RELAXATION AND ACTIVATION OF GRAVIRESPONSES IN *PARAMECIUM CAUDATUM*

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Summary

The kinetics of gravitaxis and gravikinesis in *Paramecium caudatum* were investigated by employing (1) step transitions from normal gravity ($1\,g$) to weightlessness (microgravity) and (2) turns of the experimental chambers from the horizontal to the vertical position at $1\,g$. The transition to microgravity left existing cell orientations unchanged. Relaxation of negative gravitaxis under microgravity took longer than 10 s and may be described by the time constant of the decay of orientation coefficients. Gravitaxis was started at $1\,g$ by turning the experimental chamber from a horizontal to a vertical position. Gravitaxis activated rapidly during the turning procedure and relaxed to an intermediate level after the turning had stopped. Gravity-induced regulation of swimming speed (gravikinesis) at $1\,g$ had reached a steady state after 1 min; at this point, gravikinesis counteracted the effects of sedimentation (negative gravikinesis). A step transition to microgravity initially reversed the sign of the gravikinesis (positive gravikinesis). The relaxation of this kinetic response was not completed during 10 s of microgravity. The data suggest that gravikinesis is functionally unrelated to gravitaxis and is strongly affected by the rate of change in acceleration. We present a model explaining why gravikinesis reverses sign upon the onset of a step from $1\,g$ to microgravity.

Key words: gravikinesis, gravitaxis, mechanotransduction, response kinetics, *Paramecium caudatum.*

Introduction

The graviresponses of free-swimming protists, i.e. changes in orientation and in swimming speed, have been documented under conditions of normal gravity ($1\,g$), microgravity and hypergravity (for reviews, see Machemer and Bräucker, 1992; Häder and Hemmersbach, 1997; Machemer, 1998). The investigations suggested that gravitaxis and gravikinesis are steady and do not adapt in equilibrated cells (Häder *et al.* 1995; Hemmersbach *et al.* 1996; Machemer and Machemer-Röhnisch, 1996; Köhler and Bräucker, 1997). Moreover, graviresponses change with alterations in the angle of attack and strength of the vector of acceleration. It has been proposed previously that gravisensory transduction employs mechanically sensitive membrane channels (Machemer *et al.* 1991; Lebert *et al.* 1997). The time characteristics of mechanoreceptor channels in ciliates are well known: response latencies and times of activation and relaxation of receptor currents occur in the time range of a few milliseconds (see Machemer and Deitmer, 1985). Initial studies on the mechanosensory channel properties of flagellated protists indicate potentially longer times (5–12 s) for activation (Yoshimura, 1996). The kinetic properties of mechanically

gated channels are likely to be affected by the manner in which the cytoskeleton connects to the plasma membrane (Hamill and McBride, 1997). In *Paramecium caudatum*, an intimate association between gravisensory channels and specialized cytoskeletal elements has been postulated (Machemer-Röhnisch *et al.* 1996).

The objective of the present study was to investigate the time courses of changes in gravitaxis and gravikinesis, which appear to take much longer than expected from the electrophysiological properties of mechanoreceptor channels. Do the kinetics of graviresponses give cues to gravisensory transduction and associated mechanisms? There are two major obstacles in studying graviresponse kinetics: (1) gravity is not easily stepped up or down; (2) there are lower limits to the distance (and time) a swimming cell needs to cover for its speed and orientational response to be recorded. Time resolutions between 1 and 1.5 s were obtained from a 4.6 s microgravity time in a drop tower (Machemer *et al.* 1993*a*) and, at this low time resolution, a complex relaxation of gravikinesis was apparent in *Paramecium caudatum* and *Didinium nasutum*. Previously, we used a drop facility which

provided 10 s of weightlessness following a step transition from normal gravity (1 g) to the weightless condition (microgravity). We have evaluated the data obtained from these experiments using an improved time resolution of swimming tracks.

Gravity step transitions from microgravity to 1 g (or other defined gravity levels) to activate cellular graviresponses are only feasible with great technical effort. We have therefore analyzed the onset of graviresponses of *Paramecium caudatum* at 1 g following a turn of the experimental cuvette (and the enclosed planar swimming space) from horizontal to vertical. Such onset of responses cannot be compared directly with off-responses following a 1 g-to-microgravity step. However, the data show that orientational and kinetic graviresponses start within a few seconds after turning the swimming space from the horizontal plane. Different time courses of activation/relaxation of gravitaxis and gravikinesis suggest that these types of responses are based on different mechanisms.

Materials and methods
Cultures

Paramecium caudatum Ehrenberg, line G3 (syngen 3, mating type V), was kindly provided by Dr Mihoko Takahashi, Tsukuba, Japan. Cells were reared in complex organic media in two ways: solution I contained 0.2 % (w/v) cerophyl powder (Cerophyl Laboratories, Inc., Kansas City, KA, USA) in double-distilled water, autoclaved, and buffered at pH 7.0 with Sörensen buffer (1.8 mmol l^{-1} Na$_2$HPO$_4$, 0.2 mmol l^{-1} NaH$_2$PO$_4$). Cultures in solution I were bacterized with *Enterobacter aerogenes*, cultured at 22 °C in a 14 h:10 h L:D photoperiod and harvested after 3 days in the early stationary phase. Solution II, a solution of 1.5 mmol l^{-1} CaCl$_2$, 2 mmol l^{-1} MgSO$_4$, 2 mmol l^{-1} citric acid, 1 mmol l^{-1} Mops, adjusted to pH at 7.0 using KOH, was supplemented with vitamins and lipids (in μg ml^{-1}): calcium pantothenate, 5; nicotinamide, 5; pyroxidal–HCl, 5; pyridoxamine, 2.5; folic acid, 15; thiamine–HCl, 15; D-biotin, 0.00125; DL-thioctic acid, 0.05; riboflavin, 5; stigmasterol, 5; phosphatidylethanolamine, 50). Cultures were bacterized with *Enterobacter aerogenes*, incubated at 22 °C and harvested after 7 days in the early stationary phase. Cultures in solutions I and II will be referred to as cultures I and II in the text.

Experimental solutions and equilibration

Cells from culture I were washed in the experimental solution of 1 mmol l^{-1} CaCl$_2$, 1 mmol l^{-1} K$^+$ (Cl$^-$ and OH$^-$ as anions), 0.1 mmol l^{-1} MgSO$_4$, 1 mmol l^{-1} Mops, buffered with KOH at pH 7.0, and collected using gravitactic accumulation. Washed cells were mixed in the recording cuvette with an equal volume of deoxygenated experimental solution to adjust the O$_2$ concentration to 50 % air saturation. The cell preparation was given 130 min to equilibrate in the recording device. Cells from culture II were collected by centrifugation at 245 g for 6 s, washed in the experimental solution of 0.1 mmol l^{-1} CaCl$_2$, 6.5 mmol l^{-1} KCl, 1 mmol l^{-1} Mops at

pH 7.0, suspended in the same solution and introduced into the experimental cuvette, where they were kept for equilibration times of 90–140 min. We found that healthy, equilibrated cells were important for the results; the two different preparation methods did not cause observable differences in cell behaviour. Preparations of cells in the two experimental solutions will be referred to as preparations I and II in the text.

Cuvettes for recording

Experimental cuvettes had an acrylic bottom and a glass cover leaving a depth of 2 mm. The lateral dimensions of the fluid space were 35 mm×35 mm (preparation I) and 25 mm×20 mm (preparation II); cuvettes were lined with Tygon tubing (preparation I) or silicone rubber (preparation II). In both cuvettes, the field of view for video recording was approximately 18 mm×24 mm. The cuvettes with cell preparations are referred to as cuvettes I and II in the text.

Experimental system for weightlessness

The drop shaft of the Japan Microgravity Center (Jamic, Kamisunagawa, Hokkaido, Japan) provides 490 m of free fall corresponding to 10 s of weightlessness. A high-quality microgravity (10^{-3} g after 0.4 s; 10^{-4} g after 0.6 s; 10^{-5} g after 1.3 s; JAMIC User's Guide, 1996) is obtained employing the capsule-in-capsule principle: the inner capsule encloses a rack holding several experimental modules. Both the inner and outer capsules are guided by magnetic rails. Atmospheric drag on the outer capsule is overcome by air thrusters under feedback regulation. The outer capsule is evacuated to uncouple the inner capsule mechanically. A near-simultaneous release of the inner (0 s) and outer (+40 ms) capsules induces the inner capsule to float in the vacuum. After 10 s of microgravity, rising air pressure in the shaft decelerates the drop unit within 5 s at a maximum value of −8.5 g.

Recording and experimental protocol

Experiments included up to 10 recording units per 'flight'. Each experimental unit consisted of an experimental cuvette ('chamber') containing between 100 and 300 cells in experimental solution, which was turned by 90 ° from the horizontal to the vertical position. A ring of 48 green light-emitting diodes (LEDs; 565 nm) was attached to each chamber, providing dark-field illumination at 800 lx. Video cameras (25 frames s^{-1}) and 8 mm tape recorders documented the movements of cells in the horizontal as well as the vertical plane. For the graviresponse relaxation experiments, recording started 4–5 min before the drop. After 3–3.5 min of recording in the horizontal position, up to six chambers were turned to the vertical position at a rate of 1.5 ° s^{-1} (Machemer *et al.* 1993a; Bräucker, 1994). Another 30–40 s was allowed for activation of graviresponses and recording in the vertical position at 1 g. The drop was then carried out with the chambers in the vertical position. Recording was stopped 2–5 s after the end of microgravity.

For experiments aiming at activation of graviresponses at 1 g, cells were incubated in chambers in the horizontal position,

which were turned to the vertical position. Cells swim horizontally in chambers in the horizontal position, maintaining their distance from the upper and lower walls. Viewing vertically down a horizontal chamber at $1\,\textbf{\textit{g}}$, only the horizontal components (x,y) of locomotion are seen, whereas the residual vertical component (z, parallel to the viewing direction), including sedimentation, is not recorded (Machemer and Bräucker, 1992; Machemer, 1998). The horizontal speed of *Paramecium caudatum* corresponds to cell propulsion unbiased by gravity (Machemer *et al.* 1993*a*). Horizontally swimming cells were recorded for 2.5 min. Recording continued during 60 s of turning the chamber to the vertical position and during 10 min in that position.

The chambers and modules for recording were kept at room temperature (20–22 °C) before enclosure in the drop capsule. Inside the capsule, the temperature of the air surrounding the chambers was regulated at 22 °C.

Data evaluation

Digitized video images were superimposed to obtain tracks of moving cells. Monitoring the relaxation of gravireponses after a transition from $1\,\textbf{\textit{g}}$ to microgravity requires a high time resolution in the track analysis. Tracks were subdivided into 14 segments of equal time intervals marked by different colours. Time intervals depended on digitizing time. The resolution of speed ranged between $3.8\,\mu\text{m}\,\text{s}^{-1}$ and $21\,\mu\text{m}\,\text{s}^{-1}$ depending on digitizing time and magnification. Numbers (N) per data point given in the figures refer to the number of individual tracks seen in the time segment used. Tracks including kinks from cellular reversals were not used for measurements of speed. Orientational responses of cell samples are represented by the cell orientation coefficient (r_{oC}), which describes cell orientations determined from track orientations after accounting for the sedimentation rate (Machemer *et al.* 1997). The sedimentation rate of *Paramecium caudatum* G3 has been determined previously for experimental chambers of 2 mm fluid depth to be $117\,\mu\text{m}\,\text{s}^{-1}$ (Nagel *et al.* 1997) and $118.5\,\mu\text{m}\,\text{s}^{-1}$ (Watzke *et al.* 1998). We use a value of $117\,\mu\text{m}\,\text{s}^{-1}$ for sedimentation rate.

Statistics

Non-parametric statistics were applied because Gaussian distributions were not achieved. Median values are represented with 95 % confidence intervals if applied. Tests of statistical significance of speed differences are based on the Mann–Whitney *U*-test. Coefficients of orientational distributions were determined using the Rayleigh test. Differences between orientation functions were tested using Kuiper's test. For all tests, the limit of significance was set at an error probability of ⩽5 %.

Results

Gravitaxis

Cells equilibrated for 1.5–2 h at $1\,\textbf{\textit{g}}$ showed a weak preference for upward swimming. Fig. 1A shows polar
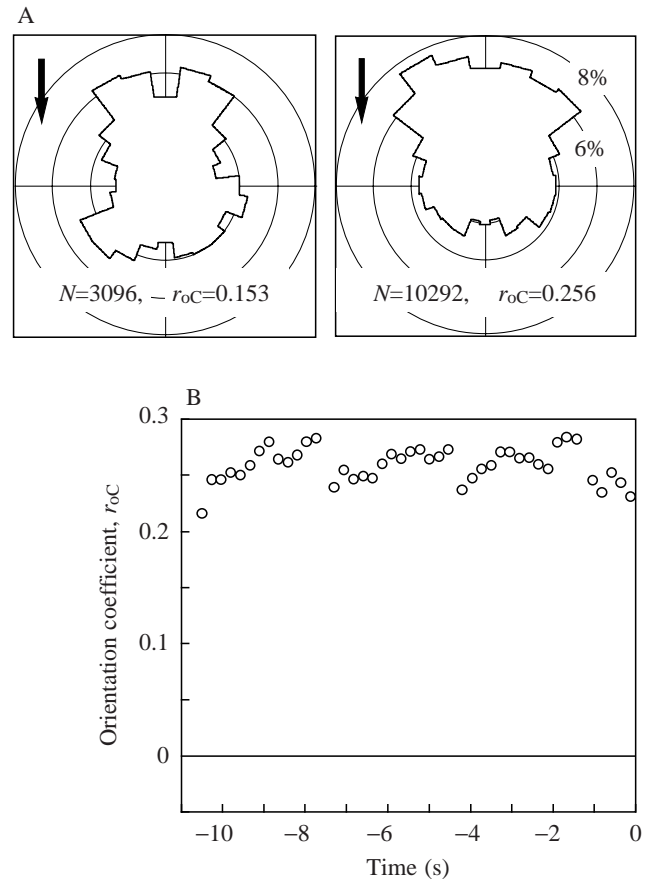

Fig. 1. Steady-state gravitaxis in *Paramecium caudatum*. (A) Circular histograms of orientation of two cell samples showing a weak negative gravitaxis at $1\,\textbf{\textit{g}}$ (cell preparation I, left; cell preparation II, right; sampling period 10 s). The histograms show cell proportions (as a percentage) inside 15° sectors of cell orientation. Arrows give the direction of the gravity vector. N, number of tracks evaluated; r_{oC}, orientation coefficients based on cell orientations. Both values are significant (see Materials and methods). (B) Time course of changes in the orientation coefficient of preparation II at $1\,\textbf{\textit{g}}$ preceding the step transition to microgravity (N ranging between 300 and 1200). The step transition to microgravity occurred at time zero.

histograms of two cell samples with orientation coefficients of 0.15 and 0.26. Applying a high time resolution (230 ms) confirmed that negative gravitaxis was maintained at a constant level until the preparation entered into microgravity (Fig. 1B).

No orientational change upon transition to microgravity

The swimming direction was very little affected by the step transition of cells from $1\,\textbf{\textit{g}}$ to microgravity, as illustrated by a plot of the frequency distribution of the angular changes between time intervals preceding and following the onset of weightlessness (Fig. 2). This virtual absence of an orientational response to the $1\,\textbf{\textit{g}}$-to-microgravity step transition suggests that, with orientational cues missing, a *Paramecium caudatum* cell continues, at least initially, to swim along its previous direction. This comparison of individual swimming directions
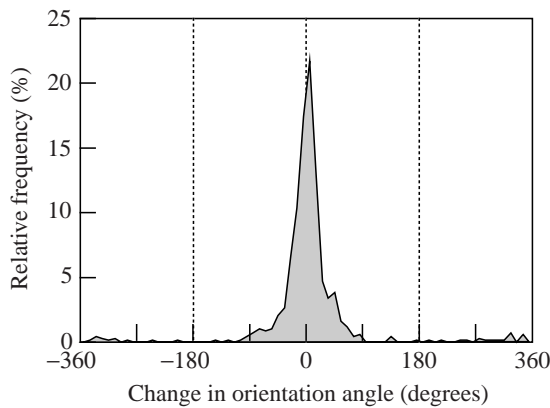
Fig. 2. Frequency distribution of changes in angular orientation of *Paramecium caudatum* following a step transition from normal gravity to microgravity. Periods of 1.4 s preceding and immediately following the step were used for the comparison. The vast majority of cells continued in the same direction as in 1 *g* (100 % = 504 tracks).

during the 1 *g*-to-microgravity step transition explains the similarities in orientational distributions of *Paramecium* before and after a change in acceleration found in previous studies (Machemer *et al.* 1992; Hemmersbach-Krause *et al.* 1993).

Gravitaxis relaxation

When the weightless condition continued for more than a few seconds, negative gravitaxis relaxed. Fig. 3A shows orientational histograms for the period between 7 and 10 s of microgravity. The orientation coefficients of 0.043 and 0.145 indicate a significant decrease in degree of orientation compared with the 1 *g* condition (Fig. 1A; $P \leqslant 0.05$ %, Kuiper's test). This is documented more clearly by the changes in orientation coefficient during the 10 s microgravity period (Fig. 3B,C). Orientational relaxation approximates a logarithmic course and may be represented by the time constant τ, the time required for decay to proceed to 1/e of the initial value. The time constant differed in different cell samples, but residual orientation was still seen after 10 s in all cases. In the present experiments, τ ranged between 4 s and 21 s (Fig. 3B,C).

Phasic and tonic activation of gravitaxis

In cells moving horizontally, the orientation coefficient fluctuates about a value of zero at any time if other modalities of stimuli (light, temperature and chemical gradients) are absent (Machemer *et al.* 1993*a*). We tested the onset of negative gravitaxis in *Paramecium caudatum* cells, whose restricted swimming space was gradually turned from the horizontal to the vertical position at a rate of $1.5\,^{\circ}\,\text{s}^{-1}$. Fig. 4 shows that gravitactic orientation increased from the beginning of turning the experimental chamber towards the vertical. The orientation coefficient increased to a maximum of approximately 0.4 by the end of the turn, then decreased again, settling at approximately 0.15 after 10 min. The relaxation of orientation from a transient peak response is not compatible with a simple logarithmic decay. The time course of gravitaxis
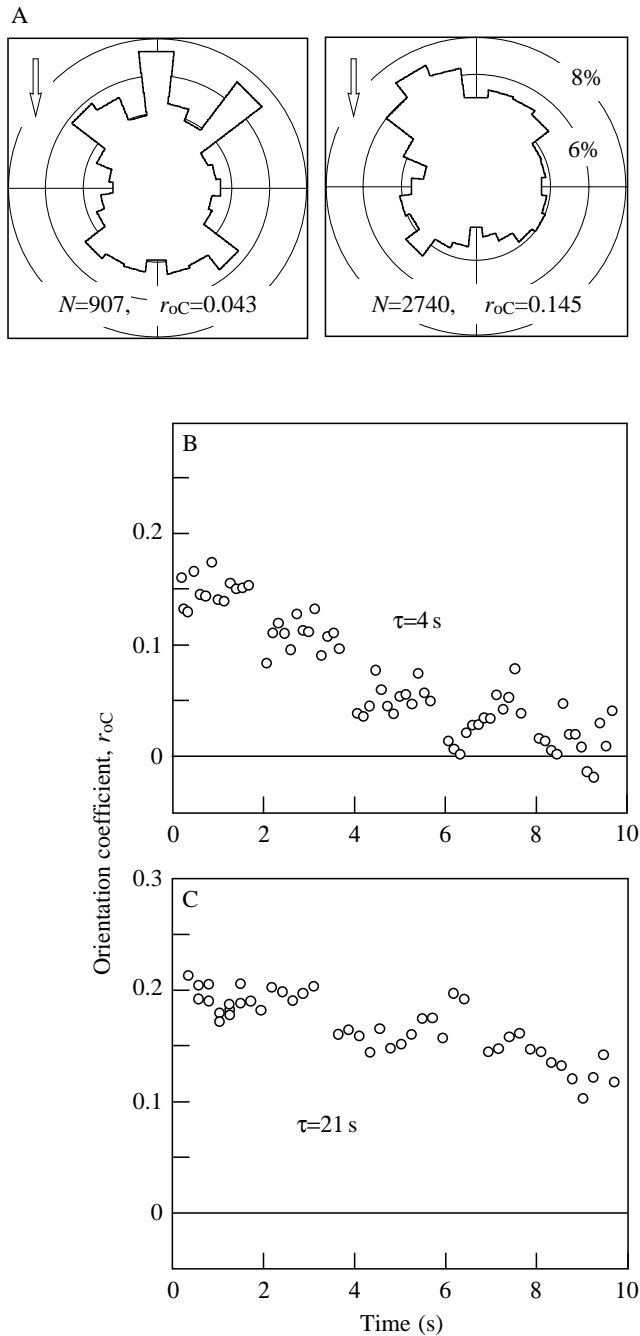

Fig. 3. Orientational distributions of *Paramecium caudatum* after an exposure to microgravity for longer than 7 s. (A) Two polar histograms of cell orientations between 7 and 10 s after the onset of microgravity (preparation I, left; preparation II, right). A comparison with Fig. 1A shows that the relaxation of gravitaxis was significant. Open arrows indicate the direction of the gravity vector during free fall. Other details are as in Fig. 1A. (B) Time course of changes in the orientation coefficient following exposure to microgravity. The preparation is the same as that shown in A (left) and in Fig. 1A (left) for 1 *g*. Preparation I; *N*=600–800. (C) Time course of changes in the orientation coefficient following exposure to microgravity. Cell samples are the same as shown in A (right) and in Fig. 1A (right) for 1 *g*. Preparation II; *N*=200–1000. The time constant (τ) of the decay of gravitaxis is calculated as the time required for decay to proceed to 1/e of the initial value.
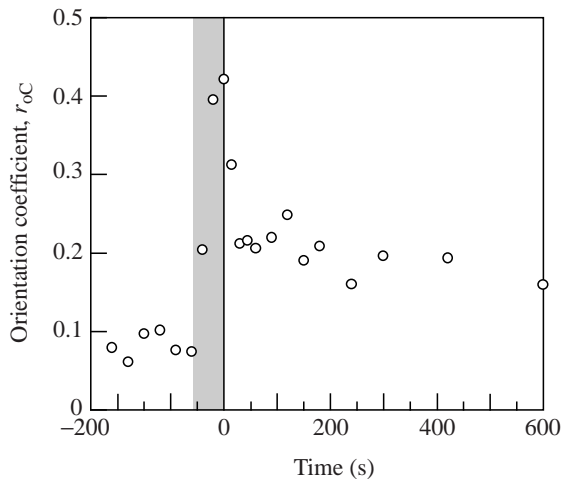
Fig. 4. Activation of gravitaxis of *Paramecium caudatum* following a turn of the experimental cuvette at a rate of $1.5\,°\,s^{-1}$ from horizontal to vertical. The shaded bar represents the 60 s period of turning. The orientation coefficient increased immediately after the onset of turning, reaching a peak value ($r_{oC} \approx 0.4$) and then decreasing to $r_{oC} \approx 0.2$ after approximately 1 min and to $r_{oC} \approx 0.15$ after 10 min. Preparation I, 100 % air saturation, 4 h equilibration time; N=2000–2500.

suggests two mechanisms: an early phasic activation and relaxation, and a late tonic response. The kinetic properties of gravitaxis activation at $1\,\boldsymbol{g}$ (Fig. 4) differ greatly from those of gravitaxis relaxation seen under microgravity (Fig. 3B,C).

Swimming rates preceding and following a transition to microgravity

At $1\,\boldsymbol{g}$, the downward swimming speeds (V_D) of *Paramecium caudatum* exceeded the upward swimming rates (V_U) by approximately $135\,\mu m\,s^{-1}$, and the speed of horizontally swimming cells (V_H) was intermediate (Fig. 5A). All these speeds varied little. A transition to microgravity caused a reduction in V_D by $70\,\mu m\,s^{-1}$ and an increase in V_U by approximately $40\,\mu m\,s^{-1}$, but V_D stabilized at a higher level than V_U. The difference in the median values of V_D and V_U remained significant during 6 s of microgravity. Beyond that time, V_D and V_U were no longer statistically distinguishable. V_H remained at a median of $540\,\mu m\,s^{-1}$ and was unaffected by the gravity transition.

Gravikinesis

The difference between V_D and V_U, after accounting for the sedimentation rate (S), is proportional to gravikinesis (Δ) (Machemer *et al.* 1991) according to the equation:

$$(V_D - V_U)/2 = S + \Delta, \tag{1}$$

where a positive value of Δ means that S and Δ act in the same direction, and a negative value of Δ indicates that they act in opposite directions. The median sedimentation rate of stationary cells of our culture line of *Paramecium caudatum* G3 ranges between $117\,\mu m\,s^{-1}$ (Nagel *et al.* 1997) and $118.5\,\mu m\,s^{-1}$ (Watzke *et al.* 1998) for experimental cuvettes

2 mm deep. The difference in vertical swimming rates at $1\,\boldsymbol{g}$ is approximately $135\,\mu m\,s^{-1}$. Using these values in equation 1 gives a negative value of gravikinesis (Δ) of approximately $-50\,\mu m\,s^{-1}$ for swimming under normal gravity. During the weightless condition, the difference in vertical swimming rates, V_D-V_U, remained significantly above zero for 6 s (Fig. 6).

Because the sedimentation component of swimming cells comes to an immediate standstill under microgravity, the remaining offset of the V_D-V_U data from zero (Fig. 6) indicates a continuation of the gravikinetic response during the first 10 s of microgravity. Fig. 7 shows that gravikinesis changed from a negative value, approximately $-50\,\mu m\,s^{-1}$ at $1\,\boldsymbol{g}$, to a positive value, $18\,\mu m\,s^{-1}$ or less, in the weightless condition, and that the value tended to decline near the end of the 10 s period of microgravity. It is likely that positive gravikinesis would have relaxed fully with longer exposure to microgravity.

Averaging gravikinesis over time intervals of 3 s (Fig. 7) masks the variation in V_D-V_U values during the relaxation (Fig. 6). The variation in the speed data resolved at 230 ms periods was less than $50\,\mu m\,s^{-1}$ at $1\,\boldsymbol{g}$; this variability increased to more than $100\,\mu m\,s^{-1}$ (Fig. 5B). The generally positive V_D-V_U values under microgravity (Fig. 6) suggest that negative gravikinesis relaxed along a damped oscillation with some 'overshooting', resulting in positive gravikinesis (see Discussion).

Gravity-induced activation of swimming rates

In horizontally swimming *Paramecium caudatum*, gravity does not affect the speed of locomotion (Ooya *et al.* 1992; Machemer *et al.* 1993*a*), in agreement with predictions from theory (Machemer *et al.* 1991). Gradual turning of the experimental chamber from the horizontal to the vertical position at $1.5\,°\,s^{-1}$ under $1\,\boldsymbol{g}$ resulted in increased V_D and reduced V_U values, with V_D-V_U eventually reaching a median value of $140\,\mu m\,s^{-1}$ (Fig. 8A). During the 60 s turn of the chamber, V_H increased by approximately $20\,\mu m\,s^{-1}$ to a median value of $725\,\mu m\,s^{-1}$ with the chamber in the vertical position (Fig. 8A).

The turning of the chamber induced downward swimmers to increase their speed by approximately $90\,\mu m\,s^{-1}$; at the same time, upward swimmers reduced their speed by $50\,\mu m\,s^{-1}$. The increase in downward swimming rates is due, in part, to an increase in effective sedimentation rate (vector component parallel to the major plane of the fluid space) towards a final value of $117\,\mu m\,s^{-1}$ (Nagel *et al.* 1997).

Activation of gravikinesis

Application of equation 1 to V_D and V_U shows that negative gravikinesis increased steadily during chamber repositioning. Gravikinesis had a median value of $-47\,\mu m\,s^{-1}$ by the end of turning; it continued to rise to a maximum value ($-77\,\mu m\,s^{-1}$) 2–4 min after turning had been completed and then settled at a value of approximately $-70\,\mu m\,s^{-1}$ after 7 min in the vertical orientation (Fig. 8B).
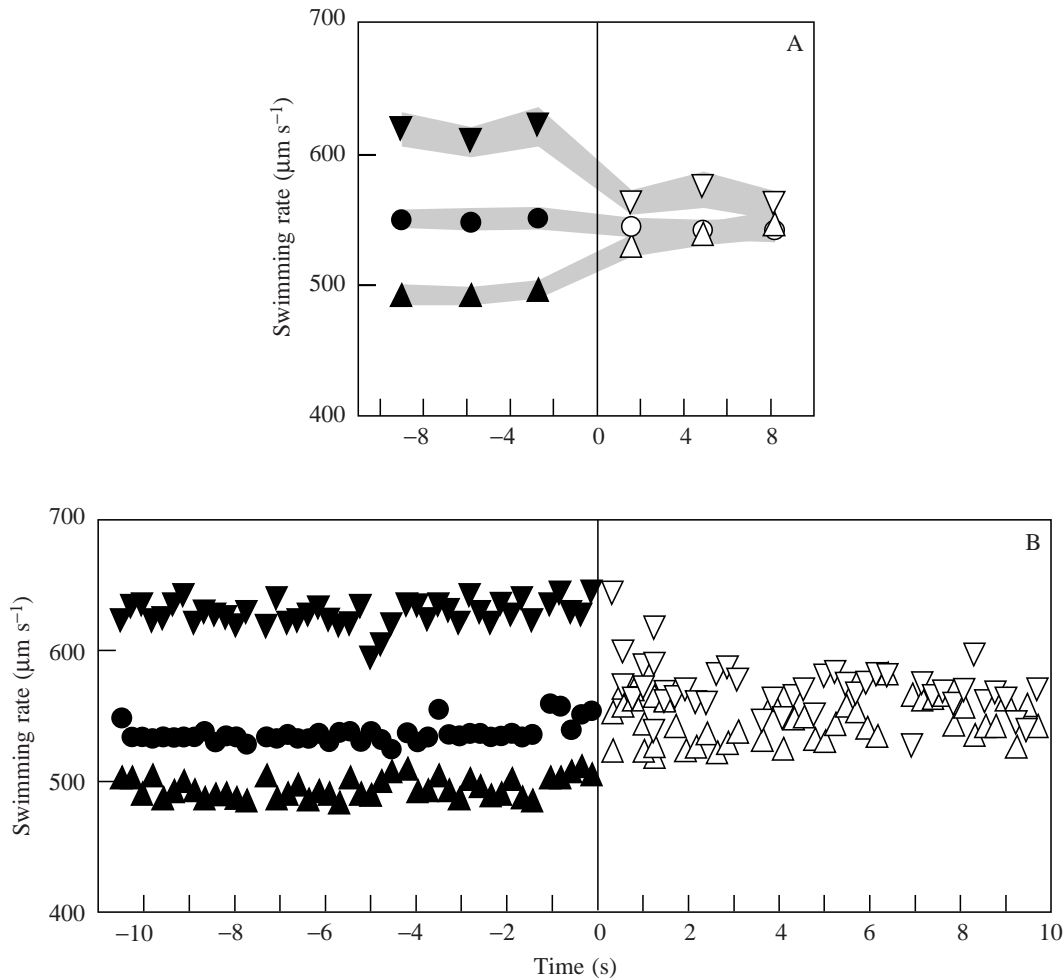
Fig. 5. Swimming rates of *Paramecium caudatum* before and after a step transition to microgravity. (A) Median downward (▼, ▽), horizontal (●, ○) and upward (▲, △) swimming speed during 10 s before (filled symbols) and 10 s following (open symbols) the 1 *g*-to-microgravity step transition. Swimming categories include ±45 °. Shaded zones give 95 % confidence intervals. (B) Swimming speeds at a higher time resolution of the cell sample shown in A reveal an increased variation in the data points compared with 1 *g*. After the transition to microgravity (at time zero), the speeds of vertically swimming cells initially continue at the previous level and then begin to converge. Speeds of horizontally swimming cells remain unchanged during the microgravity period and are omitted for clarity. Preparation II; *N*=200–1000 per 230 ms time segment.

Discussion

Gravitaxis and gravikinesis relax in the weightless condition with different time constants. The kinetics of this relaxation might be a useful tool for investigating a possible common mechanism regulating the orientational and kinetic responses of *Paramecium caudatum*. However, helical tracks of *Paramecium* cells are commonly straight in the absence of a gravitational cue (Hemmersbach-Krause *et al.* 1993). This phenomenon can be explained as follows. The swimming helix of many unicellular organisms is made up of one translational and two rotational force components. Rotation about the longitudinal axis neutralizes the effects of rotation about the transverse (right–left) axis of the cell (Naitoh and Sugino, 1984), causing the axis of the helix to be straight during unbiased swimming. A previous cell orientation (gravitaxis), therefore, will persist, whereas a gravikinetic response will eventually fail in microgravity. Because the relaxation of

orientational and kinetic behaviours may be based on different mechanisms, we discuss our results on gravitaxis and gravikinesis separately below. We conclude with a comparison between the kinetics of gravitaxis and gravikinesis.

Gravitaxis is highly sensitive to input from circumstantial stimuli

Gravitaxis relaxed in microgravity (Fig. 3B,C) and in response to turning at 1 *g* (Fig. 4) with varying time characteristics. According to previous evidence, the precision of orientation of swimming cells is a function of the strength of gravity (Bräucker *et al.* 1994; Häder *et al.* 1991, 1995) and of time (Fukui and Asai, 1985; Machemer *et al.* 1993*b*; Häder *et al.* 1997). Fig. 3B,C shows that the relaxation time constants of gravitaxis varied independently of gravitational input. What is the nature of the mechanism controlling this relaxation? We have proposed that ciliary reversals (identified as kinks in the
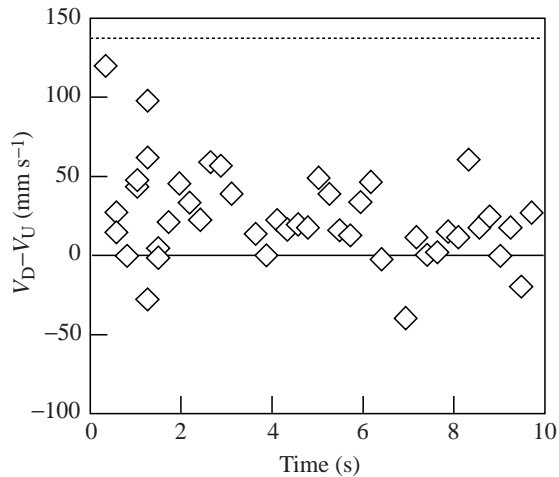
Fig. 6. Time course of the difference between downward and upward swimming rates, V_D–V_U, of *Paramecium caudatum* following a step transition to microgravity. The dashed line (approximately 135 µm s^{-1}) gives the median value of V_D–V_U at 1g. Same preparation as in Fig. 5.

swimming track; see Machemer and Bräucker, 1996) tend to randomize swimming direction and are therefore antagonists of gravitaxis (Machemer, 1998). In equilibrated *Paramecium caudatum*, one event of ciliary reversal was identified every 10 s per cell (see Machemer, 1989). Assuming that 'threshold reversals', i.e. transient cessation of forward locomotion and small redirections of the track, are as frequent as regular reversals, 20 % of an undisturbed cell sample will be engaged in redirecting the course of swimming every second. Ciliary reversals are brought about by depolarization-sensitive ciliary Ca^{2+} channels. Ionic and other conditions which favour an increase in the number of ciliary reversals (Machemer, 1989) enhance random orientation and can therefore depress the time constant of relaxation of gravitaxis. According to this view, the
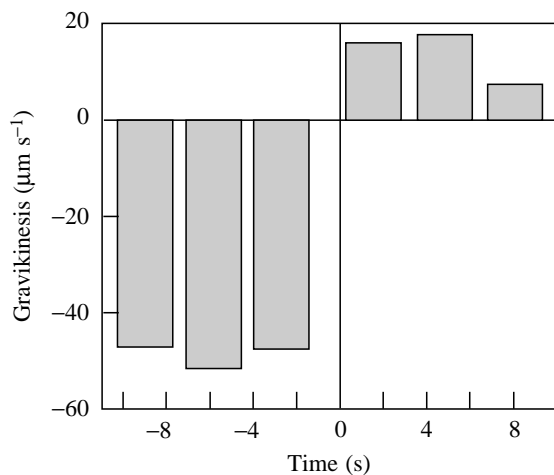

Fig. 7. Medians of gravikinesis (see equation 1) at 1g and after a transition (at time zero) to microgravity. Medians were calculated for 3 s time intervals. Same preparation as in Fig. 5.
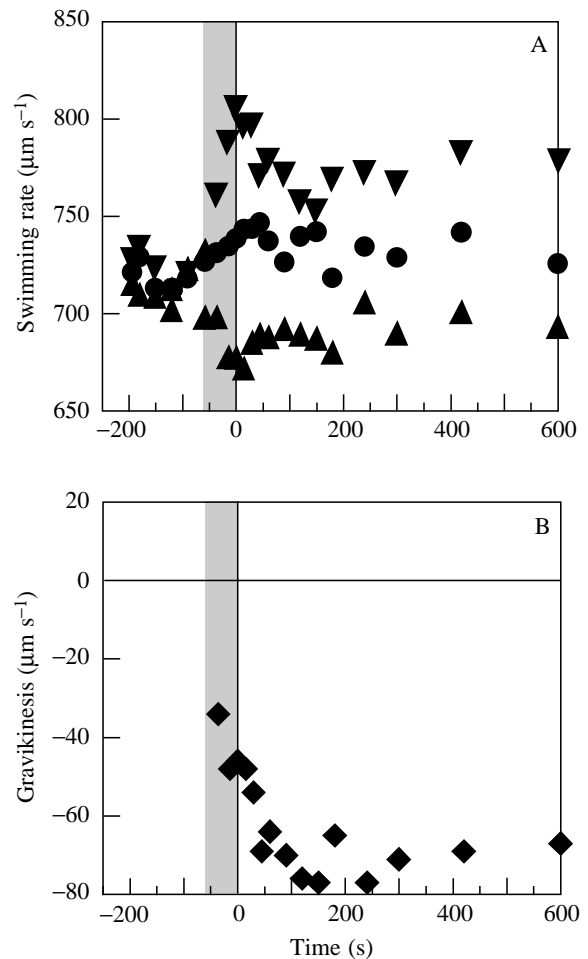

Fig. 8. Gravikinetic responses of *Paramecium caudatum* following the onset of a slow turn (at 1.5 ° s^{-1}) of the experimental cuvette from the horizontal to the vertical position at 1g. The shaded bar represents the 60 s period of turning. (A) Median downward (▼), horizontal (●) and upward (▲) swimming rates. Swimming categories include ±45 ° (also applied to the horizontal chamber). Speed changes between the horizontal and the vertical position are significant. (B) Gravikinesis (see equation 1) calculated for the data in A. Same preparation as in Fig. 4, N=2000–2500.

differences in τ seen in Fig. 3B,C are due to different rates of ciliary reversals causing cellular reorientations.

Fig. 4 shows relaxation of gravitaxis following turning under 1g. In this case, relaxation followed a steep activation of gravitaxis during slow turning of the experimental chamber to the vertical position. Turning by 90 ° causes transient angular accelerations of cells at different orientations in space. Mechanical stimuli such as a shock imposed on the chamber, or caused by pipetting the cells, are known to generate summed hyperpolarizing mechanoreceptor potentials (Machemer and Deitmer, 1985) inducing a few minutes of rapid forward swimming and a reduction in the probability of ciliary reversals (see Machemer, 1989). The observed transient increase in the speed of horizontally swimming cells following chamber turning (Fig. 8A) reflects such mechanically induced

hyperpolarization. Thus, the simultaneous occurrence of peaks in orientation coefficient and swimming speed (Figs 4, 8A) during turning is due to unmasking of gravitaxis from random cellular reorientations. In any case, the observed relaxations of gravitaxis after the turning procedure are unrelated to gravity and are therefore unsuited for investigations of gravireponses. This is in agreement with previous findings suggesting an absence of adaptation in gravitaxis (Machemer-Röhnisch *et al.* 1993; Häder *et al.* 1995; Hemmersbach *et al.* 1996).

Slow relaxation and activation of gravikinesis

Previous work using 4.6 s of microgravity in a drop tower had suggested that vertical and horizontal swimming rates of *Paramecium caudatum* equalized by 3 s of microgravity (Machemer *et al.* 1993*a*). Our present data using a more extended period of microgravity confirmed that V_D and V_U became more similar in microgravity (Fig. 5B). To our surprise, however, gravikinesis was still observed even after 10 s of microgravity, as shown by the continued disparity between the speeds of downward and upward swimmers. A slow relaxation of gravikinesis is evident in the difference V_D-V_U (Fig. 6), which is directly related to gravikinesis (see equation 1).

The sign of the vertical speed difference (V_D-V_U) was usually positive, in agreement with previous findings (Machemer *et al.* 1993*a*). Because the sedimentation rate (117 μm s^{-1}) vanishes immediately upon transition to microgravity, a simple calculation suggests that V_D-V_U should be negative in microgravity assuming that negative gravikinesis relaxes more slowly than sedimentation (see equation 1). V_D-V_U will be zero if negative gravikinesis relaxes instantaneously or is absent. A change in V_D-V_U from positive to negative was seen in the ciliate *Didinium nasutum* after a step transition to microgravity (S. Machemer-Röhnisch, R. Bräucker and H. Machemer, unpublished data). Inspection of Figs 5B and 6 suggests that V_D continued to be larger than V_U from the very beginning of microgravity. Thus, the sign of gravikinesis reversed from negative under 1 *g* to positive under microgravity (Fig. 7).

Gravikinetic relaxation reveals superposition of antagonizing mechanisms

An examination of the speed data at a high time resolution at the transition to microgravity (Figs 5B, 6) shows that the variability of V_D-V_U values increases in microgravity. This variability is not due to noise inherent in the data (see data for 1 *g*). We therefore propose that the 'noisy' relaxation of gravikinesis in *Paramecium caudatum* has a physiological basis consisting of two mechanically coupled systems of gravisensory transduction: a Ca^{2+}-dependent depolarizing activation and a K$^+$-dependent hyperpolarizing activation of gravikinesis (Machemer *et al.* 1991). The existence and topographical separation of these mechanosensory systems at the polar ends of *Paramecium caudatum* has been established previously (see Machemer and Deitmer, 1985). Here, we propose that a step transition from 1 *g* to microgravity leads to

transient asynchrony in the stimulation of gravisensory channels at the opposite ends of *Paramecium caudatum*.

A model of gravikinetic relaxation in Paramecium caudatum

The density of the cytoplasm of *Paramecium caudatum* exceeds the density of the surrounding body of water by 40 kg m^{-3} (Kuroda and Kamiya, 1989). This differential mass is effective in gravistimulation. The cytoplasmic body of a vertically swimming *Paramecium caudatum* may be modelled as a mass suspended between the membranes at the anterior and posterior ends of the cell. The viscoelastic properties of the cytoplasm including the cytoskeleton can be modelled as parallel springs and dashpots (Fig. 9). Gravity-induced outward deformation of the lower membrane activates mechanically sensitive channels in this area, whereas the steady inward pull exerted on the upper membrane by the same force is less effective on local channel activation (Machemer and Bräucker, 1992). A stepwise transition to microgravity induces the more dense cytoplasmic body to shift upwards relative to the external body of water. The upper and lower viscoelastic junctions react to this shift in the manner of a damped oscillation including outward as well as inward deformations of the mechanically sensitive membrane.

In the case of upward-swimming cells (Fig. 9A), the reduced outward deformation of the posterior membrane results in closure of the K$^+$ channels and, hence, hyperpolarization decreases. At the same time, the viscoelastic link near the anterior membrane pushes the upper membrane outwards, which opens the Ca^{2+} channels and induces a depolarizing shift in the membrane potential. In *Paramecium caudatum*, the mechanical definition of these anterior and posterior links is sufficient to generate a summed depolarizing receptor potential at the onset of microgravity in an upwardly swimming cell. The resulting inhibition of ciliary activity compensates for the depressing effect of sedimentation so that, initially, upward swimmers change speed very little (Fig. 5B).

Downward-swimming cells face conditions analogous to upward swimmers (Fig. 9B). Removal of the cytoplasmic load leads to closure of the Ca^{2+} channels at the anterior end and activation of the K$^+$ channels at the posterior end. The summation of receptor conductances generates a hyperpolarizing potential and ciliary activation which compensates for the loss of sedimentation. Therefore, the initial downward speed of these cells in microgravity resembles their speed under 1 *g* (Fig. 5B).

The initial response of the viscoelastic links between the cytoplasm including the cytoskeleton and the membrane does not persist because (1) the elastic elements of the links relax towards a new steady state under microgravity, (2) this relaxation to the new steady-state condition occurs as oscillations between extension and compression, and (3) oscillations of the upper and lower links are not in phase. The summed effect of these properties is the observed increased variability in speed superimposed on the gradual reduction towards zero of the V_D-V_U values (Fig. 6).

A

Ca²⁺ channels
Anterior

Depolarizing potential

B

K⁺ channels

Hyperpolarizing potential

K⁺ channels

Anterior

Ca²⁺ channels

$1g$ μg
Upward swimming
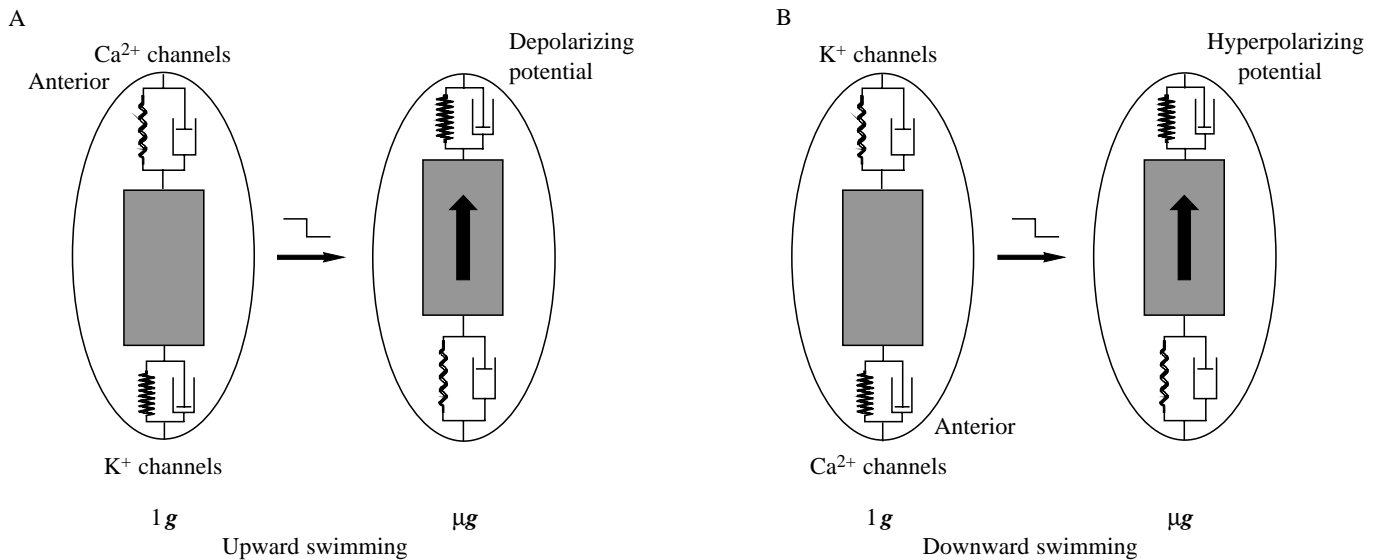
$1g$ μg
Downward swimming

Fig. 9. Schematic diagram to explain the relaxation of gravikinesis in *Paramecium caudatum* following a step transition to microgravity. Viscoelastic elements connect the cytoplasmic mass (shaded rectangular body) with the membrane, which incorporates mechanosensitive Ca²⁺ channels (anteriorly) and K⁺ channels (posteriorly). Channels in the 'lower' membrane are activated under $1g$ because outward deformation of the sensitive membrane is caused by gravity. Such outward deformation is likely to be mediated *via* compression of local viscoelastic elements of the cytoskeleton. (A) In cells swimming upwards under $1g$, K⁺ channels in the posterior membrane open and hyperpolarize the membrane and ciliary frequency increases, thus increasing upward swimming velocity (=negative gravikinesis). A step transition to microgravity removes the gravitational load from the lower spring, which moves the cytoplasmic mass upwards, charging the upper spring (the Reynolds number of cells such as *Paramecium* is 10^{-3} or less, so that the inertial drag of the mass of the cell is negligible). The resulting outward deformation of the anterior membrane activates the Ca²⁺ channels, while the posterior K⁺ channels close. The summed conductances generate a depolarizing potential, decreasing ciliary frequency and reducing the upward swimming rate (=positive gravikinesis) at the beginning of the period of microgravity. (B) Cells swimming downwards under $1g$ have their anterior Ca²⁺ channels activated by gravity, inducing a reduction in downward swimming rate (=negative gravikinesis). Removal of the gravitational load leads to an expansion of the lower spring, an upward shift of the cytoplasmic mass, opening of the posterior K⁺ channels and closure of the anterior Ca²⁺ channels. A summed hyperpolarizing potential generates augmentation of the downward swimming rate (=positive gravikinesis). The relaxation properties of the viscoelastic links determine the swimming behaviour during the subsequent exposure to microgravity.

General conclusions
Gravikinesis relaxation and activation

Our hypothetical model accounts for the inversion of the sign of gravikinesis in *Paramecium caudatum* observed after a step transition to microgravity. Results from previous experiments (Machemer *et al.* 1993*a*) and the present study suggest that gravikinesis at $1g$ neutralizes the effects of sedimentation (=negative gravikinesis) and that, upon step entry to microgravity, persisting gravikinesis acts for a limited time in an analogous way to sedimentation (=positive gravikinesis). Our experiments were designed to extend the period of microgravity studied and to increase the time resolution for analysis of the orientations and kinetic responses of cells. A major conclusion from these experiments is that gravikinetic relaxation following gravity step transitions takes longer than the 10 s used in the present study, presumably because of the viscoelastic properties of the gravisensory system of *Paramecium caudatum*.

We were able to separate gravikinetic responses from the effects of sedimentation by applying gravity step transitions. The release of the drop unit used to achieve microgravity includes a brief instability in acceleration at the beginning of

the free fall (see Materials and methods). We are unable to exclude the possibility that such instability contributed to the initial behavioural responses in *Paramecium caudatum*. However, the persistence of cell orientation during gravity transition (Fig. 2) and the persistence of the post-transition characteristics of the V_D–V_U response for 10 s (Fig. 6) suggest that this early instability of the gravity step played a minor role, if any, in the relaxation of gravikinesis.

We wish to emphasize that step-type transitions of gravity do not occur in the daily life of swimming *Paramecium caudatum* so that the paradoxical inversion of the sign of gravikinesis can be obtained under these conditions only. A free-swimming *Paramecium caudatum* that changes from upward to downward swimming, and *vice versa*, undergoes changes in linear and angular acceleration in addition to changes in the point of action of the gravity vector. Our experiments on the activation of gravikinesis by slow turning (Fig. 8B) suggest that gravikinesis in *Paramecium caudatum* is sensitive to angular as well as to linear acceleration. We have recently found that mechanical vibrations, which might occur during turning of the chamber, do not affect gravikinesis in this cell (R. Bräucker, unpublished data).

Gravikinetic activation under $1g$ conditions is a slow process. Swimming rates were near steady-state approximately 30 s after completion of turning of the chamber (Fig. 5), but Fig. 8B suggests that it took 1 min for median gravikinesis values to stabilize. A possible conclusion from these observations is that gravikinesis of *Paramecium caudatum* saturates if a cell swims in a particular direction without turns or reversals. It should be noted that the transient activation of the cilia induced by slow turning of the chamber (Fig. 8A; increased horizontal and downward speeds, decreased upward swimming speeds) did not apparently interfere with the increase in negative gravikinesis (Fig. 8B). In accordance with this finding, it has been shown previously that gravikinesis is independent of the absolute speed of cells (Machemer and Machemer-Röhnisch, 1996).

Kinetics of gravitaxis and gravikinesis

Relaxation of gravitaxis had variable time constants of up to 21 s (Fig. 3). The time constant for the relaxation of gravikinesis remains undetermined, but might be of the same order. The activation of gravitaxis was associated with a transient peak in the orientation coefficient (Fig. 4), whereas the activation of gravikinesis of the same cell sample was a continuous function of time (Fig. 8B). The time-dependence of gravitaxis (Figs 3, 4) could be explained by an antagonistic mechanism: the probability of events of cellular turning. A comparison between the kinetics of activation of gravitaxis and gravikinesis (Figs 4, 8B) provides no clues about a common basis for these mechanisms. In the absence of positive evidence from the comparison of kinetics, we conclude that the mechanisms of gravitaxis and gravikinesis are probably unrelated.

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