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GRAVITATIONAL RESPONSE OF THE SLIME MOLD *PHYSARUM*

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ABSTRACT

The acellular slime mold *Physarum polycephalum* is used as a model system to investigate the graviresponse of single cells which possess no receptors specialized for the perception of gravity. To obtain insights into the gravity-signal transduction mechanism the light response of the cell is used: Macroplasmodia of the slime mold show clear geo- and phototaxes. Gravity increases and white light decreases transiently the contraction frequency of plasmodial strands whereby both responses follow the same time pattern. Since mitochondria play a major role in changing the contraction rhythm in response to light and gravity stimuli, the simultaneous and subsequent inductions of the opposing light and gravity responses and their mutual influences on one another were investigated. The experiments were performed in weightlessness (0 g) - simulated on the fast-rotating clinostat as well as in actual weightlessness during the IML-1 Space-Shuttle mission. The results indicate that mitochondria (chondriome) are part of the acceleration-stimulus reaction chain in *Physarum*. Two models for a direct gravireceptor mechanism are discussed.

INTRODUCTION

In gravitational biology the influence of gravity on living organisms is investigated. Especially interesting is that some free living single cells use gravity - among other stimuli - for their orientation, they show a geo- or gravitaxis /1,2/. Those cells are useful model systems in considering the influences of gravity and accelerations on cells /3/, in particular those cells expressing an ameboid movement like human embryonic, nerve, and white blood cells /4,5,6/. There are indications that every cell, even if it has no special g-receptor, is able to respond to gravity, that is, there seemingly exists a general gravisensitivity of cells /6/. To investigate if and how a cell without a specialized structure for the perception of gravity can nevertheless perceive and process a g-stimulus we chose a free-living ameboid cell, the acellular slime mold *Physarum polycephalum (Myxomycetes)*, as a model system.

The Slime Mold *Physarum polycephalum*

The natural habitat of this true slime mold is the forest soil, where it grows and moves between rotting wood and leaves, where it degradates dead plant material and lives on bacteria. The single cell ordinarily varies in size between 1 and 20 cm in diameter and contains millions of nuclei, thus representing a so-called plasmodium. A plasmodium is differentiated into an advancing front zone and a rear region, the latter composed of a network of protoplasmic strands /7,8/. The strands consist of a gel-like ectoplasmic tube enclosing a channel with streaming endoplasm. In the ectoplasmic wall a system of cytoplasmic actomyosin fibrils is spatially arranged in such a way that their coordinated activity generates rhythmic contractions and dilatations of the ectoplasmic tube /7,8,9/. The rhythmic contractions in the minute range generate the motive force for a vigorous endoplasmic shuttle streaming, which is a hydrostatic pressure-flow system /10/. The highly regulated contractile activity induces an excess transport of protoplasm in one direction and thus determines the direction of the cell's

locomotion /11,12/. External stimuli modify the contraction rhythm of the strands, which leads to an altered streaming behavior, which in turn alters the speed and direction of locomotion of the whole cell. Examples of stimuli which lead to positive as well as negative taxic responses in *Physarum* are: light, temperature, humidity, chemicals, mechanical forces, and, last but not least, gravity /7,13,14,15/.

Physarum is one of the most fascinating systems for studying gravity effects - not only because at least 5 different parameters of its behavior and morphology are modified by gravity, but also because of the strand's regular oscillations which can easily be measured. Since they show immediate responses to external stimuli /16/, the oscillations are ideally suited to reveal even subtle reactions induced by only weak stimuli. - This actually was one of the main reasons we selected the slime mold for our gravitational-biology research.

Review of the Gravitational Responses of *Physarum polycephalum*

The first parameter which should be tested when looking for gravity effects on migrating cells is the direction of locomotion, to reveal whether the object displays a geotaxis, that is a gravity-oriented movement, or not. On a vertically positioned substratum plasmodia follow the gravity vector (positive geotaxis /15/). After vertically turning the plasmodia 90°, they again react with positive geotaxis - this response can be elicited several times /15/. When stimulated with a 180°-turn, plasmodia perform a U-turn in the attempt to follow the gravity vector /17/. The fact that they not just reverse their direction of locomotion, but perform a U-turn strongly indicates the active character of the response. Under submerged conditions a reversal of the sign of geotaxis can be observed: Submerged plasmodia show negative geotaxis exclusively - and this behavior does not change when the oxygen concentration is homogeneous or is raised (by pressurizing the medium with oxygen) to 21%, that is to the same level as in the atmosphere /18/; so the reversal of the sign of geotaxis during submersion cannot be attributed to a reduced energy supply. The fact that submerged plasmodia, though they are of slightly higher density than water, move upwards - even at accelerations of 20 to 40 g /19/ - also stresses the active character of the reaction.

This taxic behavior of *Physarum* allows the cell to find optimal conditions for living, if other stimuli are lacking or are constant. With the aid of the positive geotaxis it can move downwards into the humid forest soil beneath decaying wood and leaves. The negative geotaxis offers the cell the opportunity - a) to escape from submerged regions in the forest soil in search for better growth conditions, or - b) to sporulate.

As mentioned above the highly regulated rhythmic contractions induce an excess transport of protoplasm in one direction and thus determine the direction of the cell's movement. Since gravity influences the direction of locomotion of the slime mold, an influence of gravity on the contractile and streaming behavior should exist and, in fact, could be demonstrated: When checking a cell for gravisensitivity the other essential experiment, besides testing geotaxis, is to look for any cellular response upon a horizontal turn. So a plasmodium was allowed to grow under normal gravity conditions on a horizontal surface and the contraction frequency was observed in a vertical microscope. Then the plasmodium was horizontally rotated by 180° /20/. Turning a gravisensing organism upside down is expected to be a strong stimulus. Following the 180°-horizontal turn there is a striking decrease in period duration (this is an increase in frequency) followed by a slow return to the initial (baseline) values. This return to baseline values is accompanied by strong oscillations of the mean values as an expression of ongoing frequency regulation phenomena. Additionally, during the whole regulation time, remarkably high standard deviations are observed indicating a less regular contractile behavior. So the relative change in the direction of the gravity vector leads to a transient decrease in contraction period and, after an active backregulation, the periods return to their baseline values /20/.

After establishing the gravisensitivity of the cell in demonstrating its geotactic behavior and its response to a horizontal turn, the next step is to look for what would happen, if the gravity vector turns relatively, continuously, and so fast that it is no more effective. This describes the fast-rotating clinostat /21,22,23/: In a resting suspension at normal gravity conditions all particles with a density higher than that of the liquid will follow the gravity vector, i.e., they settle. In a suspension rotating around a

horizontal axis, the direction of sedimentation of dense particles is constantly changing so that they move in circular paths. During a slow rotation of 1 to 10 revolutions per minute - slow-rotating (classical) clinostat - this circular movement of particles, or dense organelles in cells, presents a constant stimulation which could damage or even destroy a sensitive gravireceptor (as is the case for plant root statocytes /24/). With increasing speed of rotation (greater than about 10 rpm), the circular paths of dense organelles become gradually smaller, until all gravity-induced movements stop - thus producing a stimulus-free condition. This is a condition of functional weightlessness, which is achieved near the axis of a fast-rotating clinostat, where centrifugal forces are below an acceleration threshold of a cell or an organism. In addition the quality of the simulation depends upon the density difference of a particular particle to the surrounding medium and the viscosity of that medium. - So, the fast rotation represents a stimulus-free condition, whereas the slow rotation represents the strongest possible disturbance that gravity can exert on cells, i.e., gravity effects found on the slow-rotating clinostat are not effects of weightlessness. Nevertheless, the slow-rotating clinostat is a valuable tool with which to identify gravisensitivity of cells and small organisms.

During simulated functional weightlessness (0 g) the contraction frequency of *Physarum* plasmodia was observed. The experiments, which provide the basis for our space experiments, revealed an immediate decrease in period duration and increase in standard deviation starting after the transition from 1 g (normal Earth's gravity) to simulated 0 g /20/. A minimum period (maximum response) is reached after 20 min. Despite continuous 0 g-conditions a backregulation of the periods to the baseline values is then performed. The whole response is very similar to but considerably weaker as the one observed after the 180° horizontal turn. This may be attributed to the fact that a relative change in direction of the gravity vector represents a stronger stimulus than the stimulus-free condition. After termination of 0 g a long lasting disturbance of the periods is observed.

An interesting phenomenon concerning the time course of the response is that the period starts to decrease immediately after the onset of simulated 0 g and reaches its minimum 20 minutes later. Then the cell performs a backregulation and subsequently the periods return to the baseline despite continuous 0 g-conditions. This clearly demonstrates the active character of the reaction. This particular type of response can be observed also in the light response of *Physarum* /16/.

In further 0 g-simulation experiments the endoplasmic shuttle streaming was registered /25/. An evaluation of the streaming velocity revealed a prominent increase after about one hour of 0 g. Here, too, oscillations of the mean values as well as high standard deviations were observed, again indicating the involvement of regulatory processes. Our D1-space experiment, where only two 5-min sequences of cinecamera registration were performed to register the streaming behavior, supports the result obtained on the fast-rotating clinostat /26/.

Two parameters which seem not to be correlated with motility and geotaxis also show graviresponses:

[1] The morphologic polarity of plasmodial strands depends on gravity: Cross sections of strands growing under normal gravity conditions on a horizontal surface revealed that the ectoplasmic wall, which encloses the streaming endoplasm, is much thinner on the lower side than on the upper side of the strand. The same can be demonstrated in strands hanging on an inversed horizontal surface /27/: The ectoplasm is still thinner on the lower side and the endoplasmic channel seems to have moved downwards in direction of the gravity vector (for hypotheses explaining this phenomenon see /28/). No experiments concerning the polarity have been performed so far in submerged or under 0 g-conditions.

[2] The time course of the synchronous mitosis of the nuclei is affected by gravity: A 0 g-simulation conducted on the fast-rotating clinostat resulted in a significant acceleration of mitosis /29/. Specifically metaphase was significantly shortened (by about 50%) compared to 1 g-controls. In contrast to the other experiments, where macroplasmodia were used (big plasmodia crawling on a surface and containing millions of nuclei) here microplasmodia were used: these are tiny plasmodia growing in a liquid shake culture and containing only a few nuclei. When put on agar and slightly squeezed, the nuclei can still be moved around by the endoplasmic streaming. During mitosis, the streaming is strongly reduced so that the mitosis of the now mostly fixed nuclei can be observed.

Summary of this short review: The gravisensitivity of *Physarum* plasmodia was established by subjecting the cells [1] to 0 g, simulated on the fast-rotating clinostat, [2] to single horizontal turns in the Earth's gravity field, and [3] to higher accelerations (provided by the NIZEMI, a slow-rotating centrifuge microscope): The experiments revealed that 5 parameters of the slime mold's behavior and morphology are influenced by gravity: *Physarum* uses gravity for its spatial orientation (geo/gravitaxis); gravity also influences the contraction frequency of plasmodial strands, the velocity of protoplasmic streaming in strands, the morphologic polarity of strands, and the time course of mitosis. - On the NIZEMI only a few experiments have been conducted so far, so the result that during higher accelerations (between 1 g and 5 g) the contraction period is prolonged, has to be regarded as preliminary.

To confirm the observed 0 g-response of the contraction rhythm experiments were performed in actual weightlessness: at first in an experiment (one measurement) conducted in 1985 during the D1-Mission (STS 61-A) /26/ and in an extended fashion in January 1992 during the STS-42 Space Shuttle mission carrying the First International Microgravity Laboratory (IML-1).

MATERIALS AND METHODS

/30/ The experiments were performed using the ESA-Biorack light microscope which could be attached to the Biorack-glove box /31/. This microscope was designed by us in cooperation with the ESA-Biorack team and built by Fokker/The Netherlands for D1 and by Bradford/The Netherlands for IML-1. In both missions a photo diode (Kunz, Bonn/Germany; the raw data were downlinked and stored in NASA computers) was attached to the microscope to register the rhythmic contractions of the strands. To record all visible movements including the protoplasmic streaming a cinecamera (in D1) and a video camera (Sony/Japan; in IML-1) respectively was attached to the microscope /26,31/. Before the IML-1 flight a final crew training (refresher training) took place two and three nights prior to launch and involved only Ulf Merbold who was going to perform the experiment in space. This greatly contributed to a successful performance of the experiment, that is, most measurements were started 5-8 min after stopping the 1-g reference centrifuge, thus allowing a registration of nearly the complete 0 g-response.

In the six hours preceding the actual flight experiment some culture/observation chambers, containing small plasmodia, were incubated on the 1 g-reference centrifuge of the Biorack. Then a *Physarum* chamber was removed from the centrifuge and exposed to actual weightlessness. The presented results are based on the complete photo-diode data provided to us in July 1992 (the preliminary evaluations based on the downlinked photo-diode data are currently in press /30/).

RESULTS AND DISCUSSION

0 g-Response

Figure 1 represents the registration and evaluation of the contraction period during actual weightlessness registered during the first of our three experiment sessions. The transition of the sample from 1 g to 0 g (Figure 1a) induces a decrease in period reaching a minimum (maximum response) 21-27 minutes after onset of 0 g. The mean period decreases from 89 to 72 seconds - then, during backregulation, the mean period rises to nearly 98 seconds. This is a stable value, which is maintained even after nearly two hours of weightlessness (Figure 1c, right part): In the time frame of 105 to 130 minutes of 0 g the mean period in the 0 g-adapted slime mold is 98 to 99 seconds. - (Figure 1a): Comparing this 0 g-response with the curve obtained during 0 g-simulation on the fast-rotating clinostat /20/ revealed that in actual 0 g the reaction is more pronounced, lasts slightly longer and is also followed by a backregulation - the overall time patterns of the reactions are identical. Thus the experiment performed in actual 0 g confirms the findings obtained during the D1-mission /26/ and during 0 g-simulations on the fast-rotating clinostat. However, the response to actual 0 g is more pronounced than on the clinostat, which can be attributed to the restricted area of 0 g-simulation on the clinostat. The size of this area depends on the clinostat's rotation speed (which determines the gradient of centrifugal forces) and the acceleration sensitivity and size of the sample. The latter probably often exceeded the critical diameter so that the outer parts of a plasmodium were subjected to centrifugal values exceeding the supposed threshold of the cell's acceleration sensitivity.

Fig. 1 a-c. Original registration and evaluation of the contraction period during actual (near) 0 g. - Abscissa: time of 0 g-exposure (t=0: stop of 1 g-reference centrifuge). - Left ordinate: changes in light intensity in the observation area of the microscope registered by a photo diode in volts. These changes in light transmission reflect the rhythmic contractions (contraction-relaxation cycles) of the strands. An intensity increase corresponds to a contraction, a decrease to a relaxation of the observed strand. The upper solid curve represents the original photo-diode values, the raw data - the superimposed dashed curve was generated partly using a computer program to smooth the raw data. This in turn allowed a computer evaluation of the period lengths and the generation of mean values and standard deviations. - Right ordinate: period lengths in seconds. - Lower solid curve: period lengths in seconds evaluated using the smoothed data. Mean period lengths of distinct intervals are plotted in the diagram together with the corresponding standard deviations.

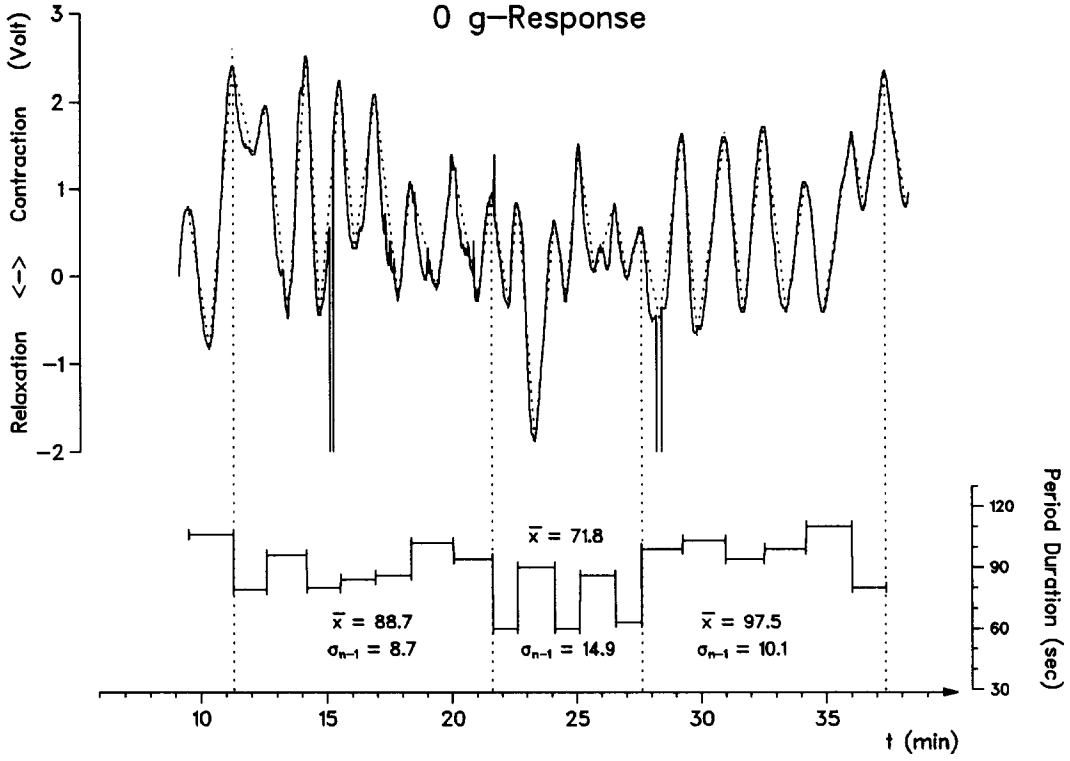

Fig. 1a. 0 g-reaction in red light. Note that the maximum response (minimum periods) is in the range of 20 min after onset of 0 g.

Direct Versus Indirect Effects of Gravity

A separate evaluation of the contraction phases and dilatation phases of the contraction-dilatation cycles of a plasmodium subjected to simulated 0 g revealed /25/ that the response to 0 g is primarily based on changes in the duration of the contraction phase. This could not only be verified in the IML-1 experiment but was also shown for other responses such as the response to light. The duration of the contraction phase depends on the highly regulated action of actomyosin, where undoubtedly mitochondria are involved, at least for providing the primary energy source (ATP) necessary for the contraction. When cellular respiration is experimentally inhibited, not only the 0 g-reaction but also other responses (light response /32/, chemotaxis /33,34/) are inhibited and also the regulation phenomena seem to be reduced /20/. These findings suggest that the mitochondria not only supply most of the energy (glycolysis plays a minor role) necessary for the contraction activity, but they also may be involved in its regulation and perhaps even in the perception of the gravity stimulus /27/. To

consider their role, it is essential to know, whether the observed graviresponses in *Physarum* are active or passive ones and whether they are based on direct or indirect effects of gravity. In a passive response, a physical (or physico-chemical) phenomenon forces the cell to move in a certain direction - for example cells sediment in a liquid medium due to their higher density. - Active responses are those, where the cells actively respond (via a physiological mechanism) to a stimulus. This is seen in *Physarum:* the response of the contraction frequency upon the 180°-horizontal turn as well as upon weightlessness is followed by a backregulation, and - the taxic response upon a 180°-vertical turn is a U-turn performed by the whole plasmodium. These active responses can be caused by effects generated within the cells or by effects resulting from g-influences on the environment of the cells: gravity can change the environment of a cell for example by causing convections and hydrostatic pressure in and stratifications of media. This active response of cells to a changed environment is often referred to as an indirect effect which had brought about some confusion. On the contrary, (for g-effects to be observed in cells) our group has proposed the following definition of g-effects based on the assumption that a cell can actively respond to direct and/or indirect effects of gravity in two different ways /35/: A direct gravity effect is induced by density differences within the cells - this could be the dislocation of a dense particle or the pressure or tension it exerts on its vicinity. The easiest way to discriminate between direct and indirect effects is submersion: During submersion these direct effects remain and the indirect effects mainly disappear - so we conducted all our 0 g-simulation experiments with submerged plasmodia to eliminate the indirect effects. In other words: indirect effects such as hydrostatic effects and deformations caused by the cell's own weight can exclusively be observed in non-submerged cells. This can be clearly demonstrated in *Physarum:* In a plasmodium growing on a vertical plane in air, due to the height of the cell, the hydrostatic gradient is superimposed on the cell-generated pressure (which induces the shuttle streaming): turning the plasmodium vertically 180° induces a distinct downwards dislocation of the endoplasm - but within the fraction of a second this downward movement is stopped by the cell, that is, it actively (or passively) compensates for the dislocation of the endoplasm. Submersion in a liquid medium of nearly the same density as that of the plasmodium totally abolishes this effect. - In all space experiments using land-living organisms direct and indirect g-effects can also not be discriminated - this discrimination is possible only when - [1] comparing flown experiments with submersion experiments conducted on the ground or - [2] by performing flown in-vitro experiments using those organ tissues, where a gravity effect has been observed in space experiments involving the whole organism - then mainly the direct effects remain.

The effects we discovered in *Physarum* are the responses of the oscillations upon the 180° horizontal turn and upon the 0 g-simulation , as well as an accelerated streaming velocity and an accelerated time course of mitosis. Since all these responses were observed in submerged plasmodia, these are all direct effects of gravity. Concerning the geotaxis, a decision between direct and indirect effects seems complicated, since *Physarum* performs in air a positive geotaxis, whereas during submersion it switches to a negative geotaxis. In case the endoplasm moves downwards due to its own weight and thus forces the whole plasmodium to move downwards, then the positive geotaxis would be a passive gravity effect. But then a positive geotaxis would be expected during submersion, too, since the densities of the ecto- and endoplasm are slightly higher than the one of water. However, during submersion *Physarum* moves actively upwards against an acceleration vector (up to 40 g was tested /19/), demonstrating that also the negative geotaxis is a direct active reaction upon gravity (because the oriented movement is not abolished by submersion). This reversal of the sign of geotaxis under changing external conditions can also be detected in other cells - as in the ciliate *Loxodes* /36/ - which also indicates the involvement of actively regulated processes. - Whether the morphologic polarity of the plasmodial strands can be attributed to a direct or indirect effect, could not be established up to now, since this experiment was performed only in air /28/.

So most of the gravity responses of *Physarum* are direct effects. Since direct effects are due to density differences in cells we now can speculate about the gravity receptors. The observed geotaxis implies the existence of a stimulus-reaction chain and a respective receptor! In general, direct gravireceptors have to be denser than the rest of the system, so that under 1 g-conditions they are able to settle or to exert a certain pressure or tension on the cytoskeleton in their vicinity. Under these aspects nuclei, nucleoli, centrioles, dense vacuoles as well as mitochondria have to be taken into account (Table 1).

0 g Versus the Light Response

To obtain information about the gravireceptor the gravity-stimulus transduction mechanism (reaction chain) was examined. In one approach the light response of plasmodia was included in the investigation /16/: An illumination with light containing the shorter wavelengths of the visible spectrum (smaller than 500 nm, i.e., also white light) induces without measurable time lag a transient increase in period duration. The light response reaches a maximum at about 20 minutes after the onset of illumination, then a backregulation is performed and the periods return to the baseline values despite continued illumination. This clearly demonstrates the active character also of the light response.

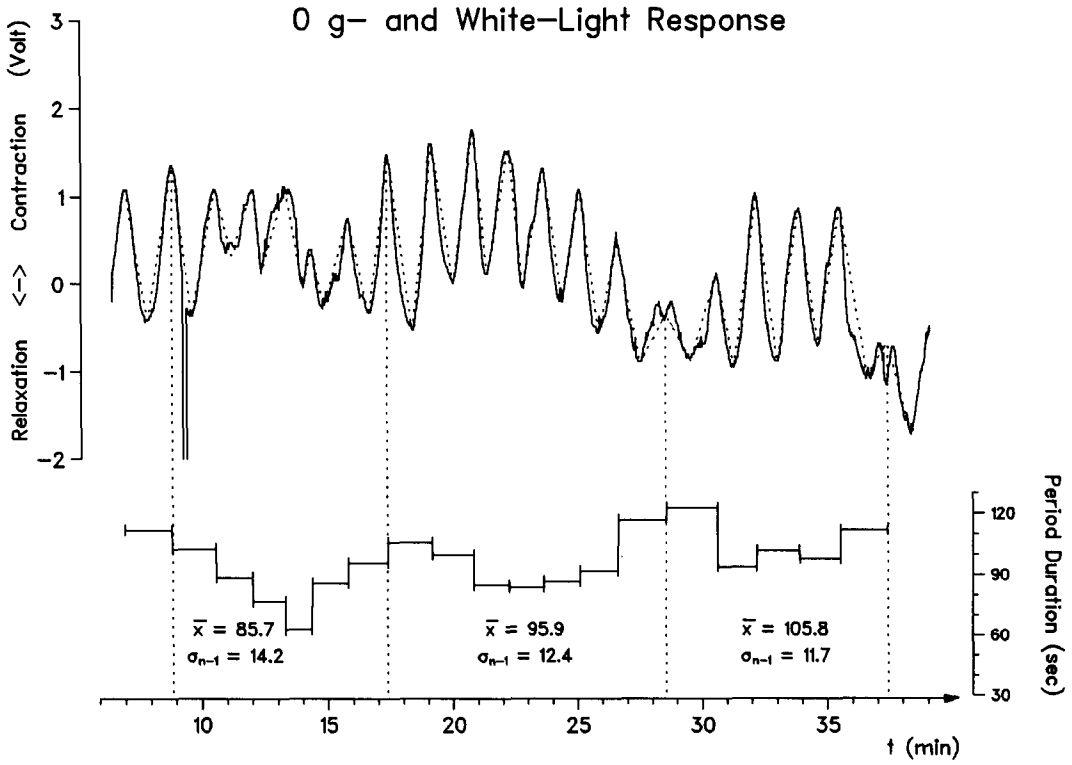

Fig. 1b. 0 g- and white-light response. Note the mutual suppressions of both responses.

It is interesting that both the 0 g- as well as the light reaction follow exactly the same time course with a maximum response after 20 minutes and a backregulation - the only difference is that the two reactions are directed into opposite directions. In addition, there are more similarities found in the two reactions: both responses are restricted to changes in the duration of the contraction phases /25,37/ and both responses can be inhibited by inhibition of respiration /20,32/ - all this indicates the involvement of mitochondria in both responses. - It is also known /38/ that the time-keeping of the contraction rhythm in *Physarum* plasmodia is related to mitochondrial activity. Mitochondria are considered to be the primary oscillator in the slime mold: Oscillating ATP and Calcium levels are responsible for the contraction-relaxation cycles /39,40/. So, if mitochondria indeed play a major role in modulating the contraction rhythm in response to external stimuli - and thus are part of any stimulus-reaction chain in *Physarum* - then any effect of an external stimulus on the contraction rhythm of actomyosin should be reflected in an altered energy production of the mitochondria. These experiments have already been conducted by Mori and coworkers /38/ as far as the light reaction is concerned, demonstrating that blue light has a strong effect in decreasing the intracellular ATP-content thus inducing a decrease in contraction frequency. We are currently starting similar experiments with regard to the gravireaction to see whether the ATP content increases and thus induces the observed increase in contraction frequency. - Concerning the light receptor of *Physarum* there exist indications (coming from experiments conducted by Korohoda /32/) that it actually may be located inside the mitochondria.

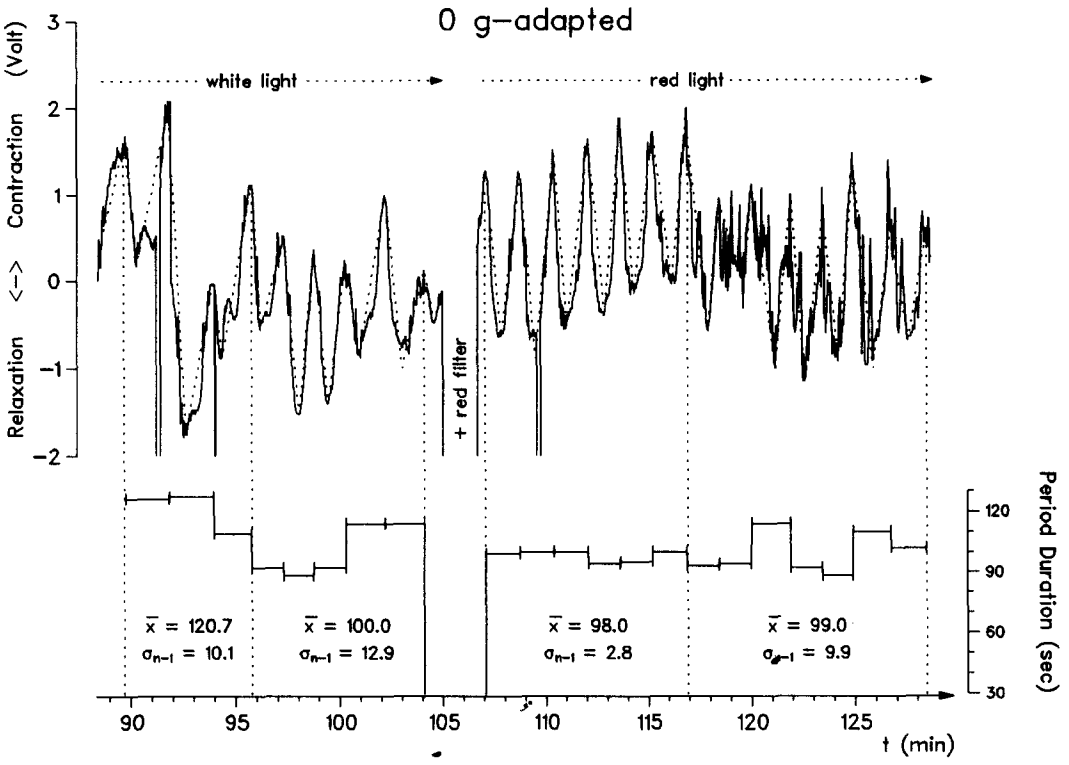

Fig. 1c. 0 g-adapted plasmodium in white light (88-105 min of 0 g) and red light (106-129 min of 0 g). Note the short light response followed by baseline values.

To gain further information about the role of the mitochondria we have simultaneously induced the opposing light- and 0 g-reactions and investigated their mutual influences on one another. At first, the experiments have been conducted on the fast-rotating clinostat, where a mutual suppression of both effects was revealed /41/. In these experiments the intensity of the white light has been so adjusted that the light reaction balanced the 0 g-reaction. - In the IML-1 mission this experiment was repeated and extended to the subsequent induction of the two opposing responses in actual weightlessness: At first the cell's response to the simultaneous 1 g to 0 g-transition and the stimulation with white light was registered. In this experiment the plasmodia were also incubated for several hours on the 1 g-reference centrifuge. Then they were subjected to 0 g, and illuminated with white light (Figure 1b, these are also data of our first IML-1 experiment session): The simultaneous inductions of the opposing light- and 0 g-reactions led to a nearly complete mutual suppression of both responses. Until about 20 minutes after onset of 0 g and white light there is a rather slight decrease in mean period followed by an increase. This poor 0 g-response can be attributed to the simultaneously occurring opposed light reaction, which slightly shows up after the 0 g-reaction has terminated; this is a delayed and strongly reduced light response. This measurement also confirms 0 g-simulation experiments performed on the fast-rotating clinostat /41/.

When the two opposing responses were subsequently induced, that is, when a seemingly 0 g-adapted plasmodium is illuminated with white light things are different: Figure 1c, left part shows the behavior of a plasmodium exposed to 0 g for about one and a half hours. Immediately after onset of white light the periods are quite long, indicating an unusual fast onset of the light response. But already after 10 minutes the periods decrease despite continued illumination. - During subsequently applied ineffective red light (Figure 1c, right part) the periods decrease only slightly further to a stable value identical to the one reached after the 0 g-reaction in red light. - The fact that the 0 g-adapted plasmodium begins and terminates its white-light reaction distinctly earlier than it normally does indicates that the time pattern of the reaction has changed. This again demonstrates the strong mutual influence of the two responses on one another, even if *Physarum* seems to have adapted to a stimulus.

Shuttle Streaming

In the IML-1 experiment also the endoplasmic shuttle streaming was observed: Although many long-lasting video camera registrations of the protoplasmic streaming had been planned, more than half of the onboard video recordings were not performed, because one Spacelab video recorder had a malfunction which was not noticed during the whole first and part of the second session of our experiment. So the streaming-velocity data of our first session are based exclusively on downlinked video sequences. This stresses the importance of redundant registration systems.

During the 0 g-response (Figure 2, upper diagram) a plasmodium shows a medium streaming velocity - maximum values are not reached during the first 40 minutes of 0 g. This is in accordance with the 0 g-simulation experiments performed on the fast-rotating clinostat. However, in the 0 g-simulations the velocity increases after about one hour of 0 g /25/ - a comparison with a 0 g-adapted plasmodium in the IML-1 experiment is presently impossible for all three *Physarum* sessions (due to still missing video sequences and other lacking information). Nevertheless, looking at the streaming registration during the simultaneously induced 0 g- and white-light responses (Figure 2, lower diagram) reveals that - compared to the pure 0 g-response in red light - the streaming velocity has remarkably increased and often reaches maximum values. This attests to the ongoing light response, where often (unpublished observation) an early increase in streaming velocity is observed.

Potential Gravireceptors

The data of the 2nd and 3rd sessions of our IML-1 experiment (contraction activity and streaming velocity) support the results of the first session. - These results allow the conclusion that the light- and acceleration stimulus reaction chains share their last members, that is, from the mitochondria onwards the two reactions follow the same biochemical pathway. - A stimulus-reaction chain implies the existence of a respective receptor. Since the light receptor actually may be located inside the mitochondria /32/, possibly also the gravity receptor could be related to this cell organelle.

Concerning the primary g-receptor, two alternative models exist based on the fact that most of the g-responses of *Physarum* are direct effects of gravity, that is, the responses are due to density differences in cells. The first model for a direct gravireceptor mechanism assumes that those cell organelles which have a higher density than the rest of the system can act as gravireceptors. The preconditions for g-perception are that the size of the organelles and their density differences compared to the surrounding medium are great enough that under 1 g-conditions the organelles are able to settle or to exert a certain pressure or tension on membranes or on the cytoskeleton in their vicinity. Calculations show that these preconditions are fulfilled by nuclei, condensed chromosomes, nucleoli, centrioles, dense vacuoles and even mitochondria (/21,22/ and Pollard cited in /22/; Table 1).

However, since the size and the density of a single mitochondrion is probably too small to induce a distinct signal, the response to gravity could be a summation effect of the chondriome (the total of all mitochondria in one cell) resulting from a weak graviperception of single mitochondria /20/. - Among the other candidates for graviperception are the condensed chromosomes. They apparently move faster in meta- and anaphases during a mitosis performed under simulated weightlessness /29/, and the size and weight of the condensed chromosomes are great enough to influence the motive forces of the spindle apparatus. But since in *Physarum* mitosis is synchronous and only occurs once about every 9 hours and lasts only a few minutes, the role of the condensed chromosomes in the above discussed graviresponse of the contraction rhythm can be ruled out. Nevertheless, the nucleoli, which show a striking eccentric location in the nuclei during interphase, might be used in graviperception during that time /42/. - Also vacuoles having a dense content, like calcium /43/ (apatite), may be candidates for graviperception. In case the polarity of a strand is due to a higher density of the endoplasm (due to a differing content of vacuoles and the lack of invaginations) as compared to the ectoplasm, the whole endoplasm would be the primary receptor /27,28/. But up to now we do not even know, if the polarity of the strands provides any information concerning the spatial orientation of a plasmodium.

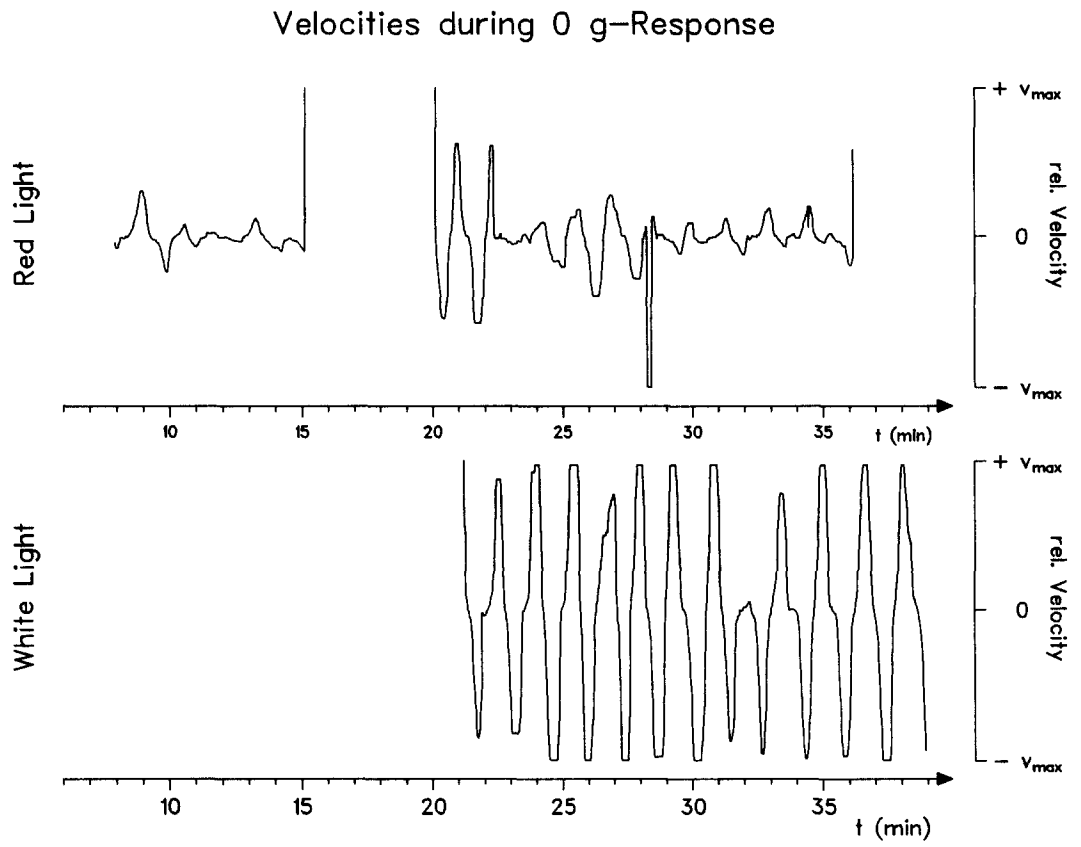

Fig. 2. Relative streaming velocity of the endoplasmic shuttle streaming. - Abscissa: time of 0 g-exposure. - Ordinate: relative streaming velocity. The shuttle streaming velocity was registered using a potentiometer: streaming velocities in direction to the leading (progressing) front zone of the plasmodium were given a positive sign, streaming velocities in direction to the rear end of the plasmodium were given a negative sign. Zero velocity is at the moment where the streaming reverses. After a reversal the streaming accelerates up to a certain velocity which is maintained for a few seconds. Then the streaming decelerates, stops again, reverses and then runs in the opposite direction. V_{max} is the maximum velocity observed. The empty spaces are LOS (loss of signal) times (where no video informations could be downlinked) or times where other experiments were downlinked. - Upper diagram: streaming velocity during the 0 g-response. - Lower diagram: streaming velocity during simultaneous 0 g- and white-light responses. Note the increase in streaming velocity during the combined 0 g- and white-light reactions.

An alternative model, proposed by members of our group /44,45/ is based on a g-dependent ion distribution along membranes. The separation of ions of different masses at membrane-solution interfaces may result in a g-sensitive system which means: bringing a membrane from a vertical to a horizontal position or vice versa, may alter its function. This may especially apply to mitochondrial membranes. - Both models restrict the site of graviperception to the gel-like ectoplasm of the plasmodium. Only here a direction-correlated gravity stimulus can be perceived.

TABLE 1 Sizes and Densities of Cell Organelles of Various Cells. - The density of the cytoplasm is estimated to be between 1.0 and 1.1 $g \cdot cm^{-3}$ /22/ and to be 1.06 $g \cdot cm^{-3}$, respectively /46/.

	diameter [μm]		density [$g \cdot cm^{-3}$]	
nucleus	6	Physarum /47/ mammals /48/	1.35	/48/
nucleolus	2-5 3	/48/ Physarum /47/	1.35	mammals /48/
mitochondrion	1.5-3.0	Physarum /47/	1.17-1.21	plants /48/
chromosomal DNA	0.035 0.25	chromatin fibre /48/ Physarum condensed chromosomes /49/	1.700- 1.702	Physarum chromosomes not condensed /50/
nucleolar DNA	0.1	/48/	1.714	Physarum /50/
mitochondrial DNA	0.007	estimate	1.686	Physarum /50/
centriole	0.2 · 0.6	/48/ (Physarum plasmodia do not possess centrioles)	> 1.5	estimate
ribosome	0.03	eucaryotes /48/ incl. Physarum /47/	1.55-1.59 1.64	eucaryotes /48/ procaryotes /48/
dense vacuoles (containing calcium)	1.0-1.5	Physarum /47/	2.22 3.14 2.9	$CaC_2O_4 \cdot H_2O$ = calcium oxalate /51/ Elodea /23/ $Ca_3(PO_4)_2$ = apatite /51/ Physarum? $CaHPO_4$ /51/

Conclusion

[1] The slime mold *Physarum polycephalum* is gravisensitive: it shows an immediate graviresponse and gravity has at least some direct effects on the cell. [2] The acceleration-stimulus reaction chain, which is the basis for the geotactic behavior of *Physarum*, is partially explained and involves the mitochondria (chondriome). [3] 0 g-simulation experiments conducted on the fast-rotating clinostat and experiments conducted in actual weightlessness supplement each other. Consequently the fast-rotating clinostat represents a valuable tool to validate and substitute other methods for investigating 0 g-effects such as drop towers, parabolic flights, sounding rockets and extended space flights. [4] The slow-rotating clinostat is a valuable tool to detect g-sensitivities of small organisms (it has nothing to do with 0 g-simulation).

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