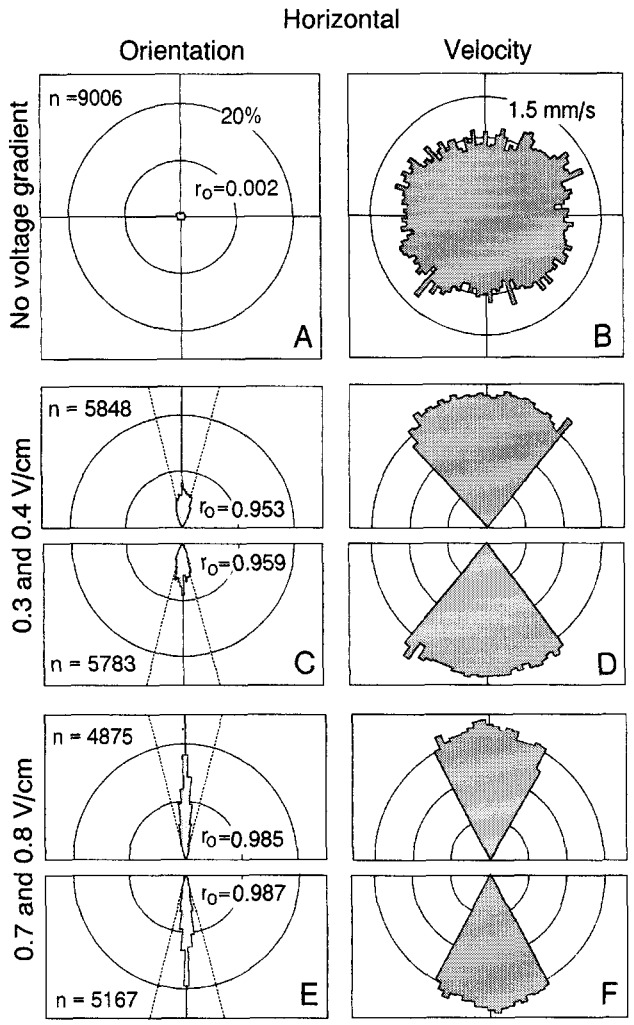


Fig. 3A–F Polar histograms of orientation (*open*) and swimming velocity (*filled*) of horizontally swimming *Paramecium* (3°-sectors of velocity including ≥ 20 data shown only). Same calibrations for comparison reasons. **A, B** No electric stimulation. Absence of bias in orientation (orientation coefficient, $r_0 = 0.002$) and velocity. **C–F** DC-field stimulation (**C, D**: medians of 0.3 and 0.4 V/cm; **E, F**: medians of 0.7 and 0.8 V/cm). Cathodal galvanotaxis is shown with polarity change (very few cells occurred in anodal directions). **C** The cells swim precisely toward the cathode (compare r_0-values). **E** Orientation even more improves with rising field strength. For DC-field stimulation, a sector of $\pm 15°$ from the field lines included the majority of cells. **B, D, F** The swimming velocity rose substantially during DC-field stimulation (galvanokinesis)

function of swimming velocity. In the following, we first consider these effects of electric stimulation.

Time-dependence of the galvanokinetic response

Cells swimming in arbitrary directions take a few seconds for galvanotactic reorientation. The time required for reorientation depends on the strength of the DC gradient and individual sensitivity. In addition to this alignment to the electric field, the membrane adapts to the persistent stimulus potential (see Machemer 1989). Only when these responses are fully developed, the swimming velocity saturates. Figure 6 shows that after the onset of the stimulus, the swimming velocities tend to rise during the first and second 30 s periods of registration. We restricted further evaluations to data obtained beyond 1 min of registration time (connected data points in Fig. 6).

Normalizing the galvanokinetic responses

Velocities of horizontally swimming *Paramecium* are unaffected by the vector of gravity (Machemer et al. 1993a) and are therefore suited to identify velocity responses unrelated to gravity. The velocities of electrically stimulated cells swimming horizontally (V_{H*}) crowd near a value of 1700 μm/s (Fig. 7). The responses of stimulated cells may be normalized to this velocity using linear regressions of the slopes of downward over horizontal rates (V_{D*}/V_{H*}) and upward over horizontal rates (V_{U*}/V_{H*}). Such treatment is even more justified, if the difference in vertical rates ($V_{D*} - V_{U*}$), which is relevant for gravikinesis, can be shown to be largely independent of velocity. The vertical velocity difference is indeed essentially unchanged over all swimming rates investigated under DC-field stimulation (Fig. 8). This is in agreement with conclusions derived from unstimulated cells with swimming rates ranging between 500 and 2100 μm/s (Machemer and Machemer-Röhnisch 1996). For further analysis, we use the values of V_{D*} and V_{U*} as normalized for a $V_{H1*} = 1700$ μm/s.

Figure 9 represents the normalized vertical velocities plotted as functions of the applied voltage gradient ($V_{H1*} = 100\%$). It is seen, in confirmation of Fig. 7, that both the downward and upward velocities are below the horizontal velocity at all gradients applied and even after accounting for the sedimentation rate. V_{D*} rises and V_{U*} decreases with increasing field strength in a linear fashion.

Gravikinesis as a function of applied external voltage gradient

Gravikinesis under DC-field stimulation ($\Delta*$) is determined from the general relationship (Machemer et al. 1991),

$$\Delta = (V_D - V_U)/2 - S \quad (1)$$

by calculating the values of V_{D*} and V_{U*} (Fig. 9) for the representative horizontal velocity of 1700 μm/s and using a median sedimentation rate (S) of 110 μm/s (Watzke 1995). Figure 10 shows the gravikinesis data as a function of the applied voltage gradients. The value of $\Delta*$ is -84 μm/s at a voltage gradient of 0.3 V/cm. It shifts toward less negative values with rising field strength. At a voltage gradient of 0.8 V/cm, the value of

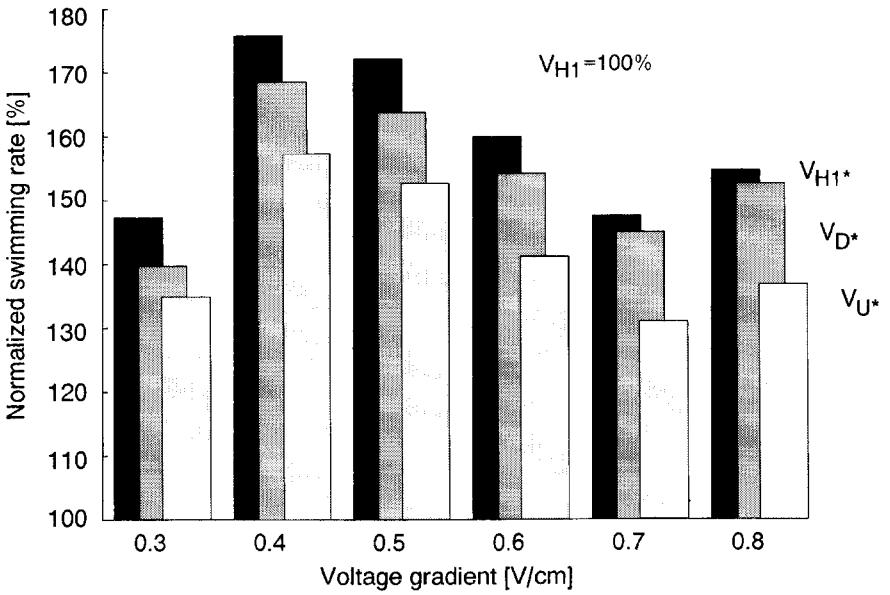


Fig. 4 Effects of voltage gradient on swimming rates. Median rates (from ± 15° of field lines) are calibrated in percent of horizontal rates of unstimulated cells (V_{H1}) to illustrate nonlinearity of the galvanokinesis. The graph indicates a maximum swimming rate (175% of V_{H1}) at a voltage gradient of 0.4 V/cm. Each histogram bar is based on data numbers ranging between 1145 and 4874 (confidence ranges of medians < 1%)

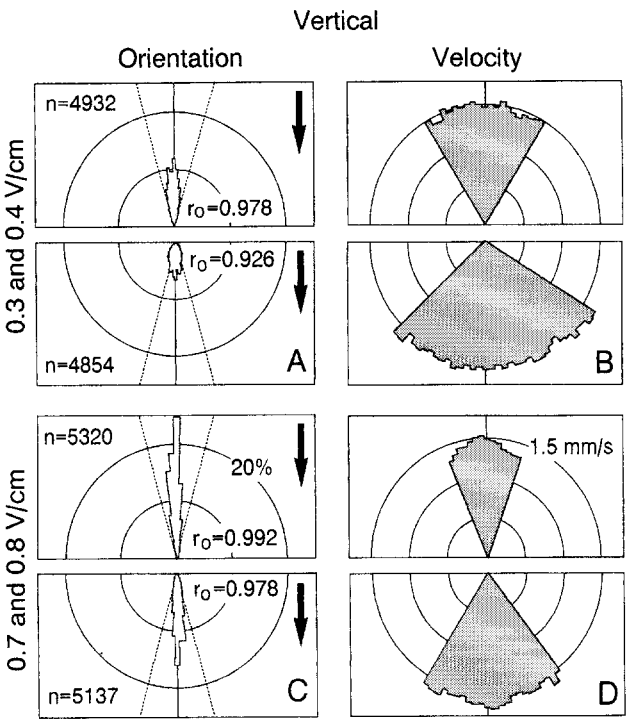


Fig 5A–D Orientation and velocity of vertically swimming *Paramecium* during DC-field stimulation (**A**, **B**: 0.3 and 0.4 V/cm; **C**, **D**: 0.7 and 0.8 V/cm). **A**, **C** Orientation improves with DC-field strength. Gravitaxis is represented in the more precise upward and less precise downward orientations (compare r_0-values with those of horizontal cells, Fig. 3C, E). **B**, **D** Both upward and downward velocities are depressed as compared to horizontal swimmers (Fig. 3D, F)

Δ^* is − 12 μm/s. The negative sign of Δ^* indicates that the velocity vector of gravikinesis is antiparallel to the vector of sedimentation ("negative gravikinesis", Machemer et al. 1991). Within the range of tested voltage stimuli, conditioned gravikinesis is a linear function of the applied field strength.

Generalized gravikinesis, Δ, is characteristic for a cell population, whereas the individual cells alternate between upward and downward swimming by chance. The rate of gravikinesis may differ in downward and upward swimming cells. The downward gravikinesis (Δ_D) results from vector summing of the rates of propulsion (P) being unrelated to gravity, sedimentation (S), and the observed downward velocity (V_D):

$$\Delta_D = V_D - P - S . \quad (2)$$

The upward gravikinesis (Δ_U) results accordingly:

$$\Delta_U = P - V_U - S . \quad (3)$$

In equations (2) and (3) a positive sign indicates a downward vector, and a negative sign indicates an upward vector. The propulsion rate of *Paramecium* closely approximates the horizontal swimming rate according to experiments done under microgravity (Machemer et al. 1993a). Therefore, the horizontal velocity (V_{H*}) can represent P. The signs of Δ_{D*} and Δ_{U*} result from summing the given parameters. Figure 10 shows that DC-field stimulation shifts Δ_{D*} to highly negative values (0.3 V/cm: − 202 μm/s), as compared to electrically unstimulated cells (− 66 μm/s, open symbol), thereby even overcompensating the sedimentation rate (+ 110 μm/s). This explains why the active downward swimming velocity ($V_{D*} - S$) is far below the horizontal velocity in the presence of voltage gradients (Fig. 9). An inverse biasing effect of electric stimulation is seen in upward swimming cells (Fig. 10): their gravikinesis (0.3 V/cm: Δ_{U*} = + 33 μm/s) shifts in the positive direction compared to unstimulated cells (− 41 μm/s, open symbol), and changes sign. For an upward swimming cell, a positive gravikinesis is equivalent to even more depression of the observed upward

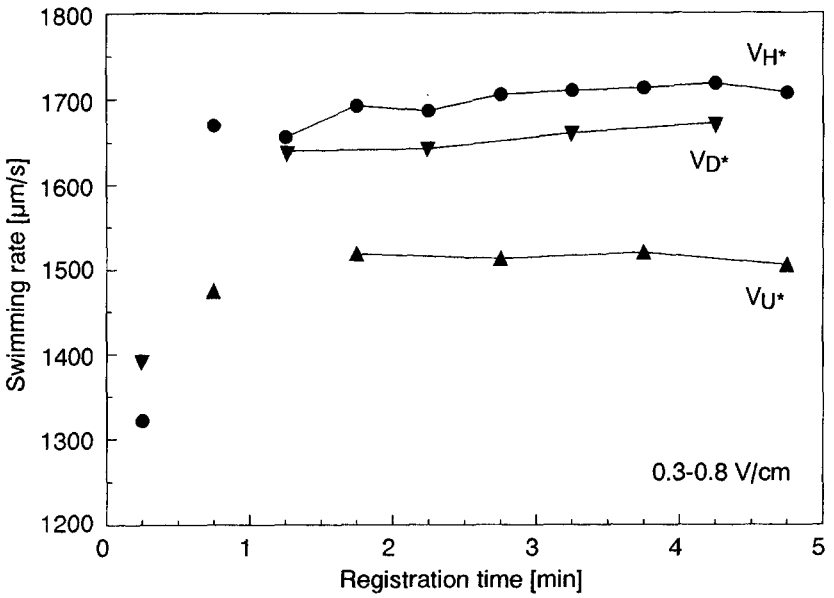


Fig. 6 Time dependence of swimming velocity of *Paramecium* during 5 min following the onset of field stimulation. *Data points* are medians from all voltage gradients (0.3 to 0.8 V/cm). Tracks were registered during 30 s periods in horizontal (V_{H*}) and vertical position of the chamber (V_{D*}, V_{U*}). Note that (i) switching of electrode polarity gave twice the number of data points during horizontal swimming as compared to upward and downward swimming, (ii) the galvanokinetic swimming rate is a saturating function of time, and (iii) transition from unstimulated to steady-state stimulated swimming lasted up to 1 min so that the first minute (*isolated data points*) is unsuited for calculation of the medians of V_{H*}, V_{D*}, and V_{U*}

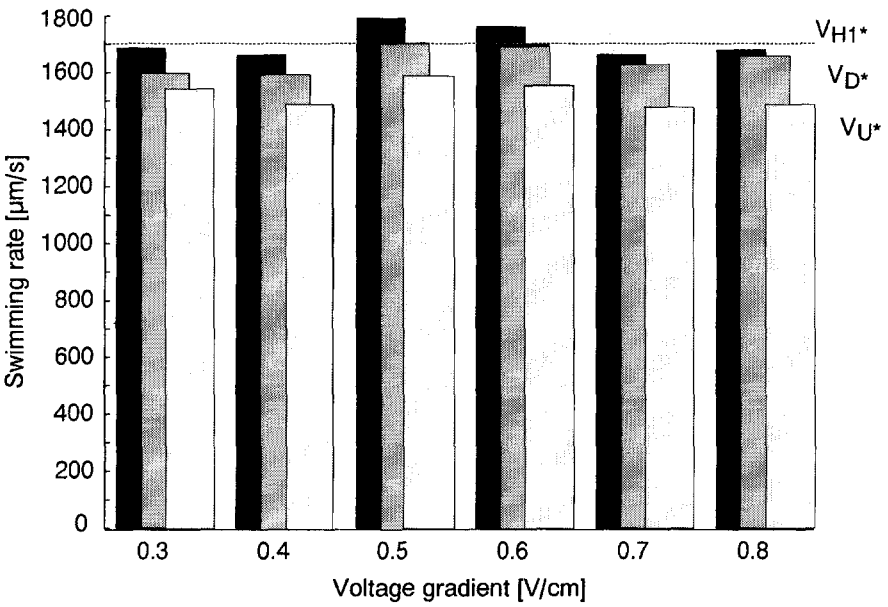


Fig. 7 Steady-state horizontal (V_{H1*}) and vertical swimming velocities (V_{D*}, V_{U*}; Fig. 1) during electric-field stimulation. Medians of data from 2nd to 5th min of registration (Fig. 6) are plotted as a function of voltage gradients between 0.3 and 0.8 V/cm. Horizontal velocities cluster near a velocity of 1700 µm/s (*dashed line*)

swimming rate, which is already diminished by sedimentation. The reduction of upward propulsion (V_{U*} + S) seen in Fig. 9 is due to a positive gravikinesis. These effects of voltage gradients on oriented swimming are not seen in the generalized term of gravikinesis (Δ^*), which is the arithmetic mean of $\Delta_{D}*$ and Δ_{U*} (Machemer et al. 1991).

Discussion

The present study provides first evidences of a highly specific effect of the membrane potential on gravikinesis: depolarization potentiates the decrease in downward swimming rate which counteracts sedimentation (= negative gravikinesis). Hyperpolarization depresses or even reverses the common upward gravikinetic response, and increases the downward velocity by adding an active component to passive sedimentation. This conditioning effect of the membrane potential depends on the size of the applied voltage gradient in a peculiar manner: rising field strength decreases negative gravikinesis in downward swimmers, and increases positive gravikinesis in upward swimmers (Fig. 10). Principally similar results were previously obtained by applying an external voltage gradient of 0.6 V/cm to *Paramecium* (Machemer et al. 1991); here, the resulting gravikinesis of upward swimmers was small as

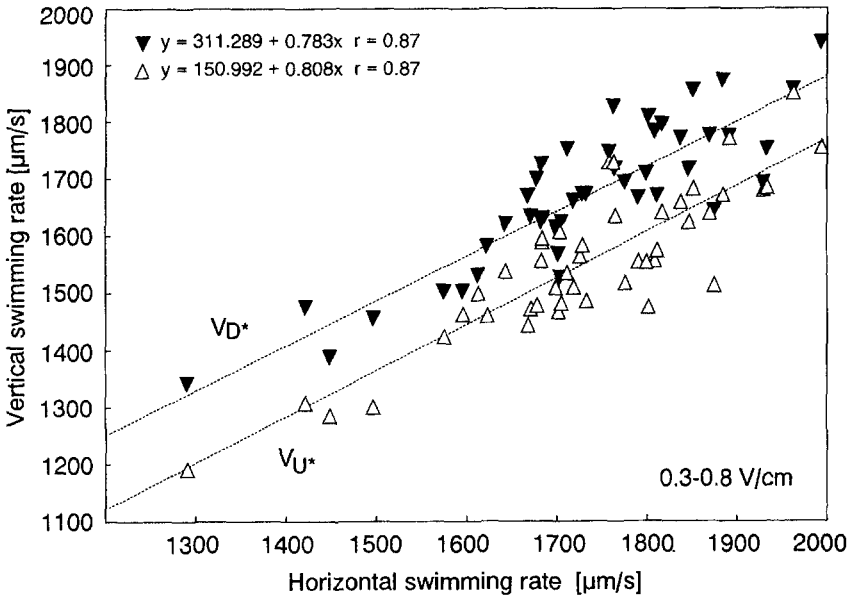


Fig. 8 Vertical velocities (V_{D*}, ▼; V_{U*}, △) correlated with corresponding horizontal velocity (V_{H*}) under electric-field stimulation (medians of data from 0.3 to 0.8 V/cm). *Open* versus *closed symbols* were chosen to enhance clarity. Data with confidence intervals $< \pm 10\%$ of medians were admitted. Slopes of the V_{D*}/V_{H*} and V_{U*}/V_{H*} relations indicate that the difference, V_{D*}-V_{U*}, is essentially unchanged with rising absolute velocity. See Table 1 for linear regressions calculated from data of single voltage gradients

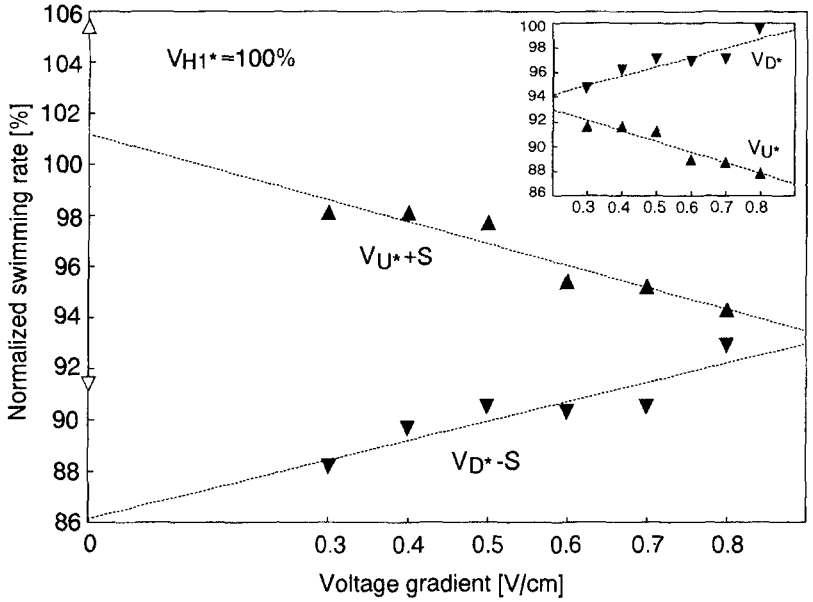


Fig. 9 The strength of the electric field affects the rates of downward swimming (V_{D*}) and upward swimming (V_{U*}). Observed vertical velocities have been corrected for sedimentation (S = 110 µm/s) and normalized (V_{H1*} = 1700 µm/s = 100%; see Fig. 7) using linear regressions of V_{H1*} data for each voltage gradient (Table 1). *Inset*: normalized vertical velocities only. In the main diagram, vertical rates in absence of voltage gradient are included for comparison (*open symbols*). It is seen that (i) a voltage gradient tended to depress both V_D and V_U (compare *closed* with *open symbols*; exception: V_{D*} at 0.8 V/cm), (ii) both V_{D*} and V_{U*} were depressed with respect to V_{H1*}, (iii) depression of downward propulsion ($V_{D*} - S$) exceeded that of upward propulsion ($V_{U*} + S$), and (iv) a rising voltage gradient increased V_{D*} and decreased V_{U*}. Linear regressions: $y = 101.2 - 8.57x$; $r = 0.95$ ($V_{U*} + S$); $y = 86.2 + 7.57x$; $r = 0.91$ ($V_{D*} - S$)

compared to gravikinesis of downward swimmers. Because these pronounced asymmetries of the gravikinetic responses are not seen using electrically unstimulated cells, an application of the galvanotactic method (Fig. 3) for the induction of precise orientation in *Paramecium* raises doubts. Here, we resume the analysis of electric-field effects on gravikinesis employing improved experimental and evaluation protocols. In the following, the physiological implications of our data will be discussed.

Exclusion of electrophoresis

Small dipoles may migrate in a constant electric field. Since *Paramecium* has an antero-posterior polar organization of mechanically sensitive Ca and K channels (Ogura and Machemer 1980), and morphogenesis in ciliates is also guided by a physiological gradient along

Table 1 Linear regressions of relationships between vertical and horizontal swimming velocities as functions of the external voltage gradient (0.3 to 0.8 V/cm). Data from sectors ± 15° of field lines (n = number of experiments; medians with confidence intervals < 10% admitted)

Gradient [V/cm]	Downward/horizontal rate (V_{D^*}/V_{H^*})	Correlation coefficient (r)	n	Upward/horizontal rate (V_{U^*}/V_{H^*})	Correlation coefficient (r)	n
0.3	y = 555.9 + 0.619x	0.82	8	y = 414.7 + 0.672x	0.92	8
0.4	y = − 61.8 + 0.997x	0.92	7	y = − 520.6 + 1.215x	0.85	7
0.5	y = 301.2 + 0.793x	0.67	8	y = 308.9 + 0.730x	0.65	8
0.6	y = 431.6 + 0.714x	0.88	7	y = 106.9 + 0.826x	0.89	7
0.7	y = 259.7 + 0.817x	0.90	7	y = 99.2 + 0.829x	0.93	7
0.8	y = 161.2 + 0.899x	0.96	8	y = 244.9 + 0.734x	0.93	8

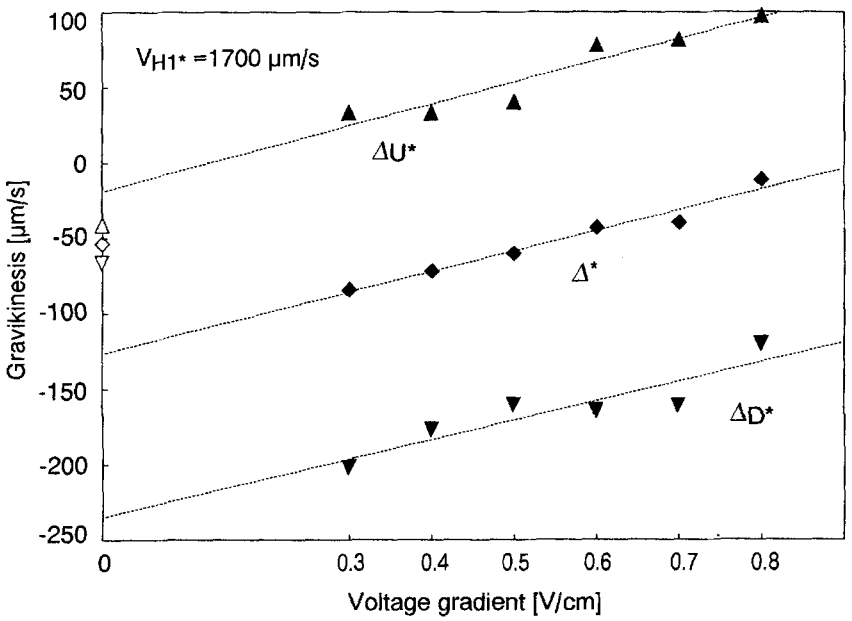


Fig. 10 Electrically conditioned gravikinesis in *Paramecium*. In general, kinesis shifts linearly toward more positive values with rising voltage gradient (*filled symbols*; correlation coefficient: 0.91). Negative sign of downward kinesis (Δ_{D^*}, ▼) indicates action antiparallel to sedimentation (= 110 µm/s). Positive sign of upward kinesis (Δ_{U^*}, ▲) shows action parallel to gravitational pull enhancing effects of sedimentation. The values of generalized gravikinesis (Δ^*, ◆) are negative throughout the applied voltage gradients. Reference gravikinesis values in electrically unstimulated cells see in 0 V/cm (*open symbols*)

the longitudinal cell axis (Jerka-Dziadosz et al. 1995), it is possible that dipole properties of *Paramecium* contribute to the observed swimming and sedimentation velocities. Tests using Ni^{2+}-immobilized *Paramecium* in a vertical chamber showed that an electric field of 0.6 V/cm did not affect the sedimentation rate (Machemer et al. 1991). Immobilized cells were not moved by fields of up to 2 V/cm (Machemer et al. 1991). An electric field of 0.6 V/cm to 4 V/cm at right angle to the vector of gravity induced a lateral digression of maximally 3 µm/s in sedimenting immobilized cells (unpublished observations). Thus a possible electrophoretic component is negligibly small at the size of *Paramecium* under the applied field strengths.

Exclusion of direct interference of voltage with gravisensory channel conductance

Sensory channels are generally thought to selectively respond to the stimulus modality, but an effect of the membrane potential on gravisensory channels cannot be excluded a priori. It has been shown previously that depolarization slightly decreases the conductance of the posterior hyperpolarizing mechanosensory channel in the ciliate *Stylonychia* (Deitmer 1981). With a mechanically induced conductance of 0.4 µS at the resting potential, this conductance decreases by approximately 4 nS/mV depolarization. Corresponding observations were made at the anterior depolarizing mechanoreceptor of *Stylonychia*: a hyperpolarization reduces the conductance seen at the resting potential (De Peyer and Deitmer, unpublished; see Machemer and Deitmer 1985). Applying these findings to gravisensation, a decrease in conductance of maximally 4% is calculated for a *Paramecium* of 250 µm length at a field strength of 0.3 V/cm, assuming that posterior mechanoreceptor channels are depolarized, or anterior mechanoreceptor channels are hyperpolarized. However, due to cathodal orientation of the anterior cell end during galvanotaxis, the membrane of *Paramecium* is inversely polarized: anterior ends depolarize and posterior ends hyperpolarize, and this polarization of the membrane does not change a mechanoreceptor conductance according

to Deitmer (1981). Apart from this inconsistency with a voltage-dependence of gravitransduction, it will be hard to explain the observed behavioural effects (Fig. 10) by a minor change in gravity-induced membrane conductance.

Alternatively, electrode current could accumulate positive charges at the external face of the anodal membrane and thereby affect the conductance of gravireceptor channels. These accumulations are much limited in *Paramecium* because the transcellular current is in the order of 50 pA (assuming a field of 0.4 V/cm, a cell size of 250 μm, and transcellular resistance of 200 MΩ). With DC-field stimulation, a potential channel screening effect by divalent cations (Ca^{2+}) at the anodal membrane will be restricted to the posterior membrane of downward as well as upward swimming *Paramecium*. The posterior soma membrane includes mechanoreceptor K channels (Ogura and Machemer 1980), which are activated by normal gravity (1 *g*) in upward swimmers only. In downward swimmers, the posterior membrane does not contribute to gravitransduction (see Machemer and Bräucker 1992), and the mechanically sensitive Ca channels of the anterior membrane face the cathode. In conclusion, a possible screening of gravireceptor conductances is difficult to reconcile with the data shown in Fig. 10, where a DC field changes the downward gravikinesis (Δ_{D^*}) even more than the upward gravikinesis (Δ_{U^*}).

Exclusion of gravity-induced modification of transcellular voltage-division

Transcellular currents driven by the electric field establish an Ohmic voltage division according to the membrane and cytoplasmic resistances passed by these currents. Assuming homogeneity of the membrane resistance in *Paramecium*, depolarization across the anterior membrane equals hyperpolarization across the posterior membrane. It is conceivable that in vertically swimming cells open gravisensory channels decrease the resistance of the "lower" membrane. Although energetic considerations suggest a much restricted number of channels activated by terrestrial gravity (Machemer 1995), these local conductances can, in principle, modify the transcellular voltage division and thereby change the proportion of depolarized and hyperpolarized membrane surface areas. Considering the electromotor coupling properties in ciliates (Machemer 1986), these hypothetical effects of gravity on galvanokinetic locomotion are roughly predictable: activated posterior receptor K channels of *upward swimming Paramecium* reduce the posterior input resistance so that the membrane area of anodal hyperpolarization decreases, and the area of cathodal depolarization increases. Therefore, the resulting forward swimming rate decreases depressing the gravikinetic rate as calculated according to equation (3). By the same reasoning, the anterior membrane resistance is reduced in a *downward swimming Paramecium*, which serves to decrease the area of depolarization-activated cilia; consequently, the downward swimming velocity rises depressing the gravikinetic response according to equation (2). It is seen that predictions along this line of argument disagree with the present data (Fig. 10), which document a substantial increase in downward gravikinesis under weak electric-field stimulation).

Electric-field stimulation generates a depolarizing bias of gravity-induced ciliary activation

The data of the present study are satisfactorily explained by assuming that depolarization-induced ciliary activation (DCA) superimposes on gravity-induced ciliary responses. A weak DCA response of *Paramecium* is a reduction in the frequency of normally beating cilia, and a corresponding decrease in forward swimming rate (Machemer 1988b).

In a downward swimming cell, anterior mechanoreceptor Ca channels of the "lower" membrane are deformed by gravity. Opening of these channels depolarizes the membrane, reduces the ciliary beating rate and decreases the active downward swimming velocity (Machemer et al. 1991). In agreement with the observations, an additional DCA component will even more reduce ciliary frequency and the rate of active locomotion of the downward oriented *Paramecium* (Fig. 9). This is equivalent to raised negative gravikinesis (Fig. 10).

In an upward swimming cell, gravity-induced activation of the posterior mechanoreceptor K channels hyperpolarize the membrane and generate a weak hyperpolarization-induced activation of the cilia (HCA), which partially offsets sedimentation of the cells (Machemer et al. 1991). Superposition of a DCA bias from the electric field can decrease the active upward swimming rate (Fig. 9). This corresponds to reduction in negative gravikinesis or even generation of a positive gravikinesis (Fig. 10).

While the DCA-bias hypothesis of gravikinesis agrees with the observations and is therefore formally correct, it needs to be demonstrated how a voltage gradient can generate the observed phenomena without directly interfering with gravitransduction (see sections above) or ciliary activation (see below). A straightforward explanation refers to evidences of a voltage-dependent Ca^{2+}-conductance of the nonciliary membrane and cellular contraction modulated by Ca^{2+}. It has been shown that a global rise above 10^{-7} *M* in somatic Ca^{2+}-concentration of permeabilized, ATP-Mg reactivated *Paramecium caudatum* induce a cellular contraction (Nakaoka et al. an 1984). In live cells, a hyperpolarizing ionic stimulation can induce a somatic contraction (Nakaoka et al. 1984;

Nakaoka and Machemer 1990). While hyperpolarization increases the driving force across a Ca^{2+} conductance and may activate hyperpolarization-sensitive Ca channels (Preston et al. 1992a), a depolarization activates voltage-sensitive ciliary and somatic Ca^{2+} conductances (Martinac and Hildebrand 1981; see Machemer 1988a). Hence, persistent DC-field stimulation of *Paramecium* may be associated with a voltage-dependent entry of Ca^{2+}, and a Ca^{2+}-dependent cytoplasmic contraction. That a DC field incurs on gravisensory transduction (Figs. 9, 10), is a first hint at a role of structural elements in gravity-sensing of *Paramecium*.

Different roles of Ca^{2+} in somatic and ciliary spaces

It might be argued that DC-field stimulation directly interferes with Ca^{2+}-sensitive ciliary motility via shifts in membrane potential (Mogami et al. 1990), thereby modulating the graviresponses. However, gravity-unrelated effects on the cilia are also seen in the velocity response of horizontally swimming cells. They do not contribute to gravikinesis (equations 1–3; see also Fig. 8). Moreover, the role of Ca^{2+} in somatic contraction is unrelated to its role in ciliary activation. Electrophysiological and ciliary motility analysis established that transient mechanoreceptor Ca^{2+} currents entering the peripheral somatic space are not "seen" by neighbouring ciliary axonemes nor ciliary membranes (De Peyer and Machemer 1978a; Deitmer 1983). Thus, if voltage gradients did strongly affect the gravikinetic behaviour, a Ca^{2+}-induced *somatic* event must interfere with mechanoreception. Our data impose two restrictions on a hypothesis of voltage- and Ca^{2+}-induced modulation of gravitransduction: (i) this effect applies to downward as well as upward swimming cells; (ii) the effect is equivalent to a DCA bias.

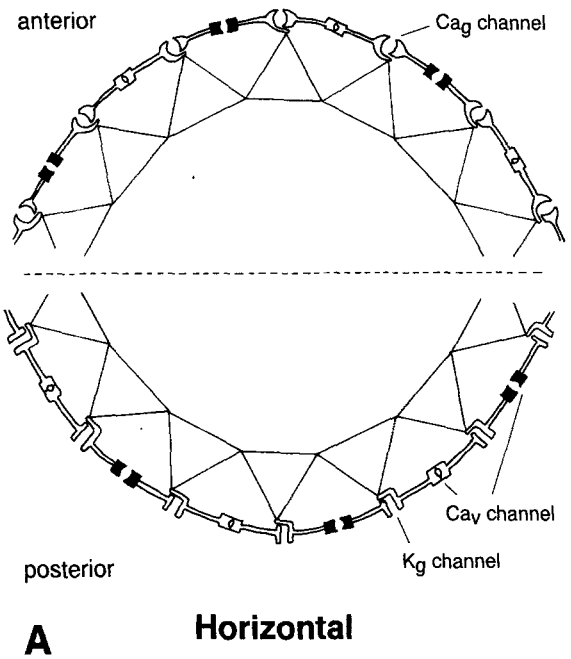
A model of gravisensory transduction

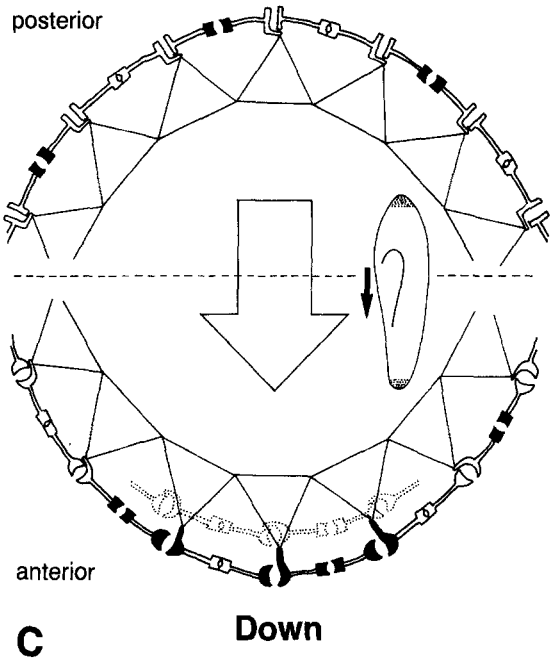
Mechanically sensitive channels, which are gated by a gravitational force of 10^{-10} N, and yet affect thousands of cilia, presumably have unique properties. We postulate that structural elements focus the gravitational force upon the gates of gravisensory channels. We assume that this occurs by means of a filamentous network. Figure 11 illustrates this view. In horizontally oriented cells, the network of filaments is in a resting state because no adequate force is transmitted to the mechanical gates (Fig. 11A). In the upward oriented cell, the gates of gravisensory K channels (K_g channels) are held in place by the filamentous complex, but downward deformation of the membrane pulls these gates open (Fig. 11B) leading to the observed hyperpolarization-dependent activation of cilia (HCA). In the downward oriented cell, a similar gravitational membrane deformation pulls the gates of gravisensory Ca channels (Ca_g channels) inducing the observed depolarization-dependent activation of cilia (DCA). This model is, in part, analogous to current views on filament-mediated gravireception in green algae (Sievers et al. 1989), fungi (Monzer 1995), higher plants (Sievers et al. 1989), and vertebrate hair cells (Hudspeth 1983, 1992). A common property of these views are actin filaments which connect acceleration-sensitive structures with the transduction sites of the plasma membrane. In the plasma membrane of hair cells, both sensitivity to acceleration and transduction site are restricted to the tips of stereovilli, which are interconnected by short filaments. Our model resembles the "gating springs" of cochlear hair cells (Hudspeth 1992; Hudspeth and Gillespie 1994) but does not necessarily incorporate filament elasticity, and excludes adaptation (Machemer-Röhnisch et al. 1993; Bräucker et al. 1994). Moreover, our model includes two types of topographically separated transduction channels to modulate the membrane potential as a function of cell orientation (Machemer et al. 1991). The model is compatible with mechanosensory properties in ciliates as established by previous work (see Machemer and Deitmer 1985). In particular, it explains why a pulsed local stimulation of the membrane of *Paramecium* induces a receptor current including long latencies (about 2.5 ms) in conjunction with an outward-bulging of the inward-deformed membrane (Machemer and Machemer-Röhnisch 1984). An interesting evolutionary perspective of a unified mechanoelectrical transduction concept is that the phasic mechanosensitivity in ciliates might have evolved at the basis of a primary system of gravitransduction.

Ca^{2+}-induced cortical tonus of contraction affects gravisensory transduction

The model of gravisensory transduction explains the observed effects of a DC field on *Paramecium* on the assumption that Ca^{2+}-sensitive contractile elements of the cortex are mechanically linked to, or identical with, the gravisensory transduction complex. We do not know, if established filamentous systems of the cortex, such as the infraciliary lattice (Allen 1988) can play this role. Figure 12 illustrates, how weak cathodal depolarization and Ca^{2+} entry through voltage-sensitive Ca channels (Ca_v channels) induces a centripetal pull via the filamentous network of gravitransduction, and how weak anodal hyperpolarization lessens a Ca^{2+}-induced tension on that network. The extra load on the transduction gates may open some gravisensory channels also in horizontally swimming cells (Fig. 12A). These possible effects are unrelated to gravitransduction in vertical swimmers and are part of the reference swimming rate (V_{H^*}; Figs. 7, 9, 10).

A cell that swims *upward* in a DC field finds its posterior membrane deformed by gravity, but





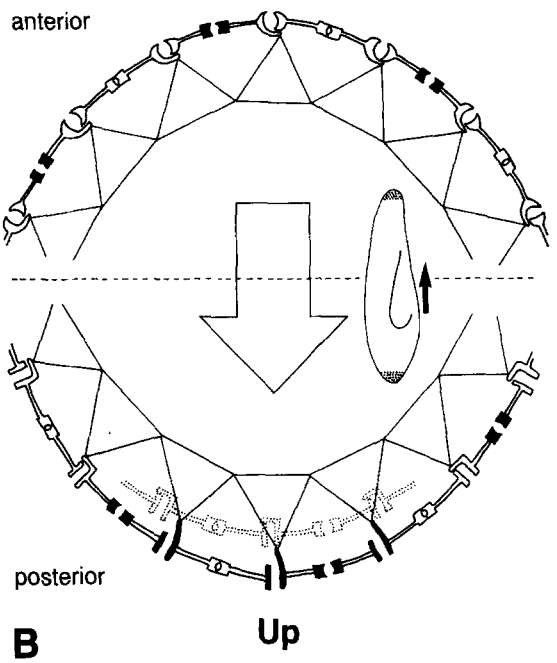


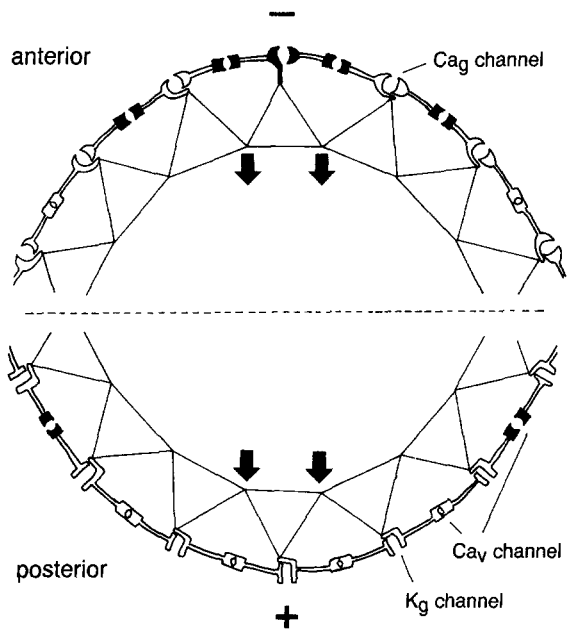
Fig. 11A–C Structural model explaining observed gravikinesis in *Paramecium* in the absence of a conditioning voltage gradient. Views of the anterior and posterior soma membrane with gravireceptor channels (Ca_g, anterior; K_g, posterior) and voltage-sensitive Ca channels (Ca_V); *black*: open channels. The conductances of Ca_V and K rectifier channels (not shown) determine the resting potential of *Paramecium*. A peripheral filamentous network connects to gravireceptor channel gates. Gravireceptor channels may open upon outward deformation of the membrane (**B**, **C**) or pull of the filaments. **A** In a horizontally swimming cell, anterior and posterior gravireceptor channels are closed. **B** In an upward swimming cell, gravity (*large arrow*) deforms the lower membrane opening K_g channels. The resulting hyperpolarization speeds up the rate of upward swimming. **C** In a downward swimming cell, the gravity-induced deformation of the lower membrane opens Ca_g channels leading to depolarization and reduction of the downward swimming rate

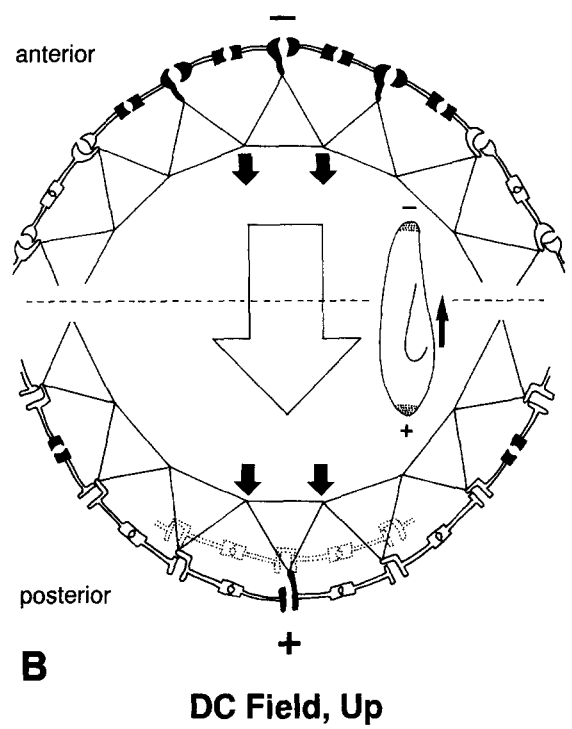
In a *downward* swimming cell, gravitational outward membrane deformation and inward contraction are cooperative in gating anterior Ca_g channels, whereas antagonism of these forces prohibits the gating of posterior K_g channels (compare Fig. 12C with 11C). It is seen that our model corresponds to the observations (Fig. 9).

hyper-polarization-induced relaxation of the contractile tonus reduces the probability of activation of K_g channels (Fig. 12B). On the other hand, contraction at the cathodal anterior cell end together with gravity-induced downward pull activates Ca_g channels. Both anterior and posterior conditioning effects of the electric field on gravitransduction are *depolarizing* (compare Fig. 12B with 11B) and depress the upward swimming rate. In analogy to these DC-field effects, conditions of hypergravity are thought to involve gravitransduction in the anterior (= "upper") membrane of upward swimming *Paramecium* and *Didinium* (Bräucker et al. 1994).

Strength of electric field shifts proportions of electric and gravity effects

Figure 9 shows that a rising voltage gradient decreases active upward swimming rates, and increase active downward swimming rates as referred to the velocity of horizontal swimming. Our crude gravitransduction model explains part of this effect in a straightforward manner: an increased Ca^{2+}-induced contraction of the anterior portion of upward swimmers (Fig. 12B) activates more Ca_g channels thereby enhancing depolarization and depressing the forward swimming rate of *Paramecium*. An increased Ca^{2+}-induced contraction





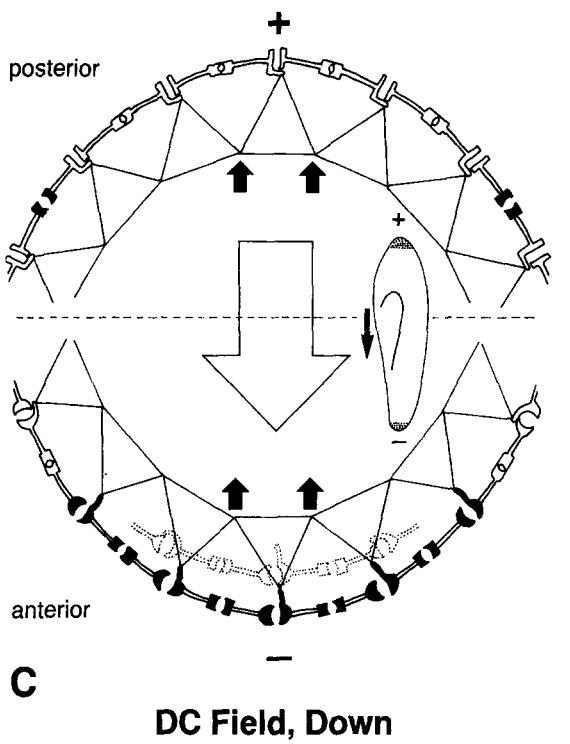


Fig. 12A–C Modeling the effects of weak DC-field stimulation on gravireception. During galvanotaxis (= heading of *Paramecium* toward cathode) the anterior cell end is depolarized, and the posterior end is hyperpolarized. A voltage-sensitive Ca channel conductance of the soma membrane (Ca_v) rises with depolarization and decreases with hyperpolarization. The extra Ca^{2+} influx induces contraction of a cortical filamentous system (*centripetal black arrows*), whereas reduction in steady-state Ca^{2+} influx reduces the tonus of this system (*centrifugal arrows*). Incorporation of gravireceptor filaments in a cortical contractile system adds effects of electric conditioning to those of gravity as observed. **A** In horizontally swimming cells, the cathodal opening and anodal closing of Ca_v channels (and induced somatic contraction and relaxation) serve as a reference for additional effects of gravity. **B** Upward swimming cell. Gravity-induced downward pull of the cytoplasm together with cathodal extra Ca^{2+} influx and contraction open anterior Ca_g channels. Anodal closing of Ca_v channels relieves the tonus of contraction so that some of the gravity-activated K_g channels close. Both Ca_g channel opening and K_g channel closing depolarize the membrane and depress the active swimming rate below the reference (compare $V_{U^*} + S$, Fig. 9). **C** Downward swimming cell. Summed effects of cathodal Ca^{2+} influx and outward deformation of the lower membrane open Ca_g channels. Anodal relaxation of tonus closes K_g channels; the resulting depolarization generates a pronounced reduction in active swimming rate (compare $V_{D^*} - S$, Fig. 9)

of the anterior portion of downward swimmers would be expected to equally reduce the downward swimming rate (Fig. 12C) because even more Ca_g channels are gated. Our data do not confirm this view: Figure 9 indicates that depression of the downward swimming rate between 0 and 0.3 V/cm reversed with steeper voltage gradients. It is possible that the rising driving force for Ca^{2+} in the more strongly hyperpolarized cell end favours an increasing Ca^{2+} influx and contraction also in the upper membrane in agreement with previous findings (Nakaoka and Machemer 1990). Such anodal contraction activates K_g channels and neutralizes depolarizing gravitransduction in the lower membrane. In addition to these presumed effects of DC-field strength on gravitransduction, the somatic Ca^{2+} influx may not rise simply in proportion to depolarization and hyperpolarization. A $[Ca^{2+}]_i$-dependent Ca^{2+}-current inactivation has been documented in ciliates for depolarization-activated Ca channels (Brehm and Eckert 1978; Deitmer 1986) and hyperpolarization activated Ca channels (Preston et al. 1992b). The difference

in DC-field dependent upward and downward swimming rates (Fig. 9) and gravikinesis (Fig. 10) suggests the possibility that, under normal conditions, voltage-dependent shifts in $[Ca^{2+}]_i$ of the peripheral soma are fine-tuned in such a way as to amplify, by means of filament contraction, the primary polarizing effect of gravisensory transduction.

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