The Journal of Experimental Biology 199, 2199–2205 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
JEB0305

GRAVIRESPONSES IN *PARAMECIUM BIAURELIA* UNDER DIFFERENT ACCELERATIONS: STUDIES ON THE GROUND AND IN SPACE

RUTH HEMMERSBACH[1,*], REGINE VOORMANNS[1] AND DONAT-P. HÄDER[2]

[1]*Institute of Aerospace Medicine, DLR (German Aerospace Research Establishment), D-51140 Cologne, Germany* and [2]*Institute for Botany and Pharmaceutical Biology, Friedrich-Alexander-University, Staudtstrasse 5, D-91058 Erlangen, Germany*

Accepted 20 June 1996

Summary

Behavioural responses to different accelerations below 1 *g* and up to 5 *g* were investigated in *Paramecium biaurelia* by using a centrifuge microscope on Earth and in space during a recent space flight. Increased stimulation (hypergravity) enhanced the negative gravitactic and the gravikinetic responses in *Paramecium biaurelia* within seconds. Cells did not adapt to altered gravitational conditions. Repetitive stimulation did not change the graviresponses. The minimum acceleration found to induce gravitaxis was between 0.16 and 0.3 *g*.

Key words: *Paramecium biaurelia*, gravitaxis, gravikinesis, hypergravity, centrifuge microscope, microgravity, threshold.

Introduction

As long ago as 1906, Jennings noted that at least some and probably many protozoa use gravity as an environmental stimulus for orientation. Some, such as *Paramecium*, are even able to control both their swimming direction (gravitaxis) and their swimming velocity in response to gravity. This gravikinesis enables the protozoa to compensate, at least in part, for sedimentation by increasing their upward swimming rate and by reducing their downward swimming rate (Machemer *et al.* 1991; Ooya *et al.* 1992). The mechanisms controlling gravitaxis and gravikinesis are still under discussion. Hypotheses based on purely physical or purely physiological reactions have been put forward to explain the graviresponses of *Paramecium*. There are many indications that a passive mechanism, such as the principle of a buoy, is insufficient to explain the variety of behavioural responses that occur (for a review, see Bean, 1984; Machemer and Bräucker, 1992). In consequence, gravitaxis and gravikinesis are thought to involve a physiological signal transduction chain similar to that involved in the perception of light (Colombetti, 1990) and chemical (Van Houten and Preston, 1988; Van Houten, 1992) stimuli. Superposition of different stimuli (light and gravity) has been demonstrated, e.g. for the flagellate *Euglena gracilis* (Häder, 1987), where such behaviour is ecologically essential for the survival of the organisms. Such superposition may involve common pathways for different signal transduction chains.

A greater knowledge of the mechanism of graviperception can be gained by studying the effects of varying the stimulus (gravity). In previous studies, it was shown that an absence of gravity resulted in random swimming of *Paramecium aurelia*, *Paramecium biaurelia* (Hemmersbach-Krause *et al.* 1993*a*,*b*) and *Euglena gracilis* (Häder *et al.* 1990) accompanied by equal swimming velocities in all directions, indicating that gravity was the sole reason for the behaviour that occurs under normal conditions of gravity (1 ***g***). In the present study, the behaviour of *Paramecium biaurelia* in response to gravity changes was investigated using a slow-rotating centrifuge microscope (NIZEMI, Dornier, Friedrichshafen, Germany) on the ground and in space. This enabled reactions to increasing and decreasing gravitational stimulation to be examined. Experiments under decreasing accelerations below 1 ***g*** were performed during the Spacelab IML-2 (International Microgravity Laboratory) mission to determine the minimum gravity level at which the transition from oriented movement (negative gravitaxis) to random swimming occurred.

Materials and methods

Cells and cuvettes

Paramecium biaurelia Sonneborn (obtained from the Culture Collection of Algae and Protozoa, Ambleside, UK) was cultivated in straw medium (pH 7.2, 22 °C). The cultures had to be enriched without centrifugation to exclude any effects of accelerations before experiments were begun. To achieve this, cell suspensions were placed in volumetric flasks, in which the protozoa assembled in the narrow neck owing to

*Present address: Deutsche Forschungsanstalt für Luft- und Raumfahrt, Institut für Luft- und Raumfahrtmedizin, D-51140 Köln, Germany (e-mail: Ruth.Hemmersbach@DLR.DE).

Dedicated to Professor Dr Andreas Sievers on the occasion of his 65th birthday.

their negative gravitaxis and positive aerotaxis. Experimental observations were performed in custom-made observation chambers, which in the case of the 15 day space experiments had to be developed to allow cultivation as well as observation of the cells. In over 20 tests, Plexiglas chambers proved well-suited for the survival of the cells for up to 15 days, but the precision of gravitaxis of *Paramecium biaurelia* was rather low in these chambers. In contrast, within 6 h of transfer into a glass chamber, *Paramecium biaurelia* showed a precise gravitaxis, but the cells died after 1 day. From previous studies, we know there is a correlation between oxygen concentration and degree of orientation, so that under hypoxic conditions the precision of orientation is high (Hemmersbach-Krause *et al.* 1991). The preliminary observations indicated that the permeability for oxygen is high through Plexiglas and low through glass, and these results were taken into account in the construction of the chambers. Experiments on the ground were normally performed in simple cylindrical Plexiglas chambers (diameter 23 mm, 0.5 or 1.0 mm depth) covered by a glass plate sealed with silicone paste. In addition, control experiments were performed in the more complex chambers designed for the experiments carried out in space. These controls showed that there was no difference in the behaviour of the cells in the different containers. The chambers used in space were divided into two parts, one for cultivation (Plexiglas, 27 mm×35 mm, 3 mm depth) and one for observation (glass, 27 mm×27 mm, 1 mm depth). The two parts of the chamber were separated by a plate with two holes which were initially sealed by silicone. On the ground, before lift-off of the space shuttle, the observation part was filled with sterile culture medium, the cultivation part with cells. Six hours before the experiments were begun, an astronaut transferred the cells into the observation part by moving a piston.

Experiments in space

The cuvettes were stored on board the shuttle *Columbia* 19 h before launching. The samples were kept at 10 °C to reduce the effects of acceleration on the cells during the ascent of the rocket. In space, the samples were incubated at 22 °C on a 1 $\boldsymbol{g}$ reference centrifuge. In preparation for the experimental run on mission day 3, one sample was transferred from the 1 $\boldsymbol{g}$ reference centrifuge to the slow-rotating centrifuge microscope (NIZEMI), accelerated to 1 $\boldsymbol{g}$ and subsequently slowed down in 10 steps to the conditions of weightlessness '0 $\boldsymbol{g}$' (NIZEMI halted) and was then taken back in a single step to 1 $\boldsymbol{g}$. Each acceleration or deceleration step lasted for 5 min. Conditions within the spacecraft are such that only near-weightlessness (microgravity) is achieved (indicated by '0 $\boldsymbol{g}$' in Fig. 6). The residual acceleration during the 'threshold experiments' did not exceed 10^{-3} $\boldsymbol{g}$ as measured by accelerometers.

Slow-rotating centrifuge microscope (NIZEMI)

The experiments were performed on a slow-rotating centrifuge microscope (NIZEMI) (Fig. 1) constructed by Dornier (Friedrichshafen, Germany) and sponsored by the German space agency DARA. Two models of the NIZEMI were used for the experiments, a ground model and a space model, differing in mass and dimensions. The NIZEMI is a horizontal microscope (Axioplan, Zeiss, Jena, Germany) mounted tangentially on a circular platform driven by an electric motor. In order to achieve good contrast for computer-controlled image analysis, the cells were observed using dark-field illumination and red light (645 nm) at an objective magnification of 1.25× or 2.5×. During rotation, the optical axis remains perpendicular to the resultant vector of gravity and the centrifugal acceleration. In the NIZEMI ground model, the effective radius is 170 mm. The maximal acceleration of 5 $\boldsymbol{g}$ was achieved at a rotational speed of 163 revs min^{-1}. In the space-adapted NIZEMI, the effective radius is 110 mm and the maximum acceleration of 1.5 $\boldsymbol{g}$ was achieved at 111 revs min^{-1}. In the space experiment, the temperature of the cuvettes was maintained at 22 °C by eight Peltier elements located around the cuvette holder. In the ground experiments, no temperature control of the cuvettes was implemented and these experiments were performed at a room temperature of 22 °C. During rotation, the microscope image was recorded by a black and white CCD camera mounted on the microscope. The video sequences were either stored on an NTSC video recorder (V-250 AB-R, TEAC, USA) in the space experiment or on an SVHS recorder for the ground experiments. The shuttle was launched from Kennedy Space Center on 8 July 1994 for a 15 day mission. During the experimental run, the video image was transmitted to the mission control center in Huntsville, Alabama, to allow control of the viewing area and focus by the experimenter.

Video analysis

Evaluation was performed by computer-controlled image analysis in real time either on-line or using video-recorded sequences (Häder and Lebert, 1985; Häder and Vogel, 1991). The measurements were started as soon as the desired acceleration was achieved. The video signal was digitized at a spatial resolution of 512×512 pixels with 256 possible grey levels each (Matrox digitizer card PIP-1024, Quebec, Canada). Four frames were taken at 80 ms intervals and stored in a video memory. The position and outline of the individual cells were determined by chain coding and were further followed through the recorded series of images. The movement vectors of all tracks were stored as the angles of deviation of the cells from the predetermined stimulus direction (acceleration vector) and as the distance an organism had moved in the time interval. A maximum of 70 cells was recorded at a time. Note that the number of cell tracks does not correspond to the number of individual cells since, during the passage of a cell through the observation field, one cell can be tracked up to four times in the worst cases. Circular histograms were calculated for the direction distribution using a resolution of 64 sectors of 5.6° each (Häder, 1985). In addition, a Rayleigh test was performed to determine the directedness of the moving organisms (Batschelet, 1965, 1981). The degree of orientation for a unimodular distribution yields a statistical value r (r-value) that indicates the precision of gravitactic orientation. The r-value

ranges from 0 (random distribution) to 1 (precise orientation of all organisms in a single direction).

$$r = \frac{\sqrt{(\Sigma\cos\alpha)^2 + (\Sigma\sin\alpha)^2}}{N}, \qquad (1)$$

where α is the angular deviation of the individual track vector from the acceleration vector and N is the number of records (cell tracks).

The preferred direction, θ, of a culture can be calculated from:

$$\theta = \frac{\arccos[(\Sigma\cos\alpha)/N]}{r}. \qquad (2)$$

As a statistical test for 'randomness' or 'orientation', the Rayleigh test was applied. The test statistic of Rayleigh's test is $z=Nr^2$ (Batschelet, 1965). Critical values of the test statistic z of the Rayleigh test for P=0.01 were taken from Batschelet (1965), adapted from Greenwood and Durand (1955).

Results

Response to hypergravity

At 1***g***, the precision of gravitactic orientation of *Paramecium biaurelia* differs from culture to culture. Under controlled conditions (closed observation chamber), negative gravitaxis is usually observed several hours after placing the cells in the observation chamber (Hemmersbach-Krause *et al.* 1991, 1993*a*). When a cell culture with a clear negative gravitaxis was accelerated (tested up to 5***g***), the cells did not sediment and negative gravitaxis persisted. Fig. 2 shows a typical example in which the *r*-value rose from 0.37 at 1***g*** (preferred direction θ=16.5°, N=521) to 0.73 at 5***g*** (θ=358°, N=509) (Fig. 2A,B). In cultures with a lower degree of orientation under 1***g*** conditions (*r*-value of 0.22, θ=20°, N=585), hypergravity of 5***g*** also induced a clear gravitactic response, demonstrated by a significant rise in the *r*-value to 0.72 (θ=1°, N=750) (Fig. 2C,D) accompanied by a closer alignment of the cells towards the ***g***-vector, as indicated by the decrease in the angle θ. The reaction occurred within seconds; the circular histograms represent the data from the first minute under hypergravity.

Repetitive stimulation between 1***g*** and hypergravity (for an example of changes between 1***g*** and 5***g***, see Fig. 3, each acceleration step lasting 2 min) revealed that the reactivity of the cells was maintained. They always showed clear responses to hypergravity (increase in *r*-value) accompanied by acceleration-dependent changes in the upward swimming velocities. In comparison with the mean 1***g*** values (599±

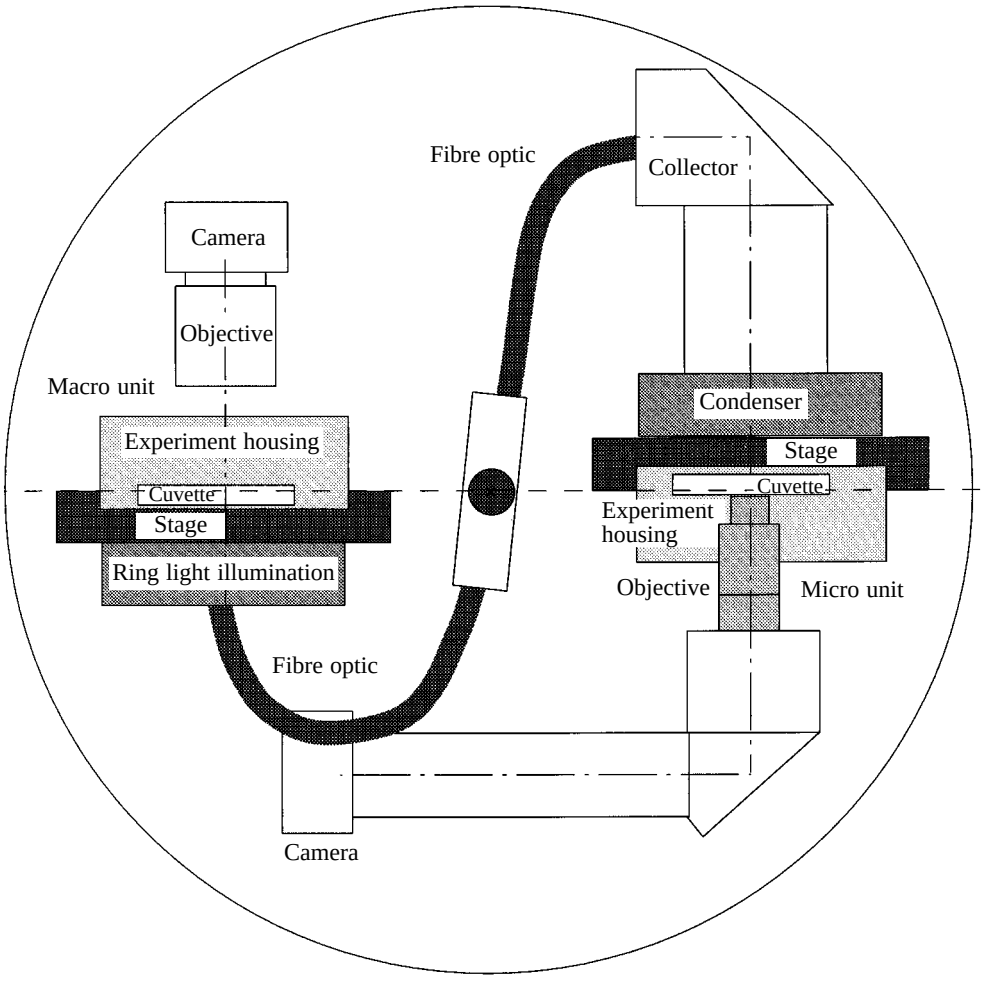

Fig. 1. Top view of the slow-rotating centrifuge microscope NIZEMI. The NIZEMI consists of a micro unit (microscope) and a macro unit (macroscopic observation). Simultaneous movement of both stages in corresponding directions compensates for any unbalancing during rotation (for further description, see Materials and methods).

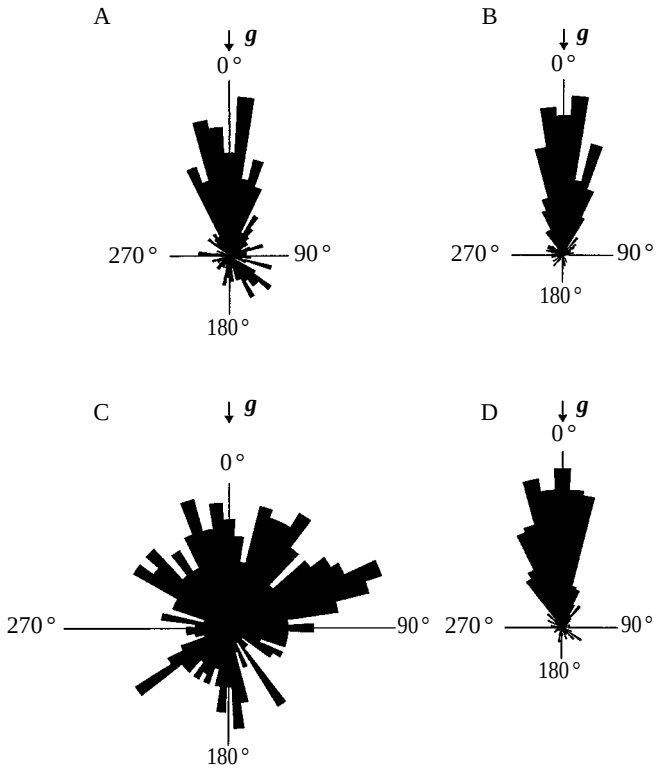

Fig. 2. Circular histograms of track orientation of *Paramecium biaurelia* at 1***g*** (A,C) and 5***g*** (B,D). During acceleration, negative gravitaxis persisted in a negative gravitactic culture (A) or was induced in a culture with weak negative gravitaxis (C). Calibration of the histograms: the largest sector in A represents 7 % of the total tracks (*N*=521); in B, 12 % (*N*=509); in C, 3 % (*N*=585); and in D, 10 % (*N*=750). The measurements were begun 30 min after adaptation of the samples in the horizontal microscope, 4 h (A,B) or 1.5 h (C,D) after putting the cells in the cuvettes. Each histogram represent a 1 min measurement.

24 μm s^{-1}, *N*=7624), the mean upward swimming velocities decreased by approximately 200 μm s^{-1} at 5***g***. This value corresponds to about 2.5 times the sedimentation velocity at 1***g*** (82±22 μm s^{-1}; Hemmersbach-Krause *et al.* 1993*b*) (see Discussion).

To determine the length of time needed for the cells to reorient with respect to a new acceleration vector and to adjust their swimming velocities, the measurements were divided into 20 s intervals, the minimum period needed for the registration of a sufficient number of cells for statistical analysis. Five experiments were carried out and a representative example is shown in Fig. 4. Immediately upon acceleration, the cells showed a reaction (Fig. 4); within 20 s, the orientation histogram revealed a clear negative gravitaxis towards the new acceleration vector, accompanied by an increased precision of orientation (*r*-value=0.96, θ=355 °, *N*=289), which subsequently decreased slightly to 0.9. Since 97 % of the cells were swimming upwards (negative gravitaxis) at 5***g***, only the mean upward (0±30 °) swimming velocities (with standard error of the means) were considered. Within the first 20 s at

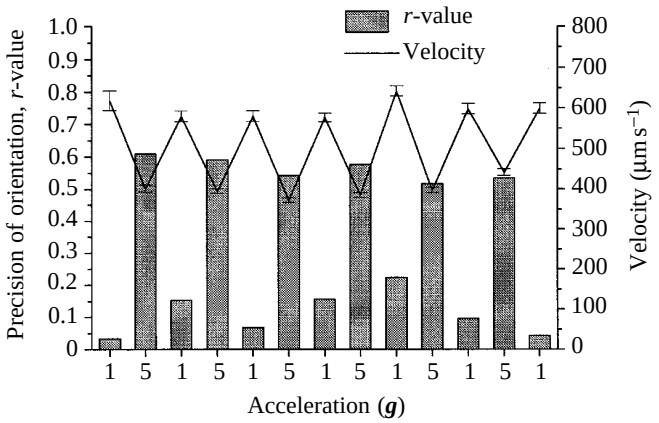

Fig. 3. Orientation of the cells (columns) and mean upward swimming velocities (line, with standard error of the means) of *Paramecium biaurelia* during repetitive stimulation at 1***g*** and 5***g***, with each acceleration step lasting for 2 min. Each point represents more than 1000 tracks.

5***g***, the upward swimming velocity decreased significantly from 642±12 μm s^{-1} (*N*=232) (1***g*** value) to 527±10 μm s^{-1} (*N*=289) and decreased further within the next 20 s to 465±10 μm s^{-1} (*N*=275). The decrease corresponds to twice the sedimentation velocity at 1***g***. When the centrifuge was stopped, the upward swimming velocity increased to 645±12 μm s^{-1} (*N*=226) within the first 20 s, a value not significantly different from the initial 1***g*** value (Fig. 4). Fig. 4 also shows that the degree of orientation was higher than the initial 1***g*** value (*r*-value 0.84, θ=26.5 °). After 60 s, the precision of orientation had declined to that of the starting conditions during the last 20 s at 1***g*** (data not shown). These results indicate that both responses (gravitaxis and gravikinesis) occurred within a few seconds.

In order to test whether cells adapted to hypergravity, they

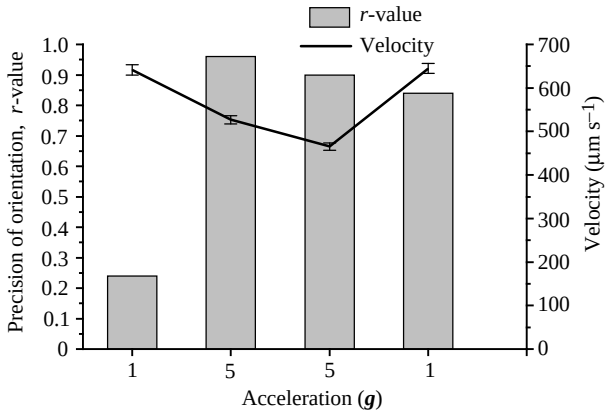

Fig. 4. Initial reaction of the orientation of the cells (columns) and mean upward swimming velocities (line ± S.E.M.) of *Paramecium biaurelia* to hypergravity (5***g***). Each measurement lasted for 20 s and represents more than 200 tracks. Note the fast responses of the cells as well as the independent occurrence of the oriented and the kinetic responses.

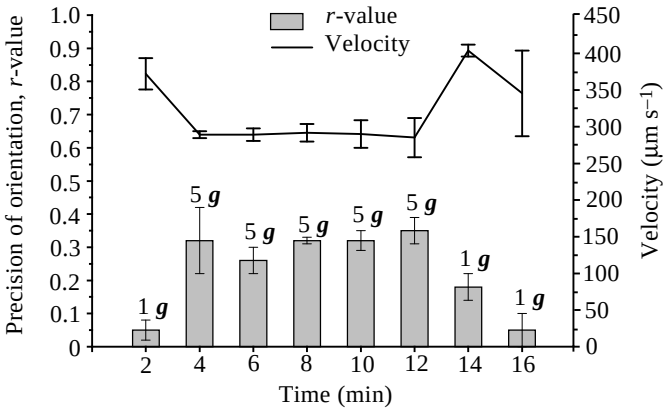

Fig. 5. Summary of the results of four experimental runs. Randomly oriented cells of a culture of *Paramecium biaurelia* were exposed to 5***g*** for 10 min. Under hypergravity (5***g***), the precision in gravitaxis (columns) increased and the mean upward swimming velocities (solid line) decreased. No adaptation occurred during the experimental runs. Each point represents more than 1000 tracks. Values are means ± S.E.M.

were exposed to 5***g*** for 10 min (Fig. 5). Hypergravity induced negative gravitaxis and a significant decrease in the mean upward swimming velocity. Both responses were maintained throughout the exposure to increased gravity.

Response to accelerations below 1 **g**

A cell culture cultivated in a 1***g*** reference centrifuge in space was transferred to the centrifuge microscope and accelerated to 1***g*** (Fig. 6) which induced negative gravitaxis, indicated by an *r*-value of 0.26 (θ=29 °, *N*=693), comparable to the values obtained on Earth (Fig. 2). During stepwise deceleration, negative gravitaxis became less precise and could no longer be measured at 0.16***g***, when the *r*-value had decreased to 0.02 (*N*=1409) (Figs 6, 7C). Randomness was tested by calculating of the statistical value *z*. In microgravity (0***g***), after stopping the centrifuge, an apparent orientation was measured. However, since the preferred direction of the culture was 177 °, this value does not provide information on the threshold for gravitaxis (Fig. 7D). The orientation might be induced by a temperature gradient (see below). Acceleration to 1***g*** in the last step re-established negative gravitaxis, indicated by an *r*-value of 0.33 (θ=26 °). The results indicate that the minimum acceleration required to induce negative gravitaxis was below 0.3***g*** and above 0.16***g***. The occurrence of thermoconvection in the cuvettes during acceleration means that the data for the swimming velocities must be considered with care. The significant difference between upward and downward velocities, which was registered at 1***g***, was no longer visible at 0.6***g*** (data not shown).

Discussion

The graviresponses of *Paramecium biaurelia* consist of two components: an oriented turn (gravitaxis) and regulation of the swimming velocity (gravikinesis). Measurements at 1***g*** revealed that the sedimentation velocity (82 μm s^{-1} for *Paramecium biaurelia*; Hemmersbach-Krause *et al.* 1993*b*) is partially compensated for in negative gravitactic cells by an increase in their active upward swimming velocity (Machemer *et al.* 1991). Bräucker *et al.* (1994) demonstrated a threefold 'quasi-linear' increase in sedimentation rate between 1***g*** and 5***g***, assuming that Stokes law does not apply completely for these large organisms since otherwise a fourfold increase would be expected. Nevertheless, our data show that the net upward swimming velocity at 5***g*** only decreased by a maximum of 2.5 times the sedimentation velocity at 1***g***, indicating that the cells had increased their active upward swimming velocity and thus their gravikinetic response.

Application of hypergravity showed that gravitaxis and

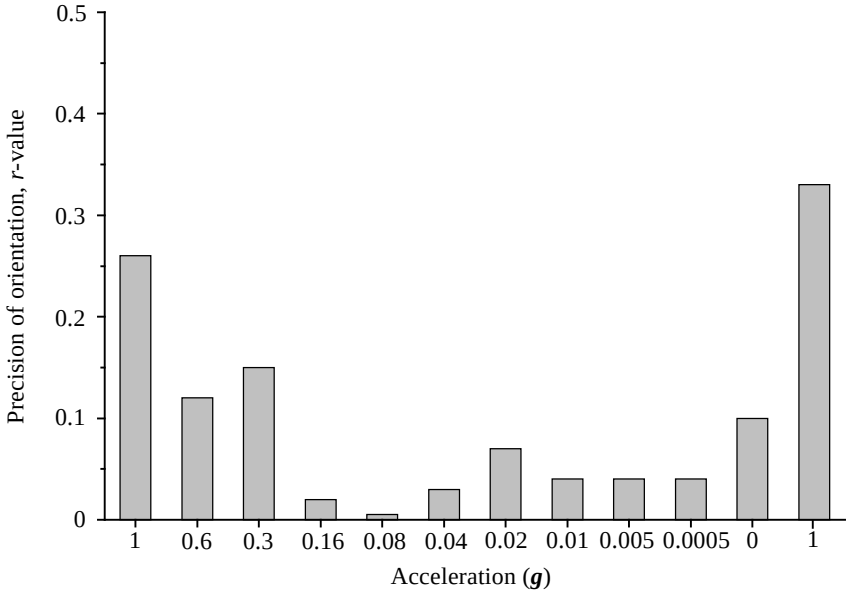

Fig. 6. The precision of orientation (*r*-value) of negative gravitaxis of *Paramecium biaurelia* in relation to a deceleration profile (***g***) ranging from 1***g*** to microgravity '0***g***'. Within each of the 5 min acceleration steps, more than 690 tracks were registered (also see Fig. 7).

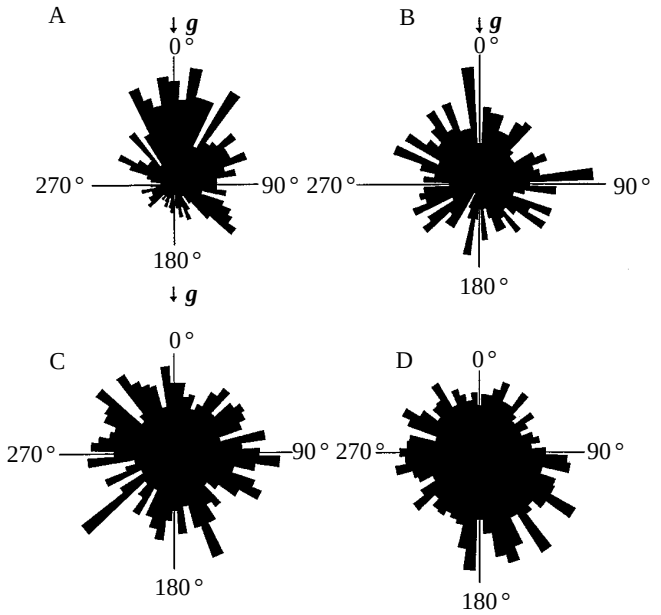

Fig. 7. Representative circular histograms of track orientation of *Paramecium biaurelia* from the experiment carried out in space at 1 ***g*** (A), 0.6 ***g*** (B) and 0.16 ***g*** (C) and '0 ***g***' (D). During deceleration (each step lasted for 5 min), negative gravitaxis decreased and it could no longer be measured at 0.16 ***g***. The largest sector in A represents 4 % of the total tracks (*N*=693); in B, 3 % (*N*=760); in C, 2 % (*N*=1406); and in D, 2 % (*N*=1705) (see also Fig. 6).

gravikinesis increased, but with obviously different time courses, supporting data from Machemer *et al.* (1993), who stated that there was no 'direct relationship between graviorientation and gravikinesis'. The question of adaptation to the stimulus (gravity) is difficult to resolve and results given in the literature are controversial. The parameters investigated by our experimental design did not adapt to changes in gravity, supporting data for *Paramecium caudatum* (Ooya *et al.* 1992), for *Loxodes striatus* (Machemer-Röhnisch *et al.* 1993), for *Euglena gracilis* (Häder *et al.* 1995) and even for vertebrates in the case of *Xenopus laevis* (Neubert *et al.* 1994). Machemer *et al.* (1993), Machemer-Röhnisch *et al.* (1993) and Bräucker *et al.* (1994) stated that gravikinesis did not adapt, but that gravitaxis did adapt, on the basis of the observation that gravitaxis declined over several hours at 1 ***g***. These results are in contrast to our observations and to those reported for *Euglena gracilis* (Lebert and Häder, 1996), because the precision of gravitaxis increased with time after filling the cells in the observation chambers. Further experiments will be necessary to show whether these differences are a result of experimental design, e.g. the materials used to construct the observation chamber and differences in oxygen permeability.

By using a centrifuge microscope in space, it was possible to observe swimming at accelerations below 1 ***g*** and to determine for the first time the lowest acceleration necessary to maintain the gravitactic response. A random distribution of *Paramecium biaurelia* occurred at and below 0.16 ***g***. This threshold value is within the range of values obtained for other species, e.g. *Euglena gracilis*, with the same experimental set-up (Häder *et al.* 1995). Use of the term 'threshold' implies that gravitaxis is not a result of buoyancy, but rather the result of a physiological signal transduction chain as indicated by previous results (see below); however, this has not been unequivocally demonstrated in *Paramecium*. A rigorous discrimination between the first sign of directivity due to the rising acceleration and the first sign of gravisensory transduction is difficult and needs further experimental or theoretical approaches.

The mechanism underlying the gravity-dependent behaviour of *Paramecium* has been under discussion for many years, with either a pure passive physical mechanism being proposed or a physiological mechanism involving a sensory transduction pathway (for a review, see Bean, 1984; Machemer and Bräucker, 1992). Electrophysiological studies revealed a characteristic distribution of specific ion channels in the membrane of *Paramecium*, with depolarizing Ca^{2+} channels in the anterior region and hyperpolarizing K^+ channels in the posterior one (Naitoh, 1984; Machemer, 1988). Mechanical stimulation of these channels induces either a decrease in the frequency of the ciliary beat (depolarization) or an increase in the frequency (hyperpolarization). This lead to the hypothesis for gravikinesis that the faster active forward swimming rate in upward-swimming cells and the decreased forward swimming rate in downward-swimming cells are the result of differential ion stimulation. It is proposed that the whole cytoplasm acts as a statolith by exerting a distinct pressure of approximately 0.1 Pa on the lower cell membrane (Machemer *et al.* 1991), thus initiating a signal transduction cascade by stimulation of specific ion channels. If stimulus saturation and, thus, maximum activation of the channels is not achieved at 1 ***g***, the increase in pressure under hypergravity should increase gravikinesis, as shown by the present data, which support the results of Bräucker *et al.* (1994). It is not clear whether gravitaxis is controlled by the same mechanism. The precision of orientation under hypergravity and the maintenance of reactivity during repetitive stimulation provide no information on the physiological mechanism involved and could be explained by the buoyancy principle. However, the variability in the degree of gravitaxis shown by different cultures, the variable orientations of sedimentating *Paramecium* cells (Kuznicki, 1968; R. Hemmersbach, personal observations), the changes in the directionality of gravitaxis in *Euglena gracilis* induced by heavy metals (Stallwitz and Häder, 1994) and the inhibition of gravitaxis by the stretch-activated channel blocker gadolinium in *Euglena gracilis* (Lebert and Häder, 1996) are strong indications that purely physical mechanisms are not sufficient to explain the complex responses to gravity. Although *Paramecium* and *Euglena* have no specific statocyst organelle, such as the Müller organelle in *Loxodes*, common cellular structures are able to take over this function as has been shown in plant systems (Björkman, 1988; Buchen *et al.* 1993; Sack, 1991; Sievers and Volkmann, 1979; Sievers *et al.* 1991). Further experiments will be necessary to identify the perception/transduction mechanism. In this context,

hypergravity experiments which increase weak graviresponses are a promising experimental approach and could be used, for example, to investigate the behaviour of genetic mutants, the effects of density-adjusted medium and the effects of channel blockers.

We are indebted to W. Briegleb both for fruitful discussions and for technical assistance. The authors thank B. Bromeis, I. Block and A. Wolke for skilful technical support. Our special thanks are due to the astronauts of the IML-2 mission for their devoted work during training and in the performance of the experiment. We would also like to thank the teams of DORNIER, MUSC and DARA for support. We thank NASA for the opportunity of performing this experiment in space.

References

BATSCHELET, E. (1965). Statistical methods for the analysis of problems in animal orientation and certain biological rhythms. In *Animal Orientation and Navigation* (ed. S. R. Galles, K. Schmidt-Koenig, G. J. Jacobs and R. F. Belleville), pp. 61–91. Washington: NASA.

BATSCHELET, E. (1981). Circular statistics in biology. In *Mathematics in Biology* (ed. R. Sibson and J. E. Cohen). London, New York: Academic Press.

BEAN, B. (1984). Microbial geotaxis. In *Membranes and Sensory Tansduction* (ed. G. Colombetti and F. Lenci), pp. 163–198. New York: Plenum Press.

BJÖRKMAN, T. (1988). Perception of gravity by plants. *Adv. bot. Res.* **15**, 1–41.

BRÄUCKER, R., MACHEMER-RÖHNISCH, S. AND MACHEMER, H. (1994). Graviresponses in *Paramecium caudatum* and *Didinium nasutum* examined under varied hypergravity conditions. *J. exp. Biol.* **197**, 271–294.

BUCHEN, B., BRAUN, M., HEJONOWICZ, Z. AND SIEVERS, A. (1993). Statoliths pull on microfilaments. *Protoplasma* **172**, 38–42.

COLOMBETTI, G. (1990). New trends in photobiology: photomotile responses in ciliated protozoa. *J. Photochem. Photobiol.* B **4**, 243–259.

GREENWOOD, J. A. AND DURAND, D. (1955). The distribution of length and components of the sum of n random vectors. *Ann. math. Stat.* **26**, 233–246.

HÄDER, D.-P. (1985). Photomovement in *Cyanophora paradoxa. Archs Microbiol.* **143**, 100–104.

HÄDER, D.-P. (1987). Polarotaxis, gravitaxis and vertical phototaxis in the green flagellate, *Euglena gracilis. Archs Microbiol.* **147**, 179–183.

HÄDER, D.-P. AND LEBERT, M. (1985). Real time computer-controlled tracking of motile microorganisms. *Photochem. Photobiol.* **42**, 509–514.

HÄDER, D.-P., ROSUM, A., SCHÄFER, J. AND HEMMERSBACH, R. (1995). Gravitaxis in the flagellate *Euglena gracilis* is controlled by an active gravireceptor. *J. Plant Physiol.* **146**, 474–480.

HÄDER, D.-P. AND VOGEL, K. (1991). Simultaneous tracking of flagellates in real time by image analysis. *J. math. Biol.* **30**, 63–72.

HÄDER, D.-P., VOGEL, K. AND SCHÄFER, J. (1990). Responses of the photosynthetic flagellate, *Euglena gracilis*, to microgravity. *Micrograv. Sci. Technol.* **3**, 110–116.

HEMMERSBACH-KRAUSE, R., BRIEGLEB, W. AND HÄDER, D.-P. (1991). Dependence of gravitaxis in *Paramecium* on oxygen. *Eur. J. Protistol.* **27**, 278–282.

HEMMERSBACH-KRAUSE, R., BRIEGLEB, W., HÄDER, D.-P., VOGEL, K., GROTHE, D. AND MEYER, I. (1993*a*). Orientation of *Paramecium* under the conditions of weightlessness. *J. Euk. Microbiol.* **40**, 439–446.

HEMMERSBACH-KRAUSE, R., BRIEGLEB, W., VOGEL, K. AND HÄDER, D.-P. (1993*b*). Swimming velocity of *Paramecium* under the conditions of weightlessness. *Acta protozool.* **32**, 229–236.

JENNINGS, H. S. (1906). *Behavior of the Lower Organisms*. London, Bloomington: Indiana University Press.

KUZNICKI, L. (1968). Behavior of *Paramecium* in the gravity field. I. Sinking of immobilized specimens. *Acta protozool.* **6**, 109–117.

LEBERT, M. AND HÄDER, D.-P. (1996). How *Euglena* tells up from down. *Nature* **379**, 590.

MACHEMER, H. (1988). Electrophysiology. In *Paramecium* (ed. H.-D. Görtz), pp. 185–215. Berlin: Springer-Verlag.

MACHEMER, H. AND BRÄUCKER, R. (1992). Graviperception and graviresponses in ciliates. *Acta protozool.* **31**, 185–214.

MACHEMER, H., MACHEMER-RÖHNISCH, S. AND BRÄUCKER, R. (1993). Velocity and graviresponses in *Paramecium* during adaptation and varied oxygen concentrations. *Arch. Protistenkunde* **143**, 285–296.

MACHEMER, H., MACHEMER-RÖHNISCH, S., BRÄUCKER, R. AND TAKAHASHI, K. (1991). Gravikinesis in *Paramecium*: theory and isolation of a physiological response to the natural gravity vector. *J. comp. Physiol.* A **168**, 1–12.

MACHEMER-RÖHNISCH, S., BRÄUCKER, R. AND MACHEMER, H. (1993). Neutral gravitaxis of gliding *Loxodes* exposed to normal and raised gravity. *J. comp. Physiol.* A **171**, 779–790.

NAITOH, Y. (1984). Mechanosensory tranduction in Protozoa. In *Membrane and Sensory Transduction* (ed. G. Colombetti and F. Lenci), pp. 113–135. New York, London: Plenum Press.

NEUBERT, J., SCHATZ, A., BROMEIS, B. AND BRIEGLEB, W. (1994). The reaction of *Xenopus laevis* Daudin (South African toad) to linear accelerations. *Adv. Space Res.* **14**, 299–303.

OOYA, M., MOGAMI, Y., IZUMI-KUROTANI, A. AND BABA, S. A. (1992). Gravity-induced changes in propulsion of *Paramecium caudatum*: A possible role of graviperception in protozoan behaviour. *J. exp. Biol.* **163**, 153–167.

SACK, F. D. (1991). Plant gravity sensing. *Int. Rev. Cytol.* **127**, 193–252.

SIEVERS, A., BUCHEN, B., VOLKMANN, D. AND HEJNOWICZ, Z. (1991). Role of the cytoskeleton in gravity perception. In *The Cytoskeletal Basis of Plant Growth and Form* (ed. C. W. Lloyd), pp. 169–182. London: Academic Press.

SIEVERS, A. AND VOLKMANN, D. (1979). Gravitropism in single cells. In *Physiology of Movements* (ed. W. Haupt and M. E. Feinleib), pp. 567–572. Berlin: Springer Verlag.

STALLWITZ, E. AND HÄDER, D.-P. (1994). Effects of heavy metals on motility and gravitactic orientation of the flagellate, *Euglena gracilis*. *Eur. J. Protistol.* **30**, 18–24.

VAN HOUTEN, J. (1992). Chemosensory transduction in eukaryotic microorganisms. *A. Rev. Physiol.* **54**, 639–663.

VAN HOUTEN, J. AND PRESTON, P. R. (1988). Chemokinesis. In Paramecium (ed. H. D. Görtz), pp. 282–300. Berlin, Heidelberg: Springer Verlag.