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Comparative studies on gravisensitive protists on ground (2D and 3D clinostats) and in microgravity

In order to prepare and support space experiments, 2D and 3D clinostats are widely applied to study the influence of simulated weightlessness on biological systems. In order to evaluate the results a comparison between the data obtained in simulation experiments and in real microgravity is necessary. We are currently analyzing the gravity-dependent behavior of the protists Paramecium biaurelia (ciliate) and Euglena gracilis (photosynthetic flagellate) on these different experimental platforms. So far, first results are presented concerning the behaviour of Euglena on a 2D fast rotating clinostat and a 3D clinostat as well as under real microgravity conditions (TEXUS sounding rocket flight), of Paramecium on a 2D clinostat and in microgravity. Our data show similar results during 2D and 3D clinorotation compared to real microgravity with respect to loss of orientation (gravitaxis) of Paramecium and Euglena and a decrease of linearity of the cell tracks of Euglena. However, the increase of the mean swimming velocities, especially during 3D clinorotation (Euglena) and 2D clinorotation of Paramecium might indicate a persisting mechanostimulation of the cells. Further studies including long-term 2D and 3D clinostat exposition will enable us to demonstrate the qualification of the applied simulation methods.

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Introduction

In order to vary the influence of gravity, different experimental and technical approaches have been followed and developed. Gravitational biological experiments can be performed on ground by means of e.g. clinostats and centrifuges and in real microgravity (e.g. during a space flight). Ground-based studies should be a prerequisite before performing an expensive experiment in space. They are essential for the preparation of space experiments and for verification of results obtained in microgravity. In order to achieve the status of functional weightlessness on ground, clinostats, which are rotating platforms, are used. However, different concepts of clinostats exist - 2D and 3D clinostats. On a 2D clinostat the object is rotated around one axis perpendicular to the force of gravity, and the maximal residual acceleration can be calculated by the diameter of the available space (which should be small) for the swimming cells with respect to the center of rotation and the rotation speed [1, 2, 3].

In a 3D clinostat the object rotates around two axes in order to provide a status of "vector averaged gravity" [4, 5]. In order to show the validity of the different types of clinostats a comparison of data obtained during the simulation experiments and under real microgravity conditions is absolutely necessary. Thus, we started to perform comparative studies with "professional gravisensing cells" in real microgravity and in different clinostat experiments on ground. According to the current theory the chosen protists – the ciliate *Paramecium* and the alga *Euglena* - perceive the gravity vector by perception of their own cell mass: their cell mass, being heavier than the surrounding medium, exerts pressure onto the lower membrane and stimulates mechanosensitive ion channels in the cell membrane, gating the ion influx, in turn changing the membrane potential and finally modifying the motility pattern of the cilia (flagella). Mechanical and gravi-stimulation of the cells can be directly visualized by changes in their swimming behavior (swimming speed, swimming direction, linearity of cell tracks) [3].

Methods

Cultures of *Paramecium biaurelia* and *Euglena gracilis* were used for the experiments as described before [6, 7].

Both clinostats were equipped with video devices; thus, the impact of accelerations on the behavior of the cells could be analyzed by real-time image analysis [3]. In the 2D clinostat microscope at DLR (Cologne) the object is rotated around one axis perpendicular to the force of gravity. Under the chosen clinostat conditions (Ø 5 mm for the observation window, 50 rpm) the maximum centrifugal force reaches 7 x 10^{-3} x g at the outer perimeter of the observed area. The 3D clinostat rotates around two axes. The maximum centrifugal force is 2 x 10^{-2} x g for the observation window (Ø 2 mm) (observation chamber: Ø 50 mm x 0.2 mm) at 50 rpm for both axes. Clinostat data were compared to data from 7 min lasting microgravity experiments on sounding rockets (TEXUS) [6 -10]. A typical flight profile of a TEXUS – rocket is the following: the ascent phase for about 6 s with a mean acceleration of 5 x g, the microgravity time of 360 s and the reentry with an acceleration of about 26 x g. The precision of orientation of the cells was quantified by the r-value, which varies between +1 (highest precision of orientation with respect to the gravity vector) and 0 (random distribution) [11].

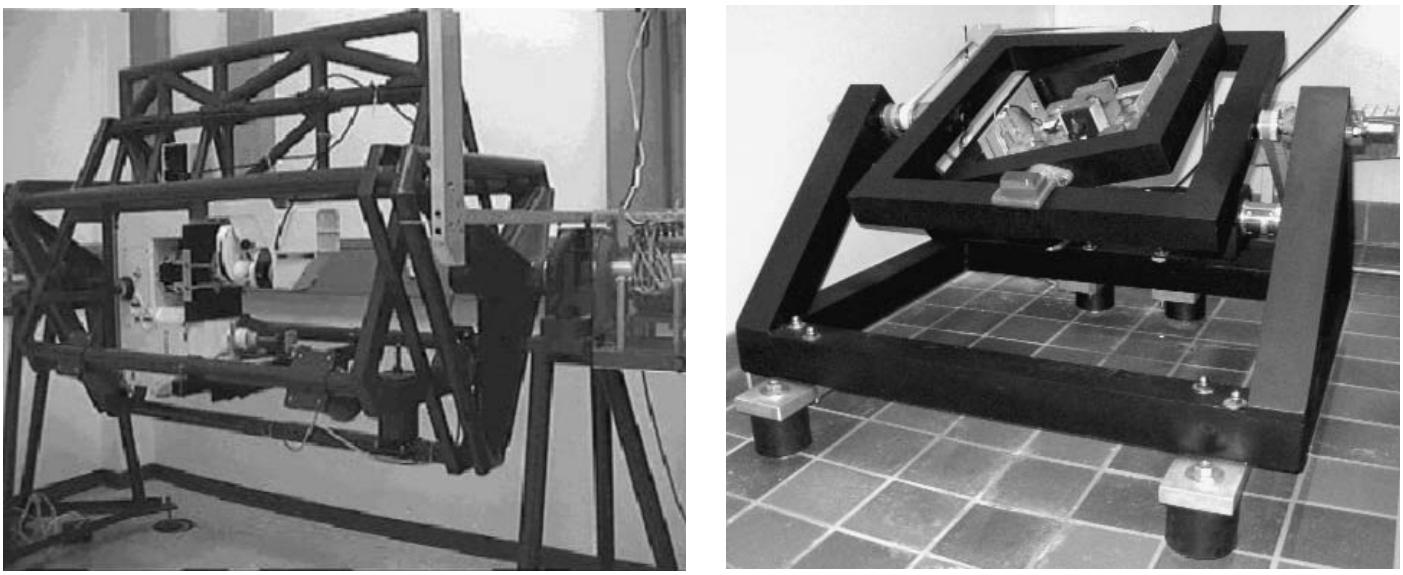

Fig. 1: 2D clinostat (DLR, Cologne, Germany) and a 3D clinostat (University of Erlangen, Germany)

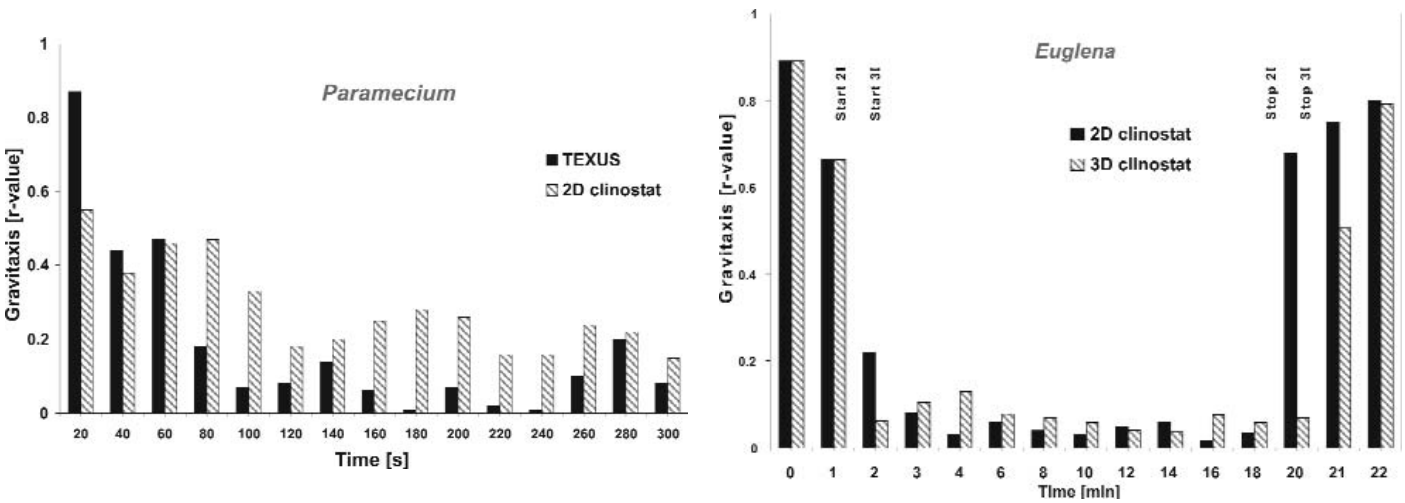

Fig. 2: Relaxation of the degree of orientation (gravitaxis) of Paramecium under real microgravity compared to 2D clinorotation (average of 5 experiments) and of Euglena in the 2D and the 3D clinostat, modified from [6, 7, 12].

Results

Gravitaxis

Under 1 x g conditions *Paramecium biaurelia* shows negative gravitaxis with a high precision of orientation as demonstrated by the r-value (Fig. 2). After 80 s in real microgravity (TEXUS) or after 120 s on the 2D clinostat random swimming of *Paramecium* was registered [6]. Similar studies with *Paramecium* still have to be performed on a 3D clinostat. *Euglena gracilis* also shows a pronounced negative gravitaxis under 1 x g, while a similar behavioral change - random distribution of the cells in the 3rd min of rotation - is observed in the 3D clinostat (r-value < 0.2) and the 2D clinostat (r-value < 0.1), as well as in real microgravity [7, 9, 10, 12], here already after about 1 min. The time course of the changes of the degree of orientation is shown in Fig. 2.

Swimming velocities

Euglena gracilis swims faster under real microgravity conditions than at 1 x g. An increased swimming velocity is also registered in the clinostat experiments, however it is less pronounced on the 2D clinostat compared to 3D clinostat conditions [12]. In the case of *Paramecium* a transient increase in the swimming velocity for about 3 min was measured in real microgravity, then the swimming velocity approached the value of the former horizontal swimming velocity under 1 x g conditions [8]. In contrast, the mean swimming velocity remained permanently increased under 2D clinorotation, even during a 2-h lasting 2D clinostat experiment [8].

Linearity of the cell tracks

Under 1 x g conditions *Paramecium* and *Euglena* show a high degree of linearity with respect to their swimming tracks. In real microgravity as well as on the 2D clinostat no significant change in the degree of linearity was measured in the case of *Paramecium* [6], however the linearity decreased under both conditions in the case of *Euglena*, indicating course corrections [7].

Discussion

On a 2D and 3D clinostat the direction of the gravity vector continuously changes with respect to the sample. Whether permanent stimulation or signal cancellation is achieved depends on different parameters, e.g., the threshold for accelerations of the perceiving system. It is mandatory to conduct a critical evaluation with all specimens on the different types of clinostats in order to meet the specific requirements and to be able to select the most suitable one. So far, our initial studies show comparable results during 2D and 3D clinorotation and real microgravity with respect to the loss of orientation (gravitaxis). However, the pronounced increase of the mean swimming velocities, especially during 3D clinorotation of *Euglena* and 2D clinorotation of *Paramecium* might indicate a persisting mechanostimulation of the cells. Using different cell systems (human cells and plant systems), a few experimenters had the possibility to perform comparative studies on 2 D, 3 D clinostats and in real microgravity [5, 3]. In case of T cells similar results have been obtained on the different experimental approaches [5]. Comparative studies on 2D clinostats and real microgravity on various systems also revealed similar results, though the response in actual microgravity appears to be more pronounced and faster [3].

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