Proceedings of the 30th Annual
Gravitational Physiology Meeting
Xi’an, China May 24-29 2009
Journal of Gravitational Physiology

A Journal of the International Society for Gravitational Physiology

At the outset, the Journal published one issue in 1994. The first number comprised the Proceedings of the 15th Annual International Gravitational Physiology Meeting, held in Barcelona, Spain in October 1993. The Proceedings of the previous 14 Annual Meetings appeared as supplements to The Physiologist from 1979 to 1993.

Each year, one issue of the Journal is devoted to the Annual Meeting Proceedings, and up to four more issues are comprised of full-length research papers. Additionally, Supplement Issues are considered by the Editorial Board as they are submitted. The Journal is published for the International Society for Gravitational Physiology by the Galileo Foundation, a 501(c)(3) nonprofit public benefit corporation.

This issue, the first number of 2009, comprises the Proceedings of the meeting of the International Society for Gravitational Physiology’s 30th Annual International Gravitational Physiology Meeting, hosted by the Fourth Military Medical School in Xi’an, China 24-29 May 2009.

Aims of the Journal

The Journal of Gravitational Physiology invites the submission of original experimental or observational papers on subjects in the field of gravitational physiology. Review articles, theoretical papers and historical or biographical articles will also be solicited by the Editor for publication.

The wide scientific span of the Journal rests on physiology as its keystone. Gravitational physiology is considered to include the effects of changes in the magnitude and directions of the gravitational force environment on cells and physiological systems and behavior of humans, animals and plants. The effects of weightlessness during space flight, high sustained G forces and chronic acceleration, vibration, impact and the various forms of simulated weightlessness are also included, as well as is consideration of the evolutionary consequences of gravity and the role of gravity in the manifestation of scale effects in animals and plants.
Information for Authors

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Acceptance of papers for publication will be determined after their review by two anonymous referees, selected by the Editor to be competent in the subject matter of the paper. If the referees differ significantly in their evaluations, the manuscript may be submitted to additional referees. If the subject matter of a particular paper is of an unusual nature, it is quite appropriate for the authors to nominate reviewers to the Editor.

Human and Animal Studies

The Journal endorses the principles embodied in the Declaration of Helsinki and insists that all investigations involving human subjects reported in the Journal be conducted in conformity with those principles. The Journal also insists that all animal experimentation reported in the Journal be conducted in accordance with the principles expressed in the “Guide for the Care and Use of Laboratory Animals” published by the Office of Science and Health Reports of the USA National Institutes of Health, Bethesda MD 20892. When reporting experiments on human or animal subjects, authors need to identify in the text that the appropriate guidelines were followed.

Further, human subjects have a right to privacy that should not be infringed without informed consent. Identifying details should be omitted if they are not essential. If such information is deemed essential for scientific purposes the subject (or parent or guardian) must provide written informed consent for publication. Informed consent for this purpose requires that a subject who is identifiable be shown the manuscript to be published. Such informed consent needs to be identified by the authors in the text.

In describing surgical procedures on animals, the type and dosage of the anesthetic agent should be specified. Curarizing agents are not anesthetics; if these were used, evidence must be provided that anesthesia of suitable grade and duration was employed.

The Editor will reject papers in which evidence of the adherence to these principles is not apparent and explicit. The Editorial Board reserves the right to judge the appropriateness of the use of animals or humans in experiments published in the Journal.
Manuscripts

Paper manuscripts shall be submitted in triplicate (original and two copies) to the Editor, or electronic copies may be emailed:

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General Instructions: All parts of the manuscript must be typewritten or printed, double spaced, on one side of bond paper. The page should have at least 1 inch margins on all sides. Each page, starting with the title page, must be numbered in the upper right corner. The right margins of pages should not be justified. Manuscripts not conforming with this format will be returned to the responsible author without review, as will articles which the Editor finds are outside the interests of the Journal.

Disk Manuscripts: The submission of disks for all accepted manuscripts is required. Microsoft Word or PDF files should be submitted either on CD-Rom or via Email to the Editor at cafuller@ucdavis.edu.

Title Page: The first page shall give (1) the full title of the paper, (2) the names of the authors, (3) name and address of each institution, (4) a short title, or “running head,” not to exceed 50 characters, and (5) the name and address of the responsible author, including telephone and fax numbers.

Abstract: The second page shall consist of an abstract of the paper, which should not exceed 200 words. The abstract should be followed by up to 6 key words not found in the title.

Text: The text should be in concise English and should conform to the general style of the Journal. The length of the text should generally not exceed 6,000 words. However, unnecessary subdivision of a study into several short articles is unacceptable. It is anticipated that the body of the text will normally be subdivided into an Introduction, Methods, Results and Discussion.

Charts, Table and Illustrations: These should be made to supplement, not duplicate the text. They must be clearly labeled, and symbols defined. Figures should be sharp, unmounted photographic quality prints or computer-generated prints on camera ready paper, not larger than 8x10 inches. On the back, the “up” position, figure number, and lead author should be indicated. Each of these should be accompanied by a legend of sufficient detail so that it is intelligible without reference to the text. Electronic copies of the figures in PDF, PICT or JPEG format is also required for accepted manuscripts.

References: Style and form should follow the format described below, with journal names abbreviated as in the Index Medicus. References should be typed separately double-spaced, arranged alphabetically and numbered serially. The references should be indicated by the number in parentheses at the appropriate location in the text. Only references essential for the reader should be included, normally a maximum of 30. References to government publications should be avoided unless their ready availability to all readers is assured. Information with the attribution “unpublished” or “personal communication,” will not appear among the references, but can be indicated in parentheses in the text. The style sheet of citations should be as follows.

Journal Articles. Last name of first author, followed by initials, initials and last names of each coauthor; title of article (only first word capitalized); name of journal, volume, pages and year.

Book References. Author(s) as above; title of book (main words capitalized); city of publication; publisher; pages and year.
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IMPORTANT ANNOUNCEMENT  
European Life Sciences Symposium 
‘Life in Space for Life on Earth’ 
31st Annual International Gravitational Physiology Meeting 

Sponsored by the  
European Space Agency  
International Society for Gravitational Physiology  
Italian Society for Space Biomedicine and Biotechnology  
European Low Gravity Research Association  

13-18 June 2010  
Stazione Marittima Congress Centre  
Trieste, Italy  

The joint 31st Annual International Gravitational Physiology Meeting, 11th ESA Life Sciences Symposium, 5th ISSBB Symposium and ELGRA Symposium will be sponsored and organized by ESA, ISGP, ISSBB and ELGRA. The meeting will be cosponsored by ASI, the University of Trieste and HE Space Operations. 

Symposia by invited speakers and slide presentations of voluntary papers dealing with the effects of changes in magnitude and direction of the force environment on the physiology and behavior of humans, animals, plants and cells. The effects of weightlessness during space flight, acute and chronic acceleration, vibration, impact, and the various forms of simulated weightlessness are also appropriate topics, as is consideration of the evolutionary consequences of gravity and the role of gravity in the manifestation of scale effects in animals and plants. 

The meeting’s plenary topics will include: 

- Current Concepts in Gravitational Physiology 
- Artificial Gravity 
- Translational Implications of Biomedical Space Research 

A workshop on Exercise Countermeasures will follow the general meeting. 

It is planned to publish the Proceedings of the Meeting, which will contain the voluntary and invited symposium papers as part of the Journal of Gravitational Physiology. 

Your participation in the Meeting is welcome. Additional Information is available at the ISGP website (http://www.isgp.org). 

Deadline for receipt of abstracts is 21 March, 2010
Proceedings of the 30th Annual International Gravitational Physiology Meeting

Organized by:
International Society for Gravitational Physiology

24-29 May 2009
Xi’an, China

The Meeting was hosted by Dr. Z.-B. Yu, Fourth Military Medical University, Xi’an, China. Support for the Meeting and publication of the Proceedings was provided by: National Aeronautics and Space Administration, European Space Agency, and the Galileo Foundation.

Papers published herein have been reviewed and approved by the Council of Trustees of the International Society for Gravitational Physiology. Publisher of the Proceedings is the Galileo Foundation.

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Future Meetings

2010 - Trieste, Italy (local organizer, Gianni Biolo)
Nello Pace Award

The Nello Pace Award was established in 1997 by the International Society for Gravitational Physiology and the Galileo Foundation to recognize individuals for performance of outstanding research in, and services to, the field of gravitational physiology. In seeking nominations for this award, many outstanding scientists who have demonstrated these characteristics were identified.

The 2009 Pace Award was presented to Dr. Li-Fan Zhang at the 30th Annual International Gravitational Physiology Meeting in Xi’ An, China. The following narratives provide a brief overview of his career and contributions to the field of gravitational physiology.
Li-Fan Zhang was born in Tianjin, China, on April 29, 1927. He studied Biology from 1944–1948 at the School of Natural Sciences, National Central University, China; firstly in Chongqing (1944–1946) and then in Nanjing (1946–1948). He subsequently received graduate training in physiology at the Department of Physiology, School of Medicine, National Central University (1948–1949)/Nanjing University (1949–1950), Nanjing, China, under the supervision of Professor Chiao Tsai, the founder of aerospace physiology and medicine in China. From 1948 to 1951, Li-Fan also worked as a teaching assistant in the same Department of Physiology. In fact he has never left this School of Medicine. In 1954, the School of Medicine was moved to the city of Xi’an and united with another medical school and renamed the Fourth Military Medical University.

In 1960, he established and developed the first Aerospace Physiology Department among the universities in China. In 1980, he led a group and attended, for the first time, the 51st Annual Meeting of the Aerospace Medical Association (AsMA) of the U.S. and visited several important laboratories in the field of aerospace physiology and medicine in the U.S. and France. He is now an Emeritus Professor of Aerospace Physiology in the Department of Aerospace Physiology, Fourth Military Medical University, Xi’an, China.

In his long professional career, Li-Fan has made important contributions in various areas. For example, he and Professor Xin Chen co-edited the book entitled “Chinese Encyclopaedia of Medical Sciences-Aerospace Medicine” that was published in 1985. He has supervised a total of 24 PhD and 22 Master students. In the 1970s and 1980s, his major research areas were altitude and respiratory physiology. He conducted research aimed at improving altitude indoctrination for flying personnel and establishing a permissible standard for aviator’s oxygen equipment. He also performed research in respiratory mechanics and regulation under respiratory mechanical loading and developed methods for assessing respiratory sensation and function and monitoring. Since 1991, his research interest has shifted to the area of gravitational physiology. In the field of high-G physiology, he performed human studies using cardiovascular signal processing to further define the influence of aerobic training on orthostatic tolerance. This work aimed to improve the aerobic training program for aircraft pilots and astronauts. He and his co-workers have studied the effectiveness and cardiovascular responses of various combined anti-G techniques under high G exposure using mathematical modeling and computer simulation. During this period, he supervised a parallel study on human centrifuge conducted at the Chinese Air Force Institute of Aviation Medicine, Beijing. In microgravity cardiovascular physiology, he and his colleagues have conducted a series of ground-based animal studies using the tail-suspended, hind-limb unloaded rat model to investigate the effect of simulated microgravity on cardiac muscle and vessels (from small resistive to medium-sized and large elastic arteries) and its underlying mechanisms. Based on these findings, they have proposed the “peripheral effector mechanism hypothesis for post-spaceflight cardiovascular dysfunction”. In recent years, he has also examined the problem of intermittent artificial gravity and reported system specificity in responsiveness to daily exposure to altered gravitational force vector during simulated microgravity. From this research he and colleagues have found that the cardiac muscles and vessels are most responsive and bone and testis are highly resistant, whereas the responsiveness of skeletal muscle is moderate.

Since 1992, he has attended more than 10 international meetings sponsored by the International Society for Gravitational Physiology, ESA Space Life Sciences, and the International Society for Adaptation Medicine to exchange ideas. In 2006, he and Professor H.C. Gunga co-chaired the first Sino-German Symposium on Space Life Sciences at Xi’an, China. In 2009, he and Dr. M.A. Custaud co-chaired a symposium on “Gravity-Induced Vascular Remodeling” during the 30th ISGP Meeting, Xi’an, China May 24–29. He is an author, co-author, or corresponding author of more than 300 journal articles, conference papers, and book chapters. Among them, a total of 72 papers have been collected in his recent book entitled “Selected Works on Environmental Physiology” published in 2006. He has served on the Editorial Board of the following journals: Chinese J Aerospace Medicine, Chinese J Applied Physiology, Space Medicine and Medical Engineering (1988–2001), News In Physiological Sciences (1988–1994), J Gravitational Physiology (since 1997), and Acta Physiologica Hungarica (since 2006). He also serves as an executive member for the International Society for Adaptation Medicine.
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Welcoming Address
Peter Norsk, Chairman of the Council of Trustees, ISGP

The 30th annual ISGP-meeting in Xi’an, China, was hosted by Dr. Zhi-Bin Yu of the Department of Aerospace Physiology in the Fourth Military Medical University. It brings pleasure to the society to note that there remains a high interest in gravitational physiology and related subjects. International scientific meetings include special symposia on gravitational physiology, thus increasing the awareness of a growing number of scientists of the effects of gravity on all levels of organismal function from molecules to cells to organisms. The inclusion of these symposia is encouraging, because it spreads the message that gravity is an important factor in most aspects of physiology.

Our annual meetings have regularly focused on areas which the Council felt were in the forefront of gravitational physiology. This was also the case in Xi’an. Remaining faithful to tradition, the meeting’s main topics included: current concepts in gravitational physiology, gravity induced vascular remodelling, the effects of gravity on development and gravity and the vestibular system.

The papers presented at previous meetings have been published by the Galileo Foundation each year as proceedings in a special issue of the Journal of Gravitational Physiology. In accordance with tradition, this was also the case in Xi’an. In this connection, I wish to express the Council’s gratitude to Drs. Charles Fuller and Tana Hoban-Higgins for their tremendous work without which, the journal issue would not have been possible to produce.

I would finally like to express the Council’s appreciation to our host and president of ISGP in Xi’an, Dr. Zhi-Bin Yu, for having organized the 30th annual meeting. His and his staff’s work were enormous and on the behalf of the society, I herewith express our sincere appreciation.

Sincerely,

Peter Norsk
Chairman
THE POSSIBLE CONTRIBUTION OF THE SUPPORT WITHDRAWAL TO THE ALTERATIONS OF VESTIBULAR FUNCTION IN MICROGRAVITY

Badakva A.M., Miller N.V., Zobova L.N.
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ABSTRACT

The gaze fixation reaction was studied in rhesus monkeys before and during thermoneutral (35°C) water immersion to the mid-chest level (3 animals) and to the neck level (2 animals). Both the eye saccade amplitude and the angular vestibulo-ocular reflex gain increased whereas the head angular velocity decreased significantly in 3 monkeys in 5 h after the start of immersion to the mid-chest level. Changes of the head movements characteristics and the angular vestibulo-ocular reflex gain were more pronounced in monkeys in 5 h after the start of immersion to the neck level. The alterations of characteristics of eye-head coordination are similar to those observed in monkeys in space flights. Similarities of effects of immersions and space flights allow to suppose that the support withdrawal contributes to the alterations of vestibular function in microgravity.

1. INTRODUCTION

Eye-head coordination during gaze shifts to lateral targets (gaze fixation reaction, GFR) was studied in rhesus monkeys in BION project [1]. The gaze fixation accuracy and rapidity depend on an interaction of three components, namely: eye saccade towards the target, head movement in the same direction, and compensatory eye counter-rotation which is based on the angular vestibulo-ocular reflex (aVOR) [2]. In the first days of space flight the gaze displacement onto lateral targets became hypermetric, and the amplitude of head movements decreased. This was accompanied by increase in the aVOR gain [3,4]. The gain increase was closely linked in time to increased responses of the vestibular nuclei neurons to canal stimulation [5].

However, similar changes in characteristics of GFR were also observed in immersed humans [6]. Since changes in gravity were not a factor in these experiments, changes in proprioceptive inputs could have contributed to or been responsible for producing changes in GFR. This suggests that vestibular overexcitability at an early stage of microgravity can be attributed to weaker inhibitory tonic of muscular and weight-bearing afferent signals [7].

Purpose of our experiments [8,9,10] was the investigation of the influence of support deprivation during water immersion to the mid-chest level and to the neck level on GFR in monkeys, using the BION flight test of GFR, to evaluate the possible contribution of the support withdrawal to the alterations of vestibular function in microgravity.

2. METHODS

Five male rhesus monkeys (Macaca mulatta) of 5-6 kg (A, B, C, D, and E) were used in our experiments. Surgical procedures included: (1) attachment of an acrylic ring to the skull, which supported a preamplifier and a protective cover carrying an angular velocity sensor, and (2) implantation of electrooculographic (EOG) electrodes bitemporally. All required surgery and all experimental procedures were approved by the Bioethics Board at RF SSC-IBMP RAS.

The animals were trained to perform eye-head-hand coordination tasks the same way as in space flight [11]. The animal could be presented with one of five small-size symbols (1°): the central (trigger) stimulus and four peripheral stimuli located at 18° or 36° to the left or to the right from the central one on the horizontal circumferential panel at a distance of 25 cm from the animal’s eyes. If the monkey touched the area surrounding the light stimulus, this stimulus turned off. When the animal turned off the trigger stimulus, one of the peripheral stimuli was presented at random as a positive or a differential sign. The stimulus remained illuminated for 0,8 s. If the monkey turned off the positive stimulus, it was rewarded with a dose of juice. If it turned off the differential stimulus, it was penalized by delaying the presentation of the next trigger stimulus.

Before experiments the monkeys were seated in primate chair which could be secured to a rigid bar of the water tank or fixed on a vestibular turntable. Trunk movements with respect to the chair were inhibited with a shoulder harness and by the shape of the chair. Horizontal neck plate allowed complete head mobility but prevented the animals from reaching equipment mounted on the head. The monkeys received a juice reward via a delivery system that was placed on this plate in such a way that required the monkeys to align their head with the central stimulus. Before the start of immersion the monkeys were placed in a cotton suit. Besides the second horizontal plate (plate 2) was secured to the primate chair at the monkey’s mid-chest level and prevented contact of animal’s hands with water during GFR testing.

The test of the passive aVOR induced by steps of velocity in darkness [12] was performed on head-restrained conditions. 30-40 steps of velocity were given by hand with animal (~800°/s² acceleration lasting ~160 ms to a peak velocity followed by a plateau of head velocity ~130°/s lasting ~600 ms). The aVOR values
were calculated during slow phases of nystagmus observed during a plateau of head velocity. The data for the rightward and leftward rotations were pooled then.

The characteristics of the GFR were studied in the monkeys A, B, and C in empty water tank (control) and during thermoneutral (35°C) water immersion to the mid-chest level once a week: animal A - 4 times, B – 3 times, and C – 2 times. Additionally, two experiments were performed: (1) the control test of the GFR after 5 hrs sitting in the empty water tank in animals C and D; (2) the test of the passive aVOR after 5 hrs immersion to the mid-chest level in monkeys C and E. Further the characteristics of the GFR were studied in the monkeys C and D during 5 hrs immersions (i1 and i2) to the neck level (two weeks between i1 and i2). The water level was lowered to the animal’s mid-chest level before GFR test at the very end of immersion to the neck level. Three weeks after i2, the imitation of immersion was performed in monkey D: the control test of the GFR in the empty water tank was performed twice but the monkey was placed in a cotton suit and the plate 2 was secured to the primate chair before the second GFR test.

Eye position signals were converted to eye angular velocity signals by digital differentiation. Velocity criteria for marking the beginning and the end of eye movements were typically 20°/s. The GFR trials were analysed only if eye and head velocities were <20°/sec during the 100 ms interval before the onset of eye movements. Data of eye and head movements to targets located at 36° to the left and to the right were pooled.

The following parameters of GFR were analyzed: latency of the eye saccade and of the head movement, amplitude and peak velocity of the eye saccade, peak velocity of the head movement, and aVOR gain as ratio of eye velocity to head velocity during the time segment (30 ms) of the eye counter-rotation. (Fig. 1).

Means ± SD were computed for all results. Statistical analysis was performed using Student’s t test with a Bonferroni correction for multiple comparison.

### 3. RESULTS

Fig. 2 illustrates the changes of GFR parameters in monkeys A, B, and C during immersion to the mid-chest level.

The peak eye saccade velocity significantly increased in 2 h of immersion by 12.9% (p<0.001) and in 5 h of immersion by 11.4 % (p<0.001) in monkey A. In monkey B the peak eye saccade velocity did not change in 2 h and increased in 5 h of immersion by 8.8% (p<0.001). The peak eye saccade velocity was unaffected by immersion in monkey C. The eye saccade amplitude significantly increased in 2 h of immersion by 14.3% (p<0.001) and in 5 h of immersion by 13.0% (p<0.001) in monkey A. In both monkeys B and C the eye saccade amplitude did not change in 2 h and increased in 5 h of immersion by 12.0% (p<0.001) and by 8.7% (p<0.001), respectively. In all three monkeys the peak head velocity was unaffected in 2 h and decreased in 5 h of immersion by 19.2% (p<0.001) in monkey A, by 19.0% (p<0.005) in monkey B, and by 27.7% (p<0.001) in monkey C. In all three monkeys the absolute value of aVOR gain did not change in 2 h and increased in 5 h of immersion by 13.3% (p<0.001) in monkey A, by 7.7% (p<0.01) in monkey B, and by 4.0% (p<0.005) in monkey C.

None of the analyzed parameters of the GFR changed significantly in monkeys C and D after 5 hrs sitting in the empty water tank.

The gain of the passive aVOR was unaffected by 5
hrs immersion to the mid-chest level in monkeys C and E (1.03±0.15 vs 1.03±0.14 in control and 0.97±0.13 vs 0.96±0.15 in control, respectively).

Fig. 3 illustrates the changes of GFR parameters in monkeys C and D in 5 hrs immersions to the neck level.

Fig. 3. Parameters of GFR in monkeys C and D in control (open columns), in i1 (gray columns), and i2 (black columns).

The peak eye saccade velocity increased in monkey C by 8.2% (p<0.05) in i1 and by 5.8% (p<0.01) in i2. In monkey D, the peak eye velocity increased by 2.8% (p<0.05) in i1 and by 10.3% (p<0.001) in i2. The eye saccade amplitude increased in monkey C by 11.0% (p<0.001) in i1 and by 10.7% (p<0.001) in i2. The eye saccade amplitude increased in monkey D by 9.0% (p<0.001) in i2 only. The peak head velocity decreased in monkey C by 17.0% (p<0.005) in i1 and by 51.9% (p<0.001) in i2. In monkey D, the peak head velocity decreased by 9.0% (p<0.05) in i1 and by 49.5% (p<0.001) in i2. The absolute value of aVOR gain increased in monkey A by 12.7% (p<0.001) in i1 and by 18.2% (p<0.001) in i2. In monkey B, the absolute value of aVOR gain increased by 14.0% (p<0.001) in i1 and by 28.8% (p<0.001) in i2.

The low peak head velocity in both monkeys in i2 was accompanied by a new control strategy of the head movements. In monkey C (Fig. 4), part of the head velocity profiles (type 1; 43%) was similar to the control profiles whereas other profiles (type 2; 57%) had an initial peak followed by a pronounced deceleration and subsequent reacceleration. On the next day after the end of i2, the peak head velocity remained reduced by 48.2% and type 2 of the head velocity profiles was observed (33%). In monkey D (Fig. 5), the head latency decreased from 125±26 to 172±44 ms (p<0.001), that is, coordinated eye-head movements were made with the head leading in i2. As a result of the imitation of immersion, the peak head velocity decreased in monkey D by 25.2% (p<0.001) and the head leading was 18.2±17.8 ms (p<0.001).

Fig. 4. Two types of profiles of the head velocity (deg/s) during GFR in monkey C in i2. Open circles – control, closed circles – type 1, and closed triangles – type 2.

Fig. 5. Head movement latency and eye saccade latency in monkey D in control (open columns), in i1 (gray columns), and in i2 (black columns).

Fig. 6 shows that the low head velocity and, respectively, low contribution of head movement to gaze shift were accompanied by the larger deviation of the eye-in-orbit at gaze shift end in both monkeys (C and D) in i1 and i2.

Fig. 6. Changes in eye and head positions during GFR in monkeys C and D in control (open circles), in i1 (black circles), and in i2 (black triangles).
4. DISCUSSION

The results of immersion to the mid-chest level indicated that support deprivation caused significant changes in characteristics of eye and head movements during GFR in monkeys A, B, and C. There were individual differences in characteristics of eye movements. Nevertheless in all three monkeys in 5 h after the start of immersion, both the eye saccade amplitude and the aVOR gain increased, whereas the peak head velocity decreased. The changes were similar to those during space flight [3,4] although there was no change in gravity during immersion. The revealed data were taken from monkeys whose bodies were restrained, yet none of the analyzed parameters of the GFR changed after 5 hrs sitting in the empty water tank.

The gain of the passive aVOR in monkeys C and E induced by steps of velocity in darkness after 5 hrs immersion to the mid-chest level was not affected, as well as the passive aVOR was not affected in microgravity [4]. It is possible that the active aVOR gain was enhanced during lateral gaze shift both in microgravity and during immersion due to a change in central programming of GFR, although the passive aVOR gain was unaffected.

The results of 5 hrs immersions to the neck level in monkeys C and D indicated that increase of support deprivation caused significant changes in characteristics of GFR. The changes in the first 5 hrs immersion were similar to those during 5 hrs immersion to the mid-chest level. However the peak head velocity dramatically decreased at the end of the second 5 hrs immersion to the neck level in both monkeys and remained reduced on the next day in monkey C. The absolute value of aVOR gain increased significantly to 1.16 in monkey C and to 1.36 in monkey D. In spite of sizeable increase of the aVOR gain, the low head velocity did not allow the eye-in-orbit deviation to remain within comfortable range in both monkeys, while complicating the redirection of gaze toward a target [13,14].

The low peak head velocity in both monkeys was accompanied by a new control strategy of the head movements: with two velocity peaks in monkey C and with the significant head leading in monkey D. Taken together, these results show that more pronounced support deprivation affects the central programming of eye-head coordination to a great extent.

Changes in strategy of the head movements were observed during GFR tests after the second immersion to the neck level: on the next day in monkey C and during the imitation of immersion in monkey D. These changes as well as more significant changes of GFR parameters in the second immersion as compared with the results of the first immersion in monkeys C and D allowed to suggest that effects of pronounced support deprivation were related to motor learning and long-term motor memory [15].

5. CONCLUSIONS

The character of changes of GFR parameters observed in monkeys in 5 h after the start of water immersion to the mid-chest level and to the neck level is similar to those during space flight: both the eye saccade amplitude and the aVOR gain increase whereas the peak head velocity decreases significantly.

Similarities of effects of immersions and space flights on eye-head coordination during the GFR in monkeys allow to suppose that the support withdrawal contributes to the alterations of vestibular function in microgravity.

REFERENCES

ALTERATION OF EYE-HEAD COORDINATION’S STRATEGY IN WEIGHTLESSNESS
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ABSTRACT
The purpose of the studies was to explore effects of long-duration SF on characteristics of the gaze fixation reaction (GFR). The study was provided with 7 cosmonauts – members of Mir missions. Test sessions were performed 4 times before launch, on flight months 1, 2, 3, 4 and 5, and after landing (R+2 and R+5). During the test sessions, the human subjects were to perform the target acquisition task in the horizontal plane on targets that appeared at a distance of 16 angular degrees in a random order up, down, right- and leftwards from the center. Characteristics of eye and head movements were recorded with the MONIMIR system (Austria). Before flight target acquisition was provided by joint eye and head movements. Time of gaze fixation amounted to 520-650 ms in all the members of the group before space flight (SF). Within the time of recording equal 1200 ms in gaze fixation on the target was successful in 97-99% of cases. During SF organization of reaction changed and the joint movement in 60% cases was divided into two separated following each other motor acts: eye movement and the movement of head. Time of gaze fixation in flight extended by 900-1000 ms and more being the longest on the 4th month of flight. The number of GFR completed in 1200 ms reduced considerably in SF. After landing, most GFR characteristics returned to baseline values, however, on R+2 the time of gaze fixation was still 680-730 ms at most, regaining the preflight values only on R+5. All the recorded alterations of the reaction’s parameters were more expressed in the vertical reactions than in horizontal ones. Changes in the GFR characteristics recorded in the course of long-duration SF point out to motor stereotype destruction and dominance of visual input in organization of gaze fixation reaction.

1. INTRODUCTION
The changes of sensormotor functions are observed in all space flights regardless of duration [1, 2, 3, 4, 5, 6]. Disturbing the activity of vestibular, support and muscle sensory systems, weightlessness provides the conditions for reorganization in motor control systems [2, 5]. One of the important sensormotor reactions is gaze fixation reaction which plays the important role in spatial orientation, behavior and performance of operational activities in man. As it was shown in the experiments with primates by Bizzi et al., the organization of this reaction is based mostly on the vestibular signals that are working closely with visual and proprioceptive signals, and that provides highly effective coordination of eye and head movements [7]. It is obvious that conditions of microgravity, that changes deeply sensory systems’ activity, the characteristics and organization of the reaction should change. This postulate was confirmed by the results of the primate experiments provided in BION flights, in which deep changes of vestibulo-ocular relationship and horizontal GFR characteristics were observed [8, 9, 10, 11]. There are no data in literature concerning effects of long duration exposure to weightlessness on GFR and characteristics of latest stages of adaptation. There are also no data about the changes of vertical gaze fixation reaction characteristic in weightlessness, though it is evident that these changes should be not less than of horizontal ones. At the same time in spite of common organization of gaze fixation reaction system in man and primates, it would not be correct to transfer directly the data that were received on monkey to a man. It explains the importance of this work, which was aimed to study mechanisms and organization of gaze control systems and their disturbances in microgravity.

2. METHODS
The studies of GFR characteristics being a part of the Russian-Austrian project MONIMIR were performed using the classic methods described by Bizzi, Barnes, Kozlovskaya and others [1, 7, 12]. 7 cosmonauts – members of crews of long duration expeditions on space station MIR - took part in inflight and postflight experiments. The subject executed the test of rapid fixation of the gaze on a target that appeared suddenly within the peripheral field of vision after the subject held gaze on the central point to which the eye had returned after accomplishing of GFR. Targets, red light-emitting diodes with a size of 0.5 angular degrees, were presented randomly in the vertical plane at a distance of 16 degrees up and down the center point. The target remained visible for 2-3 seconds. Each stimulus was presented 24 times per a test session. Eye movements were recorded electrooculographically (EOG). Head movements were recorded with videoanalysis method. Data were analyzed using software BSANALYS that was developed specially by the Austrian partners of AUSTROMIR project.

3. RESULTS
Before flight, all the cosmonauts demonstrated the classic profile of the vertical GFR. The reaction consisted of three phases. The first a saccade with a latent period of 205-230 ms occurred toward the target. 30 to 75 ms later head movement in the same direction started. During this second phase the gaze would...
acquire the target with the coherent eye and head movements.

Simultaneously, the saccade subsided and ocular counterrotation began. During the third phase, the head would continue turning to the target and the eyes would counterrotate with the speed equal to the head movement that ensure gaze stabilization at the target while both of its components were still moving. According to Bizzi et al. [7] and others, this pattern of eye and head coordination resulting in a very rapid and precise gaze fixation on a peripheral target while the motor response has not been yet finished is based on the mechanism of the vestibulo-ocular reflex (VOR).

Exposure to microgravity altered the reaction parameters. During the initial months of flight the time for gaze fixation in the subjects, one and all, significantly increased. Latencies of the eye movement didn’t change significantly, at the same time the head movement latent periods increased by 100 ms. The amplitude of saccades in most of the cases showed a trend upward during the first month of flight; however, the parameters decreased during the subsequent in-flight test sessions and at the end of flight didn’t differ from the control values. The results of data analysis showed the existence of two groups of the cosmonauts that differed by the direction of eye and head velocity changes during and after space flight. In the first one that was consisted of mostly engineer-cosmonauts the early days of flight were associated with the high saccade amplitudes and velocities; the head velocity decreased in this group. In group 2, consisted mostly of pilot-cosmonauts, on the opposite, the peak velocity of saccades markedly reduced. The peak head velocity in group 2, decreasing also at the beginning of flight, later increased and remained high during the whole flight. The counterrotation velocity in this group increased also even more than the velocity of the head movement. As the result, the coefficient of VOR increased greatly (Fig.2). Its values at the late stage of flight decreased and raised again after landing, remaining increased even on the 5th postflight day.

The similar but even larger expressed changes there were observed in the system of vertical GFR. Latencies of the vertical eye movement didn’t change significantly. The same, as in the horizontal GFR, the head movement’s latencies raised by 100-120 ms. Consequently, differences between the head and eye latencies on the average reached 140 ms. The time for gaze fixation significantly extended that was seen already in the date of the first in-flight session (Fig.3).

Gaze fixation required additional 200-300 ms as compared with pre-flight baseline values. Further into flight, this parameter continued to increase and on flight months 4-5, gaze fixation took almost twice as long, i.e. 1000-1150 ms. Most of described characteristics returned to the control values after landing, however the overall time of reaction remained increased.

Great changes were observed in flight in the vertical GFR kinematic parameters. At the initial stage of flight the head velocity decreased a little while the counterrotation velocity increase twice and more. Later the counterrotation velocity showed the tendency to decrease and on the 5th month of flight it reached the
values lower than the head peak velocity. After landing the counterrotation velocities again increased greatly, the head velocities were close to the control values. Correspondingly, in the first month of flight $K_{VOR}$ grew in three and more times. Later in flight, it slowly decreased and increased again after landing (Fig. 4).

The structure of the reaction altered as well in the course of flight. In horizontal and vertical GFR as well the number of reaction in which the head movement started only after the main saccade was accomplished grew subsequently.

These changes were also larger expressed in the vertical GFR. So the joint GFR during space flight transformed into two separated reactions that were performed following each other: first by the saccades, and then – by the head movement. After landing these changes disappeared quite fast: on the 2nd postflight day the pattern of the reaction was close to that on the Earth. The described changes in structure and parameters of the gaze fixation reaction caused the sharp decline in precision of the test implementation (Fig.6).

The number of reactions in which gaze fixation on the target was accomplished within 1200 ms during flight decreased progressively. In the vertical GFR this decrease were especially significant. After flight this parameter recovered very quickly, and already on R+2 was close to 90%.

4. DISCUSSION

By the results of analysis of kinematical and amplitude changes in vertical GFR the cosmonauts were divided into two groups. On this point, our results of inflight and postflight experiments are in good agreement with the earlier findings of Barmin et al. who supposed that type-1 adaptation can be attributable to pilots skilled to slow down the head movements on duties and “to release the brake” in weightlessness [13]. Type-2 adaptation is a feature of flight engineers, on the contrary, slowing down the head movements due to sensory disturbances in microgravity. The growing variation range was the primary and consistent GFR response to space flight. It should be said, that this increase in variation ranges of as motor, so vegetative reactions, as well as loss of the motor and autonomous response stereotype due to spaceflight and simulated microgravity have been reported by some other investigators [14, 15]. We can make a suggestion that with altered activities of the gravity-dependent sensory systems the normal program control of GFR precision becomes impossible. Deprived of programmability, GFR relies predominantly on feedback which explains the sharp increase of time for task implementation. The study shows that, as it was suggested, weightlessness deeply changes the organization and structure of GFR. The changes in vertical GFR were more expressed than in horizontal one. The results show the existing of two stages of adaptation to microgravity differing by the direction of parameters’ changes (Fig.7).
The first, early stage was characterized by the hypermetry of saccades, increased KvOR and head movement inhibition, and in the whole was analogous to those registered in short term flights in monkeys. At the second stage of adaptation there were observed the processes of slow decrease in VOR loop. However even in this case the accuracy of reaction didn’t recover because its organization remained changed. These occurrences of “uncompleted adaptation” were observed through the whole flight.

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5. REFERENCES
ABSTRACT
To determine the role of the support-proprioceptive factor in the functioning of the vestibular system, in particular the static torsional otolitho-cervico-ocular reflex (OCOR), comparative OCOR studies with videooculography recording were performed after a 7-day “dry” horizontal immersion and after a prolonged exposure to microgravity (126 to 195 days). The first time it was demonstrated that the removal of support afferentation and minimization of the proprioceptive one may result in the inversion of static torsional OCOR and the development of positional nystagmus with an inverted reflex. A comparative OCOR data analysis of cosmonauts after prolonged microgravity and of subjects after immersion revealed similar responses. However changes in OCOR after immersion were noted in only 60% of subjects, while after space flight, in 90% of cosmonauts. Post-flight changes were more marked and long-standing.

1. INTRODUCTION
The absence of gravity during space flight results in altered afferentation from gravity-dependent sensory inputs: otolith receptors and support zones of the foot, which is the reason for the in-flight sensorimotor disturbances [1-3]. The necessity of understanding the role of a given etiological factor in the development of sensorimotor disturbances which appear during microgravity requires comparative studies in simulation experiments. It is known that immersion has no direct effect upon the vestibular receptor but does create support unloading by muscle activity minimization [4]. Absent support during immersion leads to an altered nature of the functioning of multisensory vestibular nuclei which are the sites of convergence of afferent signals of varying sensory modalities: visual, vestibular, proprioceptive, support [5]. An altered functioning of the vestibular nuclear complex due to the absent support and minimized muscular afferentation can, via efferent bonds, influence vestibular responses dependent on the vestibular receptor function.

The purpose of this study was to determine the role of the support-proprioceptive factor in the vestibular system functioning, in particular the OCOR.

2. MATERIAL
The OCOR study involved 13 cosmonauts before and after a prolonged exposure to microgravity (126 to 195 days) and 16 subjects before and after a 7-day “dry” horizontal immersion. The subjects were aged 25 to 45.

3. METHOD
OCOR parameters (i.e. – amplitude of the compensatory torsional eye counter-rolling during head tilt) were measured by a videooculography. Baseline studies were performed pre-flight and before immersion, post-flight – on days 1(2), 4(5) and 8(9), after immersion – on days 1, 3(4) and 7. The OCOR was measured during 30º head tilt to the shoulder (controlled by an angle-measuring device) and head was held in this position for 16 sec. To record horizontal, vertical and torsional eye movements subject used Chronos Vision ETD (Germany) [6] helmet equipped with infrared video cameras with frequency of recording: 200-400 shots/sec. Processing of videooculographic records was performed by ETD Iris Tracker software with accuracy of the eye position detection: 0.25º

4. RESULTS
4.1. Torsional static OCOR after space flights.
The pre-flight amplitude of the compensatory eye counter-rolling was within the 4º to 8º range. The reflex was symmetrical. One cosmonaut was an exception: the angle of eye counter-rolling during head tilt to the left was 4º, to the right – 8º.
On days 1(2) post-flight (Fig.1) 4 cosmonauts had absence of the torsional compensatory eye counter-rolling; 3 – an inverted OCOR (the torsional eye counter-rolling was directed unilaterally during the head tilt); 4 – a 50% decrease of the eye counter-rolling amplitude; only 2 cosmonauts had practically unchanged OCOR. On days 4(5) and 8(9) post-flight 5 cosmonauts demonstrated decreased amplitude of the torsional compensatory eye counter-rolling, remaining subjects had it closed to the baseline.

Fig.1 OCOR pre-flight and on 2-day post-flight
1 – horizontal VOG; 2 – vertical VOG
3 – torsional VOG; ↑- moment of head tilt
4.2. Torsional static OCOR after immersion.
For most subjects before immersion static torsional OCOR was symmetrical and within the physiological normal range (4º to 8º). However 1 subject had 2º decrease of the static torsional OCOR, while 3 had asymmetry of OCOR (the difference in compensatory eye counter-rolling during head tilt to the right and to the left shoulders was from 3º to 6º).
After immersion OCOR remained within the physiological normal range and was symmetrical during head tilt both to the right and to the left for 6 subjects on all days of the study. On day 1 after immersion 4 subjects had 50% decrease of OCOR; 1 – increase of OCOR by 40% to 80%; 2 – 50% asymmetry of OCOR. On days 3 and 7 after immersion OCOR normalized for all of these 7 subjects.
Another 4 subjects had atypical features of OCOR (Fig.2). On day 1 after immersion 2 subjects had inversion of the first phase of OCOR and then absence of the compensatory eye counter-rolling during head tilt to the left (a compensatory saccade was directed unilaterally with the head tilt and then eye returned to the zero position and remained there throughout the head tilt). On days 1 and 3 after immersion another 1 subject had similar OCOR features but in several seconds after returning to the zero position eye counter-rolled from 4 to 6º counterlaterally with the head tilt). Also all these 3 subjects had a positional nystagmus after head tilt. OCOR during head tilt to the right was always normal. The last subject of this group on day 1 after immersion had a delayed OCOR (after head tilt there was an inverted compensatory saccade then eye returned to the zero position, and only towards the end of the test OCOR became normal).

5. CONCLUSION
A comparative OCOR data analysis of cosmonauts after prolonged microgravity and of subjects after immersion revealed similar responses. However OCOR changes after immersion took place in 60% of the subjects, while after space flight, in 90% of cosmonauts. OCOR changes after flight were more marked and prolonged. Absence or a sharp decline of OCOR after space flight indicated a profound inhibition of the otolith function resulting from a central deafferentation of the vestibular afferent signal distorted in microgravity. Immersion, having no direct effect upon vestibular receptors however, changing the level and nature of support, tactile, and proprioceptive afferentation, via central integrative multisensory structures of the central neural system which carry on convergence of afferent signals of varying sensory modality (primarily visual, vestibular, support, and motor) may lead to a change of the nature of functioning of multisensory vestibular nuclei. An altered functioning of the vestibular nuclear complex related to the removed support afferentation leads to a changed nature of central intersensory interactions and, via efferent relations, influences the function of the vestibular receptor. The nature of OCOR parameters is determined by the combined effect of various afferent signals and their ratio.

REFERENCES
Expression of Calcitonin Gene-Related Peptide in Efferent Vestibular System and Vestibular Nucleus in Rats of Motion Sickness

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Abstract
Recent studies showed that the efferent vestibular neurons contain calcitonin gene-related peptide (CGRP). The CGRP immunoreactivity (CGRPi) fibers of the efferent vestibular system could modulate primary afferent input into the central nervous system. To elucidate the relationship between motion sickness and CGRP, the effects of CGRP on the vestibular efferent nucleus and the vestibular nucleus were investigated in rats with motion sickness. The number of CGRPi neurons in the vestibular efferent nucleus and expression levels of CGRP in the vestibular nucleus increased significantly in rats with motion sickness. Administration of anisodamine decreased the expression of CGRP within the vestibular efferent nucleus and the vestibular nucleus in rats subjected to rotary stimulation. In conclusion, CGRP plays a role in motion sickness, and its mechanism is worth further investigation.

Keywords: calcitonin gene-related peptide, anisodamine, motion sickness, vestibular system

INTRODUCTION:
Motion sickness presents a challenge for medicine due to its high incidence and unknown pathogenesis although it is a known fact that a functioning vestibular system is essential for the perception of motion sickness. Recent studies showed that the efferent vestibular neurons contain CGRP. The CGRPi fibers of the efferent vestibular system could modulate primary afferent input into the central nervous system [1]. Thus, it is likely that CGRP plays a key role in motion sickness. To elucidate the relationship between motion sickness and CGRP, the effects of CGRP on the vestibular efferent nucleus and the vestibular nucleus were investigated in rats with motion sickness.

METHODS:
Rotary Stimulation Equipment and Method:
A unit is composed of a generator and an arm with two suspended plexiglass cages. The cages were accelerated at $10^3/s^2$ to a peak speed of $240^\circ/s$, and rotated at peak speed for 5 minutes. Then the cages were decelerated at $10^2 /s^2$ to $0^\circ /s$. After that, the clockwise rotation alternated with the counterclockwise rotation. This stimulation lasted 30 minutes [2].

Verification of animal model with motion sickness by conditioned taste aversion:
Conditioned taste aversion (CTA) tests were performed to verify that the animal model with motion sickness induced by the rotary stimulus would be available [3]. The four groups were: A: stimulation group that was undosed, B: stimulation group saline dosed, C: stimulation group anisodamine dosed, and D: a control group. Rats in groups A, B and C were all subjected to rotary stimulation for 30 minutes. The group C were administered anisodamine orally while the group B were administered saline orally 30 minutes before rotary stimulation. The control group had no rotary stimulation. Immunohistochemistry
Fifty rats were divided into five groups (10 per group) using a random digits table. The groups were: I: triple rotary stimulation undosed, II: single rotary stimulation undosed, III: single rotary stimulation saline dosed, IV: single rotary stimulation anisodamine dosed, and V: a control group. Rats in group I were subjected to rotary stimulation 3 times, and groups II, III and IV were subjected to rotary stimulation once. The control group was not subjected to rotary stimulation. Rats in group IV were administered anisodamine orally 30 minutes before rotary stimulation while rats in group III were administrated saline orally 30 minutes prior to rotary stimulation. The transverse serial sections (30µm) were sliced through the brainstem and incubated with rabbit anti-CGRP. Antibody-antigen binding was visualized using an avidin-biotin-peroxidase complex.
RESULTS:

Rotary stimulation and conditioned taste aversion:
The intake of saccharin solution was reduced in the group A, B and C after the rotary stimulation, compared to that prior to the stimulation (Fig.1).

Expression of CGRP in the vestibular efferent nucleus and the vestibular nucleus.
The number of CGRPi neurons in the vestibular efferent nucleus and the level of immunoreactivity in the vestibular nucleus were increased significantly in groups I and II compared with group V. The increase in group I was significantly greater than that in group II.

The number of CGRPi neurons and the level of immunoreactivity was significantly decreased in group IV compared with that of groups I and II, while there was no significant difference between groups II and III (Fig.2).

DISCUSSION

In our experiments, the intake volume of 0.15% saccharin solution was significantly reduced after motion stimulation, and this antidipsia could be decreased by administration of anisodamine, an anticholinergic drug currently used to prevent and treat motion sickness[4]. The results of our experiments indicated a successful establishment of animal model with motion sickness by rotary stimulation for 30 minutes.

The number of CGRPi neurons in the vestibular efferent nucleus and the level of CGRP fiber immunoreactivity in the vestibular nucleus increased significantly in rats after rotary stimulation. Moreover, the increase in rats with rotatory stimulus 3 times was greater than that of the rats with rotatory stimulus once. Our results showed that anisodamine administered orally before stimulation can alleviate the symptoms (i.e., CTA) of motion sickness (Fig.1) and also significantly reduce the expression of CGRP in the vestibular efferent nucleus and the vestibular nucleus in rats with motion sickness (Fig.2). This suggested that CGRP may have a potential role in motion sickness.

REFERENCES

AN ULTRASTRUCTURAL STUDY ON HIPPOCAMPI NEURONS IN HINDLIMB UNLOADING RATS

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ABSTRACT
A variety of evidence suggests that neuronal function is altered during microgravity and in response to alterations in input which are coming from overall sensory system. To gain insight into the structural changes, electron microscopy was used to study structural elements of hippocampi neurons in hindlimb unloading rats decapitated in 1, 2, 3 and 4 weeks after simulated microgravity. Analysis of the revealed ultrastructural changes on the base of morphofunctional correlations leads to the following conclusions: compared with control group, multiplied and swollen mitochondria were observed in each experiment group. The structure of neurons from the 4 weeks experimental group was shown to be severely damaged, the changes involved the cristae of mitochondria reduced even disappeared, some mitochondria be, Irregular nuclear and increased heterochromatin were frequently observed in 1 week experiment group. These results demonstrate that the ultrastructure of hippocampi neurons maybe damaged by tail suspension, and the changes of neuronal function not only attributed to physiological but also pathological changes in hindlimb unloading rats.

1. INTRODUCTION
Mammalian brain has been studied during space flight aboard NASA orbital laboratory Spacelab-2. The main ultrastructural differences in the somatosensory cortex of the brain fixed under microgravity conditions and after landing include an increased number of degenerating presynaptic axon terminals after landing[1]. A variety of evidence suggests that nervous system function is altered during microgravity, however, assessing changes in neuronal physiology during space flight is a non-trivial task. Some people have used a rotating wall bioreactor with a high aspect ratio vessel, which simulates the microgravity environment, to investigate the how the viability, neurite extension, and signaling of differentiated neuron-like cells changes in different culture environments[2]. Otherwise, some people have used hindlimb unloading animal model to simulate microgravity to study. In this paper, a tail-suspension rat model was used to study the ultrastructural changes of hippocampi neurons.

2. MATERIALS AND METHODS
Wronski and Morey-Holton tail-suspension rats model were used[3]. 25 Adult male Sprague-Dawley rats weighing 250 to 280 g were used and were divided into five groups: one control (Con) group and four tail-suspended (SUS) groups. Con and SUS rats were killed at 7, 14, 21 and 28 days. Rat brain tissue was perfused with normal saline solution followed by phosphate-buffered 2% glutaraldehyde and 4% paraformaldehyde. The brain was carefully removed and immersed in the same fixative, and 2-mm coronal slices were prepared. Three approximately 1- to 2-mm cubes of representative brain tissue were obtained from hippocampi (gray matter). Each tissue sample was then postfixed in 1% potassium-ferrocyanide–reduced osmium tetroxide, dehydrated in graded acetones, and embedded in Epon 812. Semithin (0.5 um) sections were cut from tissue blocks and stained. sue for subsequent thin sectioning. The sections were observed and
photographed using a electron microscope who was instructed to find neurons as identified by a typical nucleus and surrounding perikaryon.

3. RESULTS AND DISCUSSION

Neurons in the control group have a normal-appearing nucleus and cytoplasm without signs of edema or injury, as illustrated in Fig.1. A, Organelles within the perikaryon appear normal, including the mitochondria, which demonstrate no evidence of swelling or injury; there is a typical homogeneous staining pattern of the matrix without excessive pallor or increased density or any signs of electron-dense precipitants. The surrounding neuropil also appears normal. After 7 days of hindlimb unloading, the neuronal cytoplasm is mildly swollen. The mitochondria within the perinuclear region of the perikaryon are moderately swollen, as seen in Fig.1. B, and C, compared with control. After this length of hindlimb unloading, the swollen mitochondria appear to have an intact inner and outer membrane with some disruption of the cristal structures, as illustrated in the representative areas of the cytoplasm in Fig.1. D(14 days) and E(21 days). In addition, the 28-days group (Fig.1. F) is significantly swollen, creating cytoplasmic vacuoles, demonstrating swollen matrix and cristae of mitochondria reduced even disappeared some mitochondria became elongate, and other organelles of the cytoplasm, for example, tough endoplasmic reticulum were enlarged. Irregular nucleus and increased heterochromatin were observed in 7 days group, but no karyopycnosis or karyorrhexis was found. There results demonstrate that the ultrastructure of hippocampi neurons may be damaged by tail suspension, and the changes of neuronal function not only attributed to physiological but also pathological changes in male SD rats by hindlimb unloading.

Reference

ABSTRACT
In this study, we analysed the eye movements of flatfish for linear acceleration and body tilting. The fish was fixed in the water sealed aquarium on a tilting table or a linear accelerator controlled by computer. The eye movements for acceleration along and perpendicular to longitudinal body axis were video-recorded. The vertical eye rotations were analysed frame by frame. In normal flatfish, vertical eye movements were observed for 0.1 G sinusoidal linear acceleration. After removal of left utriculus, response amplitude to tilting and to linear acceleration decreased. Response amplitude induced by linear acceleration did not coincide with responses predicted from data obtained by body tilting. This discrepancy implies that tilt and translation are not equivalent as stimulation producing vertical eye movements on Earth.

1. INTRODUCTION
Eye movement serves to hold the gaze steady or to shift the gaze to an object of interest. On Earth, signals from otolith organs can be interpreted either as linear motion or as tilt with respect to gravity. In microgravity, static tilt will no longer give rise to changes in otolith activity. However, linear acceleration as well as angular acceleration stimulates the otolith organ. Therefore, during adaptation to microgravity, otolith-mediated response such as eye movements would alter. Flatfish provide a natural model for the study of adaptive changes in the vestibulo-ocular reflex. During metamorphosis, vestibular and oculomotor co-ordinate of flatfish displaced 90 degrees about the longitudinal body axis [1]. Therefore, it is expected that microgravity induce the sensory mismatch in adult flatfish. Purpose of this study is to clarify visual-vestibular adaptation of flatfish.

2. METHODS
We performed experiments using left-eyed flatfish (*Paralichthys olivaceus*) with a body length up to 14.0 cm and a body weight of 8 to 10 g. Experiments were conducted in accordance with the principles expressed in the "Guide for the Care and Use of Laboratory Animals" published by the Office of Science and Health Reports of the USA National Institutes of Health, Bethesda MD 20892. After experiments using normal fish, we removed a utricle of left side and performed the same experiments. Fish were gently restrained in the horizontal position in a tank filled with aerated water (3.5 l). The mouth was fitted to the end of a small pipe that could serve water by respiration of the fish. We sealed the water-filled tank and endeavored to remove air bubbles. The fish tank was fixed in a light-tight box on a tilting device or linear accelerator.

Vertical eye movements were video recorded with CCD cameras attached in front of the fish tank. All recordings were done in lighting by a LED light mounted at both sides of the fish tank. However, the visual scene did not move during motion because the fish tank was fixed in the light-tight box. First, the fish tank was fixed on the stimulating device so that the longitudinal body axis was aligned with the direction of acceleration (direction-1; acceleration along the rostral-caudal axis). We then altered the fish position in the yaw plane 90 degrees (direction-2; acceleration perpendicular to the longitudinal body axis). We recorded the eye movements during sinusoidal sled movement with peak accelerations of 0.05, 0.1, 0.15, 0.2, and 0.5G and during body tilting angle of 30, 60, 90, 120, and 180 degrees. We distinguished the response to the acceleration of direction-1 from the caudal-to-rostral (Acc.-A) or the rostral-to-caudal (Acc.-B) and right-to-left (Acc.-A) or left-to-right (Acc.-B) for the acceleration of direction-2. In this experiment, the vertical eye rotation angles were calculated from the images digitized by computer.

3. RESULTS and DISCUSSION
Acceleration applied leftward or rightward in fish may be most effective in inducing vertical eye movement. In Fig.1, time course of vertical eye movements in normal fish (left column) and in otolith removed fish (right column). Three cycles of responses (thin lines) and the average of these responses (thick lines) are superimposed. Positive response corresponds to counterclockwise rotation and negative response corresponds to clockwise rotation. In Fig. 1 a, examples of vertical eye movement of normal flatfish for linear acceleration of direction-1 were shown. The direction of eye rotation was in agreement with the direction of compensatory eye movements when fish were tilted. Head-up body tilting of 90 degrees produced a counterclockwise rotation in the left eye and a clockwise rotation in the right eye (Fig.1 c). On the other hand, for the tilting perpendicular to longitudinal body axis (direction-2), left eye showed counterclockwise rotation and right eye showed clockwise rotation (Fig.1 e). After removal of left utricle,
the response amplitude to linear acceleration and body tilting decreased (Fig. 1b, d, f). From the results that reduction of responses to the acceleration of caudal-rostral direction, it was suggested that utriculus may play a role mainly for the changes of acceleration along the longitudinal body axis. With increasing tilt angle, the response amplitude increased up to 90 degrees and saturated about 120 degrees for both head-up and head-down body tilting.

In common with most vertebrate, fish hold their eyes more or less horizontal when their body rotates around the longitudinal or transverse axis [3]. In linear acceleration, the resultant force calculated from gravity and linear acceleration may be exerted on the otolith organs. Stimulation of the otolith by linear acceleration is thought to be equivalent to stimulation produced by tilting the head. In this experiment, the direction of vertical eye movements produced by linear acceleration was the same as expected from body tilting. The eye rotation angle for linear acceleration should coincide with the angle of deviation from the vertical (gravity) due to resultant force.

In Fig. 2, we plotted the response amplitudes to body tilting and to linear acceleration against the tilt angle. Magnitude of acceleration was converted to angle using a relation between gravity and applied acceleration. Straight lines in Fig. 2 indicated response amplitude for linear acceleration estimated from data obtained by body tilting experiments. For equal shearing acceleration applied to the otolith, which was produced by linear, sled motion or body tilting, it is expected that may induced the same amplitude of eye movements. However, there was only partial compensation for all magnitudes of acceleration. This discrepancy implies that tilt and translation are not equivalent as stimulation producing vertical eye movements on Earth.

The property of vertical eye movements was resembled to that of goldfish [2]. In goldfish, vertical and torsional eye movement relating to gravity can be explained by input only to utriculus. In flatfish, however, sacculus and/or lagena may play some roll in controlling eye movements [1]. Changes of eye movement after removal of the otolith on one side should be investigated in the future.

Fig. 1. Time course of vertical eye movements for linear acceleration and body tilting in normal (left column) and otolith removed fish (right column). Three cycles of responses (thin lines) and the average of these responses (thick lines) are superimposed.

Fig. 2. Response amplitude of vertical eye movements for linear acceleration and body tilting in normal fish, (a) (b) and otolith removed fish (c) (d).

4. REFERENCES
ABSTRACT
The present study focused on the mechanisms underlining the reduction in the activity of Na+-dependent glutamate transporters in brain synaptosomes isolated from rats exposed to hypergravity conditions (10 G for 1 hour). The treatment of synaptosomes with 200 nM bafilomycin A₁, which is a highly specific inhibitor of V-type ATPase, essentially decreased the initial velocity of L-[¹⁴C]glutamate uptake from 2.5 ± 0.2 nmol x min⁻¹ x mg⁻¹ protein to 1.1 ± 0.05 in control, whereas under hypergravity this value lowered from 2.05 ± 0.1 nmol x min⁻¹ x mg⁻¹ protein to 0.9 ± 0.05 (P ≤ 0.05). Bafilomycin A₁ inhibiting glutamate uptake did not eliminate difference in uptake between control and hypergravity. Thus, it may be suggested that a decrease in acidification of synaptic vesicles, which is known to be coupled with neurotransmitter loading, is not the main cause of weak Na⁺-dependent uptake by nerve terminals under hypergravity conditions.

1. INTRODUCTION
Proper glutamate synaptic transmission is essential for basic neuronal communication, synaptic plasticity and is important for learning and memorizing, attention, control of mood, stress and anxiety. Abnormal glutamate homeostasis contributes to neuronal dysfunction [1]. Exposure of humans and animals to microgravity environment of space flight or to hypergravity conditions is accompanied by changes in mental efficiency, altered cognitive abilities, learning and language skills abnormalities that may be associated with changes in glutamatergic neurotransmission.

Low extracellular concentration of glutamate is normally maintained between episodes of exocytotic release of glutamate to prevent continual activation of glutamate receptors protecting neurons from excitotoxic injury and to ensure a high signal-to-noise ratio for neurotransmitter from synaptic vesicles to cytosol. Glutamate transporters use the Na⁺/K⁺ electrochemical gradient as a driving force for uptake, which is the main cellular mechanism for maintaining low extracellular glutamate concentration. Vesicular glutamate transporters utilize the electrochemical proton gradient across the synaptic vesicle membrane (∆µH⁺) generated by a V-type H⁺-ATPase to accomplish glutamate accumulation into synaptic vesicles. This vesicular glutamate uptake system, in contrast to Na⁺-dependent plasma membrane one, has a low affinity for glutamate and is stimulated by physiologically relevant concentrations of chloride. The H⁺-ATPase uses energy produced by the hydrolysis of cytosolic ATP to direct a flow of H⁺ into the interior of synaptic vesicles, pH of the vesicle interior is more acidic establishing pH gradient (∆pH) across the vesicle membrane and the vesicle interior is more positive establishing a corresponding membrane potential (Δψ). The sum of these two events is the electrochemical proton gradient (∆µH⁺), which provides the coupling between ATP hydrolysis and glutamate uptake. Since, acidification of vesicles is associated with neurotransmitter loading, the dissipation of the proton gradient lead to the inhibition of glutamate uptake and in some cases to the leakage of neurotransmitter from synaptic vesicles to cytosol.

The study focused on the interrelation between transporter-mediated glutamate uptake and the ability of synaptic vesicle to accumulate the neurotransmitter under hypergravity.

2. EXPERIMENTAL PROCEDURES
2.1 Isolation of rat brain synaptosomes
Wistar rats (males 100–120 g body weight), either control (1 g for 1 hour in the cage without rotation) or centrifuged (at 10 G / 60 rpm for 1 hour) were maintained in accordance with the European Guidelines and International Laws and Policies. The cerebral hemispheres of decapitated animals were rapidly removed and homogenized in ice-cold 0.32 M sucrose, 5 mM HEPES-NaOH, pH 7.4 and 0.2 mM EDTA. Synaptosomes were prepared by differential and Ficoll-400 density gradient centrifugation of rat brain homogenate according to the method of Cotman (1974) with slight modifications [2]. The standard salt solution was oxygenated and contained (in mM): NaCl 126; KCl 5; MgCl₂ 1.4; NaH₂PO₄ 1.0; HEPES 20; pH 7.4 and d-glucose10. The Ca²⁺-supplemented medium contained 2 mM CaCl₂. Protein concentration was measured as described by Larson et al. [3].
2.2 Uptake experiments

Uptake of L-[14C]glutamate by synaptosomes was measured as follows: samples (125 μl of the suspension, 0.2 mg of protein/mL) were pre-incubated in standard salt solution for 10 min at 37 °C. Uptake was initiated by the addition of 10 μM L-glutamate supplemented with 420 nM L-[14C]glutamate (0.1 μCi/mL), incubated for 0–20 min at 37 °C and then rapidly sedimented in a microcentrifuge (20 s at 10,000 × g). Uptake was measured in aliquots of supernatant (100 μl) and pellets by liquid scintillation counting with scintillation cocktail ACS (1.5 mL). Nonspecific binding of the neurotransmitter was evaluated in cooling samples sedimented immediately after addition of radiolabeled glutamate. Results were expressed as mean ± S.E.M. values. Statistical analysis used two-tailed Student's t-test. Differences were considered significant when P≤0.05.

3. RESULTS AND DISCUSSION

Recently, we have demonstrated that activity of Na+- dependent uptake of glutamate was decreased [4], whereas transporter-mediated glutamate release was augmented after hypergravity loading [5]. Flow cytometric analysis with acridine orange, a pH-sensitive fluorescent dye, which is selectively accumulated by synaptic vesicles (acidic compartments of synaptosomes), have demonstrated a decrease by 10% in mean intensity of acridine orange fluorescence at steady state level in hypergravity synaptosomes as compared to controls. Flow cytometry showed the changed ability of synaptic vesicle to accumulate the neurotransmitter in hypergravity that may be one of the causes influencing transporter-mediated uptake of glutamate. To test the suggestion of a possible interrelation between transporter-mediated glutamate uptake and the ability of synaptic vesicle to accumulate the neurotransmitter under hypergravity, the experiments with bafilomycin A1 were performed.

Bafilomycin A1 is a highly specific inhibitor of V-type ATPase, which impacts accumulation of the neurotransmitters into synaptic vesicles by disturbance of the proton gradient generation across synaptic vesicle membrane. We have found that the treatment of synaptosomes with 200 nM bafilomycin A1 essentially decreased the initial velocity of L-[14C]glutamate uptake from 2.5 ± 0.2 nmol x min-1 x mg-1 protein to 1.1 ± 0.05 nmol x min-1 x mg-1 protein in control (P≤0.05), whereas under hypergravity this value lowered from 2.05 ± 0.1 nmol x min-1 x mg-1 protein to 0.9 ± 0.05 nmol x min-1 x mg-1 protein (P≤0.05) (Fig.1). Bafilomycin A1 similarly inhibited glutamate uptake by plasma membrane transporters in control and hypergravity by means of specific disturbance of synaptic vesicle proton gradient, but did not eliminate difference in glutamate uptake between control and hypergravity.

4. REFERENCES


**EFFECTS OF WATER IMMERSION TO THE NECK LEVEL ON EYE-HEAD COORDINATION IN RHESUS MONKEYS**

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**ABSTRACT**

The gaze fixation reaction was studied in two rhesus monkeys before and during thermoneutral (35°C) water immersion to the neck level. Changes of the peak head velocity and the angular vestibulo-ocular reflex gain were more pronounced in both monkeys in 5 h after the start of immersion to the neck level as compared with previous results in monkeys immersed to the mid-chest level. The low peak head velocity in both monkeys was accompanied by a new control strategy of the head movements. Thus, more pronounced support deprivation affects the central programming of eye-head coordination to a great extent.

**INTRODUCTION**

The gaze fixation reaction (GFR) was studied in monkeys during spaceflight [1,2] and during water immersion to the mid-chest level [3,4]. The reaction of rapid fixation of the gaze at targets appearing unexpectedly in the peripheral visual areas is provided by a coordinated interaction of three components, namely: eye saccade towards the target, head movement in the same direction, and compensatory eye counter-rotation that is based on the angular vestibulo-ocular reflex (aVOR). Similarities of effects of immersions and space flights on the characteristics of eye-head coordination during GFR in monkeys allowed to conclude that water immersion can be used as an adequate model simulating the effects of support deprivation occurring in microgravity.

However, changes in GFR parameters in 3 monkeys were somewhat modest in 5 h after the start of immersion to the mid-chest level [4]. Purpose of the present study was to evaluate the influence of more pronounced support deprivation on the characteristics of vestibulo-ocular-motor interactions in two monkeys during GFR in 5 h after the start of water immersion to the neck level.

**METHODS**

Two male rhesus monkeys (Macaca mulatta) of 5-6 kg were used in this study. Surgical procedures included: (1) attachment of an acrylic ring to the skull, which supported a preamplifier and a protective cover carrying a head angular velocity sensor (Watson), and (2) implantation of electrooculographic (EOG) electrodes bitemporally. All required surgery and all experimental procedures were approved by the Bioethics Board at RF SSC-IBMP RAS.

The monkeys were pre-trained to perform eye-head-hand coordination task. The animal was required to touch with its hand finger a light stimulus (1°) presented on the horizontal circumferential panel at a distance of 25 cm from the animal’s eyes. After the animal touched the trigger (central) stimulus, it was presented at random with one of the lateral stimuli (±18° or ±36°) in the form of a positive or a differential sign for no longer than 0.8 s. The ratio at which positive and differential stimuli were presented was 2:1. If the monkey turned off the positive stimulus, it was rewarded with a dose of juice. If it turned off the differential stimulus or missed the positive stimulus, it was penalized by delaying the presentation of the next trigger stimulus.

The task was performed by monkeys before and after 5 hrs thermoneutral (35°C) water immersions (i1 and i2) to the neck level (two weeks between i1 and i2). During experiments the monkeys were seated in primate chair which could be secured to a rigid bar of the water tank. Trunk movements with respect to the chair were inhibited with a shoulder harness and by the shape of the chair. The water level was lowered to the animal’s mid-chest level before test of motor task at the very end of immersion to the neck level.

Digitized (500 Hz) data of eye and head movements to targets located at 36° to the left and to the right were pooled. The GFR trials were analysed if eye and head velocities were <20°/s during the 100 ms interval before the onset of eye or head movements. Averaging of data was performed in sync with the pre-selected in each trial points and the corresponding time segments broken into bins of 10 ms in duration. The aVOR gain was calculated as the ratio of eye velocity to head velocity during the time segment (30 ms) of the eye counter-rotation. The EOG was calibrated using eye saccades to targets located at ±18°.

Means ± SD were computed for all results. Statistical analysis was performed using Student’s t test with an Bonferroni correction for multiple comparison.

**RESULTS**

Fig. 1 illustrates the changes of GFR parameters in monkeys A and B in two 5 hrs immersions to the neck level. The peak eye saccade velocity increased in monkey A by 8.2% (from 616±66°/s in control to 667±81°/s; p<0.05) in i1 and by 5.8% (to 652±57°/s; p<0.01)
in i2. In monkey B, the peak eye velocity increased by 2.8% (from 717±77°/s in control to 737±85°/s; p<0.05) in i1 and by 10.3% (to 791±145°/s; p<0.001) in i2. The eye saccade amplitude increased in monkey A by 11.0% (from 27.4±3.0° in control to 30.4±3.7°; p<0.001) in i1 and by 10.7% (to 30.3±3.2°; p<0.001) in i2. The eye saccade amplitude increased in monkey B by 9.0% (from 28.7±4.2° in control to 31.3±7.4°; p<0.001) in i1 only. The peak head velocity decreased in monkey A by 17.0% (from 80.8±14.1°/s in control to 67.1±20.3°/s; p<0.005) in i1 and by 51.9% (to 38.9±21.7°/s; p<0.001) in i2. In monkey B, the peak head velocity decreased by 9.0% (from 100.2±35.5°/s in control to 91.2±34.3°/s; p<0.05) in i1 and by 49.5% (to 50.6±24.4°/s; p<0.001) in i2. The absolute value of aVOR gain increased in monkey A by 12.7% (from 0.99±0.13 in control to 1.1±0.28; p<0.001) in i1 and by 18.2% (to 1.16±0.38; p<0.001) in i2. In monkey B, the absolute value of aVOR gain increased by 14.0% (from 1.05±0.20 in control to 1.26±0.42; p<0.001) in i1 and by 28.8% (to 1.36±0.47; p<0.001) in i2.

The low peak head velocity in both monkeys in i2 was accompanied by a new control strategy of the head movements. In monkey A (Fig. 2), part of the head velocity profiles (type 1; 43%) was similar to the control profiles whereas other profiles (type 2; 57%) had an initial peak followed by a pronounced deceleration and subsequent reacceleration. In monkey B (Fig. 3), the head latency decreased from 125±30 ms in control to 98±46 ms (p<0.001) in i2 whereas the eye saccade latency increased from 125±26 ms to 172±44 ms (p<0.001), that is, GFR was made with the significant head leading in i2.

**DISCUSSION AND CONCLUSIONS**

The results indicate that decrease of the peak head velocity and increase of the aVOR gain were more pronounced in both monkeys in 5 h after the start of the second water immersion to the neck level as compared with results in monkeys immersed to the mid-chest level. The low peak head velocity was accompanied by a new control strategy of the head movements in both monkeys. Thus, more pronounced support deprivation affects the central programming of eye-head coordination to a great extent.

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FOURFOLD COMPARATIVE POSTUROGRAPHY

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ABSTRACT
A novel posture evaluating platform and multi-parametric analytic software has been introduced for experimental and clinical practice. The spontaneous (small) standing straddle and a fourfold test battery is proposed for both clinical and experimental posturometry. The comparative evaluation of the four sections provides a sensorimotor-context-based diagnosis.

INTRODUCTION
Posturography is widely accepted as the relevant representation of postural performance. Nevertheless, a broad sensorimotor context (e.g. visuomotor coordination, cerebellar adaptation, holistic extrapyramidal control, motor state) should be considered as well for an extended functional evaluation [2,3,4,5].

A) THE POSTURE EVALUATION FACILITY
Our Posture Evaluation Platform (PEP®) recently has been introduced in laboratory and clinical measurements. The high resolution and stability of this equipment has been completed by a fully automatized evaluating software package. As a continuation of our former experimental activity [1], this computer program has been developed in close cooperation with clinical specialists. An optional set of the large variety of the calculated parameters (time-course, amplitude-distribution, frequency spectrum both of left/right and front/back component of body sway) are displayed as an actual standard analytic panel (Fig. 1.).

This display of data can be arranged by the clinical specialist's interest concerning disorders in posture, proprioceptive afferentation, sensorimotor centers of CNS or in the functional state of motor organs.

B) THE ANALYTIC PROCESS
a) Separating the distinct anteroposterior and left-right body sways
b) Separating for a lower and higher frequency range
c) Compound diagram of body sway (dispersion of amplitudes, SwU- sway unit) indicating the differences referring the above mentioned parameters (Fig. 2).

d) For some clinical syndromes - as nystagm-like body shifts at central type balance disorders - time dependent analysis has been elaborated. Regression lines of ascending and descending phases on a given filtered record were faced to recognize the less steep direction (as the neurally driven shift) in contrast of the more steep one - similarly to that of slow and fast components of the ocular nystagm respectively.

C) CLINICAL TRIALS
After preliminary measurement sessions in our laboratory carried out on healthy young people (university students of both gender), clinical tests were organized at an otoneurology unit on a wide spectrum of vertigo/dizziness patients [6]. Soon it became clear, that the PEP facility could be utilized far beyond the standard Romberg test, the latter is being a rather forced double provoking state by the closed legs and eyes.

a) Spontaneous small standing straddle proposed for physiological posture
At the preliminary laboratory test series different conditions were practiced. We propose four of them for a future clinical test battery [7].

b) The fourfold test session aiming comparative analysis of postural control
The next four sections should be performed continuously and analyzed at closing of the session:
i. spontaneous small standing straddle as physiology-based posture paradigm, open eyes
ii. spontaneous small standing straddle - closed eyes
iii. the rather constrained Romberg position, open and closed eyes.

**c) Comparative analysis of posture control**
The common display of the four sections as a proposed standard allows us to reveal basic functional differences concerning standing positions, visual reference, also slow and fast components of body sway, all distinguished for the antero-posterior and left-right postural control.

**d) Comparison of visuomotor impacts**

![Fig. 3. Romberg a) closed, b) open eyes. Standing straddle a) closed, d) open eyes](image)

This display provides the facility to observe the role of the visual reference on the body sway, particularly the possible sensorimotor conflict (see Fig. 4. d) syndrome in the acute phase and in cerebellar adaptation at chronic state.

**e) Adaptive changes in body sway**
The exact follow-up of changes in the course of a given syndrome and the effect of the therapy and also the development of adaptation and/or rehabilitation can be performed.

**CONCLUSION**

A battery of four records relating different conditions is proposed for posturography clinical tests: a, for a physiology-based posture paradigm we propose the normal (small standing straddle) for standard instead of the rather constrained Romberg position. b, for the same reason open and closed eye measurement should be performed. The comparison of the latters provides the facility to observe the sensorimotor conflict syndrome in the acute and cerebellar adaptation at chronic state. The proposed fourfold posturography together with the relevant comparative analysis as a standard test can promote the sensorimotor-context-based diagnostic work both in otoneurology and clinical research.

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ARTERIAL PRESSURE RESPONSE TO SHORT PERIOD OF \(\mu\)G IN RATS
Hironobu Morita, Chikara Abe, Chihiro Iwata, Kunihiko Tanaka
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ABSTRACT
Acute pressure responses to short period of \(\mu\)G were examined in rats. Microgravity was induced by free drop. When anesthetized rats were fixed in 30° head-up position, microgravity elicited pressor responses, which were characterized as a slight increase in abdominal aortic pressure (AP), a significantly more increase in carotid arterial pressure (CAP: 22 ± 3 mmHg), and a slight but significant increase in jugular venous pressure (JVP, 15 mmH2O). All of these pressor responses were not observed, if rats were fixed in horizontal flat position. In contrast to the pressor response in anesthetized rats, it in conscious rats was mainly mediated via the vestibular system. In freely moving conscious rats, \(\mu\)G elicited a large increase in AP by 38 ± 4 mmHg. This increase was significantly reduced by vestibular lesion (VL: 20 ± 2 mmHg) or body stabilization (27 ± 2 mmHg), and completely abolished by combination of VL and body stabilization. These results indicate that acute hemodynamic response to short period of microgravity in rats was modified by anesthesia. In anesthetized rats, hemodynamic response to microgravity was triggered by a disappearance of gravitational pressure difference; however in conscious rats, role of the vestibular system in AP control became more significant.

INTRODUCTION
Gravity, not only a change in amount of gravity but also a change in direction of gravity, is one of a major stress for the cardiovascular system. The key issue in acute cardiovascular responses to gravitational change is thought to be blood redistribution induced by changes in gravitational hydrostatic pressure difference. For example, during \(\mu\)G, hydrostatic pressure difference disappears and fluid shifts from the lower body to thoracic and cephalic organs, which induce increases in venous return and cardiac output, and then arterial pressure (AP) increases. These hemodynamic changes are thought to be buffered by baroreflex. However, recent study from our laboratory demonstrated that the vestibular system also has a significant role in cardiovascular response to gravitational changes. Thus, in this paper I will focus on the role of these three factors, i.e., hydrostatic pressure, baroreflex, and vestibular system, in the AP response to gravitational changes.

EXPERIMENTS
Male Sprague-Dawley rats weighing 300-360 g (10-14 wk old) were used and maintained in accordance with the Guiding Principles for Care and Use of Animals in the Field of Physiological Science of the Physiological Society of Japan. The Animal Research Committee of Gifu University approved the experimental protocol.

1. Effect of Hydrostatic pressure [3]
Hemodynamic responses in the cephalic region to free drop-induced (MGLAB, Toki, Japan) microgravity were examined in urethane and \(\alpha\)-chloralose (300 and 50 mg/kg, ip) anesthetized rats. To evaluate the effect of the disappearance of the hydrostatic pressure gradient on the hemodynamic responses, experiments were performed on two positions, flat prone position and 30° head-up whole body-tilt (HU) position. My hypothesis was that in the HU rats, the disappearance of the hydrostatic pressure gradient in the head-to-foot axis would cause the carotid arterial pressure (CAP) and jugular venous pressure (JVP) to increase by the same extent, which is equivalent to the gravitational pressure difference between the heart and the neck, and thus cephalic perfusion pressure (CPP=CAP-JVP) would not change during \(\mu\)G.

Fig. 1. Typical recordings of G-level, CAP, and JVP responses to free drop-induced \(\mu\)G in anesthetized rats in the 30° HU and flat position. Modified from reference [3].

Fig 1 shows typical G-level, CAP, and JVP responses to free drop in HU and flat rats. The G-level smoothly decreased from 1 G to \(\mu\)G and \(\mu\)G lasted 4.5 s. In a HU
rat, the CAP increased at the onset of μG. In contrast, in a flat rat, the CAP and JVP did not change during μG. Averaged data of 6 rats in each group shows that the CAP and JVP in HU rats significantly increased from 106 ± 3 to 129 ± 4 mmHg and from 0.4 ± 0.7 to 1.7 ± 0.8 mmHg, respectively; the increase in CAP was significantly larger than that in JVP, thus calculated CPP significantly increased by 23 mmHg. Images of the microvessels of the iris were obtained using a charge-coupled device camera installed on a microscope with a ×4 objective lens. During the control 1 G period, there was no difference in the capillary diameter and flow velocity between the HU and flat groups (12 ± 1 vs 13 ± 1 µm, and 185 ± 22 vs 168 ± 16 µm/s). In the HU group, both the flow velocity and diameter increased during μG (Fig. 2). In contrast, in the flat group, these variables did not change during μG.

2. Role of the baroreflex during μG [4-6]

The key issue in understanding the increase in JVP is that it was observed only in the HU position. The JVP change in HU rats on going from 1 G to μG was caused by the disappearance of the gravitational pressure gradient in the head-to-foot axis, the observed change in JVP was 15 mmH2O (1.1 mmHg), which approximately corresponds to the height difference between the heart and the jugular vein in HU rats. The marked increase in the CAP seen in the HU rats was unexpected. To understand the different extent of increase in CAP and JVP, the static component (generated by gravity) and dynamic component (generated by the heart pumping) of the pressure should be considered.

The importance of the baroreflex in the acute hemodynamic responses to μG became evident, if the arterial pressure (AP) were measured with renal sympathetic nerve activity (RSNA). At the onset of μG, the AP did not change, however RSNA decreased to background noise levels (Fig. 3). This decrease in RSNA was completely abolished by sinoaortic baroreceptor denervation and vagotomy, suggesting that the decrease in RSNA was mediated via baroreceptors. Furthermore, the afferent nerve activity from the aortic baroreceptor increased with no change in the AP. To understand this phenomenon, transmural pressure of the aorta (AP - intrathoracic pressure (ITP)) and aortic diameter were measured (Fig.4). At the onset of μG, the AP did not change, while the ITP decreased by 2.4 mmHg. Accordingly the calculated transmural pressure increased, and then aortic diameter increased by 25 µm.

The major findings of this study are: 1) The AP did not increase on entry into μG, while the ITP decreased and the transmural pressure of the baroreceptors increased. 2) Thus, the baroreceptors were stimulated, and then RSNA was reflexively suppressed during μG.

3. Role of the vestibular system in μG [8]

Vestibular lesion (VL) had no effect on acute AP and RSNA responses to μG in anesthetized rats (1). However, role of the vestibular system became evident, if the AP response was examined in conscious rats. The AP responses to free drop-induced μG are shown in Fig.
5. In a freely moving rat, µG induced a marked pressor response, which was somewhat suppressed if a vertical body movement was restricted by an internal septum (Stab), and completely abolished by combination of VL and Stab. Average data of the pressor responses were 38 ± 4 mmHg in freely moving rats, 27 ± 2 mmHg in Stab rats, 20 ± 2 mmHg in VL rats, and 7 ± 5 mmHg in VL+Stab rats, respectively. Thus, the pressor response in conscious rats was mediated via the vestibular system and non-vestibular system, which was blocked by body stabilization.

4. Role of baroreflex and vestibulo-sympathetic reflex in hypergravity [2]
The role of the baroreflex and vestibulo-sympathetic reflex in maintaining AP during hypergravity was examined in chronically instrumented conscious rats. Fig. 6 shows typical AP and RSNA responses to hypergravity. A rapid increase in gravity from 1 to 3 G was induced by centrifugation. In an intact rat, the AP and RSNA started to increase at the onset of gravitational change, and then remained above the baseline level. These responses were modified by VL and/or SAD. In a VL rat, the RSNA increase was attenuated, and the pressor response was completely abolished. In contrast, in a SAD rat, the AP and RSNA increases were markedly augmented. In a VL+SAD rat, the AP decreased, while RSNA did not change. In this study, the roles of baroreflex and vestibulo-sympathetic reflex were assessed by lesion experiments. Because RSNA did not change in response to hypergravity in rats lacking functional baroreceptor and vestibular receptors (i.e., VL+SAD rats), these receptors can be considered the two major components affecting RSNA under the conditions of this study. The RSNA response observed in rats with functional vestibular receptors, but no baroreceptor activity (i.e., SAD rats), indicates that vestibular input elicited a marked RSNA increase in response to hypergravity. In contrast, the role of baroreflex in controlling RSNA is complex. In intact rats, hypergravity elicited an increase in RSNA, which was significantly augmented by SAD, suggesting that baroreflex suppressed the RSNA increase in intact rats. In VL+SAD rats, RSNA was not affected by hypergravity, but in VL rats, i.e., rats with a functional baroreceptor, but no functional vestibular receptors, RSNA increased in response to hypergravity, suggesting that baroreflex augmented RSNA. These opposing effects of baroreflex on RSNA may be due to the bidirectional input to the baroreceptor. The baroreceptor is loaded in rats with functional vestibular receptors due to the pressor effect of the vestibulo-sympathetic reflex but is unloaded in rats lacking functional vestibular receptors.

Based on these experimental observations, simple block diagram can be depicted (Fig. 7). At the onset of hypergravity, this gravitational change is detected by the vestibular receptors, which reflexively control the AP before it decreases due to blood redistribution. Thus, the vestibular system acts as a feedforward AP controller.
against hypergravity. However, the AP increase observed in intact rats indicates that the AP was overcompensated rather than compensated, and this effect was more readily seen in SAD rats lacking the baroreflex. While feed forward control has the advantage of a short response delay, the major disadvantages are the instability of the response and the overcorrect error. Compensation of the AP response in the intact and SAD rats showed that the overcompensated AP was compensated by the baroreflex. Thus, the AP control system during hypergravity is a combination of the vestibular feedforward system and the baroreflex feedback system.

**PERSPECTIVE**

Although the vestibular system plays a significant role in the AP response to gravitational changes, the physiological significance of this response remains unclear. Hypergravity causes fluid shift from the intrathoracic component to the legs; this could result in reduced venous return and cardiac output, followed by a decrease in the AP. Thus, the vestibular system-mediated pressor response counteracts the hypergravity gravity-induced hypotension. In this regard, the vestibular system acts as a regulator of the AP in order to prevent hypotension. On the other hand, µG causes a headward fluid shift that could result in increased venous return and cardiac output, followed by an increase in the AP. Thus, if the vestibular system has a physiological significance in the control of the AP during gravitational changes, it should induce a depressor response under µG condition. Conversely, however, it induces a pressor response; i.e., the vestibular system induces a pressor response irrespective of the direction of the changes in gravity and the AP. These results suggest that the vestibular system-mediated pressor response is a type of stress response but not a purposeful response. This pressor response is, however, effective in preventing hypotension under hypergravity condition and on posture transition from recumbency to upright standing [7].

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**REFERENCES**

Simulated microgravity induced activation of BK$_{Ca}$ channel associated with increased apoptosis in cerebrovascular smooth muscle cells

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1. ABSTRACT

Cerebral arterial remodeling is one of critical factors in occurrence of postspaceflight orthostatic intolerance. We hypothesize that large conductance calcium-activated K$^+$ (BK$_{Ca}$) channels in vascular smooth muscle cells (VSMCs) are involved in regulating cerebrovascular apoptotic remodeling during microgravity exposure. Sprague-Dawley rats were subjected to 1-week hindlimb unweighting to simulate microgravity. Alterations of BK$_{Ca}$ channels in cerebral VSMCs were investigated by patch clamp and Western blotting, apoptosis was assessed by electron microscopy and TUNEL. To evaluate the correlation of BK$_{Ca}$ channel and apoptosis, BK$_{Ca}$ channel protein and cell nucleus were double-stained. Findings of the present study strongly support that simulated microgravity induced activation of BK$_{Ca}$ channel associated with increased apoptosis in cerebrovascular smooth muscle cells.

2. INTRODUCTION

Postspaceflight orthostatic intolerance has been regarded as one of the major adverse effects of microgravity exposure, and there are still no effective countermeasures. In the past decades, human studies from bed rest and animal studies with tail-suspended rat have revealed that simulated microgravity induced function and structure remodeling in cerebrovascular adaptations.

The large conductance calcium-activated K$^+$ (BK$_{Ca}$) channels are highly expressed in VSMCs and play an essential role in the regulation of various functions. Activation of BK$_{Ca}$ channel has been considered to regulate vascular tone in a negative feedback manner which limits cerebrovascular constriction or prevents vasospasm. Besides its function in vascular relaxation, BK$_{Ca}$ channel has also been reported to be implicated in apoptosis of cultured pulmonary artery smooth muscle cells (PASMCs). Based on previous studies, we hypothesized that BK$_{Ca}$ channels of cerebral VSMCs may have an important role in mediating cerebrovascular apoptotic remodeling during microgravity.

Therefore, we designed the present studies to investigate whether activation of BK$_{Ca}$ channel has been involved in apoptosis cerebral VSMCs in short-term (1-wk) simulated microgravity rats. We specifically investigate the alterations of BK$_{Ca}$ channels by comparing the whole-cell current densities, single-channel properties, and expressions of BK$_{Ca}$ α-subunit in cerebral VSMCs obtained from control and simulated microgravity rats. Furthermore, double-staining of BK$_{Ca}$ channel α-subunit and cell nucleus were performed to evaluate the correlation of BK$_{Ca}$ channel and apoptosis in cerebral VSMCs. Finally, we assessed the apoptosis by transmission electron microscopy and in situ terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling (TUNEL) in cerebral VSMCs from the same rat strains.

2. Materials and Methods

Animal model

A total of 80 male Sprague-Dawley rats were randomly assigned into two groups (n=40/group): 1-week tail-suspension (SUS) and simultaneous control (CON).

Isolation of cerebral VSMCs

Enzymatic isolation of single VSMCs was carried out as previously described.$^1$

Electrophysiological measurements

Whole-cell and single-channel of BK$_{Ca}$ currents were recorded as previously described.$^{1,2}$

Evaluation of BK$_{Ca}$ protein expression by Western blotting

Protein samples of cerebral arteries were prepared according to the published methods.$^3$
Immunocytochemistry double-staining
Double-staining was performed with BK$_{\text{Ca}}$ α-subunit antibody and nuclear dye to evaluate the relationship between BK$_{\text{Ca}}$ channel and apoptosis in cerebral VSMCs.

Transmission electron microscopy
The basilar artery sections were prepared and examined with a transmission electron microscopy.

2.2 Statistical analysis
The data are expressed as means ± SE. A one-way ANOVA was used to determine the overall differences. A value of P ≤ 0.05 was considered to be statistically significant.

3. RESULTS
Augmented activities of BK$_{\text{Ca}}$ in cerebral VSMCs isolated from SUS rats
Whole-cell BK$_{\text{Ca}}$ currents recorded from cerebral VSMCs in CON rats showed a smaller but consistent component of traces, whereas currents recorded from cerebral VSMCs in SUS rats showed a noisier and larger component of traces. Plots of open probability and current amplitude against membrane potentials are compared between CON and SUS rats.

Increased expression of BK$_{\text{Ca}}$ channel α-subunit in cerebral arteries from SUS rats
Averaged data expressed as percentage of the β-actin signal showed a 2-fold increase in BK$_{\text{Ca}}$ channel α-subunit expression in SUS cerebral arteries as compared with that in CON. In agreement with the Western blotting, immunocytochemistry double-staining of isolated cerebral VSMCs with anti-BK$_{\text{Ca}}$ α-subunit antibody and DAPI demonstrated the enhanced red-fluorescence intensity in SUS rats as compared with those from CON rats.

Increased apoptosis in cerebral VSMCs from SUS rats
To investigate cerebral VSMC apoptosis in qualification, basilar arterial sections were examined by transmission electron microscopy. In the medial area, some cerebral VSMCs displayed chromatin condensation or fragmentation, which is the morphological characteristic for VSMC undergoing apoptosis. To access the cerebral VSMC apoptosis in quantitation, cerebral VSMCs with a brown-yellow nuclear labelling were defined as TUNEL-positive. Averaged data indicated that TUNEL-index in the media of basilar arteries significantly increased by 5-fold in SUS as compared with that in CON.

4. DISCUSSION
The present study demonstrates that activation of BK$_{\text{Ca}}$ channel is associated with increased apoptosis in cerebral VSMCs of 1-wk simulated microgravity rats. Our specific new findings show that 1) Activities of BK$_{\text{Ca}}$ channel were enhanced during 1-wk simulated microgravity in cerebral resistance arteries, which represents a fundamental adaptive mechanism to buffer acute or chronic hypertension in cerebral arteries; (2) Apoptosis is increased suggesting that apoptotic remodeling has been attributed to regression in cerebral arterial adaptations during simulated microgravity; (3) Activation of BK$_{\text{Ca}}$ channels is positively correlated with increased apoptosis in cerebral VSMCs. These results suggested that BK$_{\text{Ca}}$ channel may be a critical mechanism involved in arterial structural remodeling. Our results also suggest that BK$_{\text{Ca}}$ channel-mediated apoptosis would be a novel way for the prevention or treatment of hypertension.

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REFERENCES
ROLE OF THE VESTIBULAR SYSTEM IN ARTERIAL PRESSURE CONTROL DURING POSTURE TRANSITION IN HUMANS
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ABSTRACT
Galvanic vestibular stimulation (GVS) is known to create an imbalance in the vestibular inputs, thus it is possible that the simultaneously applied GVS obscures adequate gravity-based inputs to the vestibular organs, and then impairs the vestibular-mediated response. To examine this, the arterial pressure (AP) response to gravitational change was compared among rats with or without GVS and rats with vestibular lesion (VL), and found that the effects of GVS on the AP response were qualitatively and quantitatively similar to that caused by VL. Using this method, role of the vestibular system in AP control during 60° head-up tilt (HUT) was examined in young and aged subjects. In young subjects, the AP did not change during posture transition from supine to HUT. When GVS was applied, AP immediately and significantly decreased by 17 ± 2 mmHg upon HUT. In contrast, in aged subjects, the AP decreased during HUT regardless of whether GVS was applied. These results indicate that the vestibular system plays an important role in initial AP control during posture transition in young subjects. However, this function might be impaired in aged subjects.

The role of the vestibular system in controlling the arterial pressure (AP) has been the focus of many studies on both humans and animals, and in these studies on animals, the AP response to gravitational change was compared between intact animals and animal with vestibular lesion (VL) [2, 4]. In human studies, however, such invasive and irreversible method like VL could not be employed. Thus, in human studies, an alternative method for acutely interrupting the vestibular-mediated AP response is required in order to examine the role of the vestibular system in controlling AP during gravitational change. Galvanic vestibular stimulation (GVS) is known to create an imbalance in the vestibular inputs, thus it is possible that the simultaneously applied GVS obscures adequate gravity-based inputs to the vestibular organs or modifies an input-output relationship of the vestibular system, and then impairs the vestibular-mediated response. This possibility was examined, and if it was true, then the role of the vestibular system in controlling the AP during posture transition was examined in human subjects.

1. Effect of GVS on vestibular-mediated AP response to microgravity [1, 4]
The AP response to free drop-induced µG was examined in chronically instrumented conscious rats. Free drop experiments were performed in four different conditions: free movement (FM) without GVS (GVS(off)FM), FM with GVS (GVS(on)FM), and with restricted vertical movement with or without GVS (GVS(off)Stab and GVS(on)Stab). Fig. 1 shows the typical responses of the AP obtained from a rat in the GVS(off)FM and GVS(on)FM groups. The pressor responses seen in a GVS (off) rat was suppressed by GVS (on). The pressor response was significantly suppressed by GVS in both FM and Stab groups, and the effect of GVS was quite similar to that of VL (Fig.2). Thus, GVS could be used for acute interrupting vestibular-mediated pressor response.

Fig. 1. Typical responses illustrating the AP of a free moving rat without GVS and with GVS. Modified from reference [1].

Fig. 2. Effects of VL (left) and GVS (right) on µG-induced pressor response. *P<005, significantly different from intact or GVS(off). Modified from references [1, 4].

2. Role of the vestibular system in controlling AP upon posture transition [3]
In this study, we recruited 15 healthy young subjects (12
males and 3 females; age, height, and weight: 22 ± 0.5 years, 169.6 ± 1.5 cm, and 64.0 ± 3.0 kg, respectively) and 10 aged subjects (5 males and 5 females; age, height, and weight: 83 ± 3 years, 156.3 ± 3.2 cm, and 52.6 ± 5.0 kg, respectively). The young subjects had no history of any illness related to the vestibular or cardiovascular system. Of the aged subjects, 3 were prescribed treatment for hypertension, but they had no history of any illness related to the vestibular system. This study was approved by the institutional review board of Gifu University and conformed to the Declaration of Helsinki. Written informed consents were obtained from all subjects after they were thoroughly acquainted with all the aspects of the experiment.

Fig. 3 shows representative responses of AP in a young subject during 60º head-up tilt (HUT) with and without GVS. When GVS was not applied, the AP was well maintained; however, the AP decreased during the change in the posture from the supine position to HUT when GVS was applied, and gradually returned to normal.

The averaged data of AP response to HUT in young and aged subjects are summarized in Fig. 4. The AP was well maintained at the pre-HUT control level when the HUT was performed without GVS in young subject, although a slight non-significant dip was observed at 15 s. However, when GVS was applied, AP immediately and significantly decreased by 17 ± 2 mmHg upon HUT. On the other hand, in aged subjects without GVS, the AP decreased during HUT unlike the observation in HUT in young subjects. When GVS was applied, the AP also decreased during posture transition. The change was similar to that during HUT without GVS, and did not return to control during the measurement.

These results demonstrates that the vestibular system plays an important role in controlling AP during posture transition from the supine position to HUT, since GVS attenuated AP control during posture transition. This vestibular system-mediated AP control during posture transition was impaired in aged subjects, and a decrease in AP was observed.

Fig. 3. Typical responses of the AP in a young subject during HUT with or without GVS. Modified from reference [3].

Fig. 4. Summary of the data on the changes in AP in young and aged subjects with or without GVS. Modified from reference [3].

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REFERENCES
DAILY SHORT-PERIOD GRAVITATION CAN PREVENT CHANGES IN A\textsubscript{0} AND AT\textsubscript{1}R EXPRESSIONS IN ELASTIC ARTERIES OF SIMULATED MICROGRAVITY RATS.

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1. ABSTRACT
The aim of the present study was to investigated whether intermittent gravitational loading by daily 1-h STD can also prevent differential changes in mRNA and protein expressions of both A\textsubscript{0} and AT\textsubscript{1}R in the common carotid artery and abdominal aorta of medium-term (28-d) simulated microgravity (SUS) rats by in situ hybridization and immunohistochemistry. In situ hybridization and immunohistochemistry revealed the presence of mRNA and protein expression of A\textsubscript{0} and AT\textsubscript{1}R in the media, adventitia, and perivascular tissue. SUS induced an up- and down-regulation in A\textsubscript{0} and AT\textsubscript{1}R expression in the common carotid artery and abdominal aorta, respectively. Daily 1-h STD over 28 days fully prevented the L-RAS changes in the large elastic arteries that might occur due to SUS alone. These Findings of the present study strongly support that vascular L-RAS may play a pivotal role in differential vascular adaptation to microgravity.

2. INTRODUCTION
Our previous work has shown that daily 1-h standing (STD), which mimics the physiologic effect of IAG, is sufficient to prevent differential adaptive changes in function and structure of small muscular arteries in different anatomic regions due to simulated microgravity [1]. These findings substantiate in general the hypothesis first raised by Hargens et al [2] that microgravity-induced chronic changes in regional vascular transmural pressures due to the removal of hydrostatic pressure gradient may well initiate differential adaptation of vessels in different anatomic regions. Furthermore, these results from animal studies are also consistent with recent findings from ground-based and space human studies. We further showed that certain elements, like angiotensinogen (A\textsubscript{0}) and angiotensin \textsuperscript{1} type 1 receptor (AT\textsubscript{1}R) of arterial local renin-angiotensin system (L-RAS), were also differentially regulated suggesting an important role of arterial L-RAS in vascular adaptation to microgravity [3].

In the present study we investigated using in situ hybridization and immunohistochemistry whether 1 h/d STD can also prevent differential changes in mRNA and protein expressions of both A\textsubscript{0} and AT\textsubscript{1}R in the common carotid artery and abdominal aorta of SUS rats.

2. METHODS
2.1 Rat model simulated microgravity and in situ hybridization
A tail-suspended rat model was used to simulate weightlessness on the ground [4]. Fifteen male Sprague-Dawley rats weighed 260–290g were divided into three groups: 4-wk tail-suspension (SUS), synchronous control groups (CON), tail-suspension for 23 h/day and standing for 1 h/day (SUS+STD). Immunohistochemical staining of sections from carotid artery and superior mesenteric artery tissues. was performed as described previously [5]. Primary polyclonal antibodies used were anti- A\textsubscript{0} (IgG, Department of Aerospace Physiology FMMU), and anti-AT1R (Santa Cruz Biotechnolgy, Santa Cruz, CA). Negative controls were prepared by using non-immune rabbit serum instead of the primary antibody. The degree of A\textsubscript{0} and AT1R immunoreactivity was determined as relative greyness. The in situ hybridization of both A\textsubscript{0} and AT1R mRNA in common carotid artery and abdominal aorta tissues were performed as described previously [6]. The complementary DNA fragments of the rat A\textsubscript{0} and AT1R cDNA (GenBank accession. NM 134432 and NM 030985) were cloned into the vector pCR\textsuperscript{2}-TOPO (Invitrogen, Carlsbad, CA, USA). Using this plasmid as a template, sense and antisense single-strand RNA probes were synthesized with a digoxigenin labeling kit (Roche Diagnostics, Mannheim, Germany). The degree of A\textsubscript{0} and AT1R hybridization was determined as relative greyness by an image analysis system (Leica Q500MC, Germany).

2.2 Statistics
All experimental data analysis are presented as means ±
SE. A paired Student’s t-test was used for statistical analysis of data between different groups.

3. RESULTS

Stained \( A_0 \) and AT1R are in brown color and mainly located in adventitia and perivascular tissue, but less in media. In the wall of the common carotid artery from a SUS rat, more intensive \( A_0 \) and AT1R immunoreactivity were detected in the media and adventitia as compared with that of a CON rat. On the contrary, in the wall of the abdominal aorta from a SUS rat, these immunoreactivities were scarcely detected as compared with that of a CON rat. Statistical analysis further confirmed that, in the wall of common carotid artery from SUS rats, the protein expression of \( A_0 \) and AT1R in the adventitia and media obviously increased, respectively; as compared with CON group. On the contrary, in the wall of abdominal aorta from SUS rats, the protein expression of \( A_0 \) and AT1R in the adventitia and media obviously decreased, respectively; as compared with CON group. However, daily 1-h STD completely prevented these changes due to suspension alone. There were no significant differences in \( A_0 \) and AT1R immunoreactivity of the common carotid artery and abdominal aorta between SUS + STD1 and CON groups. The specific signals of the antisense probe were located in the media and adventitia of the two kinds of vessels. In the wall of the common carotid artery from a SUS rat, more intensive \( A_0 \) and AT1R mRNA signals were detected in the media and adventitia as compared with that of a CON rat. On the contrary, in the wall of the abdominal aorta from a SUS rat, these mRNA signals were scarcely detected as compared with that of a CON rat. Statistical analysis further confirmed that the intensity of \( A_0 \) and AT1R mRNA signal in the wall tissue of the common carotid artery from SUS rats significantly increased in the media and adventitia, respectively, as compared with that of CON rats. On the contrary, in the wall tissue of the abdominal aorta from SUS rats, the \( A_0 \) and AT1R mRNA signal significantly decreased in the media and adventitia, respectively, as compared with CON rats. However, daily 1-h STD completely prevented these changes due to SUS alone. There were no significant differences in the intensity of \( A_0 \) and AT1R mRNA signal of the common carotid artery and abdominal aorta between SUS + STD1 and CON groups.

4. DISCUSSION

Our previous works had confirmed that simulated microgravity can induce differentially structural and functional changes in cerebral and hindquarter arteries. The in situ hybridization and immunohistochemical results of the present work showed that mRNA and protein expressions of \( A_0 \) and AT1R of elastic arteries in fore and hindquarter location accordingly altered. This results strongly support that vascular L-RAS may play an important role in differential vascular adaptation to microgravity. Furthermore, it further suggest that the L-RAS mechanism might be involved in mediating the surprising effect of daily short-period gravitation in counteracting microgravity-induced differential changes in arteries in different anatomic regions.

REFERENCES

The plastic alteration of the vestibulo-cardiovascular reflex induced by hypergravity environment

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Previously, we have demonstrated that the vestibular system has a significant role in arterial pressure (AP) response during gravitational change. The vestibular system is known to be highly plastic, and on exposure to different gravitational environments, the sensitivity of the vestibular system-mediated AP response might be altered. In order to test this hypothesis, rats were maintained in a 3 G or a normal 1 G environment for 2 weeks, and then the AP responses to free drop-induced microgravity were determined. In 1 G rats, the AP was increased by 37 +/- 3 mm Hg during microgravity; this pressor response was significantly attenuated in 3 G rats (24 +/- 2 mmHg). Furthermore, there was no difference between 1 G and 3 G rats in response to air jet stimulation-induced pressor response. These results indicate that pre-exposure to 3 G for 2 weeks induces plastic alteration of the vestibulo-cardiovascular reflex.

Previous study from our laboratory has demonstrated that the vestibular system has a significant role in arterial pressure (AP) response during gravitational change [1, 4]. The vestibular system is known to be highly plastic; i.e., the sensitivity of the vestibular system is altered if subjects are maintained in a different gravitational environment. This plasticity is considered to be one of the mechanisms underlying post-space flight orthostatic intolerance [6]. Accordingly, the aim of the present study was to examine whether gravitational alteration induced plasticity of the vestibulo-cardiovascular reflex in conscious rats. The rats were maintained under 3 G environment for 2 weeks, and their microgravity-induced pressor responses were compared with those of 1 G rats.

Seventeen days before the free drop experiment, we induced vestibular lesion (VL) in 26 of 40 rats, while in the remaining 14 rats we performed a sham treatment. After 2 days of recovery from the VL or sham treatment, the rats were divided into four groups: 1G (sham rats reared under a 1 G environment, n = 7), VL-1G (VL rats reared under a 1 G environment, n = 14), 3G (sham rats reared under a 3 G environment, n = 7), and VL-3G (VL rats reared under a 3 G environment, n = 12). These rats were reared under each environment for 2 weeks. One day before the free drop experiment, all rats were implanted the catheter into the abdominal aorta via the femoral artery. On the day of the experiment, VL-1G and VL-3G rats were further subdivided into two groups: free movement (FM) group and body stabilization (STAB) group. Thus, six groups of rats were studied: 1G-FM (n = 7), VL-1G-FM (n = 7), VL-1G-STAB (n = 7), 3G-FM (n = 7), VL-3G-FM (n = 7), and VL-3G-STAB (n = 5). To further examine the AP response to different types of stressors, all rats were subjected to a brief jet of compressed air, and their AP was recorded.

The summarized data for the AP responses that were used in the among group comparison are shown in the Fig. 1. In the 1G-FM group, AP increased by 34 +/- 3 mmHg in response to microgravity. This AP response was significantly suppressed in the VL-1G-FM group, and was completely blocked in the VL-1G-STAB group. In the 3G group, VL and STAB prevented the pressor
The pressor response in the 3G-FM group was significantly smaller than that in the 1G-FM group. In contrast, the pressor response was similar between the VL groups (VL-3G-FM vs. VL-1G-FM or VL-3G-STAB vs. VL-1G-STAB). In contrast, there was no difference in the air jet stimulation-induced pressor response among groups.

We recently described plastic alterations in AP responses mediated by the nonvestibular system following exposure to hypergravity [1]. VL was performed 1 day before the free drop experiment for estimating the AP response mediated by the nonvestibular system. Our data demonstrated that the AP response of VL rats was attenuated in 3G rats compared with 1G rats. In the present study, VL was performed before the 3G loading (17 days before the free drop experiment), and the AP response did not differ between VL-3G and VL-1G rats. These findings indicated that the vestibular system is indispensable for induction of the plastic alteration of the nonvestibular-mediated pressor response to free drop.

The cause of plastic alterations in the vestibulo-cardiovascular reflex is not clear, but two possible mechanisms have been proposed. First, a hypergravity environment increases static input to the vestibular system, which may in turn down-regulate receptor expression in the central vestibular system [5]. Second, phasic input recognized as an acceleration in the central vestibular system may be reduced because of decreased body movement in a hypergravity environment [2, 3]. In turn, this reduced phasic input may elicit use-dependent plasticity. These possibilities have to be examined in the future study.

In conclusion, 3G environment induces the plastic alteration of both the vestibular- and nonvestibular-mediated pressor response to free drop, and the vestibular system is indispensable for induction of the plastic alteration of the nonvestibular-mediated pressor response

Fig. 1. The summarized data of the free drop-induced pressor response (Filled bar shows the 1 G group and open bar shows the 3 G group)

CONTRASTING EFFECTS OF SIMULATED MICROGRAVITY WITH AND WITHOUT DAILY–GX GRAVITATION ON FUNCTION OF MIDDLE CEREBRAL ARTERY AND MESENTERIC SMALL ARTERY IN RATS

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ABSTRACT
This study was designed to test the hypothesis that a 28-day tail-suspension (SUS) could enhance myogenic and vasoconstrictor reactivity in the middle cerebral arteries (MCAs), whereas decrease myogenic and vasoconstrictor responses in mesenteric third-order arteries (MSAs), And the n to examine the effects of daily 1-h dorsoventral (~Gx) gravitation by restoring to standing (STD) posture to the alteration of the two kinds of arteries. The results showed that SUS induced an enhancement of the myogenic tone and vasoconstrictor responsiveness in the isolated middle cerebral artery but a depression of those in the mesenteric small artery. Daily STD for 1 h prevented the depression of myogenic tone and vasoconstrictor responsiveness in the mesenteric small artery, but did not prevent the functional enhancement in the middle cerebral artery. These data suggest that a short-term simulated microgravity may result in differential alterations in the function of the cerebral artery and the resistance vessel in the hind-body. Moreover, only the functional changes in these resistance vessels, not in the cerebral arteries, can be prevented by such a countermeasure of daily STD for 1 h.

1. Introduction
Humans exposed to microgravity often exhibit signs of cardiovascular deconditioning marked by orthostatic intolerance and reduced exercise capacity on re-exposure to gravity. The impaired cardiovascular response to standing after return from space might be among the highest risks to the safety, well-being, and performance of astronauts. This study was designed to test the function alteration of resistance artery after simulated microgravity.

2. Results
2.1 Increased myogenic tone in middle cerebral artery after 4-week simulated microgravity.
After 4-week hindlimb unweighting, the myogenic tone in the suspension group was increased significantly compared to that in the control group, with 1 hour per day standing, the augmentation of myogenic tone was not effected.

Fig.1 Myogenic tone developed (A) in middle cerebral arteries isolated from CON, SUS, and STD rats at intraluminal pressure of 0–125 mmHg. n=8 animals per group. ** P <0.01; NS, not significant (two-way ANOVA); ## P <0.01, CON vs. SUS; ++ P <0.01, CON vs. STD.

2.2 Decreased myogenic tone in middle cerebral artery after 4-week simulated microgravity.
In mesenteric small artery, after 4-week hindlimb unweighting, the myogenic tone in the suspension group was decreased significantly compared to that in the control group, with 1 hour per day standing, the attenuation of myogenic tone was fully recovered.

Fig.2 Myogenic tone developed (B) in mesenteric third-order small arteries isolated from CON, SUS, and STD rats at intraluminal pressure of 0–125 mmHg. n=8 animals per
2.3 Increased vasoconstriction in middle cerebral artery after 4-week simulated microgravity.

With the concentration of serotonin and KCl increased, the relative change of vessel diameter in suspension group was enhanced compared to that in control group, and in standing group, this enhancement was not prevented. There was no significant difference between suspension and standing groups.

2.4 Decreased vasoconstriction in middle cerebral artery after 4-week simulated microgravity.

With the concentration of phenyllphrine and KCl increased, the relative change of vessel diameter in suspension group, was attenuated compared to that in control group, and in standing group, this attenuation was fully prevented. There was no significantly difference between suspension and control groups.

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MODELING THE RECOVERY OF THE SYMPATHETIC AND PARASYMPATHETIC MODULATION IN ASTRONAUTS AFTER SHORT AND LONG DURATION SPACE MISSIONS


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ABSTRACT
The influence of microgravity on cardiovascular control was confirmed in this study. Therefore, linear HRV parameters were clustered in groups according to their physiological meaning. At early post flight, parts of the autonomic modulation of heart rate were significantly influenced, different for day and night periods, but the recovery was already complete 30 days after return.

1. INTRODUCTION
Does weightlessness in space disturb the human cardiovascular control system in astronauts? This question was already examined multiple times in literature, but the answer is still not clear. The clinical hallmark post-spaceflight orthostatic intolerance with postural tachycardia suggests that the autonomic nervous system (ANS) might be involved. The ANS affects heart rate by a continuous interaction between the sympathetic and parasympathetic branch. The sympathetic pathways speed up the firing rate of the sino-atrial (SA) node and consequently the heart rate while the parasympathetic or vagal pathways lower the heart rhythm [1]. The main interest of measuring cardiac sympathetic and vagal activity lies in its prognostic value in cardiovascular risk [2, 3].

Heart rate variability (HRV) is used as a noninvasive marker to investigate the autonomic modulation of heart rate. Low HRV levels and slow HR recovery are two important indications of impaired vagal activity. The aim of this study is to investigate how the several aspects of autonomic nervous system are influenced by microgravity when astronauts return on Earth. Also the recovery afterwards is examined. Therefore, different HRV parameters relating to the same aspect of autonomic modulation will be grouped in one model. In addition, day and night periods will be investigated separately.

2. METHODOLOGY
2.1 Data acquisition
24h Holter recordings from 8 astronauts were used. 5 went to the International Space Station (ISS) for a long term mission of several months while the other 3 have only been in space a short time. Each astronaut was measured at three different time moments, namely pre flight (L-30), early post flight (R+5) and late post flight (R+30)

2.2 Linear HRV analysis
After preprocessing the RR interval time series, all linear parameters described by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [1] were calculated. In the time domain, HRV Index and TINN are used as geometric measures while mean RR, SDNN, SDANN, SDSD, SDNN-Index, RMSSD and pNN50 are the statistical parameters. After resampling the tachogram at 2 Hz, the power spectral density (PSD) was computed by using the Welch method. In the frequency domain, very low frequency power (VLF: 0.003 – 0.04 Hz), low frequency power (LF: 0.04 – 0.15 Hz), high frequency power (HF: 0.16 – 0.40 Hz) and total power (0.01 – 1.00 Hz), as well as the ratio of low over high frequency power (LF/HF), were calculated. In addition, the power can be expressed in absolute values (ms²) or in normalized units (n.u.).

To evaluate all aspects of cardiovascular control by ANS, HRV parameters were combined. SDNN and total power (TP) are both measures for the total variability in heart rate, while rMSSD, pNN50 and HF power represent vagal modulation. LF power is mainly, but not only, influenced by sympathetic influence. LF/HF and LF (n.u.) reflect typically the sympathovagal balance.

2.3 Statistical analysis
Combining multiple parameters in one model is only possible by using z-scores. Then, different parameters could be considered as repeated measure of the same physiological phenomenon. Repeated Measures Multivariate ANOVA offers a solid testing method to determine whether the weightlessness had a significant influence on a certain group of parameters and therefore on a specific part of the ANS. The P value was obtained by the Wilks’ Lambda test statistic. P < 0.05 was considered statistically significant.

3. RESULTS
The differences between day and night were as expected for most parameters, with higher values during the night for all statistical time domain measures except for SDANN. Also TP, VLF and LF increased during the night as did the HF due to the respiratory sinus arrhythmia (RSA).
At early post flight (R+5) the geometric parameters did not change in comparison with the pre flight (L-30) condition. All statistical measures showed a significant or nearly significant decrease at R+5 during the day and night except SDNN, SDANN and SDNN-Index at night. On the contrary, mean RR showed no evolution at all. All effects seemed to be disappeared 30 days after return (R+30).

Fig. 1 shows the time evolution for several HRV parameters, averaged over the complete population and clustered in three groups. All parameters belonging to the same group indicate a similar evolution. Microgravity caused a fall in the total variability (TP, SDNN) although only significant during day (p=0.007). While the vagal modulation decreased significantly (day: p=0.004, night: p=0.01) at early post flight, the sympathovagal balance decreased slightly during daytime (p=0.328), but increased strongly (p=0.01) at night short after returning to Earth.

4. DISCUSSION

All HRV parameter values obtained pre flight are in the range expected for healthy male test subjects as found by Ramaeckers et al. [4], except for SDNN due to different signal lengths in both studies. LF and HF differed as well due to a different way of calculating the PSD although the normalized parameters corresponded.

With respect to the influence of microgravity on the autonomous control of HRV, evolutions during day and night should be discussed separately. Some days after returning, a general decrease in modulation of the heart rate by the ANS was found during the day, as vagal influence dropped significantly and the sympathovagal balance did not change, which indicates that also sympathetic influence dropped. After 30 days, there seems to be an almost complete recovery. At nighttime, the sympathovagal balance increased early post flight, probably mainly caused by a fall in vagal modulation. This means that the sympathetic modulation of heart rate during the night increased relatively in comparison with the values pre flight. All parameters seemed to be restored after 30 days.

5. CONCLUSION

The influence of microgravity on cardiovascular control was confirmed in this study. During daytime a general decrease in modulation of heart rate was found early post flight compared to pre flight while at night an increase in sympathovagal balance was observed indicating that microgravity caused a relatively higher sympathetic influence in the autonomic modulation. All effects seemed to be disappeared 30 days after return.

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EFFECTS OF 24 H CLINOROTATION ON EXPRESSION AND PROMOTER ACTIVITY OF INOS IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

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ABSTRACT
Background Evidences from human and animal studies in both real and simulated microgravity strongly suggested that alterations in the regulation of nitric oxide (NO) could contribute to postflight orthostatic intolerance. The aim of this study was to investigate the changes of inducible nitric oxide synthase (iNOS) in human umbilical vein endothelial cells (HUVECs). Methods Cells were divided into 3 groups: stationary control without rotation, horizontal rotation control and clinorotation. Cells were cultured in clinorotation condition for 24 h. RT-PCR and western-blot were performed to measure the level of mRNA and protein of iNOS, respectively. Luciferase activities were obtained by analyzing the total cell extract after transfecting with iNOS promoter-driven reporter system. Results The level of iNOS mRNA and protein was significantly increased in clinorotation group. And the level of iNOS mRNA and protein maintained very low level in stationary and rotational controls. Reporter gene assays showed 2.29-fold induction of luciferase activity in clinorotation HUVECs transfected with iNOS promoter-driven reporter system compared with that in controls. Conclusion The iNOS expression and promoter activity can be induced by 24 h simulated microgravity in HUVECs.

INTRODUCTION
Individuals exposed to extended periods of microgravity during spaceflight or prolonged 6° head-down tilt often experience postflight orthostatic intolerance (POI) when returning to Earth or upright posture, respectively. It can adversely affect the performance of physical work. Although a lot of works had been done, the underlying mechanism remains to be fully elucidated. Evidences from human and animal studies indicate that vascular contractile hyporesponsiveness is a valuable contributor in the occurrence of POI [1]. Endothelial cells release substances acting directly on vascular smooth muscle cells, causing either contraction or relaxation. The studies in the Hind-limb unloading (HLU) rodents model had clearly shown that NO synthesis was strongly enhanced and NO released from the endothelium is an important factor inducing vasodilation. However, to our knowledge, the alterations of the iNOS and the possible mechanisms underlying the regulation of iNOS in ECs cultured in real or simulated microgravity remain to be determined. The aim of this study was to investigate the changes of inducible nitric oxide synthase (iNOS) in HUVECs.

METHODS
Clinoration to simulate microgravity. The HUVECs were divided into 3 groups: stationary control without rotation, horizontal rotation control and clinorotation. RT-PCR analyses. Reverse transcription-polymerase chain reaction was used to measure the steady-state levels of mRNA using primers for human iNOS and GAPDH induced by the predetermined periods of clinorotation system. The housekeeping gene GAPDH was used for normalization. The ratios of the emissions incorporated into the PCR products of iNOS mRNA to the GAPDH products were calculated to evaluate relative changes in the mRNA expression levels of iNOS. Western blot analysis. Western-blot was performed to measure the level of protein of iNOS. The intensity of each band was quantitatively determined using UN-SCAN-IT™ software and the density ratio represented the relative intensity of each band against those of controls in each experiment. Transfections and reporter gene assays. Cells were plated at 60% to 70% confluence in 6-well plates before transfection. 3 µg of plasmid phiNOS(7.2)Luc was transfected by Lipofectamine Plus following the manufacturer’s protocol. To control for transfection efficiency between groups, 150 ng of pRL-TK (a plasmid encoding Renilla luciferase) was added to each well. 24 hours after cotransfection, cells were exposed to clinorotation for 24 h and relative luciferase activities were ob tained by analyzing the total cell extract. Statistical analysis. The data were shown as Mean ± SEM. All the experiments were performed three times with similar results for each time. Differences between 3 or more groups were analyzed by repeated-measures one-way ANOVA and Fisher’s LSD. Statistical significance was accepted at p < 0.05.

RESULTS
Induction of inducible nitric oxide synthase expression in clinorotation. In this study, we performed RT-PCR and Western-blot to evaluate the changes of iNOS expression after 24 h clinorotation.
Fig. 1A shows the upregulation of iNOS protein in HUVECs in the clinostat for 24 h, while the level of iNOS protein maintained very low level in stationary and rotational controls. Subsequently, RT-PCR for iNOS mRNA also shows markedly higher expression of iNOS mRNA in clinostat group than that in controls (Fig. 1B). These results indicated that the expression of iNOS mRNA and protein can be induced and was statistically higher in the simulated microgravity exposed cells contrast with the control cells ($P<0.05$).

**Induction of iNOS promoter-driven luciferase activity in clinorotation.** The inducible isoform of NOS is mainly regulated on the level of expression, with transcriptional, post-transcriptional and translational mechanisms involved. To characterize the molecular mechanisms for the upregulation of the iNOS mRNA in the transcriptional level, iNOS promoter-driven luciferase activity was studied. Construction of phiNOS(7.2)Luc, a plasmid contains 7.2 kb of upstream 5' flanking DNA linked to the luciferase reporter gene, has been described previously [2]. Reporter gene assays showed a 2.29-fold induction of luciferase activity in cells transfected with phiNOS(7.2)Luc in clinorotation compared with that in controls (Fig. 1C). These results indicated that the iNOS activity is regulated by 24 h clinorotation at transcriptional level.

**DISCUSSION**

Recent works showed that the vasoactive substance such as NO from vascular system may account, in part, for the orthostatic hypotension and the HU-induced hyporesponsiveness of vessels to norepinephrine [3]. NO, released from the endothelium following activation of NOS, acts in a paracrine fashion to stimulate smooth muscle cells via cGMP messenger to induce vascular relaxation of the blood vessels. Since simulated microgravity stimulates NO synthesis, we hypothesized that activities of NOS would be enhanced under clinorotation conditions. Here we confirmed our hypothesis by observing an upregulation of iNOS at both protein and mRNA levels, and an upregulation of iNOS mRNA and protein expression after 24 h clinorotation in respect to controls. Our data obtained are in agreement with the results obtained in HU rats. For example, functional and morphological evidences that upregulation of iNOS expression associated with NO-induced hypotension have been found in many cardiovascular and noncardiovascular tissues from rats subjected to HU. It has been demonstrated, by using both a vessel ring bioassay and expression data, that iNOS is increased in the aorta of HU rats.

In conclusion, the results in this paper document show that the iNOS expression and promoter activity can be induced by 24 h simulated microgravity in HUVECs. Our finding might help to know the possible mechanism of the vasculature dysfunctions induced by microgravity and provide some theoretical basis to the protection of postflight orthostatic intolerance.

**REFERENCES**

PERIPHERAL ARTERIAL and VENOUS RESPONSE to TILT Test after a 60 DAY BEDREST WITH AND WITHOUT COUNTER-MEASURES (ES-IBREP).

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Abstract: We quantified the impact of a 60-day head-down tilt bed rest (HDBR) with countermeasures on the arterial & venous response to Tilt.

Method: Twenty-one men (25–40y): 7 Control (Con), 7 Foot Vibration (Vib) and 7 Chinese Herb (Herb), all subjects were allowed to have a daily 10 min squat period for toilets. The subjects were identified as finisher (F) or non finishers (NF) at the post HDBR 10min Tilt. The Cerebral (MCA), Femoral (FEM) arterial flow velocity and leg vascular resistance (FRI), the Portal vein section (PV), the flow redistribution ratios (MCA/FEM; MCA/PV), and the Tibial (Tib), Gastrocnemius (Gast), Saphenous (Saph) vein section were measured by Echography & Doppler. Arterial and venous parameters were measured at 3 min pre Tilt in supine position, and at one minute before the end of the Tilt. Results: At post HDBR Tilt: FEM reduced less and FRI increased less in Con & NF only - PV (Portal vein flow) decreased less in Con, Herb, NF while it increased similarly in Vib - MCA decreased similarly as pre in all gr - MCA/FEM increased less compare to pre in the Con & NF while it still increased in Vib and Herb - MCA/PV increased in the Vib, while it decreased in Con, Herb, F, and NF - % Gast section was higher in all gr - % Tib section was higher in Con, and Herb only. Conclusion: In control the vasoconstriction was reduced in both the leg and Splanchnic areas. Vibration contributed to maintain vasoconstriction both in splanchnic and leg areas, while Herb contributed to maintain vasoconstriction in the leg but not in the splanchnic one. Vein distensibility was higher in all gr.

(Keywords: Artery, vein, countermeasure-Bedrest)

INTRODUCTION: Several bed rest and space flight studies have reported a significant lack of increase in vascular resistance at the leg level and or a lack of Portal flow reduction in response to fluidshift downward the feet as provoked by Stand up, LBNP, or Tilt test. Such observation were correlated with orthostatic intolerance and interpreted as a deficit in vasoconstriction in these territories. Moreover the deficit in leg arterial vasoconstriction was not related to a reduction in sympathetic activity as measured by micro-neurography in the Peroneal nerve but to regional vascular responsiveness (2). On the other hand percent increase in Tibial and Gastrocnemius vein section during Tilt, LBNP or Stand tests was found in non finisher (NF) subjects after Head down bedrest (HDBR) (1). Only aerobic exercise coupled to LBNP (as counter measure; CM) was found to prevent efficiently the lack of vasoconstrictive response at the leg and splanchnic levels and also the increase in leg vein distensibility after HDBR (1,2). Despite their efficiency such CM are time consuming and are not necessarily adapted for preventing the degradation of other systems like the neurosensorial one (otolith, muscle proprioceptors, eyes 3D visual reference…) which may also limit the capacity of the subject to stand, walk, exercise, and control his posture. The CM that looks the most adapted is the artificial gravity as obtained with a short arm centrifuge, the head being on the rotating axis and the feet at the extremity of the rotating arm (4:5). Our hypothesis was that (a) a squat of 10min per day (exposure to 1g during 10 min during the toilet period) should be sufficient for reducing significantly the alteration of the arterial and venous response to a fluidshift towards the leg Post HDBR, (b) that the effect of daily feet vibration or Chinese Herb ingestion should also reduce the HDBR deleterious effect on the arterial and venous system. The objective of the present study was to quantified the impact of a 60-day HDBR with the CM cited above on the arterial and venous response to Tilt test.

METHOD: Twenty-one men (25–40y) divided into 3 groups : Control (Con), Foot Vibration (Vib) and Chinese Herb (Herb) underwent a 60 day HDBR. All subjects were allowed to have a daily 10min squat period for toilets. The subjects were identified as finisher (F) or non finishers (NF) at the post HDBR 10 min Tilt test. The Cerebral (MCA), Femoral (FEM) arterial flow velocity and vascular resistance (FRI), Portal vein section (PV), flow redistribution ratios (MCA/FEM; MCA/PV), and Tibial (Tib), Gastrocnemius (Gast) and Saphenous (Saph) vein cross section areas (CSA) were measured by Echography and Doppler. Blood pressure (BP) was measured by arm cuff and fingerpress. These parameters were measured 3 min pre Tilt, supine, and 1min before end of Tilt.

RESULTS: At Post HDBR Tilt: MCA tended to decrease more post HDBR compare to pre in all gr except in F gr (p<0.05) - FRI increased less and FEM reduced less in Con and NF gr only (p<0.05) - MCA/FEM dropped in the Con and NF gr only - PV decreased less in Con,
Herb and NF gr - MCA/PV still increased in the Vib gr and F, while it decreased in Con Herb and NF. Tib and Gast vein CSA supine & in tilt were not significantly different pre & post HDBR in all gr. At post HDBR tilt %Tib section was higher in Con, Herb, F and NF gr but not in Vib gr. %Gast was higher in all gr and %Saph was not changed.

**DISCUSSION:** In control gr the vasoconstriction was reduced by the HDBR in both the leg and Splanchnic areas, and consequently the flow redistribution towards the brain became less efficient (FRI, MCA/FEM & MCA/PV increased less at post HDBR Tilt). Foot Vibration contributed to maintain vasoconstriction both in splanchnic and leg areas, and Herb contributed to maintain vasoconstriction in the leg but not in the splanchnic area. Thus one may suggest that both Vibration and Herb CM should have contributed to prevent partially post Bedrest orthostatic intolerance. Nevertheless the daily squat-up period may also have contributed to prevent the deterioration of the vasomotor response both in the leg and splanchnic areas in all groups which contributed also to maintain orthostatic tolerance (only 23% of the subject non finishers) (3). HDBR induce higher Tibial vein distension at post HDBR Tilt in all gr. Only Vib gr showed a similar distention of Gast vein pre and post. The arterial and venous changes in relation with HDBR and Vib and Herb CM remained tight, and should have been minimized or hidden by daily squat-up period. In conclusion we suggest that Daily 10min squat in 1G counterbalance the HDBR effects as even the controls are more tolerant to Tilt and that the Vib and Herb contribute to protect the vascular system as the vascular response in these groups is better maintained.

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NONLINEAR HEART RATE VARIABILITY ANALYSIS OF ASTRONAUTS BEFORE AND AFTER SPACEFLIGHT BY MEANS OF HOLTER RECORDINGS.


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ABSTRACT
Having Holter recordings of 8 astronauts before and after spaceflight, nonlinear HRV methods were applied to study the dynamics in cardiovascular regulation. The goal is to examine how the cardiovascular system is influenced by microgravity. The significant differences present some days after returning were disappeared after one month.

1. INTRODUCTION
Even after more than 600 people who have been in microgravity environment, it is still not clear how this influences the human body and more specific the cardiovascular system. Orthostatic intolerance and postural tachycardia indicate a change in the autonomic nervous system (ANS) and therefore the cardiovascular control. The ANS consists of a sympathetic and parasympathetic branch, always interacting each other. While the sympathetic network increases heart rate, the parasympathetic or vagal pathways cause a decrease of the heart rhythm.

Heart rate variability (HRV) has proven to be a good noninvasive tool to address the modulation by the ANS [1] and therefore HRV parameters are used to study the changes in cardiovascular control induced by microgravity. Not only standard HRV analysis will be used, but especially many nonlinear parameters since it has been shown that the ANS control underlies the nonlinearity and the possible chaos of normal HRV [2]. Nonlinear HRV methods were rarely applied on ECG data of astronauts because of the need of long term recordings while most studies only had 5 minute measurements. However, nonlinear parameters give additional information about the nonlinear dynamics in the cardiovascular system which can not be reflected by standard HRV analysis. Moreover, it enables us to examine the time evolution separately during day and night, as well as the day-night differences.

2. METHODS
2.1 Data.
24h-recordings of 8 astronauts were used of which five who have been in space for long duration missions and three who did short-term assignments. ECG is measured one month before launch (L-30), some days after returning (R+5) and one month after returning to earth (R+30).

2.2 Analysis
First, some basic filtering and preprocessing was applied to correct for missing and ectopic beats. Linear HRV parameters were obtained in agreement with the standards of measurement, proposed by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [2]. Mean and standard deviation (SD) of the tachogram, the square root of the mean of the sum of the squares of differences between consecutive RR intervals (rMSSD) and the percentage of intervals that vary more than 50 ms from the previous interval (pNN50) were calculated in the time domain. After resampling the tachogram at 2 Hz, the power spectral density (PSD) was computed by using the Welch method. In the frequency domain, low frequency power (LF: 0.04 – 0.15 Hz), high frequency power (HF: 0.16 – 0.40 Hz) and total power (0.01 – 1.00 Hz), as well as the ratio of low over high frequency power (LF/HF), were calculated. In addition, the power can be expressed in absolute values (ms\(^2\)) or in normalized units (n.u.).

Nonlinear HRV parameters do not describe the amount of modulation as such, but are able to describe the scaling and complexity properties of the signal. Often used parameters which study the scaling of the system are 1/f slope, fractal dimension (FD) and detrended fluctuation analysis (DFA \(\alpha_1\) & \(\alpha_2\)). In order to address the complexity of the signals, the correlation dimension (CD), maximal Lyapunov exponents (LE), sample entropy (SampEn) and Noise Limit (NL) are calculated. An overview of these methods is recently given by Aubert et al. [4].

Statistical analysis was done by the nonparametric Wilcoxon Signed Rank test to compare, for each HRV parameter, pairwise between the different time moments. \(P < 0.05\) was considered statistically significant. The Pearson Correlation Coefficient 'r' was calculated to examine the similarity between the HRV parameters.

3. RESULTS & DISCUSSION
All statistical parameters showed a significant or nearly significant decrease at R+5 which was more pronounced during day than night. On the contrary, mean RR showed no evolution at all as shown in Fig. 1. All effects seemed to be disappeared 30 days after return.
In general, the nonlinear parameters did not show any consistent alignment or time evolution. Therefore one has to remark that the different nonlinear parameters address different aspects of the scaling and complexity of the signal and should be evaluated independently.

4. CONCLUSION

This study examined the evolutions of different aspects of the cardiovascular regulation before and after a stay in a microgravity environment by using nonlinear HRV parameters. Some showed significant differences short after returning to Earth, but the dynamic behaviour of the cardiovascular control is completely recovered after one month. However they did not show any consistent alignment or time evolution, many of them seemed useful in the study of the physiological changes caused by microgravity. They can be a supplement for parameters who express vagal modulation or the sympathovagal balance. In order to use them separately more research and standardization is needed.

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ABSTRACT
The aim of the present study was to further elucidate the mechanism of vascular adaptation to microgravity and its gravity-based countermeasure by a biomechanical approach. Active (the dissected vessel segment was perfused with PPS) and passive (while it was perfused with Ca2+-free PPS) biomechanical properties of mesenteric third-order small arteries and middle cerebral arteries isolated from 3-day simulated microgravity (SUS), countermeasure (STD), and control (CON) groups of rats were studied. Results from the analysis of active biomechanical properties revealed the contribution of VSM tone during the early adaptation to microgravity: (1) For the middle cerebral arteries, active circumferential relation of the SUS group was shifted to the left side of the passive curve and kept at an early constant level with the corresponding being at its normal range; furthermore, the enhanced myogenic tone responsiveness was not prevented by daily short-duration -Gx. (2) For mesenteric small arteries, active circumferential curve of the SUS group was comparable with that of the passive vessels, indicating that the function of VSM to restore the normal stress distribution is compromised; however, this mal-adaptation was fully prevented by the countermeasure of daily 1-h -Gx gravitation.

1. Introduction
Orthostatic intolerance (OI) is common in astronauts after they return to earth. In previous study, we had found decreased contractility in small arteries. In this article, we test the mechanism of vascular adaptation to microgravity and its gravity-based countermeasure by a biomechanical approach.

2. Results
2.1 In mesenteric small arteries, active circumferential curve of the SUS group was comparable with that of the passive vessels, indicating that the function of VSM to restore the normal stress distribution is compromised; however, this mal-adaptation was fully prevented by the countermeasure of daily 1-h -Gx gravitation.
however, this mal-adaptation was fully prevented by the countermeasure of daily 1-h -Gx gravitation.

2.4 Reduced responsiveness of working heart to ISO after 4-week tail-suspension

3. Conclusion
The above results suggested remodeling changes in matrix components of different types of vessels, which might be significant if the exposure duration was further prolonged.

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ANTI-G MANEUVERS TRAINING WITH UTILIZATION OF LBNP METHOD

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ABSTRACT

Our LBNP (lower body negative pressure) device is utilized to the practical exercise of so-called anti-g manoeuvres. The proper pursuance of these manoeuvres during flights in highly agile combat aircraft substantially increases tolerance of the organism to the +Gz acceleration. The method of the efficient training of these protective manoeuvres was developed. At the same time it was developed testing method of their right mastery based on responses of the continual peripheral blood pressure (BP) monitored by the Portapres device. This new LBNP examination method is also an important training stage before the centrifuge training.

1. INTRODUCTION

The full exploitation of all flight properties of modern highly agile and powerful aircraft requires an application of all accessible anti-g safety and protective means and techniques. Well-timed and correct implementation of anti-g manoeuvres belong to one of very important protecting elements. The exercise of these manoeuvres is as a rule initialized at the beginning of the flying training in jet training aircraft. The new LBNP examination method serves successfully for Czech Army cadets’ training of already mentioned anti-g manoeuvres.

The original method of our LBNP examination was developed for the pre-selection of pilots with low +Gz tolerance [2]. This examination method was again improved for the pilot’s testing with low tolerance to the so-called Push-Pull (PP) effect [1, 3]. This phenomenon is characterized by the rapid and progressive decrease of BP accompanied by a slower return to normality during +Gz load following immediately after –Gz load. Identical BP responses approve a correspondence of the PP effect during LBNP expositions with the PP effect in real flights. BP changes during LBNP exposition correspond very well to in-flight PP effect.

Anti-g straining manoeuvre (AGSM) means the strain and breathing exercise.

2. METHOD

The anti-g manoeuvre technique is primarily practiced without any LBNP load in the seat but after the theoretical introduction. The strain manoeuvres are practiced apart as the first and then the breathing manoeuvres again separately. The coordinated whole of both parts of this protective manoeuvre is drilled in the second training phase. After mastered these parts of the training it follows using of an LBNP method.

32 Czech Air Force pilots were in our examined group. All pilots were exposed to an LBNP load in the sitting position at the negative pressure of −70 mmHg with achievement of this value in one second. It means the negative pressure onset is 70 mmHg per second. A proper practical exercise of complete anti-g manoeuvres is initiated upon an indication of cardiovascular system regulation insufficiency at the beginning of the pre-collapse state. The strain manoeuvres involve correct tension of the appropriate leg muscles and abdominal muscular groups. The breathing manoeuvres represent the Valsalva manoeuvre or forcible exhalation against a closed glottis. The complete anti-g straining manoeuvres (AGSM) cover a combination of muscle tensing and the Valsalva manoeuvre performed rhythmically every three to four seconds. To gain maximum protection from the manoeuvre, muscle-tensing should be sustained throughout the acceleration exposure and not relaxed during breathing. Exhalation and subsequent inhalation should be performed as rapidly as possible.

Objective criteria for anti-g manoeuvres initiation were assessed according to statistical evaluation of blood pressure (BP), heart rate (HR) and ear photoplethysmogram (EP) behaviour. These are progressive BP and HR decrease after short duration of tachycardia and significant reduction of the EP amplitude or smoothing of both one-sided and two-sided EP signal. BP significant improvement, recovery of EP pulsation and stopping of the collapse state progress mean that anti-g manoeuvres were mastered well.

Fig. 1. Typical blood pressure course during a practical exercise of anti-g manoeuvres in the event of their correct execution.
3. RESULTS

6 pilots were unsuccessful in these examinations from the whole group of 32 pilots. The average systolic value of BP of successful pilots in an instant of anti-g manoeuvres beginning was increased by 55.2 % from 97.3 to 149 mmHg. The average diastolic value was increased by 46.7 % from 74.1 to 107.1 mmHg and pulse pressure (differential value between systolic and diastolic pressure) by 96 % from 23.1 to 42.1 mmHg. The EP pulsation was recovered in all cases by 178 % respectively 137 % on the average from both ears.

Results at the group of 6 unsuccessful pilots were as follows: The average systolic value was increased only by 4.3 % from 102 to 107 mmHg, the average diastolic value by 6.7 % from 74.5 to 81.5 mmHg and pulse pressure was even decreased by -5.2 % from 27 to 25.1 mmHg. The EP pulsation was also recovered in all cases but only by 42.4 % respectively 24 % on the average from both ears.

4. DISCUSSION

BP course during a practical exercise of anti-g manoeuvres in the event of their correct execution is shown in Fig. 1 and Fig. 3. Continuous BP was measured by the Portapres device. Even if the BP measurement is substantially fault-tolerant to active move artifacts we can see specific BP signal distortion (Fig. 1.) at intervals of intensive anti-g manoeuvres. Nevertheless BP measurement is still sufficiently reliable as we can see in Fig. 1. The same statement stands for a measurement of the ear photoplethysmogram signal as we can see in Fig. 3.

Persons with low level tolerance to the gravitational acceleration can be endangered of the collapse state in the case that the complex of anti-g manoeuvres was mastered insufficiently. The LBNP exposition is immediately finished and the LBNP device is tilted backwards to the horizontal position.

5. CONCLUSIONS

It can be emphasized that all successfully accomplished practices were characterized by an immediate and rapid BP increasing and by EP pulsation recovery. The collapse state didn’t come into being on any event. Utilization of the LBNP load method appears, at lacking human centrifuge, a suitable opportunity for an evaluation of the anti-g manoeuvres training efficiency and also for the anti-g manoeuvres practice. This method is undemanding, reliable, safe and financially acceptable.

It offers unequivocal results with high decisiveness for tested pilots. It is possible to repeat easy this LBNP examination at any time in the event of the incorrect anti-g manoeuvres accomplishment. Of course a new training of the proper technique of manoeuvres has to precede a new LBNP examination.

REFERENCES

IMPAIRED RELAXATION FUNCTION MEDIATED BY β-ADRENERGIC RECEPTOR REDUCES CARDIAC PERFORMANCE IN TAIL-SUSPENDED RAT
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ABSTRACT
The aim of this study was to observe changes of contractile function and responsiveness to isoproterenal (ISO) in the cardiomyocytes and working hearts in 4-week tail-suspended rat. Compared with control group (CON), the unloaded shortening amplitude decreased in both left (LV) and right ventricular cardiomyocytes (RV) in tail-suspended group (SUS). During perfusion with ISO, the unloaded shortening amplitude had less increment in tail-suspended group compared with control group. The ISO increased the intrinsic heart rate of working heart in control and tail-suspended groups. The cardiac output was decreased and left ventricular end-diastolic pressure was increased significantly in 4-week of tail-suspended rats compared with control. The above results suggest that the unloaded shortening amplitude and responsiveness to ISO decrease in cardiomyocytes after 4-week of tail-suspended rat, and impaired left ventricular relaxation function mediated by β-adrenergic receptor reduces cardiac performance in tail-suspended rat.

1. Introduction
Orthostatic intolerance (OI) is common syndrome in astronauts after returning to earth. Decrease in cardiac contractility may contribute to OI. In the previous study, we had found a decreased contractility in papillary muscles \(^{[1]}\). The mechanisms remain unclear.

2. Results
2.1 Deceased contractility in cardiomyocytes after 4-week tail-suspension
Compared with control group, the unloaded shortening amplitude decreased by 12.2% and 10.9% in left ventricular cardiomyocytes \((P<0.05)\), and 16.5% and 16.3% in right ventricular cardiomyocytes \((P<0.05)\) at 1.0 and 2.0 Hz, respectively, in tail-suspended group (Fig. 1). But there was no significant difference at 4.0 Hz between tail-suspended and control group.

Fig.1 Percentage of unloaded shortening amplitude in left and right ventricular cardiomyocytes from control and 4 weeks of tail-suspended rats. CON, control. SUS, tail-suspended. LV, left ventricle. RV, right ventricle. * \(P<0.05\) vs. control.

2.2 Reduced responsiveness of cardiomyocytes to ISO and forskolin after 4-week tail-suspension
The unloaded shortening amplitude had a 10.63±0.83%, 35.06±5.22% and 71.64±6.83% increase in control cardiomyocytes, but 5.75±0.76%, 23.97±4.50% and 26.38±8.13% increase in tail-suspended group with 1, 5, and 10 nM ISO treatment \((P<0.05)\). During perfusion with 10, 50, and 100 nM forskolin, the unloaded shortening amplitude had a 3.04±0.27%, 9.81±2.66%, and 20.2±3.47% increase in control cardiomyocytes, but 1.42±0.53%, 3.83±1.71%, and 5.49±4.08% increase in tail-suspended group \((P<0.05)\) (Fig. 2).
2.3 Decreased contractility in working heart after 4-week tail-suspension

There was no difference between cardiac output from control and tail-suspended group at the preload of 5 mmHg and 10 mmHg. At the preload of 15 mmHg, cardiac output in tail-suspended group was much lower than control group \( (P<0.05) \). As to left ventricular end-diastolic pressure (LVEDP), there was significant difference between tail-suspended and control group at preloads of 5 mmHg, 10 mmHg and 15 mmHg \( (P<0.05) \) (Fig. 3).

2.4 Reduced responsiveness of working heart to ISO after 4-week tail-suspension

The increment of cardiac output was 20.8±1.64%, 32.5±1.97% in control group, but 14.3±1.46%, 20.04±1.75% in tail-suspended group with 10 and 20 nM ISO perfusion \( (P<0.05) \). There was no difference between control and tail-suspended group with 1 nM ISO perfusion. As to the left ventricular end-diastolic pressure (LVEDP), there were significant difference between tail-suspended and control group during with 1, 10 and 20 nM ISO treatment \( (P<0.05) \) (Fig. 4).

3. Conclusion

The above results suggest that the unloaded shortening amplitude and responsiveness to ISO decrease in cardiomyocytes after 4 weeks of tail-suspended rat, and impaired left ventricular relaxation function mediated by \( \beta \)-adrenergic receptor reduces cardiac performance in tail-suspended rat.

4. References


This study was supported by a NSFC grant (30370538).
ABSTRACT  Our previous work has suggested that vascular channel remodeling might be among the important mechanisms that mediate/modulate differential adaptive changes of cerebral and hindquarter arteries during simulated microgravity [1–3]. The aim of this study was to specify the time course of differential changes in function and expression of L-type calcium channels (CaL) in the cerebral and hindquarter arterial vascular smooth muscle cells (VSMCs) during a 4-wk simulated microgravity and their reversal after release from suspension. Tail suspension (SUS) for 3 and 28 days was used to simulate cardiovascular effects of acute and medium-term microgravity exposure. In addition, the reversibility of CaL changes were examined with rats recovered for 3 d after a 28-d SUS. Whole-cell recording mode was used to record current densities of CaL and Ba++ was used as charge carrier. Protein expression of α1c subunit of CaL was examined by Western blotting.

Whole-cell recording showed that the current densities of CaL of the cerebral and small mesenteric arterial VSMCs significantly increased and decreased, respectively, after 3-d and 28-d SUS. These changes due to a 28-d SUS were completely and partially recovered in cerebral and mesenteric arterial VSMCs after a 3-d recovery. Protein expression of α1c subunit was examined by Western blotting. Whole-cell recording showed that the current densities of CaL of the cerebral and small mesenteric arterial VSMCs significantly increased and decreased, respectively, after 3-d and 28-d SUS. These changes due to a 28-d SUS were completely and partially recovered in cerebral and mesenteric arterial VSMCs after a 3-d recovery. Protein expression of α1c subunit was significantly increased and decreased in cerebral and mesenteric arteries after a 28-d SUS. The findings indicate that the differential adaptational change in calcium channel function is an immediate and early adaptive response which persists during the simulated microgravity. The differential protein expression was manifested only after a medium-term simulated microgravity. Though these changes are reversible, the function of cerebrovascular CaL recovered more rapidly.

INTRODUCTION  Our previous work has shown that simulated microgravity induced increased CaL and BKCa currents and decreased Kv current associated with membrane depolarization in cerebral arterial myocytes[1,2]. Therefore, we designed aims were to clarify the time course of differential changes in function and expression of L-type calcium channels (CaL) in the cerebral and hindquarter arterial vascular smooth muscle cells (VSMCs) during a 4-wk simulated microgravity and their reversal after release from suspension.

MATERIALS AND METHODS  Tail-suspended hindlimb unloading rat model with modification from our laboratory was used to simulate the cardiovascular deconditioning effects of microgravity in rats. Electrophysiological measurements were performed by whole-cell recording with a amplifier and a version interface using patch clamp techniques. Whole-cell CaL currents were measured using conventional voltage-clamp configuration. The VSMCs from the cerebral and small mesenteric arteries were tested. Protein expression of α1c subunit of CaL was examined by Western blotting. The data are expressed as means ± SE. A one-way ANOVA was used to determine the overall differences Ca2+ current density between different groups. Student’s t-test was used to determine the differences in body weight, soleus wet weight, and Cm between different groups. A value of P<0.05 was considered to be statistically significant.

RESULTS  There were no significant differences in final body weight and soleus wet weight among CON and SUS groups. After 3 days of SUS, the CaL current densities of cerebral arteries and mesenteric arteries VSMCs were significantly increased and decreased, respectively, compared with the CaL current densities from CON rats(Fig 1), but the protein expression were not changed (the data were not showed). As compared with that of the simultaneous control rats, the CaL current densities of cerebral arteries and mesenteric arteries VSMCs were significantly increased and decreased, respectively, after a 28-day simulated microgravity. The CaL current densities of cerebral and mesenteric arteries VSMCs were completely and partially restored to their control levels after a 3-day release from a 28-day simulated microgravity, respectively (Fig 2 and 3).
DISCUSS The findings that a 3-d simulated microgravity can induce differential changes in Ca_L current densities in VSMCs isolated from cerebral and the small mesenteric arteries, respectively, indicate that the functional change of Ca_L channel might be an immediate early response while the rats were exposed to simulated microgravity. Simulated microgravity for 28 days results in differential regulation of Ca_L current as well as protein expression in VSMCs from cerebral and mesenteric arteries. Though these changes are reversible, the function of cerebrovascular Ca_L recovered more rapidly. In the work, animal experimentation was conducted in accordance with the guiding principles in the care and use of animals. This work is supported by a NSFC grant (No.30470649 and No. 30800545).

References
TEMPORAL AND CEREBRAL HEMODYNAMIC RESPONSE DURING THE LAST MINUTE OF A TILT TEST, AFTER A 60 DAY BEDREST (ES-IBREP).

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Abstract: Objective: Identify an early hemodynamic predictor of syncope at the Tilt test performed on subjects submitted to a 60-day head-down tilt bed rest (HDBR) with countermeasures. Method: Twenty-one men (25–40y) divided into 3 groups [Control (Con), Foot vibration (Vib) and Chinese Herb (Herb)] underwent a 60 day HDBR. The subjects were allowed to have a daily 10 min squat period for toilets. Post HDBR 20min Tilt identified Finishers (F) and Non Finishers (NF). Cerebral (MCA), Temporal (TEMP), Femoral (FEM) flow velocity, were measured by Doppler during the Tilt. Blood pressure (BP) was measured by arm cuff and fingerpress. Arterial parameters were measured at 3 min pre Tilt in supine position, and at 1 min and 10s before the end of the tilt. Results and discussion: Four of the 21 subjects were NF at the post HDBR Tilt test (Con gr:2, Vib gr: 1, Herb gr: 1). At 1 min and 10s before end of Tilt in NF gr, FEM flow decreased less and MCA decreased more at post HDBR Tilt compared to pre (p<0.05), while in the F gr they changed similarly as pre. In NF gr: TEMP flow decreased more at post HDBR Tilt compared to pre, but only at 10s before the end of Tilt (P<0.05). During the last 10s the diastolic component disappeared and a negative (reverse) flow appeared which induced a drop in mean flow velocity. In F TEMP decreased similarly at pre and post HDBR Tilt. Conclusion: The decrease in TEMP flow preceding the decrease in MCA flow confirm that the TEMP flow is not as accurately controlled as the MCA flow (No autoregulation) thus this territory respond more directly and instantaneously to the cardiac output redistribution in response to fluidshift than the brain. Keywords: Temporal, cerebral, orthostatic, bedrest.

INTRODUCTION: Presently the decision to interrupt an orthostatic test is made on the basis of the clinical signs of intolerance, the heart rate, and the blood pressure (BP, arm cuff and fingerpress). Previous HDBR showed that the most frequent and reliable sign preceding the onset of syncope is the drop in blood pressure but in some cases the cerebral flow velocity dropped some cycles earlier or in parallel with the BP drop, or a bradycardia preceded the BP drop by some seconds. Nevertheless even in case of non efficient cardiac output redistribution among the major vascular territories (Splanchnic and lower limb) due to an insufficient reduction of flow volume in both area (1) the cerebral flow velocity does not expresses that immediately due to the autoregulation which contribute to prevent any significant diastolic flow drop. Thus cerebral flow drops when all compensatory process have been used which could explain why it drops lately. Our hypothesis was that the Temporal flow measured at the same level of altitude as the cerebral one during a stand or tilt test should be affected by the abnormal cardiac output redistribution earlier than the cerebral flow because it is not as acutely protected as the cerebral one by the autoregulation process. The objective of the present study was to monitor simultaneously and continuously the cerebral and temporal flows by Doppler during a Tilt test before and after a 2 months bedrest.

METHOD: Twenty-one men (25–40y) divided into 3 groups [Control (Con), daily 30 min Foot vibration (Vib) and Chinese Herb (Herb)] underwent a 60 day HDBR. The subjects were allowed to have a daily 10 min squat period for toilets. Post HDBR 10 min Tilt identified Finishers (F) and Non Finishers (NF). Cerebral (MCA), Temporal (TEMP), Femoral (FEM) flow velocity, were measured continuously by Doppler during the Tilt. Blood pressure (BP) was also measured by arm cuff and fingerpress. Arterial parameters were measured at 3 min pre Tilt in supine position, at (-1 min), and (-10s) before the end of the test.

RESULTS: Four of the 21 subjects were NF at the post HDBR Tilt test (Con gr:2, Vib gr: 1, Herb gr 1) (2) - In NF gr: FEM flow decreased less at post HDBR Tilt compared to pre, both at 1min & 10s before the end of the Tilt (P<0.05). In F gr, FEM decreased both at 1 min and at 10s, but similarly at pre and post HDBR Tilt - In NF gr MCA flow decreased more at post HDBR Tilt compare to pre, both at 1min and 10s before the end of the Tilt (P<0.05). In F gr, MCA decreased similarly at pre and post HDBR Tilt - In NF gr: TEMP flow decreased more at post HDBR Tilt compared to pre, but only at 10s before the end of Tilt (P<0.05). During the last 10s the diastolic component disappeared and a negative flow (reverse) appeared which induced the drop in mean flow velocity (Fig 1). In F, TEMP decreased similarly at pre and post HDBR Tilt - In NF: MCA/FEM ratio decreased more post HDBR both at 1min and 10s before the end of Tilt, compare to pre HDBR Tilt. (P<0.05). In F, MCA/FEM changed similarly at pre and post HDBR Tilt - In NF: TEMP/FEM ratio decreased more at post HDBR Tilt, only at 10 s before the end of Tilt, compare to pre HDBR Tilt. (P<0.05).
In F TEMP/FEM changed similarly at pre and post HDBR Tilt. BP drop induced Tilt arrest.

**DISCUSSION:** In the NF gr (a) FEM reduces less because of insufficient leg vaso-constriction (b) MCA reduces more because of insufficient reduction in lower limb (femoral) and splanchnic flow (c) TEMP decrease markedly (compare to end Tilt -1 min) but only around 10s before the subject faint while the FEM & MCA remained at the same level as at 1 min even some sec prior to fainting. During the last min of the Tilt, FEM & MCA did not change except in the last 2-3s before the tilt stop and thus did not provide any useful information for predicting fainting during this period. Decrease in MCA/FEM means insufficient flow redistribution towards the brain, but does not mean that the subject is about to faint as there was no difference in MCA/FEM response from 1min to 10s before end of Tilt. Conversely TEMP/FEM indicate several second before fainting an opposite change compare to pre HDBR thus the TEMP flow and TEMP/FEM ratio are earlier indicator of fainting than MCA, FEM, and MCA/FEM. We suggest that the earlier drop in TEMP flow is related to the fact that this area is not as accurately controlled as the MCA flow (No autoregulation) and thus respond more directly to changes in other regional flows. Moreover the change in TEMP flow Doppler trace (monophasic to biphasic) and the earlier drop in TEMP mean flow are new and valuable parameters for anticipating the syncope several second prior to BP or MCA drop. Such conclusion should have to be confirmed on a larger number of subjects.

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**Références**


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**Figure 1:** (Left) BP, TEMP, MCA flow normal (at -1min). (Right) BP, MCA flow normal, TEMP with negative diastole (at -10s)

**Figure 2:** FEM (femoral) flow at 1min and 10s before end of Tilt in F (finisher) and NF. Similar change pre / post HDBR.

**Figure 3:** MCA (Cerebral) flow at 1min and 10s before end of Tilt in F (finisher) and NF. Similar change pre / post HDBR.

**Figure 4:** TEMP (Temporal) flow at 1min and 10s before end of Tilt in F (finisher) and NF. TEMP drops 10s before Tilt arrest.

**Figure 5:** Temporal/Femoral flow ratio at 1min and 10s before end of Tilt in F (finisher) and NF. The ratio drops only 10s before Tilt arrest, while FEM is similarly decreased at 1min and 10s.
ABSTRACT
To analyze of microgravity-induced cellular structural and functional changes in soleus (Sol), medial gastrocnemius (MG) and tibialis anterior (TA) muscles, we performed 12-day hindlimb suspension protocol for Mongolian gerbils according to standard procedure of Morey-Holton. It was shown that in Sol skinned single fibers peak isometric tension was reduced by 31%; the calcium sensitivity also significantly reduced. In TA and MG fibers, we detected no significant changes of Ca sensitivity, but found decrease of the peak tension in fibers of these muscles by 20% and 25% consequently. Lateral stiffness of contractile apparatus for half-fibers of these muscles by 20% and 25% consequently. Contractile properties of skinned single fibers were evaluated by means of the standard technique described by L. Stevens et al. [6]. We'd evaluated the following parameters - peak isometric tension and calcium sensitivity, the sensitivity was driven from modified Hill equation.

1. INTRODUCTION
It is well known that gravitational unloading causes significant changes in functional properties of postural muscles [1, 2]. These alterations could be considered as the result of structural changes at the level of single muscle cells. Changes, accompanied with reduction of peak tension and Ca$^{2+}$-sensitivity, could be associated with Ca-dependent protein degradation [3]. Although the exact cause of Ca$^{2+}$ accumulation in muscle fibers during unloading leaves unknown, there is a number of speculations about probable mechanisms of this phenomenon [4]. The experiments, held in the scope of 12-day spaceflight of satellite FOTON-M3 (2007, Russian Federation), with Mongolian gerbils aboard, showed, that these animals are more resistant to atrophy of human muscles during space missions.

2. MATERIALS AND METHODS

16 two month old male Mongolian gerbils were used in this study. Were randomly divided into two groups: «Cage control» (average weight 44.7±1.1 g) and «12-day hindlimb unloading» (average weight 47.5±2.7 g), the unloading was performed in accordance with Morey-Holton procedure. All the experimental procedures were performed in accordance with the international regulations for animal care and treatment and approved by the Institutional Bioethical Committee. We analyzed the following muscles: «slow» m. Soleus (Sol) (main postural muscle), its «fast» synergist – m. Gastrocnemius medialis (MG), and its antagonist – m. Tibialis anterior (TA). Single fibers were extracted from these muscles.

Contractile properties of skinned single fibers were evaluated by means of the standard technique described by L. Stevens et al. [6]. We'd evaluated the following parameters - peak isometric tension and calcium sensitivity, the sensitivity was driven from modified Hill equation.

Transverse regional fiber stiffness was measured by means of atomic force microscopy (Solver-Bio, NT-MDT, Russia) by means of technique described in [7]. Measurements were held in isometric mode along the longitudinal fiber axis in the areas of Z-disc, M-band and between them (half-sarcomere) both in glycerinated and skinned fibers in three different conditions: relaxed R (20 mM MOPS, 170 mM Ca propionate, 2.5 mM Mg acetate, 5 mM K$_2$EGTA, 2.5 mM ATP), Ca-activated A (20 mM MOPS, 172 mM K-propionate, 2.38 mM Mg- acetate, 5 mM Ca-EGTA, 2.5 mM ATP) and in rigor state Rg (20 mM MOPS, 170 mM K propionate, 2.5 mM Mg acetate, 5 mM K$_2$EGTA).

To evaluate the Ca$^{2+}$ concentration we placed extracted muscle into standard Ringer solution for warm-blooded animals with 2.5 mM ATP for 40 minutes, then for 40 min into relaxing solution R, with 4% v/v of formaldehyde, under the temperature of 37°C. After this part of procedure the isolation of single fibers was held. Isolated fibers were incubated with fluorescent probe Fluo-4 in accordance with the instructions of the Molecular Probes Company. Then fibers were immediately photographed with Leica fluorescent microscope. Leica Digital software was used to analyze the data obtained. Statistical analysis was held with Sigma Plot software.

3. RESULTS AND DISCUSSION
After 12 day of gravitational unloading we found reduction of muscle mass in Sol and GM (at 28% and
34% respectively), wet mass of TA hasn’t demonstrated any changes. Evaluation of single fiber contractile properties showed that calcium sensitivity decreased only in Sol (pCa50 changed from 5.74±0.03 to 5.57±0.028), at the same time the reduction of peak tension was detected in both three muscles (see table 1).

Table 1. Peak force (mN) of single fibers of shin muscles of Mongolian gerbils after 12 day hindlimb unloading.

<table>
<thead>
<tr>
<th>muscle</th>
<th>group</th>
<th>Cage control</th>
<th>12-day unloading</th>
</tr>
</thead>
<tbody>
<tr>
<td>MG</td>
<td>0.200±0.008</td>
<td>0.160±0.010*</td>
<td></td>
</tr>
<tr>
<td>Sol</td>
<td>0.130±0.011</td>
<td>0.090±0.004*</td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>0.220±0.027</td>
<td>0.170±0.003*</td>
<td></td>
</tr>
</tbody>
</table>

* - p<0.01 in comparison with control

AFM measurements of transverse stiffness showed that after 12 days of unloading the significant drop of stiffness of sarcolemma (between Z-disc and M-band) in all muscles in relaxed state (by 66% in Sol, and 49% - at MG, by 47% - in TA), approaching the level of plasma membrane stiffness in non-muscle cells (0.3 – 0.5 pN/nM). The stiffness of contractile apparatus itself (measured in Triton-treated preparations) at the area of half-sarcomere (between Z-disc and M-band) reduced only in Sol (by 49% at relaxed state, by 58% - at activated state and by 66% - at rigor state. Also we found significant decrease of stiffness of mechanotransductional nodes (Z-disc and M-line) (fig. 1).

It is known that the main components of M-line and Z-band are proteins which are specific substrates of calpains [8]. Since calpains are Ca-activated proteases, we considered reasonable to evaluate the abundance of Ca2+-ions in the fibers of muscles under study (table 2).

Table 2. Concentration of Calcium ions (in relative units of fluorescence intensity) in single fibers of different muscles after 12-day unloading.

<table>
<thead>
<tr>
<th>muscle</th>
<th>Cage control</th>
<th>12-day unloading</th>
</tr>
</thead>
<tbody>
<tr>
<td>MG</td>
<td>1.10±0.23</td>
<td>2.3±0.4**</td>
</tr>
<tr>
<td>Sol</td>
<td>1.09±0.28</td>
<td>3.3±0.6**</td>
</tr>
<tr>
<td>TA</td>
<td>1.94±0.25</td>
<td>3.4±0.5**</td>
</tr>
</tbody>
</table>

** - p<0.05 in comparison with control

The comparative analysis of the data on the fiber contractile properties and the stiffness data, reflecting the mechanical state of the fiber cytoskeleton allows us to speculate that calcium sensitivity could be associated mainly, with the state of contractile apparatus in the area of half sarcomere, meanwhile peak tension might depend on structural integrity of mechanotransduction nodes (Z-disc and M-line).

Acknowledgments

Authors want to express their gratitude to Dr. O.I. Orlov for technical and administrative management of the study. This work was supported by program of Branch of Biological Sciences of RAS 6006/4 and by grant of RFBR 07-04-12153-ofi.

4. REFERENCES

“CALCIUM LEAK” OF SARCOPLASMIC RETICULUM INDUCES DEGRADATION OF TROPONIN I IN SKELETAL MUSCLE FIBERS

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INTRODUCTION

Troponin I subunit (TnI) was as a molecular marker to explore the relationship between highly resting intracellular Ca$^{2+}$ concentration and myofibril degradation in muscle fibers. The isolated soleus muscle strips of rats were treated by caffeine, an opener of sarcoplasmic reticulum (SR) calcium release channel in contraction phase [1], and H$_2$O$_2$, inducing a calcium leak of SR calcium release channel in relaxation phase [2]. The TnI, a marker molecular of somatic muscle damage [3], was detected by Western Blot technology.

RESULTS

In the following experiments, muscle strips were perfused by caffeine and H$_2$O$_2$ for 40 min composed of 10-min balance, 5-min fatigue and 20-min recovery. Figure 1 showed that resting tension of isolated soleus muscle strip had no significant change in 40-min calcium-free Krebs perfusion. Low concentrations of caffeine (1 and 5 mM) perfusion only induced a transient increase in resting tension during fatigue period, High concentrations of caffeine (10 mM) perfusion induced a continued increase in resting tension of muscle strip, especially during fatigue period.

After 5-min fatigue, tetanic tension of soleus was decreased to 7.6% of initial tetanic tension with calcium-free Krebs solution perfusion, but significantly decreased to 4.2%, 10.7% and 15.0% in 1, 5 and 10 mM caffeine-treated soleus, respectively (Fig.2A).

The tetanic tension could recover 61.7% with calcium-free perfusion at the twenty-fifth minute after fatigue, and the recovery rate was increased in 1 mM caffeine-treated soleus strips. The recovery rate had no significant change in 5 mM caffeine-treated soleus strips compared with control at the fifteenth minute, but descended slowly. Tetanic tension could only recover to 22.0% at the ninth minute in 10 mM caffeine-treated soleus strips and then descended to 4.8% (Fig.2B).

Fig.1 Changes in resting tension with and without caffeine treatment in soleus muscle strips.

Values are means±SEM, n=6 muscle strips in each group.

Fig.2 Effect of caffeine on fatigability and recovery rate after fatigue in soleus strips.

Values are means±SEM, n=6 muscle strips in each group.

The tetanic tension could recover 61.7% with calcium-free perfusion at the twenty-fifth minute after fatigue, and the recovery rate was increased in 1 mM caffeine-treated soleus strips. The recovery rate had no significant change in 5 mM caffeine-treated soleus strips compared with control at the fifteenth minute, but descended slowly. Tetanic tension could only recover to 22.0% at the ninth minute in 10 mM caffeine-treated soleus strips and then descended to 4.8% (Fig.2B).

Fig.3 Changes in resting tension with and without H$_2$O$_2$ treatment in soleus muscle strips.
Values are means±SEM, n=6 muscle strips in each group.

The resting tension slightly declined to 90.8% during the first 10 min, then upgraded to 113.9% at the end of fatigue period, continuing to ascend to 180.0% till the end of recovery stage with 1 mM H2O2 perfusion (Fig.3). The resting tension upgraded to 198.4% at the end of fatigue period, reached maximal value of 247.9% at the thirtieth minute, then declined to 176.9 % at the end of recovery period with 5 mM H2O2 perfusion. The resting tension increased to 109.2% at the tenth minute, 176.9% after fatigue period, and maximal 358.9%, then declined to 308.5% at the end of the recovery period with 10 mM H2O2 perfusion. The resting tension of muscle strip increased progressively by a dose-dependent manner. H2O2 perfusion did not influence the extent of muscle fatigue compared with the control group (P>0.05). After 5- min fatigue, tetanic tension was decreased to 7.5%, 9.0% and 13.1% with 1, 5 and 10 mM H2O2 treatment, respectively (Fig.4A).

After 5-min fatigue, the developed tension showed a quick resumption and then declined rapidly during the H2O2 perfusion (Fig.4B). At the fortieth minute, the developed tension is 72.4% which was higher than the control group in 1 mM H2O2-treated soleus strips, but significantly declined to 46.7% and 36.9% in 5 and 10 mM H2O2-treated soleus strips.

Figure 5 showed that no degradation of TnI was detected in soleus muscle strips with calcium-free Krebs’ solution, low concentration of caffeine (1 and 5 mM) and 1 mM H2O2 perfusion, but there was a detectable degradation in TnI with 10 mM caffeine, 5 and 10 mM H2O2 perfusion. Degradation of TnI with 10 mM H2O2 perfusion was more than that with 5 mM H2O2 perfusion.

CONCLUSION

Since the resting tension is depended on the intracellular Ca2+ concentration, the above results suggest that SR Ca2+ leakage from calcium release channel in relaxation phase cause a degradation of TnI, indicating sarcomere damage. The sarcomere damage may be one of the factors to reduce the recovery rate after fatigue.

REFERENCES


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Indirect evaluation of sarcoplasmic reticulum Ca$^{2+}$ release function in skeletal muscle strips*  

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1. OBJECTIVE  
Multiple chemical and biophysical methods are available to study sarcoplasmic reticulum (SR) function. However, most of them are somewhat limited because the purified preparations of SR or channels disrupt the natural environment and may affect their function. The aim of this study was to set up a simple method to measure the SR Ca$^{2+}$ release function in skeletal muscle strips.

2. METHODS  
The time from peak tension to 75% relaxation (TR$_{75}$) was prolonged rapidly, and then shortened slowly during fatiguing tetanic contraction. The ratio of maximal TR$_{75}$ to initial TR$_{75}$ (R-TR$_{75}$) indicates a balance between SR Ca$^{2+}$ release and uptake. If the SR Ca$^{2+}$-ATPase (SERCA) activity and inhibition extent of SERCA in fatiguing contraction are identical, the R-TR$_{75}$ may be an index for SR Ca$^{2+}$ release function.

3. RESULTS  
Figure 1A is a typical recording of intermittent tetanic contractions for 5 min. The developed tension degraded gradually. Figure 1B is a time course of change in TR$_{75}$ measured in 10-second interval during 5 min fatigue. TR$_{75}$ was prolonged rapidly at first, and then shortened slowly. It suggests that the metabolites may inhibit the activity of SERCA, then the SR Ca$^{2+}$ release function in fatigued soleus.

![Fig. 2 Stimulation voltage or Caffeine treatment affects ratio of maximal TR$_{75}$ to initial TR$_{75}$ in control soleus muscle strips.](image)  
Values are mean ± SEM, n=6 muscle strips in each group. **P<0.01 vs. control value.

![Fig. 3 Relationship between stimulation frequency and ratio of maximal TR$_{75}$ to initial TR$_{75}$ in control soleus muscle strips.](image)  
Values are mean ± SEM, n=6 muscle strips in each group.

The developed tension of tetani reached maximal value in control soleus strips at 26V of stimulation voltage. But the mean R-TR$_{75}$ was 2.6 and 3.0 at 26 V and 46 V in control soleus strips, respectively (P<0.01, Fig. 2). In addition, the R-TR$_{75}$ increased significantly (P<0.01) by 3.0 at 26V with perfusion of 5mM Caffeine, which...
increases the SR Ca\(^{2+}\) release channel open probability \[2\].

The R-TR\(_{75}\) was 2.5, 2.7 and 2.3, respectively, at 100Hz, 120Hz and 140Hz. The stimulation frequency did not affect R-TR\(_{75}\) (\(P>0.05\), Fig. 3)

The R-TR\(_{75}\) was 2.6 at the fifth minute of first fatigue in control soleus muscle strips. The R-TR\(_{75}\) was 2.4 at the second fatigue after 60-min recovery (Fig. 5). But when the recovery interval shortened to 5 min and 10 min, the R-TR\(_{75}\) significantly reduced to 1.4 and 1.9, respectively (\(P<0.01\)).

The soleus muscle strips did tetanic contraction at 25V, 100Hz, and 30% duty cycle. The R-TR\(_{75}\) showed a significant decrease after 15 min and 60 min perfusion with 5 mM magnesium sulfate, an inhibitor of SR Ca\(^{2+}\) release (\(P<0.01\), Fig. 4).

The TR\(_{75}\) of tetanic contraction was 133.9±3.25 ms in control soleus, and 125.3±5.40 ms in 2-week unloaded soleus. There was no difference in TR\(_{75}\) of tetanic contraction between control and 2-week unloaded soleus (\(P>0.05\), Fig. 6). But the R-TR\(_{75}\) was increased by 2.9 in the unloaded soleus, which is significantly higher than 2.4 in control soleus (\(P<0.05\)).

4. CONCLUSION

The above results suggest that R-TR\(_{75}\) during fatiguing tetanic contraction can be considered a functional index of SR Ca\(^{2+}\) release. The enhanced R-TR\(_{75}\) indicates an increase in SR Ca\(^{2+}\) release function in 2-week unloaded soleus.

REFERENCES


*This study was supported by Grant 30770805 from NSFC.
EFFECT OF SHORT-TERM SUPPORTLESSNESS UPON PERFORMANCE OF REGULAR LOCOMOTION TEST MO-3 AT STABLE AND UNSTABLE TREADMILL

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ABSTRACT
Performance of regular locomotion test MO-3 at an unstable treadmill is associated with a decrease of impact loads and of total way covered by a subject. Energy cost of locomotion has a tendency for an increase. EMG-activity of m. soleus and m. vastus lateralis increases during running at an unstable treadmill as well. After 6 hours dry immersion the maximal speed of locomotion at the unstable treadmill is decreased and impact loads are enforced. The energy cost of locomotion at the unstable treadmill after immersion is considerably increased in comparison with the stable treadmill. Simultaneously a significant increase of EMG-activity for m. soleus and a tendency for an increase for m. vastus lateralis at the unstable treadmill are revealed. Thus the changes in energy cost of exercise at unstable treadmill and after immersion may be connected with redistribution and increase of activity for different muscle groups under conditions of instability and as a result of short-term period of supportlessness.

1. INTRODUCTION
Step-wise locomotion test MO-3 with voluntary selection of speeds is used for evaluation of physical fitness of cosmonauts. Test consists of 4 steps with rising intensity at a passive treadmill. Speed at each step is selected by the subject individually according to following wording: walking, jogging, moderate running, fast running. According to the technical requirements for ISS equipment the treadmill is to be vibro isolated from the body of the Station, that is the treadmill is unstable. The aim of the study was to evaluate the efficacy of locomotion exercise at the unstable treadmill under control conditions and after 6 hours of “dry immersion” mimicking the conditions of supportlessness.

2. METHODS
8 healthy young male subjects gave their informed consent to perform test MO-3 at the stable and unstable treadmill both under control conditions and after 6-hours dry immersion. During test locomotion speed, total distance covered, energy expenditure (V229, SensorMedics), impact loads by means of strain gauge insole (Diasled, DiaService), mean amplitude of EMG-activity (MG42, Medicor) of m. soleus and m. vastus lateralis and perceived exertion were recorded.

3. RESULTS AND DISCUSSION
Total way covered by a subject during test was significantly (by 7±2%) lower at unstable treadmill than at stable. After dry immersion the total way covered at a treadmill did not change in comparison with corresponding value under control conditions, but at unstable treadmill it was significantly (by 7±3%) lower than at a stable treadmill under control conditions. Maximal running speed at the unstable treadmill in comparison with stable was unchanged under control conditions and was decreased by 12±5% after immersion, in comparison with control trial at an unstable treadmill it was decreased as well. Speeds dispersion (coefficient of variation) at an unstable treadmill was lower two fold than at a stable treadmill, which apparently means more tough pattern of locomotion control under complicated conditions (Fig. 1).

The impact loads significantly (by 22±8%) decreased at an unstable platform apparently because of damping. Dry immersion did not affect impact loads at the stable treadmill, but significantly (by 19±6%) enforced them at an unstable treadmill (Fig. 1).
Sensation of the perceived exertion during test did not change in all cases but one: fast running at an unstable treadmill after immersion was easier to perform than in control conditions. Insensitivity of perceived exertion to the impacts used is understandable as the subject is choosing the speeds according to his perceived exertion.

Energy cost of exercise was evaluated as an increments of energy expenditure in response to corresponding increments of locomotion speed during jogging-running part of the test. Energy cost of locomotion had a tendency for an increase during exercise at an unstable treadmill. After immersion the energy cost of exercise at unstable treadmill increased in comparison with the costs of all other trials (Fig. 1). Simultaneously after immersion the HR increments related to corresponding increments of speed during jogging-running significantly (by 44±9%) increased at unstable treadmill compared to stable. Thus after immersion the difference between energy costs of running at stable and unstable treadmill became more pronounced. To understand why it was so the changes of individual muscles participating in the movements were studied.

![Graph](image)

**Fig. 2.** EMG activity of m. soleus and m. vastus lateralis during running at the stable and unstable treadmill in control conditions and after dry immersion. * and # - significant (p<0.05) difference from the corresponding value of IS- and CS-trial accordingly. For abbreviations see fig. 1

EMG-activity of m. soleus and m. vastus lateralis did not change significantly during exercise at unstable treadmill compared with control. After short-term dry immersion EMG-activity at a stable treadmill had a tendency to a decrease for m. soleus and to an increase for m. vastus lateralis compared with control trial. On the contrary at unstable treadmill EMG-activity of m. soleus was significantly increased compared with activity after immersion at stable treadmill and in control trial (only for fast running).

Summing up the obtained data one can say that after short-term immersion subject keeps balance and moves at a stable treadmill by increasing to some extent activity of muscles operating at a knee joint. In other words during movements at a stable treadmill after immersion the accent is carried over from muscles of calf to the muscles of thigh and control of m. soleus is somewhat decreased. Our interpretation of the obtained data is based at the earlier data of considerable decrease of tone (transverse stiffness) of m. soleus already after 2-6 hours of supportlessness (1, 2). Apparently, in a simple motion at a stable treadmill the nervous system is trying to choose an easier way of maintaining balance through strengthening of the knee joint. During movement at an unstable treadmill after immersion this is not enough and strengthening of an ankle joint is added.

Taking together the data obtained at an unstable treadmill under control conditions and after short-term immersion show that the locomotion at an unstable treadmill is quite possible. That means that Russian counter-measure program may be used at a treadmill with vibroisolation without substantial changes.

One finding prevents us from discarding hesitation about efficiency of counter measure locomotion at an unstable treadmill – a decrease of reaction forces during exercising at an unstable platform. The impact loads during locomotion have been shown to have a principal role in overcoming deteriorating effect of weightlessness upon organism/motor system (3).

Thus changes in energy cost of exercise at unstable treadmill after immersion may be connected with redistribution and increase of activity for different muscle groups under conditions of instability and as a result of short-term period of supportlessness.

The study was supported be the Russian Ministry of Education and Sciences, Governmental Contract # 02.522.11.2004

4. REFERENCES


Effect of antagonist muscle inactivation on energy substrate content in soleus slow- and fast-twitch fibers under conditions of rat hindlimb suspension

Tavitova M.G., Fokina N.M., Shenkman B.S.

NOTE:

ABSTRACT

The study was performed to analyze the effect of the flexor muscle tenotomy on energy substrate content in the slow- and fast-twitch soleus fibers under conditions of 7 day rat hindlimb suspension. It was shown that after 7 days of hindlimb suspension glycogen content significantly decreased in both types of soleus fibers: at 49% and 18% in the slow- and fast-twitch fibers respectively. At the same time the flexor tenotomy completely prevented the glycogen depletion in both fiber types and even led to increase of the glycogen content. As for triglyceride content it did not change neither in slow-, nor in fast-twitch soleus fibers and antagonist muscle tenotomy had no influence on this parameter.

1. INTRODUCTION

The present study is focused on the analysis of the energy substrate content in the slow- and fast-twitch soleus fibers under conditions of gravitational unloading. The alterations of the energy substrate content in muscle fibers under conditions of gravitational unloading may be associated with the hormonal changes (growth hormone level, insulin sensitivity etc) and changes in the contractile activity as well. The muscle contractile activity is controlled by the interaction of the central motor drive, deep skin afferent input and proprioceptive input from postural extensor agonist and flexor antagonist muscles and tendons [1] The impact of the antagonist proprioceptive input on the muscle fiber characteristics including energy substrates content is not clear yet. Earlier it was shown that flexor tenotomy allows to attenuate the slow-to-fast shift of myosin heavy chain isoform distribution in unloaded soleus muscle [2]. Also the tenotomy brought about to reduced atrophy development and decreased titin and nebulin degradation in m. soleus during hindlimb unloading[2].

The main objective of the study was to evaluate the effect of flexor tenotomy on energy substrate content in the m. soleus under conditions of 7 day rat hindlimb suspension.

2. METHODS

Forty eight male Wistar rats with mean body weight of ~200–250 g were used in the experiment. Rats were housed in cages and supplied with food and water ad libitum. All animal procedures were approved by the Committee for Biomedical Ethics of the IBMP (Russian Academy of Sciences, Moscow). Animals were divided on 6 groups with 8 rats in each group. There were 2 control groups, 2 groups of hindlimb suspension and 2 groups of hindlimb suspension with calf flexor tenotomy. Ten days before the end of experiment bilateral tenotomy of calf flexors (m. tibialis anterior, m. extensor digitorum longus and m. extensor hallicis longus) was performed under nembutal anaesthesia in 2 groups. Animals of another 4 groups were sham operated. Three days after operation rats of 4 groups were hindlimb suspended [3] for 7 days. After the end of hindlimb suspension muscle samples were taken under anesthesia with nembutal (40 mg/kg) from left hindlimbs and frozen in liquid nitrogen.

The corresponding cryostat muscle sections were double stained for different types of myosin heavy chains (MHC-I and MHC-II) and triglycerides (Oil Red O) [4] or glycogen [5]. The quantitative evaluation of the energy substrate content was done by means of the computerized fluorescence microscope with different filters. We analyzed the mean cross sectional area (CSA) and triglyceride and glycogen content in the slow- and fast-twitch fibers of m. soleus. All values are reported as mean ± SE.

3. RESULTS AND DISCUSSION

The CSA of type I and II fibers is shown in Tab. 1. The major effect of the 7-day hindlimb suspension was a significant decrease of CSA of both fiber types in m. soleus. Flexor tenotomy had no effects on cross sectional area of soleus fibers of both types (Tab. 1).

It was shown that after 7 days of hindlimb suspension glycogen content significantly decreased in both types of soleus fibers: at 49% and 18% in the slow- and fast-twitch fibers respectively. Previously we analyzed energy substrate content in the slow- and fast-twitch fibers of m. soleus under conditions of 3 and 14 days of rat hindlimb suspension. It was shown that glycogen content significantly decreased both in the fast- and slow-twitch fibers of m. soleus after 3 days of unloading, but returned to the control level on 14th day of hindlimb suspension [6].

The glycogen depletion in soleus fibers may be caused by the development of insulin resistance at the early stage of unloading, which in turn may bring about to increase of glycogen degradation [7]. At the same time there are data on the increased insulin-dependent glucose transport into rat soleus fibers after 3 days of unloading [8]. This phenomenon may result in the glycogen content recovery by the 14th day of hindlimb unloading.

At the same time it can not be excluded than the altered contractile activity could influence upon the
energy supply turnover in muscle fibers during unloading. In our study the tenotomy of antagonist muscles was performed in order to attenuate the proprioceptive input which was believed to inhibit the soleus activity. The flexor tenotomy completely prevented the glycogen depletion in both fiber types and even led to increase the glycogen content (Fig. 1). This fact may be the evidence that the increased flexor activity could modify the energy substrates turnover in soleus fibers during unloading. Estimating triglyceride content in the slow- and fast-twitch soleus fibers we found that in slow-twitch fibers triglyceride content almost did not change under conditions of 3 and 14 day hindlimb suspension, whereas in fast-twitch fibers there was a tendency to triglyceride accumulation after 3 days of suspension and to triglyceride depletion after 14 days [6]. According to results of this experiment triglyceride content did not change neither in slow-, nor in fast-twitch soleus fibers and antagonist muscle tenotomy had no influence on this parameter. So it was shown that the flexor tenotomy under conditions of 7 day rat hindlimb suspension prevents glycogen depletion in slow- and fast-twitch soleus fibers. Therefore, the influence of increased flexor activity equally with hormonal level changes could be one of the mechanisms that lead to the changes in m. soleus under conditions of unloading.

This research was supported by grant from Russian foundation for basic research # 07-04-01608.

REFERENCES

Table 1

<table>
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<th></th>
<th>Control</th>
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<th>Tenotomized</th>
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<td>MHC I</td>
<td>2527±142</td>
<td>1350±95*</td>
<td>1482±163*</td>
</tr>
<tr>
<td>MHC II</td>
<td>1828±115</td>
<td>1328±68.6*</td>
<td>1300±100*</td>
</tr>
</tbody>
</table>

*– significantly differ from control

Fig. 1. Glycogen content in soleus muscle fibers contained MHC-I (slow) or MHC-II (fast) in control (Con) or hindlimb suspended (7HS) and tenotomized groups.

Fig. 2. Triglyceride content in soleus muscle fibers contained MHC-I (slow) or MHC-II (fast) in control (Con) or hindlimb suspended (7HS) and tenotomized groups.
AN ACTIVE RESISTANT EXERCISE SUPERIOR TO PASSIVE MOTION ON PREVENTING BONE LOSS IN HINDLIMB UNLOADING RATS

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ABSTRACT
The existing exercise methods were not adequate to fully prevent muscle and bone loss during spaceflight. The absence of gravity might change the active exercise to passive motion in space in some degree. A device has been developed by our group for training tail-suspended rat to do gravity independent active or passive resistant exercise. Twenty SD rats were randomly divided into four groups---tail-suspended (TS), tail-suspended plus passive motion (TS+PM), tail-suspended plus active exercise (TS+AE) and control (CON) On 0d and 21d, bone mineral density (BMD) of femurs were measured by Dual Energy X-ray Absorptiometry (DXA). At the end of experiment, the mechanical properties of femurs were measured by using three-point bending test. The results showed BMD and the mechanical properties of femurs in TS+AE were most similar to CON among three experimental groups. These indicate the effect of active exercise on preventing bone loss induced by hindlimb unloading is better than that of passive motion.

1. INTRODUCTION
Weightlessness-induced osteoporosis could prove hazardous to astronauts when exposure to microgravity and the space environment during short- and long-duration space missions [1-3]. Exercise had generally been considered to be a safe and efficient countermeasure of osteoporosis without side effects. But the existing data on bone loss in the space program which were collected on individuals who were already participating in an exercise countermeasure program showed exercise had not fully prevented bone loss [4]. The reason might be that muscle contractions which were kept body balance on Earth were not needed during spaceflight, and exercise in space was more similar with the passive motion on the ground, so muscle contractions would not provide the highest skeleton loads as on the ground because of the weightless limbs [5]. In order to testify the hypothesis, we designed a rat active and passive resistant exercise training device base on rat tail-suspension model to compare the effects of active exercise and passive motive on preventing bone loss in simulated microgravity environment.

2. METARIALS AND METHODS
Twenty female eight-week old SD rats were randomly divided into four groups: tail-suspended (TS), tail-suspended plus passive motion (TS+PM), tail-suspended plus active exercise (TS+AE) and control (CON) On 0d and 21d, bone mineral density (BMD) of femurs were measured by DXA. At the end of experiment, the mechanical properties of femurs were measured by using three-point bending test. The results showed BMD and the mechanical properties of femurs in TS+AE were most similar to CON among three experimental groups. These indicate the effect of active exercise on preventing bone loss induced by hindlimb unloading is better than that of passive motion.
3. RESULTS
As table 1 shown, BMD of femurs decreased significantly in group TS and TS+PM compared with CON. However, there was no significant difference between TS+AE and CON.
As table 2 shown, tail suspension decreased most of the parameters of the mechanical properties except breaking load while breaking load increased in both TS+PM and PS+AE. Both passive and active exercise could ameliorate the mechanical properties of femurs in tail suspension rats and the effect of active exercise was better than that of passive motion according to energy absorption at breaking load point and stiffness.

4. DISCUSSION
The form of exercise could be mainly divided into active exercise and passive motion. Active exercise was induced by contracting muscle with the control of mind, and passive motion is induced by moving limbs with the help of instruments or manpower. A device was design and made by our group, using which rats could be trained by both active and passive exercise.

Table 1. Bone mineral bone of rat femurs by DXA

<table>
<thead>
<tr>
<th>Group</th>
<th>BMD growth rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS+AE</td>
<td>-2.60 ± 7.90</td>
</tr>
<tr>
<td>TS+PM</td>
<td>-10.65 ± 9.06*</td>
</tr>
<tr>
<td>TS</td>
<td>-13.67± 4.82*</td>
</tr>
<tr>
<td>CON</td>
<td>4.51 ± 7.98</td>
</tr>
</tbody>
</table>

BMD growth rate = (BMD_{21d} - BMD_{0d}) / BMD_{0d}.

* indicates significant difference compare with CON.

Table 2. Mechanical properties of rat femurs by three-point bending test

<table>
<thead>
<tr>
<th>Group</th>
<th>Breaking load (N)</th>
<th>Energy absorption (mJ)</th>
<th>Maximum load (N)</th>
<th>Energy absorption (mJ)</th>
<th>Stiffness (N/mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS+AE</td>
<td>77.16± 36.31</td>
<td>5.12#</td>
<td>86.30± 36.42</td>
<td>4.34#</td>
<td>226.17± 9.80</td>
</tr>
<tr>
<td>TS+PM</td>
<td>72.36± 36.75</td>
<td>5.12#</td>
<td>78.95± 36.42</td>
<td>4.91#</td>
<td>210.28± 9.80</td>
</tr>
<tr>
<td>TS</td>
<td>44.98± 18.23</td>
<td>5.17#</td>
<td>67.28± 18.74</td>
<td>5.91#</td>
<td>180.99± 9.80</td>
</tr>
<tr>
<td>CON</td>
<td>53.26± 18.75</td>
<td>5.17#</td>
<td>87.04± 18.74</td>
<td>1.11#</td>
<td>232.01± 9.80</td>
</tr>
</tbody>
</table>

* indicates significant difference compare with CON.
# indicates significant difference compare with TS.

The results in this study showed that both active exercise and passive motion were useful to counter tail-suspension induced bone loss. However, it seemed that active exercise was superior to passive motion on preventing bone loss of hindlimb unloading rats. This maybe useful when exercises as countermeasure of microgravity-induced bone loss is designing. However, further experiments need to be done to support this conclusion.

Acknowledgement
This work was funded by grants from the National Natural Science Foundation of China (No. 10672014).

References
DYNAMIC MECHANICAL PROPERTIES ALTERNATIONS IN RAT FEMUR DURING SIMULATED MICROGRAVITY

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ABSTRACT
The aim of this paper is to investigate the relationship of the quasi-static and dynamic mechanical properties of rat bone changed with the simulated microgravity term. In this paper, thirty male Wistar rats were equally and randomly assigned to either tail-suspension groups (SUS) or control group (Con). The microstructure of rat femur was detected by scanning electron microscope (ESEM). With the help of split Hopkinson pressure bar (SHPB), the rat femur dynamic responses were observed. The relationship of the two kinds mechanical properties between the simulated microgravity term were obtained. Contrasts with Con, both the quasi-static and dynamic mechanical strength of SUS were decreasing during the microgravity term. The strain rate sensitivity of the rat femur bones increased with the rising strain rates. In conclusion, effects of simulated microgravity term on the quasi-static mechanical properties changes for rat femur are larger than that of the dynamic.

1. INTRODUCTION
There were many reports in the literatures that long term spaceflight or simulated microgravity experiments would results in a reduction in bone mass of the human or rats skeleton, which could ulteriorly degrade the structural integrity and mechanical strength [1,2]. However, most of their work with focus on the influence or mechanism of mechanical unloading from the aspect of gravitational physiology and quasi-static mechanics, the effect of simulated microgravity on the dynamic mechanical properties of bone still remains unclear. When a manned spacecraft descends to an Earth landing by parachute or in case of emergency escape procedures, the crewmen were exposed to the ground impact environment because of an abrupt deceleration, even face the more serious condition, such as unusual landing when the failure of the braking rockets ignition. This could have catastrophic consequences if the response of mission operations to impact force over his dynamic compression strength [3]. In this paper, a tail-suspension rat model was used to study the effect of skeletal unloading on bone, and with the help of SHPB, the dynamic properties of the loss microgravity bone under high strain rate were detected.

2. MATERIALS AND METHODS
In present study, Wronski and Morey-Holton tail-suspension rats model were used [4]. Thirty 4-month-old Wistar rats were divided into five groups: one control (Con) group and four tail-suspended (SUS) groups. Con and SUS rats were killed at 7, 14, 21 and 28 days. Femurs were removed and used for multiple studies.

Quasi-static compression tests (10^{-3}s^{-1}) were conducted on a standard material testing machine (WDW-5) under displacement control. The microstructure of bone and change of calcium (Ca) content were imaged and determined by using environment scanning electron microscopy (ESEM, FEI-Quanta 600) coupled with energy spectrum analysis (EDS, Oxford INCA-sight 6427). To study the effect of strain rate on the compression properties of rat bone, the aluminum pressure bar and piezoelectric PVDF (polyvinylidene fluoride) gauges was used. Data are reported as means ± SD.

3. RESULTS AND DISCUSSION
The ESEM pictures of rat femur shown that the sizes of pores in SUS group were obviously larger than that in Con. For example, the pores size of Con and 21d SUS are 13.4 ± 3 μm and 32.6 ± 4 μm respectively. The energy spectrum analyse results shown that there was a huge drop in calcium weight during the microgravity course. The content of calcium of Con group is 20.04%,
SUS(d=28) reduced to 8.25%.

There was a steady decline in the quasi-static and dynamic mechanical strength of rat femur during the microgravity term. According to the experiments, the relationship between quasi-static fracture load with microgravity term were fitted as following equation:

\[ F = e^{(5.03-0.05 \times \text{days})} \]  

Typical stress-strain curves of rat femur derived from SHPB tests were shown in Fig. 1. It can be seen that the dynamic strength increased with the increasing strain rates. This implied that the femur was representative viscoelastic material with obvious rate-dependent mechanical behavior. The dynamic strength after microgravity were shown in Fig. 2, which at a strain rate of 1100/s, and the relationship between dynamic strength and microgravity term could be fitted as the following equation:

\[ \sigma = \ln(14690.48-445995) \times \text{days} \]  

The behaviour of porous material under dynamic conditions can be assessed by a micromechanical approach. In this approach, the representative volume element for the porous material is usually defined as a hollow sphere. Using an approximation of the velocity field and the principle of virtual work, an explicit relationship was found between the macroscopic stress and strain rate. Numerous authors have studied the effect of the void size, results shown that a large initial void radius contribute to enhance void collapse [5]. And the void collapse can be governed solely by viscosity (“low” loading rate combined with “consequent” viscosity) or was governed by viscosity at the beginning and by inertia in the late stage (“high” loading rate with “low” viscosity), i.e. it has a different sensitivity of loading rate.

In macro-scale, strain rate sensitivity increase with strain rate (see Fig. 1.). With the prolong of microgravity term, the increase pores or void size of cancellous bone lead to the increase of strain rate sensitivity.

ACKNOWLEDGMENTS

This research program is supported by the Advanced Space Medico-Engineering Research Project of China, projects number SJ200803. The authors gratefully acknowledge the technical assistance of China Astronaut Research and training Center for the hind limb unloading procedures and animal care.

REFERENCES


ABSTRACT
It was studied the streaming potential (SP) of fresh exited moist femur of 24 adult male Wistar rats after normo- and hypokinesia and different mechanical loading (ML) levels. The ML corresponded to 30 - 50 and 100% of animal body mass was carried out in axial direction. The experimental rats were in state of hard low space hypokinesia for 28 days. Control rats were in standard vivarian condition. It was set that amplitude of SP which arises up in bones at the ML depends on the level this loading. However there is a certain optimum of the ML which provides the maximal increase of SP. This optimum is in the range of physiology level loadings. In hypokinetic rat's bone SP diminished most substantially in the range of physiology level loadings also. It may be one of the reasons of standard physical loadings low efficiency in state of microgravitation and slow renewal of bone tissue after hard hypokinesia and long space mission.

INTRODUCTION
The conversion of ML to bioelectric signals in bone has been suggested to control the bone remodeling and reparative regeneration. It was shown that this mechanism in wet bone is connected with the SP generation [1]. Mechanical forces cause electrical signals due to motion of an ion carrying extracellular fluid in bone matrix. SP can be fixed on the bone surface both in vivo and in vitro [2]. Suppose that SP is taken important role in regulation of the bone tissue remodeling and reparative regeneration. It was shown that between the size of SP and degree of bone deformation there is direct cross-correlation dependence. Maximal SP amplitude on unit of loading diminishes at the decline of deformation gradient. However much researches of the last years testify that connection between electric potential and loading is not such simple, as appeared before [3]. Suppose that not only intensity but also character of loading influence on the SP generation processes [4]. The aim of this study was to determine if hypokinesia affects the SP of compact rat's bone.

METHODS
The objects of the study were the fresh exited wet rat's femur. There were 2 groups of adult male Wistar rats. The 1st – 12 normokinetic rats (control) and 2nd- 12 hypokinetic rats (experimental) were exposed in the special low space containers. The duration of hypokinesia was 28 days. The ML on bone was carried out in axial direction in special device. The size of loading was expected coming from the terms of physiological partition of gravimetric loading between front and back rat's extremities (40% - on front extremities, and 60 % - on back). All researches were conducted at 3 degrees of loadings: 30 % (loading 1), 50 % (loading 2) and 100 % (loading 3) from rat's body mass. In all experiments Ag-AgCl electrodes with a salt bridge from 0,9% NaCl in 2% agar were utilized [5]. Electrodes were disposed in the middle of femur diaphysis. Thus an electrode, located on the concave side of bone, was connected to the inverting entrance, and electrode of opposite side – to the uninverting entrance of strengthener. During experiment bone was saved in the moist state at 25°C. The rats were decapitated by guillotine in state of profound ether narcosis. All animal experiments were conducted in accordance with principles expressed in the "Guide for Care and Use of Laboratory Animals" (Office of Science and Health Reports of the USA NIH, Bethesda MD 20892). We used standard parametric and non-parametric statistical methods for analysis of results.

RESULTS AND DISCUSSION
It was set that hypokinesia caused changes in SP which combine with the decreasing of bone mass, volume, size of cortical area and bone tissue density. The mean of SP had high dependence on the loading level (fig. 1).

![Graph showing SP amplitude in normo- and hypokinetic rat's bone at different loadings levels.](image)
After hypokinesia SP diminished most substantially in the range of physiology level loadings also. In hypokinetic rats the mean values of SP per unit of loadings 1 and 2 were less on 35-37% accordingly (fig.3). But there were no differences in SP between control and experimental rats at loading 3.

The first researches of SP, which arising up in bone at the ML, shown that their character considerably varied depending on frequency, size and duration of deforming influence. However some clear conformity to law set it was not. The results of our researches testified that SP amplitude of rat's femur was largely determined by size of this loading. However there was a certain optimum of loading provided the maximal increase of SP. It corresponds to the physiological level loadings (30-50 % from body mass). Electric potentials, arising up in a bone at the functional loading, are examined as one of major messengers, providing its connection with the processes of the bone remodeling. Considered that in the mechanism of bioelectric potential generation in moist bone a dominant role is played by the electrokinetic phenomena [1]. SP arises up in bone tissue because of existence the electrical double layer between the solid phase and liquid phase in bone cavities and pore [6]. Consider that it reflects not only the features of liquid motion in the lakuno-canaliculer system, but participates in maintenance of homeostasis and control of ionic exchange between the different bone departments. Suppose that SP is a major information generator for bone cells about the state and structure of bone matrix and changes of the biomechanics loading on a bone [2]. Bioelectric mechanisms participate not only in the processes of the physiological remodeling but also reparative regenerations of bone. As the electrokinetic phenomena, closely related to the processes of polarization and bone tissue conductivity, undoubted interest is presented by complex research of physiological and biophysical conformities to law of SP changes and passive electric properties of bone in different statokinetic terms.

CONCLUSION
Our results suggested that amplitudes of SP were in direct dependence on the magnitude of the loading. But there was a certain optimum magnitude of the ML, provided the maximal increasing of SP in the physiological condition. After the long and severe hypokinesia the efficiency of SP generation decreased most considerably in the range of physiological level loadings also. Decline of the level bioelectric regulation of bone remodeling because of SP diminishing may be one of the reasons low efficiency of standard physical loadings in state of microgravitation and slow renewal of bone tissue after hard hypokinesia and long space mission.

REFERENCES
IN VIVO PHYSIOLOGICAL EXPERIMENTS IN MODELED MICROGRAVITY (RPM) ON RAT BONE MARROW CELLS MINERALIZATION

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ABSTRACT

It is well known that microgravity leads to modifications of several physiological processes. This study aimed to further contribute to the validation of Random Positioning Machine (RPM) for “in vivo” experiments by studying bone marrow cells mineralization in the rat. Bone marrow cells were obtained from femora of rats exposed to RPM for 72 h and cultured for 13 days in osteoblast differentiation medium. Calcified matrix was analyzed by Alizarin Red assay demonstrating that calcium deposition was significantly decreased in RPM samples. Moreover, we determined alkaline phosphatase (ALP) activity, a marker of osteoblastic phenotype, using a fluorimetric assay. The data obtained showed a dramatic downregulation of ALP in RPM samples. Cellular area distribution and F-actin stained were also determined. The results are compared with in vitro experiments in bone marrow stromal cells and osteoblasts exposed to the RPM.

INTRODUCTION

Several investigations demonstrated that exposure to weightlessness affects many fundamental cellular functions which include cell proliferation and differentiation, gene expression, loss of muscle mass and bone, reduced immune response and cardiovascular decondition. The limited access to experimentation in real microgravity in space strongly suggests to use ground-based facilities for long or short duration exposure of humans (e.g. bed rest) or small-sized mammals to modeled microgravity conditions. The threedimensional clinostate Random Positioning Machine (RPM) has been used so far as a suitable facility for in vitro cellular physiology studies. In a recent investigation in rats exposed to modeled microgravity conditions in RPM, we observed that response to carrageenin or PGE_2-induced paw edema, thermal hyperalgesia and intestinal transit were significantly reduced [1]. Moreover, the manufacture of Mouse Habitat on RPM (MHHOR), a system which can permit to house 1 or 2 mice or rats on a RPM for a time interval up to 20 days with a minimum involved of personnel, is currently in progress. Investigations from many laboratories have indicate that microgravity causes decreased bone formation in combination with increased resorption leading to calcium and mineralized bone loss [2]. The progressive loss of calcium and bone may be the most critical biomedical obstacle that astronauts in extended duration space flights will encounter. The present study was designed to further contribute to the validation of the RPM as facility for in vivo biological and physiological studies; therefore bone marrow cells mineralization in young male rats exposed to simulated microgravity has been investigated.

METHODS

Male albino Wistar rats weighing 150-175 g were kept in a perspex semicylinder fixed to the inner frame center of clinostat for 72 hours. The first ground control group (Control RPM, CR) was kept individually in a perspex semicylinder on the basement of RPM and exposed to the same experimental conditions, while a second ground control group (Control Cage, CC) was kept in standard cage. All animals were given standard laboratory diet and water available ad libitum three times per day. The rat experiments were performed in accordance to the Italian law (D.L. 116, 1992), which allows experiments in laboratory animals only after submission of a research project to the competent authorities, and in accordance to the "Principles of laboratory animal care" (NIH publication no. 80-23, revised 1996). After rats sacrifice with massive dose of urethane anesthetic (1,5 g / Kg), bone marrow cells were obtained from the femora bone shafts and cultured for 13 days in α-Minimum Essential Medium supplemented with 15% fetal bovine serum, 10 mM HEPES, 10 mM β-glycerophosphate (β-GP), 0,2 mM L-ascorbic acid 2-phosphate magnesium salt (AA). Production of calcified matrix was detected by Alizarin Red staining for calcium deposition. Cells were fixed in 10% neutral-buffered formalin and stained with 40 mM Alizarin Red (pH 4.2). The precipitate was solubilized using 10% (w/v) cetylpyridinium chloride (CPC) in 10 mM phosphate buffered saline and the ARS concentration was determined by absorbance measurement at 550 nm. Moreover, alkaline phosphatase (ALP) activity, a marker of osteoblastic phenotype, was determined using a fluorimetric assay. Analysis of cell area and F-actin network was performed by immunofluorescence technique after culturing cells in a gas-permeable cell culture disks (OptiCell).

F-actin staining: cells were fixed with 4%
paraformaldehyde, labeled with phalloidin-TRITC-conjugated.

Cell area distribution: cells were fixed with 4% paraformaldehyde, labeled with primary monoclonal anti β-tubulin followed by a secondary FITC-conjugated antibody. Cell area was calculated with the Image J software and the statistic analysis was performed by MYSTAT software.

RESULTS AND DISCUSSION

The results indicated that simulated low g inhibited alkaline phosphatase activity and bone nodule formation in rat osteoblasts differentiated from bone marrow stromal cells. Alkaline phosphatase appeared downregulated by modeled microgravity with a significant decrease by 54% (p< 0.05) compared to the cage control (GC) and by 35 % (p< 0.05) compared to the RPM control (CR) (Fig.1). Also calcified matrix deposition was dramatically decreased by 95% (p<0,001) in modeled microgravity conditions compared to the cage control (GC) and by 36 % (p< 0,001) compared to the RPM control (CR) (Fig. 2). Because the alkaline phosphatase is a well known marker and key regulator of osteoblast differentiation, our results suggest that microgravity inhibits the differentiation of osteoblasts. Cell area distribution showed a significant decrease in low gravity conditions. In both controls we observed that cells were distributed as a heterogeneous population including both small and big cells, whereas cells from rats exposed to modeled microgravity showed a homogeneous population with significantly smaller cells. Moreover, the fluorescence staining of F-actin filaments showed a remarkable decrease in the filamentous biopolymer density. This result confirm the notable role of cytoskeleton in the mechanotransduction of gravity signaling. Similar changes in the actin network, especially a reduction of the stress fibers, were also observed in osteoblasts [3], HUVEC cells [4] and J111 monocytes [5] exposed to low g. In conclusion, our results can be considered a further contribute to the validation of the RPM and of the oncoming MHOR for in vivo physiological studies in modeled microgravity conditions.

In vitro experiments in cultured bone marrow stromal cells exposed to modeled microgravity g for 72 h and cultured for two weeks in α-medium supplemented with AA and β-GP demonstrated a decreased mineralization (- 52%) and ALP activity (-68%) in RPM samples. Also in vitro experiments in cultured osteoblasts (first subculture) obtained from rat bone marrow cells and exposed to modeled microgravity for 9 days confirmed a strongly decreased calcified matrix deposition (-60%) and ALP activity (-48%).

Acknowledgments

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REFERENCES

A LOOK AT THE IMMUNE RESPONSE UNDER MICROGRAVITY:
THE “ROALD” EXPERIMENT

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The supposed cause of a reduced immune response in microgravity has been addressed to lymphocytes apoptosis but, to date, there is no evidence for a specific signalling cascade responsible for this process. We have previously demonstrated that 5-lipoxygenase (5-LOX) pathway can have a central role in apoptosis upon simulated microgravity condition; in fact, experimental data highlighted an increase of the enzyme activity both in parabolic flight and in simulated microgravity [1, 2]. The “ROALD” experiment, aimed at evaluating the Role Of Apoptosis in Lymphocyte Depression, was launched on board Soyuz 17S in the frame of the BIO4 mission. The goal of ROALD was to ascertain whether immune cell apoptosis and 5-LOX pathway might be altered under microgravity.

Peripheral blood lymphocytes (PBL) were purified from human blood and were placed in customized hardware, designed by Kayser Italia, until their final integration in KUBIK incubator on the International Space Station (ISS). The experimental activities were stopped at different time points (3, 24 and 48 hours) and the experimental containers were stored in the Minus Eighty Degrees Celsius Laboratory Freezer for the ISS (MELFI) facility.

Post flight analysis demonstrated that 5-LOX mRNA expression had a similar trend in both 0g and 1g samples, with no appreciable statistical difference between the two groups (**p<0.05) (Fig. 1). Western blot analysis demonstrated the presence of a single immunoreactive band (78 kDa), of roughly the same intensity in 0g samples and 1g controls. Therefore, 5-LOX mRNA and protein expression was unaltered in microgravity (Fig. 2).

On the other hand, the content of the 5-LOX product leukotriene B\textsubscript{4} (LTB\textsubscript{4}), measured in 0g samples versus 1g controls, increased already after 3 hours of exposure to 0g (data not shown).

Moreover, our results showed that PBL exposed to microgravity undergo apoptosis in the 24-48 hours time-window, as demonstrated by RT-PCR analysis of specific genetic markers of apoptosis, such as calpain, p53 and poly (ADP-ribose) polymerase (PARP).

The ground control experiment was performed in the presence of specific 5-LOX inhibitors: MK886, an inhibitor of 5-LOX activating protein (FLAP), and ETYA, that blocks 5-LOX directly. Table 1 shows that both compounds were able to block LTB\textsubscript{4} synthesis (i.e., 5-LOX activity) and to reduce the expression of calpain, p53 and PARP in PBL exposed to simulated microgravity in the random positioning machine (*p<0.05, **p<0.01).
Table 1 Effects of various compounds on 5-LOX activity and apoptosis

The “ROALD” experiment demonstrates the engagement of a specific signalling cascade as a cause of lymphocyte apoptosis in real microgravity. In fact, the altered levels of p53, PARP and calpain, the increased production of leukotrienes B₄, detected during the post-flight analysis in 0g versus 1g samples, and the effect of selective inhibitors are a clear proof of a misregulation of the normal cell life survival under the control of 5-LOX. In the perspective of space exploration and colonization, these findings seem to provide new insights in the immune response of peripheral blood lymphocytes to an extreme environment, and suggest new markers of apoptosis that could be useful to ensure the safety of International Space Station crewmembers on future missions.

ACKNOWLEDGMENTS
This investigation was supported by the Italian Space Agency (ASI) and by Fondazione Banco di Sardegna.

REFERENCES
MICROTUBULAR NETWORK RECOVERY IN HUMAN MONOCYTES IN MODELED LOW GRAVITY AFTER PRETREATMENT WITH NOCODAZOLE

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ABSTRACT

Early investigations in space and in simulated low gravity have shown that gravity changes affect important cellular functions, including, but not limited to, proliferation, gene expression, cytoskeletal architecture and motility. Changes in cell architecture are responsible for a different response to their environment. In this work we have studied the reorganization of microtubules in J-111 monocytes in modelled low gravity after they have been first depolymerized by exposure to the antimicrotubule agent nocodazole. Analysis by immunofluorescence technique and a quantification of β-tubulin fluorescence intensity revealed remarkable differences in microtubule pattern reorganization in cells pretreated with nocodazole and exposed to modelled low gravity for 1 and 4h, but an evident recovery after 24h, compared to 1g controls.

1. INTRODUCTION

Since the first flights of humans into space it is known that the immune system is depressed after their return to Earth. With the planned human exploration of space, including Moon and Mars, there is an increasing concern of the effects of long duration space flight on the immune system of space travelers. Our previous studies at the cellular level in modelled and in real low gravity revealed a dramatic depression of the in vitro mitogenic T-cells activation, changes in lymphocytes and monocytes differentiation and motility [1-2]. Both cell shape and cytoskeletal components such as microtubules are affected by altered gravitational conditions, compared to 1g; especially a reduced and incomplete arborisation of β-tubulin has been observed [3]. Cells rely on intact microtubule network for their structure, transport of organelles and cell division.

The objective of this work was to investigate on monocytes J-111 microtubules reorganization in modelled low gravity conditions and at 1g after they have been first depolymerized by exposure to the antimitotic agent nocodazole. Earlier studies indicated that alterations in microtubule dynamics are likely mediated through a reversible and relatively rapid tubulin dimer-nocodazole interaction [4].

2. METHODS

Cell monolayers of J-111 cells, a monocyte macrophage cell line, were: pre-treated with 20 mM nocodazole (methyl [5- (2-thienylcarbonyl) - 1H-benimidazol 2yl-carbamate), whereas fresh medium was added to control samples (Fig.1c), exposed to simulated microgravity on RPM (Dutch-Space), as earth-based model of spaceflight (Fig.1b) and placed onto the RPM supporting static frame (control samples) in order to expose them to the same vibrational stress (Fig.1a). After 1h, treated and non-treated cell chambers, completely fluid-filled with fresh culture medium, were positioned on the RPM (low g samples) at 37°C for 4 and 24h respectively and control samples were placed in static position.

2.1 Microscopic analysis

Microtubular structures were observed by fluorescence-inverted microscope (Olympus) 100 X magnification. Pictures were acquired by F View II Image camera with CCD (2/3”) sensor and 1376 X 1032 at 8 bit resolution coupled to the software “Analysis” (Soft Imaging System GmbH-Olympus).

2.2 Microtubules staining

After 4% paraformaldehyde fixative, β-tubulin staining was performed using a primary monoclonal antibody against b-tubulin (isotype Ig G1 mouse, clone TUB 2.1, SIGMA) and a secondary FITC-conjugated goat anti-mouse globulin, (IgG Fc specific). Nucleuses staining was performed by 4’,6-diamidino-2-phenylindole hydrochloride (DAPI).

![Figure 1](image1.png)

![Figure 2](image2.png)

Fig.1 Disruption of the microtubular network in J-111 monocytes treated with 20μm nocodazole for 1h (1c) compared to untreated cells (1a) and cells exposed to 1h simulated low g in RPM (1b).

Fig.2 Quantitative analysis of β-tubulin fluorescence intensity in J-111 monocytes treated with nocodazole (Noco) compared to untreated cells (Control) and to cells exposed to 1h of simulated low g (RPM). Mann-Whitney test comparation showed statistical significant differences between untreated cells v/s exposed cells to RPM (P<0.0001) and mostly v/s nocodazole treated cells (P<0.0001) There was also a difference between RPM exposed cells v/s nocodazole treated cells (P<0.045)
2.3 Quantitative analysis
Fluorescence intensity of β-tubulin filaments was detected by Data Analysis (Soft Imaging System GmbH-Olympus) algorithm [5].

3. RESULTS
3.1 Morphological changes induced by nocodazole or modelled low gravity in microtubules.
Control samples showed a well organised microtubule network, appearing orderly radiated from the microtubule organizing centre to the plasma membrane, with β-tubulin filaments abundant and well arranged into cytosolic bundles and in the elongated and extended filopodia (Fig.1A). Treatment for 1h with nocodazole disrupted microtubules structures (Fig.1C). In fact, tubulin was no longer detected in fine elongated fibers but appeared as patches distributed within the cytosol. Thus nocodazole hinders the polymerization of αβ-tubulin dimers. Conversely, β-tubulin network in J-111 cells exposed to 1h of RPM modelled low gravity (Fig.1B) showed a remarkable decrease in the filamentous density and appeared to be highly disorganized and shortened. Microtubules appeared packed in perinuclear position with a surrounding arborization organized with very short prolongations to the plasma membrane. Quantitative analysis of β-tubulin fluorescence intensity is shown in Fig.2.

3.2 Effects of modelled low gravity on microtubules recovery

![Fig.3](image-url)
Fig.3 Microtubular network recovery in J-111 monocytes exposed for 4-24h to simulated low g in RPM clinostat (B, D left panel) and to 1g earth normal gravity (A, C left panel) after 1h pre-treatment with 20μM nocodazole (treated, right panel) compared to untreated cells (control, left panel).

![Fig.4](image-url)
Fig.4 Quantitative analysis of microtubule recovery in J-111 monocytes, pre-treated with nocodazole after 4h (top) and 24h (bottom) of exposure at modelled low g (RPM-Noco) and to 1g earth gravity (GC-Noco) compared to un-treated cells exposed to low g (RPM) and to 1g (GC).

Mann-Whitney test comparison showed statistical significant differences in β-tubulin fluorescence intensity recovery of GC 4h v/s GC-Noco 4h (P<0.0396), GC 4h v/s RPM 4h (P<0.0132), GC 4h v/s RPM-Noco 4h (P<0.0002), GC-Noco 4h v/s RPM 4h (P<0.0669), GC-Noco 4h v/s RPM-Noco 4h (P<0.0418). Conversely no significant differences were observed within 24h in modelled low gravity indicating a following reorganization of the microtubules.

Analysis by immunofluorescence technique and a quantification of β-tubulin fluorescence intensity revealed remarkable differences in microtubule pattern reorganization in pre-treated cells exposed to modelled low gravity for 4h (Fig.3B, right panel) compared to 1g (Fig.3A, right panel). Moreover, despite the difference in the microtubules structure arborization an evident recovery was observed after 24h at modelled low gravity conditions (Fig.3D, right panel), although they are not completely reorganized compared to cells exposed for 24h to 1g (Fig.3C, right panel) where β-tubulin filaments appeared completely recovered, as in untreated cells at 1g (Fig.3D left panel).

Quantitative analysis of microtubule fluorescence intensity recovery is shown in Fig. 4.

4. DISCUSSION
In this work we could confirm the earlier data [3] on the disturbances of the microtubules in J-111 monocytes by a short time exposure to modelled low gravity conditions and the following reorganization of the microtubules within 24h in modeled low gravity. Furthermore microtubules first depolymerized by nocodazole, showed an evident recovery after 24h at modelled low gravity, although to a lesser extent than samples exposed to 1g. Thus modelled low gravity has an impact on the complete reorganization of the microtubules in cells pre-treated with nocodazole. The described reorganization of β-tubulin in modelled low g might represent an adaptive mechanism of the cells and might have relevance in the process of cell adaptation to gravitational unloading.

5. ACKNOWLEDGMENTS
This research was supported by the Italian Space Agency (ASI) and by Fondazione Banco di Sardegna.

6. REFERENCES
ABSTRACT

Peculiarities of culture growth, morphology and ultrastructure of cells of widespread blue-green alga *Anabaena cylindrica* Lemm. under clinorotation were studied. The formation of greater quantity of heterocysts, as well as special spores – akinets in culture was shown under clinorotation. The latter were not observed in the control variant even under prolonged clinorotation. Presence of different types of cells (vegetative cells, heterocysts and akinets) in the trichoms was established during long-time clinorotation of *A. cylindrica* culture. Heterocysts were observed only in the laboratory control. The ultrastructural organization of vegetative cells and heterocysts in the trichoms of *A. cylindrica* in both variants were generally similar. However, the changes in the topography of thylakoids, as well as localization and number of cytoplasmic inclusions were observed under clinorotation. The influence of clinorotation on the form of *A. cylindrica* colonies, appearance of akinets in trichoms, as well as changes in ultrastructure organization of cells were discussed.

1. INTRODUCTION

Alga cells are repeatedly used for evaluation of the influence of space flight factors on their structural and functional peculiarities. It was shown (1-3), that microgravity causes serial rearrangements in submicroscopic organization and physiological properties of alga cells. The blue-green alga *Anabaena* with well studied structure of cells and facility for cultivation under laboratory conditions is very convenient for experiments under clinorotation. The presence of different cell types in trichomes of *Anabaena* (i.e. threads surrounded by mucous cover) made of this species a very convenient object for study of both morphological and structural changes in cells, as well as formation of colonies under influence of clinorotation.

2. OBJECT AND METHODS

As an object of our research we used the culture of widespread blue-green alga *Anabaena cylindrica* Lemm. It was grown on solid nutrient medium Drju with addition of 1, 8 % agar under regime light/dark (12h: 12h). Intensity of illumination was kept at the level of 200 μmol/photon/m²/sec. Growth of this alga under clinorotation continued during 30 days. Experimental material was taken every 10 days.

Methods of light-optical and electron transmission microscopy were used for morphological and ultrastructural research respectively. The fixation of alga material was performed according to technique worked out earlier (1). Ultrathin sections of cells were investigated and photographed in electron transmission microscope Jem 1230 EX (Jeol, Japan).

3. RESULTS AND DISCUSSION

Growth of *A. cylindrica* trichoms was accomplished, mainly, due to apical cell. As a result, the colonies in a form of dense flat tendrils of different diameters up to 3 mm were produced in control variant (Fig 1).

![Fig. 1. Colony of *A. cylindrica*. Control.](image)

Only single heterocysts in the trichoms of *A. cylindrica* culture in control variant usually were observed. They were connected with neighboring vegetative cells by plasmodesms.

The typical colonies of *A. cylindrica* with clear configuration were not observed under clinorotation. Predominantly separate trichomes in a form of chaotic thread were revealed in this condition (Fig 2).

![Fig. 2. Trichoms of *A. cylindrica*. Clinorotation. Heterocysts (black arrows), akinet (grey arrow).](image)
As a rule, under clinorotation the quantity of heterocysts increased. An interesting phenomenon in this condition was observed in culture, in particular, the appearance of the special spores – akinets in contrast to the control variant (Fig 3). The quantity of akinets under long-time clinorotation (over 20 days) strongly increased.

The typical peculiarities of akinets were the presence of very thick cell wall with rough surface covered by papillae, as well as big lipid drops (Fig. 4). Besides, these cells don’t have any thylakoids and assimilation pigments accordingly.

The protoplasts of A. cylindrica cells contained nucleoplasm zone with DNA threads, ribosomes and RNA, but thylakoids were equally distributed in total cytoplasm volume. Other cytoplasmic inclusions (carboxisomes, cyanoficine granules, small vacuoles and lipid drops were also observed in the cells (Fig. 5).

The submicroscopic organization of A. cylindrica cells under clinorotation was different from that of control cells in some respects, firstly, in thylakoid topography (Fig. 6). They were located in parallel with the cell wall in peripheral zone of the cells. Besides, cytoplasm inclusions, such as angular polyedral bodies, small lipid drops and vacuoles were predominantly observed in central part of the cells (Fig. 6).

Revealed changes in morphology of the colonies coincide with early obtained data on the modification of Staphylococcus colonies in microgravity (4). The colonies took on the elongated form instead of globular one in the control variant.

The increase of the number of heterocysts and especially the appearance of akinets testify about the negative influence of clinorotation on culture growth and its metabolism. Formation of akinets takes place only under unfavorable condition and they remain vitality due to presence of large quantity of lipid drops and thick wall. Therefore, appearence of akinets testifies about acceleration of culture ageing.

In general, obtained results showed the negative influence of clinorotation on culture growth, as well as morphological and ultrastructural peculiarties of different types of cells in alga culture.

LITERATURE

CLINOROTATION-RELATED CHANGES OF PEA ROOT MITOCHONDRIA

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ABSTRACT
Effects of clinorotation on the mitochondrial ultrastructure in cells of meristematic, distal (DEZ) and central (CEZ) elongation zones of pea 5-day-old etiolated seedling roots were studied. It was shown that mitochondria in cells of examined root growth zones revealed a different sensitivity to clinorotation. The ultrastructure of mitochondria in the meristem and CEZ cells did not substantially change in comparison with stationary control. At the same time, changes in the mitochondrial ultrastructure were observed in the DEZ under clinorotation, namely: a decrease in the mitochondrial size, an increase in the matrix electron density and cristae volume. It is supposed, that changes in the mitochondrial ultrastructure under clinorotation display the disturbance of energy metabolism in DEZ cells in these conditions.

1. INTRODUCTION
Plant cell bioenergetics is strongly affected by abiotic stresses. Plant mitochondria may control reactive oxygen species generation by means of energy-dissipating systems. Therefore, mitochondria may play a central role in cell adaptation to abiotic stresses, which are known to induce oxidative stress at cellular level [1].

There are a significant number of publications, in which the data on the changes in the structural and functional organization of mitochondria in cells of lower and higher plants are presented [2, 3]. At the same time, the majority of such data is related to mitochondria in root meristem and cap cells [4, 5]. The aim of our work was to investigate the ultrastructure of mitochondria in cells of roots different growth zones under horizontal clinorotation.

2. MATERIALS AND METHODS
Pea (Pisum sativum L.) seeds germinated in moist tubes from filter paper in containers placed on the slow horizontal clinostat (2 rpm) and seedlings grew for 5 days in darkness at temperature 25°C and 70% humidity.

Root apices were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, for 2 h at room temperature, postfixed with 1% osmium tetroxide in the same buffer for 2 h at 4°C, dehydrated in a graded ethanol series, then in acetone and afterwards embedded in the epon-araldit mixture. Ultrathin sections (50 – 70 nm) were cut on a RMC MT-XL ultramicrotome, stained with uranyl acetate and lead citrate [6] and examined with transmission electron microscopes JEM 1200EX and JEM 1230EX at either 60 kV. Morphometry was done with UTHSCSA Image Tool 3.0. The significance of differences between mean values was determined by a non-parametric Mann-Whitney U test. Differences at p ≤ 0.05 were considered significant.

3. RESULTS AND DISCUSSION
An analysis of the mitochondrial ultrastructure in meristematic cells under clinorotation has shown the absence of essential changes in comparison with the control (Fig. 1). A shape and a size of mitochondria were significantly variable on the sections. Mitochondria had mainly an oval and roundish shape, sometimes, organelles were elongated and branched. The mitochondrial matrix expressed an average level of electron density usually with electron transparent zones with contained DNA fibrils. Mitochondrial cristae of tubular or lamellar shape with a narrow intramembrane space were observed. Cristae disposed without certain order.

Fig. 1 Fragments of pea root meristematic cells with mitochondria in stationary control (a) and clinorotation (b). Bar = 0.5µm

Fig. 2 Fragments of pea root DEZ cells with mitochondria in stationary control (a) and clinorotation (b). Bar = 0.5µm
At the same time, certain changes in the mitochondrial ultrastructure were observed in DEZ cells under clinorotation. Under the influence of clinorotation, the mitochondrial population in a cell was more homogeneous in comparison with the control. In the most cases, mitochondria became condensed (Fig. 2). A size of organelles was shown to reduce (Fig. 4). An increase in both the matrix electron density and relative volume of cristae was noted to occur due to crista swelling (Table 1). The crista quantity did not increase. Electron transparent zones in the mitochondrial matrix were found out much less often than in control.

![Fig. 3 Fragments of pea root CEZ cells with mitochondria in stationary control (a) and clinorotation (b). Bar = 0.5 µm](image)

In cells of the CEZ, a size of mitochondria was smaller than in the control, though the internal organization did not differ (Fig. 3).

**Table 1. A relative volume of mitochondrial cristae in pea root growth zones, %, * – significant difference (p<0.05).**

<table>
<thead>
<tr>
<th>Root growth zone</th>
<th>Control</th>
<th>Clinorotation</th>
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<tbody>
<tr>
<td>Meristem</td>
<td>10.08±4.28</td>
<td>10.03±3.59</td>
</tr>
<tr>
<td>DEZ</td>
<td>10.5±4.46*</td>
<td>16.4±4.87*</td>
</tr>
<tr>
<td>CEZ</td>
<td>9.63±2.98</td>
<td>7.48±2.12</td>
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Thus, the data obtained by us testify the changes in the mitochondrial ultrastructure only in DEZ cells. It is known that DEZ is characteristic by the high metabolic activity. It is supposed that actively metabolizing cells are the most sensitive to altered gravity [2] and many aspects of cell functioning change in microgravity and under clinorotation [7, 8]. So gradually development of free radical oxidation [7] and time-dependent HSP90 and HSP70 overexpression in pea root cells under clinorotation were reported [8]. The high metabolic activity of DEZ cells is connected with their specific physiological properties as well as with their function to provide the formation of specific enzyme systems for fast growth of cells in the CEZ. Responses of DEZ cells to various exogenous and endogenous signals such as auxin, mechanical impedance, electrotropic stimulation and gravity, differ from CEZ cells, and they can be frequently opposite [5].

**Fig. 4 Size of mitochondria in the meristem (1,2), DEZ (3,4), and CEZ (5,6) of pea roots in control (1, 3, 5) and clinorotation (2, 4, 6)**

Thus, our results demonstrated that clinorotation affects the mitochondrial ultrastructure of pea root DEZ cells. Described structure rearrangements are supposed to reflect the changes in the functional activity of mitochondria in altered gravity.

4. REFERENCES

Ca\textsuperscript{2+} IONS ARE INVOLVED IN PLANT CELL GRAVISENSITIVITY

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ABSTRACT
Despite the circumstantial evidence for a role of Ca\textsuperscript{2+} ions in plant cell gravising and graviperception, direct measurements for its changes remain ambiguous. Recently, we showed the increased Ca\textsuperscript{2+} concentration in cortex cells of the Beta vulgaris root distal elongation zone under clinorotation as compared to the control. Under gravistimulation of Lepidium sativum seedlings in the combined magnetic field (CMF) with the frequency resonance to the cyclotron frequency of calcium ions, a positive root gravitropic reaction changed on a negative, and calcium ions accumulated in the upper site of a root curvature in the elongation zone unlike a positive gravitropic reaction. Amyloplasts-statoliths displaced to the upper longitudinal cell wall in the root cap statocytes, i.e. in the direction opposite to a gravitational vector. Displacement of amyloplasts, which contain the abundance of free Ca\textsuperscript{2+} in the stroma, was accompanied with Ca\textsuperscript{2+} redistribution and increasing in the cytosol in the same direction.

1. INTRODUCTION
Calcium as a second messenger is known to play a crucial role in stimulus – response coupling for many plant cellular signaling pathways. Its messenger functions are realized by transient changes in the cytosolic ion concentration induced by a variety of internal and external stimuli such as light, hormones, temperature, salinity, and gravity. There are numerous experimental data concerning the changes in calcium balance and distribution in plant cells under the influence of altered gravity and gravistimulation [1-5] indicating its role in gravising and graviperception. Though some questions on calcium biological functions and significance of the calcium balance changes in altered gravity and under gravistimulation are discussed and direct measurements for its changes remain ambiguous. Therefore, the aim of our work was to determine the calcium distribution and relative content in the root distal elongation zone (DEZ) and cap statocytes under clinorotation and gravistimulation in the steady magnetic field (SMF) and in the combined magnetic field (CMF) with the frequency resonance to the cyclotron frequency of calcium ions.

2. MATERIAL AND METHODS
Three-day old table beet (Beta vulgaris L.) and cress (Lepidium sativum L.) seedlings were used for the investigation. Dry seeds of B. vulgaris wrapped in a moist filter paper germinated in the stationary conditions and on the slow horizontal clinostat in darkness. Straight-grown roots of L. sativum were placed horizontally (=gravistimulated) in a moist chamber and placed into a shielded space where the SMF and CMF with the frequency resonance to the cyclotron frequency of calcium ions were created [6]. A specific fluorescent dye fluo-4 was used as the concentration of calcium ions in a cell correlates directly with an intensity of complex fluorescence at the corresponding emission wavelength [7] Observations were carried out with confocal microscope LSM 5 Pascal at the excitation wavelength 494 nm and emission wavelength 516 nm. A potassium antimonate method [8] was also used for cytochemical identification of calcium ions in root cap statocytes with an electron microscope JEM 1230.

3. RESULTS AND DISCUSSION
We revealed significant increase in a concentration of calcium ions and alterations of calcium localization in the cells in altered gravity. It was shown the increased Ca\textsuperscript{2+} relative volume in cortex cells of the table beet root distal elongation zone under clinorotation as compared to the control, that confirms the earlier data on Ca\textsuperscript{2+} increasing in plant cells in altered gravity [4]. Under gravistimulation of cress roots in CMF with the frequency resonance to the cyclotron frequency of calcium ions, where roots change their positive gravitropism on negative ones (Fig. 1), the paradoxical amyloplast displacement and Ca\textsuperscript{2+} redistribution occurred in columella cells.

Fig. 1 Cress seedlings after 30 min of gravistimulation in SMF (a) and in CMF (b).

It is of a peculiar interest the displacement of amyloplasts-statoliths in the root cap statocytes to the upper longitudinal cell wall, i.e. in the direction opposite to a gravitational vector. Amyloplasts contain the abundance of free Ca\textsuperscript{2+} in the stroma (Fig. 2). Displacement of amyloplasts is accompanied with Ca\textsuperscript{2+} redistribution in the cytosol in the same direction, that has been also demonstrated using the fluorescent calcium indicator fluo-4 (Fig. 3). Under gravistimulation in the CMF, the calcium relative
content increases (Table 1). Made calculation of precipitate deposits in statocytes at electron micrographs showed its increasing in roots under gravistimulation in the CMF nearly ten times in comparison with the SMF, especially in the hyaloplasm around the amyloplasts.

**Fig. 2. Fragment of a statocyte after 30 min of gravistimulation in CMF.** Electron micrograph. Bar=500 nm.

In the DEZ, calcium ions accumulated in the upper side of a root curvature unlike a positive gravitropic reaction, i.e. at the same direction as amyloplasts. It is known that the concentration of wall Ca²⁺ is higher in the region of lower growth and the converse is true for the region of higher growth rate [8]. This redistribution is likewise observed in a root cap [1].

**Fig. 3. Fluorescence intensity of Ca²⁺ + Fluo-4 complex in cress statocytes from: a – the straight-grown root, b, c – after 15 min of gravistimulation in SMF (a) and in CMF (c). The arrow points to the scanning direction.**

Gravistimulation initiates a rapid Ca²⁺ movement to the “lower” part of a root and the relocation of Ca²⁺ in cell walls of the elongation zone. Whether this difference in wall Ca²⁺ is sufficient to alter growth asymmetrically is a key question which has not been satisfactorily answered by current research. Are the observed differences sufficiently large to cause unequal growth and thus initiate bending [3]. The obtained data open new approach to search the answers on these questions. On a role of Ca²⁺ in plant cell gravisensing and graviperception, and possible participation of Ca²⁺ in the unusual direction of amyloplast movement under gravistimulation in the CMF.

**Table 1** Fluorescence intensity of Ca²⁺ + Fluo-4 complex in statocytes scanned in parts along the long axis from the basal to apical end after 15 min of gravistimulation, rel. un.

<table>
<thead>
<tr>
<th>Part</th>
<th>SMF</th>
<th>CMF</th>
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<tbody>
<tr>
<td>1st</td>
<td>77.30± 1.04</td>
<td>124.29± 1.75</td>
</tr>
<tr>
<td>2nd</td>
<td>93.34± 1.17</td>
<td>142.44 ±1.87</td>
</tr>
<tr>
<td>3rd</td>
<td>101.17± 1.19</td>
<td>169.30± 2.03</td>
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A question arises also what causes such paradoxical movement of amyloplasts and calcium ions in statocytes in the CMF in the direction opposite to a gravitational vector. Three assumptions are possible: 1) the influence of CMF and Ca²⁺ on the cytoskeleton, 2) the influence of CMF and Ca²⁺ on the electric field in statocytes and 3) the influence of CMF on energy and direction of Ca²⁺ ions rotation accordingly to the model of ion cyclotron resonance. The cyclotron frequency of Ca²⁺ ions is the formal frequency of ion rotation in the static magnetic field. Cyclotron resonance may be produced any time there is a steady magnetic field combined with an oscillating electric or magnetic field acting on a charged particle. Cyclotron resonance has the ability to transfer energy to these ions and to cause them to move more rapidly. These effects change the functional properties of living cells that has been called “ion cyclotron resonance in biosystems” [9]. Simultaneous applying the altering magnetic field with the same frequency can provoke auto-oscillation in the system and consequently change the rotation rate and/or the direction of Ca²⁺ ion flow in the biological objects. In our experiments, such phenomenon could lead to surprising rapid Ca²⁺ movement to the “higher” side of a gravistimulated root resulting in paradoxical ion redistribution in statocytes and ultimately in negative root gravitropism.

Realization of root negative gravitropic reaction in CMF with the frequency resonance to the cyclotron frequency of Ca²⁺ ions is an effective model for future research of the mechanism of plant gravitropism, including a Ca²⁺ role in plant physiological growth reactions.

**4. REFERENCES**

ADAPTATION REACTIONS OF VIRUS-INFECTED PLANTS EFFECTED BY SIMULATED MICROGRAVITY

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ABSTRACT
The role of the simulated microgravity in forming of adaptation plant reactions is presented in the research. Studied influence of the simulated microgravity on the maintenance of viruses – representatives to the genus of Carlavirus in the potato plants. Determined the key moments of change in viral infection flowing under the system infection.

INTRODUCTION
Researches of viral particles accumulation in the leaves of the system infected plants-owners testify of viruses reproduction can be repressed as a result of reactions limitations which are conditioned in the varied nocifensors of plants [1]. The level of virus accumulation and its spreading over plant tissues serve the indices of plant resistance to a given virus [2]. The influence of abiotic factors, particularly that of simulated microgravity on viral pathogenesis remains practically unknown. Our studies of gravisensitivity of a system virus-host plant allowed us to elucidate some aspects of its influence on Wheat streak mosaic virus reproduction in clinostating [3]. M.-potato virus (MPV) belongs to the genus of Carlavirus and is a damage-causing pathogen infecting potato plant. Effection of unsteady varieties of soft cultures even can result in the decline of harvest on 10-18% [4, 5]. In plant tissues the MPV is often present in a company of other potato viruses and very often the researches notice its combined infection with another representative of the genus Carlavirus, the S.-potato virus (SPV). The isolates of SPV under monoinfection do not cause noticeable symptoms on many sorts of potato. The S.- potato virus is easily passed mechanically in a way from the infected plants to healthy that, makes sure, a danger at cultivation of potato in artificial ecosystems. Morphology of these two viruses is similar in many aspects. The viral particles of SPV have sizes of 650 x 12 nm, virions lines or slightly out bowed [5]. The viral particles of MPV have sizes of 650 x 13 nm mostly, also meet 100-150 and 250-300 x 13 nm [6]. Research of dynamics change of viruses maintenance of at the joint infection of two family viruses at plants of potato in the conditions of the simulated microgravity not conducted before. Therefore the aim of our work was to study the dynamics of reproductive changes of MPV and SPV in their combined infection within potato plants in simulated microgravity (clinostating) and to envisage the possibility of plant adaptation to such conditions.

MATERIALS AND METHODS

The objects of our research included potato varieties Zdabytak and Krymchanka, systematically infected with MPV and SPV. Changes in plant orientation relative to gravity vector were obtained in Cycle-2 and KG-8 clinostats with horizontal and vertical axes of rotation with and speed of 2 revelations per minute (rpm). The plants were growing in containers with artificial substratum and illuminated of 15000 luxes; the plant received periodic nutrition – the solutions of micro- and macro salts. The time of simulated microgravity action was within 21 – 83 days. The virus was detected using das-ELISA and electron microscopy (JEM-1230, JEOL) techniques. For diagnostics, the test-system of Bioreba (Switzerland) was employed. The optical density of immuno enzymic reaction products was determined on and reader (Termo Labsystems Opsis MR with Dynex Revelation Quicklinck Software, USA) at wavelengths of 405/630 nm. The statistical processing of data is conducted with the software package of Microsoft Office Excel.

RESULT AND DISCUSSIONS
Our research results gave an evidence that for the MPV reproduction in Zdabytak potato variety there was a reduction in viral antigen content during 83-day's of cultivation. A critical point in viral reproduction under “on earth” conditions was achieved at 65th day of growth; for clinostat species, it was achieved at 67th day. In this period there was a drastic reduction in virus antigen content in plant tissues (Fig. 1).

![Graph showing dynamics of MPV antigens maintenance in potato plants](image)

**Fig.1.** Dynamics of MPV antigens maintenance in the potato plants (S.tuberosum), variety of Zdabytak. VC –
vertical clinostating; HC – horizontal clinostating; MC – motionless control.

The dynamics of SPV maintenance had a reverse tendency as compared to reproduction of MPV in plant tissues of potato Zdabytak sort. To 65 days of experiment observed relatively low concentration of SPV antigens both in motionless control and in those which were under the conditions of clinostating (Fig.2.). It should be noted that at plants which were in the conditions of horizontal clinostating, maintenance of antigens to 67 days of cultivation had a tendency to decline. On 65 days of cultivation maintenance of SPV antigens in the plants of this variant was in 2 times below, than under vertical clinostating and in 1.5 times below, than at plants which reared in surface terms.

Fig.2. Dynamics of SPV antigens maintenance in the potato plants (S. tuberosum), variety of Zdabytak. VC – vertical clinostating; HC – horizontal clinostating; MC – motionless control.

At the plants of Krymchanka variety after 35 days of cultivation the fall-off of MPV antigens maintenance was marked, thus in the conditions of clinostating their concentration was below, than in motionless control (Fig.3).

Results analysis shows that the action of microgravity on plants has a mechanism similar to a “rate-effect” condition. It was found that microgravity can confirm the organisms resistance to a given pathogen. Receipt of potato tubers harvest the Krymchanka sort from the virus infected plants in the conditions of clinostating is a proof of adaptation reactions. For today there is a little information about influence of microgravity on the systems «pathogen-owner». It is exposed, that during cultivation of bacterium Salmonella enterica in the conditions of microgravity its activity increases [7]. Role of microgravity at mutual relations of the system «virus – host-plant», which has a substantial value for the biotechnology of virus free cultivation of plants, But this requires additional research activities.

CONCLUSIONS
Thus, the clinostating favored both the reduction of viral reproduction at and certain stage of plant growth and an increase of it. All depends on the extent of variety resistance to infection and microgravity, its adaptation potential. The sensitivity of and system “Virus-host plant” to microgravity also depends on pathogen’s genetic specificity.

REFERENCES
The aim of the present study is to investigate the effects of high magnetic gravitational environment (HMGE) on production of avermectin B1a in *Streptomyces avermitilis*. A special designed large gradient superconducting magnet can produce three types of HMGE, namely 0 g (12 T), 1 g (16 T) and 2 g (12 T). After the industrial strain 3-115 was mutated, mutant PE1 screened from microgravity group with high yield of avermectin B1a was isolated. Results showed that the production of avermectin B1a of PE1 was dramatically increased more than 60%, which greatly increased efficiency and reduced the cost of industrial production. Another mutant strain, designated as PE162, derived from the parent strain, produced a new compound, which was proved to be the aglycon of avermectin A1a. The filamentous morphology of the experimental groups is also different from that of the control group. These results demonstrated that the HMGE could induce the changes in some physiological characteristics of microbes. Based on the mutation rates of the experimental groups, the optimal conditions were determined.

1. INTRODUCTION

Gravity plays an important role in evolution of life on Earth and provides the required living environment for life activities. Space environment, such as microgravity, cosmic radiation and vacuum, have been reported to stimulate microbial growth [1]. It has been reported that space culturing can improve the production of secondary metabolites [2]. However, because the limited real space flight times and conditions made the intensive and extensive research hard to be studied, ground-based simulated experiments have great practical significance.

It has been reported that a superconducting magnet with a high magnetic force field can make diamagnetic materials including cells be levitated instead of using a spacecraft [3]. Moreover, it is much less expensive to use magnetic force rather than a spacecraft to obtain a weightless condition.

Avermectins are a class of widely used as anathematic and insecticidal agents produced by *Streptomyces avermitilis* [4], and B1a is the most efficient component [5].

In this paper, mutant libraries of *Streptomyces avermitilis* were constructed by ground-based simulated experimental platform for gravitational biology. We screened a potential industrial avermectins high-yield mutant, evaluated the effects of gravitational environment on production of avermectin B1a, and found a mutant with extraordinary metabolites.

## 2. MATERIALS AND METHODS

### 2.1 Microorganism

*S. avermitilis* 3-115, high-producing industrial strain, was stored in our lab and grown on YMG agar medium.

### 2.2 Cultivation conditions

Spores of parental strain were grown on YMG agar medium. Cultivations were performed in two stages: spore germination and the actual cultivation. For the spore germination stage, mycelium from a YMG agar medium plate were inoculated into 250-ml flasks containing 40 ml seed medium and incubated at 28°C and 220 rpm on a shaker incubator (5cm shaking diameter, 50/300, Long March, China) for 40 h. Then, fermentation medium were inoculated with seed cultures and incubated at 28°C and 220 rpm for 10 days [6].

### 2.3 Mutant libraries construction

The HMGE mutagenesis libraries were prepared from *S. avermitilis* 3-115. The spore suspension was prepared in sterile water. Four groups were designed as follows: control group, 0 g (12 T), 1 g (16 T), 2 g (12 T) [7].

### 2.4 Analytical methods

For the quantification of avermectins, 1 ml of culture broth was extracted with 9 ml of methanol. The quantities of the major avermectin components B1a were determined by HPLC (Agilent 1200, USA) with a Waters C18 reverse phase column, UV detection at 245 nm, and mobile phase methanol-water (90:10, v/v) at 1 ml/min flow rate.

## RESULTS

### 2.5 Mutation induced by HMGE

Two hundred colonies generated from each of the three experimental groups were picked out. The mycelium morphology of experimental and control groups was observed. We found that after 240 h culture, the mycelium morphology of control changed from filamentous to coccoid, while the mycelium morphology of the mutant groups remained filamentous. And the mycelium of control was more abundant than that of experimental groups, as shown in Fig. 1.
2.6 Screening for high producing strains
The productions of avermectin B1a by control and experimental groups were analyzed by HPLC. One mutant, PE1 (CGMCC No. 2712), with highest yield was obtained in 0g (12T) group. The average yield of PE1 was increased from 4234.5 mg/l in parent strain to 5419.0 mg/l in the mutant. The production of PE1 remained stable during five generations of flask culture. The strain was also cultivated in 360 tons scale-up fermentations in fermentor (Fig. 2). Comparing to the parent strain, PE1 exhibited remarkable avermectin B1a titer and productivity increased more than 60%. The results implied that the mutant could be applied on the industrial scale and well-adapted to industrial fermentation processes, which will greatly elevate the productivity of industrial fermentation of avermectins.

2.7 Studies on the mutant PE162
During the process of screening for avermectin B1a high-yield mutant strains, it was found that the mutant strain PE162, which came from the parent strain in the 2g (12T) group, produced a new compound as compared to the parent strain (Fig. 3), and the new compound had UV absorptions similar to those of avermectins.

From the results of several analyses of the compounds produced by the mutant PE162, performed by nuclear magnetic resonance, and mass spectrum, the compound was determined to be the aglycon of avermectin A1a [8](data not shown).

3. DISCUSSION
In this study, we examined the effect of HMGE on the production of avermectin B1a by S. avermitilis. The findings indicated that the simulated weightless environment by means of diamagnetic levitation significantly affected cell morphology and production of antibiotic.

This experiment adds empirical support to the hypothesis that microgravity is the most important mutagen factor in space flight [9]. Microgravity may disturb the system of DNA repair, which blocks or delays the repair of DNA strand breakage. Although the mechanism of space-simulated experiments is not elucidated, this research suggests that they are valuable mutagen sources. It could be widely applied to the microbe breeding and could improve the selection efficiency.

4. ACKNOWLEDGEMENTS
This work was supported in part by grants from National Natural Science Foundation of China (No 30700015), National 863 project (2006AA09Z402 and 2007AA09Z443), National Key Technology R&D Program 2007BAI26B02, the National Science & Technology Pillar Program (No. 200703295000-02), Important National Science & Technology Specific Projects (No. 2008ZX09401-05). L.Z. was an awardee for Hundred Talents Program.

5. REFERENCE:
ASSESSING THE POTENTIAL OF INDUCED MUTATIONAL STRATEGY TO ELICIT HIGH-YIELD AVERMECTINS PRODUCING STRAINS

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ABSTRACT
Induced mutagenesis is an important and classical approach to develop high-yield producing strains. To find the best way of breeding for high-yield avermectins producing strains, mutant libraries of Streptomyces avermitilis were constructed by three induced mutagenesis methods, UV (ultraviolet), NTG (N-methyl-N’-nitro-N-nitrosoguanidine) and HMGE (High magnetic gravitational environment). Then for each population, the distribution of average phenotypic distance was calculated, and the correlation between the distribution of average phenotypic distance and the percentage of improvement was investigated. HMGE could introduce a larger phenotype distribution than UV and NTG. Microgravity environment produced by HMGE was found to be a better method for breeding high producing strains.

1. INTRODUCTION
Secondary metabolites produced by microorganisms have wide application in healthcare, pharmaceutical, chemical, food and agricultural industries. Induced mutagenesis of the microorganisms exhibits various advantages, including the improvement of production capability of strains, the optimization of product quality and the simplification of production techniques. New approaches of induced mutagenesis emerged with the development of biotechnology, and space-induced mutagenesis had led to great progress in strain improvement. In space, cosmic radial, high vacuum, intense magnetic field and microgravity induce chromosome aberrance and further lead to genetic mutation of microorganisms[1][2][3]. However, it is difficult to carry out space-induced mutagenesis extensively, so ground-based simulated experiments, such as cosmic radial, intense magnetic field and microgravity, have great practical significance. Avermectins and its analogues, produced by Streptomyces avermitilis, are major commercial anti-parasitic agents[4]. A variety of mutagenesis methods had been developed to increase its productivities. For evaluating induced mutagenesis approaches Klein-Marcuschamer and Stephanopoulos presented a metric based on the quantification of phenotypic diversity to evaluate strain improvement approaches [5].

In this study, mutant libraries of S. avermitilis were constructed by three induced mutagenesis methods, UV, NTG and HMGE. Then for each population, the distribution of average phenotypic distance was calculated. The correlation between the distribution of average phenotypic distance and the percentage of improvement was analyzed. In this way, we compared the potential of producing mutations among different induced mutagenesis approaches, to find the most effective one and optimize the experiment techniques for S. avermitilis breeding.

2. MATERIALS AND METHODS
2.1 Strain and medium
S. avermitilis 3-115, high-producing strain, is used as parent strain. YMG agar medium, the seed medium and the fermentation medium were prepared.

2.2 Culture conditions
Spores generated by induced mutagenesis of parental strain were grown on YMG agar medium. Cultivations in both solid micro-culture and flask were incubated at 28 °C for 10 days.

2.3 Mutant Libraries Construction
The mutant libraries were prepared from S. avermitilis 3-115 with three different mutagenesis approaches.

The spore suspension was prepared and treated by UV, NTG and HMGE. After treatment, the samples were serially diluted in the same buffer and plated over YMG.

2.4 Phenotype Selection

The optical density at 245 nm was chosen as the phenotype for measuring diversity. More than 165 clones from each library were screened. The phenotypic distributions of five different populations (including the control) were quantified.

2.5 Diversity Quantification

We used optical density at 245nm as the phenotype for diversity quantification of libraries generated by the previously mentioned mutagenesis methods.
All data were analyzed with MATLAB (MathWorks). Average phenotypic (Euclidean) distance was calculated as

\[ d_{ij} = \sum_{i}^{n} | P_i - P_j | \]  

(1)

Where the brackets indicate an average over all pairs of members of the population and \( P_i \) is the phenotype of colony \( i \):

\[ P_i = \ln O_i \]  

(2)

### 2.6 Characterization of Improved Mutants

To determine the mutants with improved production, the strains from improved wells with respect to the control OD245 were cultured in shake flasks. The production of avermectins was determined by high-performance liquid chromatography (HPLC, 1200, Agilent, USA) with a Waters C18 reverse phase column, UV detection at 245 nm, and mobile phase of methanol-water (90:10, v/v) at 1 ml/min flow rate.

### 3. RESULTS

#### 3.1 Phenotypic distribution of different induced mutation libraries

![Figure 1: Distributions of the average phenotypic distance (\( d, \) Eq. 1) of five different populations and the control. A histogram that reflects the probability that the ‘true’ average phenotypic distance has a certain value.]

#### 3.2 Phenotypic Distribution Correlations with the Probability of Finding Improved Mutants

![Figure 2: The correlation of divergence and percent improved. A sigmoidal fit was used.]

### 3.3 Isolation of Improved Mutant

![Figure 3: The relative production of parent Strain 3-115 and selected mutant from microgravity library (0g 12T) in 360 t fermentor. The max production of strain 3-115 was taken as 100%.

### 4. CONCLUSION

HMGE-induced mutagenesis enhanced average phenotype distance and diversity better than UV and NTG mutagenesis, for S. avermitilis. Microgravity introduced the most mutations in the genome of S. avermitilis under HMGE conditions. For medium divergence (0.6<\text{divergence}<0.8), improved diversity increased the probability of isolating mutants with improved phenotype.

### 5. ACKNOWLEDGEMENTS

This work was supported in part by grants from National Natural Science Foundation of China (No 30700015), National 863 project (2006AA09Z402 and 2007AA09Z443), National Key Technology R&D Program 2007BA126B02, the National Science & Technology Pillar Program (No. 200703295000-02), Important National Science & Technology Specific Projects (No. 2008ZX09401-05). L.Z. was an awardee for Hundred Talents Program.

### 6. REFERENCE

THE EFFECTS OF SPACE FLIGHT ON THE PRODUCTION OF AVERMECTIN BY

STREPTOMYCES AVERMITILIS

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ABSTRACT

Avermectins were produced by Streptomyces avermitilis, especially B1a exhibited an excellent potency and broad spectrum against a variety of nematode and arthropod parasites with quite a low level of side effects on the host organism. To improve the productivity of avermectin, an avermectin-producing strain was carried into space by a manned spaceship, ‘Shenzhou VII’ (Divine Vessel VII) on Sep 25, 2008. Samples for space flight and their corresponding ground controls were similarly prepared using identical media and inoculums. After samples were rescued, the mutants were screened for high avermectin producing strains monitored by high performance liquid chromatography (HPLC). Post flight analyses indicated that space flight had increased the productivity of avermectin. One mutant, F-556 could produce 18.95% more avermectin B1a comparing to control strains. Another two mutant strains, designated as F-158, showed significantly reduced productivity of avermectin B1a and exhibited changes in morphological characteristics. All of the results demonstrated that the space flight could induce the changes in some physiological characteristics of microbes. The divergence was also calculated, and compared to that of mutant library induced by high magnetic gravitational environment.

1. INTRODUCTION

Avermectins are a class of anistic and insecticidal agents produced by Streptomyces avermitilis [1, 2]. They are featured by a 16-membered pent acyclic with a disaccharide of methylated deoxysugar L-oleandrose polyketides [3]. The structural characterization of eight major avermectin compounds are different mainly at the C5, C22-C23 and C26, and in which, B1a is the most efficient component [4].

Space conditions, such as microgravity, cosmic radiation and vacuum, have been reported to stimulate microbial growth [5, 6]. It has been reported that space culturing can improve the production of secondary metabolites [5, 7, 8]. Although the specific underlying mechanisms remain largely unknown, a majority of the findings reported in the literature that microorganisms are affected in a variety of ways as a result of space flight. In Sep 2008, a pilot study was undertaken to investigate the effects of actual space flight on the production of the avermectin by S. avermitilis.

In this paper, we report two post-flight mutants, mutant F-556 and mutant F-158. F-556 was a high-yield avermectin mutant and F-158 showed significantly reduced productivity of avermectin B1a with changes in morphological characteristics.

2. MATERIALS AND METHODS

2.1 Microorganism and medium

*S. avermitilis* 3-115, high-producing industrial strain, was stored in our lab and grown on YMG agar medium. The seed medium contained (per liter): corn starch 30 g; soya flour 8 g; peanut meal 10 g; yeast extract 4 g, CoCl2 0.03 g, and α-amylase 0.04 g. The pH of the seed medium was adjusted to 7.0 with NaOH before autoclaving. The fermentation medium contained (per liter): corn starch 140 g; α-amylase 0.1 g; soya flour 28 g; yeast extract 10 g; Na2MoO4·2H2O 0.022 g; MnSO4·H2O 0.0023 g; (NH4)2SO4 0.25 g; CoCl2 0.02 g; and CaCO3 0.8 g. The pH value was adjusted to 7.5 with NaOH before autoclaving.

2.2 Cultivation conditions

Spores of parental strain were grown on YMG agar medium. Cultivations were performed in two stages: spore germination and the actual cultivation. For the spore germination stage, mycelium from a YMG agar medium plate were inoculated into 250-ml flasks containing 40 ml seed medium and incubated at 28°C and 220 rpm on a shaker incubator (5cm shaking diameter, 50/300, Long March, China) for 40 h. Then, fermentation medium were inoculated with seed cultures and incubated at 28°C and 220 rpm for 10 days.

2.3 Space flight

The matured spores of 3-115 were inoculated to the slant agar medium, which was contained in a 0.5 milliliter centrifuge tube, and incubated at 28°C for 5 days. The slants were divided into space flight and ground control group. The space flight group was placed in the returning module of the manned spaceship, ‘ShenzhouVII’. ‘ShenzhouVII’ was launched at 21:10 Beijing time on September 25, 2008 from the Jiuquan Satellite Launching Center in Gansu Province, People’s Republic of China. 20 minutes after blast-off, the spaceship entered its preset orbit and successfully orbited the earth 45 times in a period of 68 h. The orbit inclination angle was 42.4°, altitude of the apogee was 336.7 km and altitude of the perigee was 330.8 km. The returning module landed safely in the central Inner Mongolia Autonomous Region at 17:27 Beijing time on September 28, 2008. The package containing the samples of 3-115 was in good condition when removed.
from the returning module. The samples were transported to the laboratory and stored at 4°C.

2.4 Analytical methods
For the quantification of avermectins, 1 ml of culture broth was extracted with 9 ml of methanol. The quantities of the major avermectin components B1a were determined by HPLC (Agilent 1200, USA) with a Waters C18 reverse phase column, UV detection at 245 nm, and mobile phase methanol-water (90:10, v/v) at 1 ml/min flow rate.

3. RESULTS

3.1 Mutation induced by space flight
728 colonies generated from experimental group were picked out, and mutation rates were calculated. The positive mutation rate was 14.97%, and the negative mutation rate was 20.19%.

The production of avermectin B1a by control and experimental groups was analyzed by HPLC. One mutant, F-556, with highest yield was obtained. The average yield of F-556 was increased from 5139.5 mg/l in parent strain to 6113.0 mg/l. The production of F-556 remained stable during six generations of flask culture (Table 1).

Table 1. The genetic stability of the mutation S. avermitilis F-556

<table>
<thead>
<tr>
<th>Generation</th>
<th>Avermectin yield, mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6113</td>
</tr>
<tr>
<td>2</td>
<td>5891</td>
</tr>
<tr>
<td>3</td>
<td>5862</td>
</tr>
<tr>
<td>4</td>
<td>5950</td>
</tr>
<tr>
<td>5</td>
<td>6007</td>
</tr>
<tr>
<td>6</td>
<td>6132</td>
</tr>
</tbody>
</table>

3.2 Studies on the mutant F-158
During the process of screening for avermectin B1a high-yield mutant strains, it was found that the mutant strain F-158 were not only reduced in their productivity of avermectin B1a (Fig. 1), but also had a changed color of their fermentation broth. After 240 h of incubation of F-158 in fermentation medium with shaking at 28°C, for F-158, the color of the broth changed from yellow to purple, whereas the broth of the ground control 3-115 still remained yellow, as shown in Fig. 2.

4. DISCUSSION
In this study, we examined the effect of space conditions on the production of avermectin B1a by S. avermitilis. Production of avermectin B1a using F-556 was constant throughout 5 generations. Avermectin B1a yield of the mutant strain F-556, and the changes that occurred with regard to morphology and culture characteristics of the mutant strain F-158 and F-24 demonstrate that space conditions can cause mutations of microorganisms.

5. ACKNOWLEDGEMENTS
This work was supported in part by grants from National Natural Science Foundation of China (No 30700015), National 863 project (2006AA09Z402 and 2007AA09Z443), National Key Technology R&D Program 2007BA126B02, the National Science & Technology Pillar Program (No. 200703295000-02), Important National Science & Technology Specific Projects (No. 2008ZX09401-05). L.Z. was an awardee for Hundred Talents Program.

Reference:
COLD SHOCK (25°C) AND RE-WARMING (37°C) AFFECT CELL PHENOTYPE: IMPACT ON SPACE BIOLOGY EXPERIMENTS

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ABSTRACT

The preparation of cell cultures for space flight experiments is submitted to technical constraints which often oblige the investigators to keep cells at a suboptimal temperature (22-25°C) for several days before launch and re-warming at 37°C when experiencing microgravity. Here we report that maintaining cell cultures at 25°C followed by a rising of temperature to 37°C affects the expression of a number of genes and proteins involved in the cellular and oxidative stress response. The expression of most of the tested genes (IL-8, matrix metalloproteinase-1, HSP70, Bcl-2, Bcl-X and VEGF), as well as the phosphorylation of p53, Akt, JNK and γH2AX and the production of LC3 (II/I), a marker of autophagy, were significantly modulated. These effects indicate that cellular stress and survival pathways as well as DNA integrity are affected. Therefore our data suggest that keeping cells at suboptimal temperature must be avoided when possible. These effects should be considered while studying the impact of microgravity to avoid misinterpretations of the data.

1. INTRODUCTION

Constraints as late access to space engines, absence of cell biology facilities close to launch sites, energy restriction and delay between launch and transfer of samples on ISS impose that cells are kept at low temperature (22-25°C) before warming to 37°C and experiencing microgravity. An evoked advantage of such a low temperature is to maintain cells in a non-proliferative and somehow “sleeping mode” thought to make them insensitive to vibrations and hyper-gravity levels during transportation and launch.

Our laboratory participated to the FOTON M3 campaign (launched on 14 September 2007 from Baikonour, Kazakhstan). A delay of 5 days at 25°C was imposed between the preparation of the samples at the laboratory (Liege, Belgium) and the launch. During the preparation of the experiment, we observed that this procedure affected cell morphology, some cells looking apoptotic.

We investigated the potential regulation of expression of a number of genes and of the level of phosphorylation of signalling proteins during storage of the cells at 25°C followed by a rising of temperature to 37°C for increasing period of time.

2. MATERIAL AND METHODS

WI26 cells (SV-40 transformed human lung fibroblasts) were seeded at 40000 cells/cm² in Petri dishes. They were grown in Dulbecco’s Modified Eagle’s Medium supplemented with 10% Foetal Bovine Serum and buffered with 25mM HEPES. Cells were maintained 1 to 5 days at 25°C and transferred to 37°C for 1 to 24 hours. Proteins phosphorylation and mRNA of selected genes were quantified by Western-Blot and RT-PCR, respectively.

3. RESULTS

The expression of cytokines (IL-1β, IL-6 and IL-8), of proteins involved in extracellular matrix (ECM) homeostasis (matrix metalloproteinase-1 (MMP-1) and α1 chain of collagen type I), of heat shock protein HSP70, of the apoptotic factors Bcl-2, Bax, Bcl-X and of VEGF has been investigated at the mRNA level (Table I). The expression of MMP-1, IL-8, HSP70, Bcl-2 and Bax was reduced at 25°C to reach a minimum level after 5 days, while IL-1β, IL-6 and collagen type I were not or less affected. Although the overall expression of Bcl-X and VEGF remained constant, the splicing of their pre-mRNA was progressively altered as the expression of the pro-apoptotic Bcl-XS and of the newly identified VEGF111 (1) isoforms were increased at the expense of other isoforms.

<table>
<thead>
<tr>
<th>Gene</th>
<th>1D 25°C</th>
<th>5D 25°C</th>
<th>8h 37°C</th>
<th>24h 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1β</td>
<td>0.4 ± 0.02</td>
<td>0.9 ± 0.06</td>
<td>0.7 ± 0.03</td>
<td>1.1 ± 0.04</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.5 ± 0.01</td>
<td>1.1 ± 0.06</td>
<td>0.7 ± 0.05</td>
<td>0.6 ± 0.02</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.3 ± 0.03</td>
<td>0.1 ± 0.06</td>
<td>0.2 ± 0.01</td>
<td>0.4 ± 0.02</td>
</tr>
<tr>
<td>MMP-1</td>
<td>0.5 ± 0.03</td>
<td>0.5 ± 0.03</td>
<td>0.4 ± 0.1</td>
<td>0.3 ± 0.02</td>
</tr>
<tr>
<td>Collagen α1(I)</td>
<td>1.1 ± 0.35</td>
<td>1.3 ± 0.2</td>
<td>0.5 ± 0.3</td>
<td>0.2 ± 0.07</td>
</tr>
<tr>
<td>HSP70</td>
<td>0.3 ± 0.11</td>
<td>0.6 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>1.7 ± 0.05</td>
</tr>
<tr>
<td>Apoptotic genes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bcl-2</td>
<td>0.2 ± 0.09</td>
<td>0.2 ± 0.05</td>
<td>0.4 ± 0.06</td>
<td>1.2 ± 0.13</td>
</tr>
<tr>
<td>Bax</td>
<td>0.8 ± 0.08</td>
<td>0.3 ± 0.02</td>
<td>0.5 ± 0.03</td>
<td>0.8 ± 0.03</td>
</tr>
<tr>
<td>Bcl-X (pro-apo)</td>
<td>3.6 ± 0.01</td>
<td>10.1 ± 0.1</td>
<td>10.6 ± 0.1</td>
<td>5.5 ± 0.05</td>
</tr>
<tr>
<td>VEGF111</td>
<td>0.7 ± 0.01</td>
<td>19.2 ± 0.4</td>
<td>40.5 ± 0.4</td>
<td>60.5 ± 3.7</td>
</tr>
</tbody>
</table>

Table I: Effect of cooling and re-warming the cells on the expression of various genes. RNA was extracted prior to cooling (T0), after 1 at 25°C (1D 25°C) or 5 days at 25°C prior (5D 25°C) or after re-warming at 37°C for 8 (8h 37°C) and 24h (24h 37°C). The factor of induction is relative to cells harvested at T0.
Following the rise in temperature to 37°C, the expression of Bel-Xs, IL-6 and collagen type I decreased while that of HSP70 and most notably VEGF111 increased.

The modulation of the expression of cytokines and HSP70 and of the of Bel-Xs to Bel-Xl mRNA ratio suggests that cellular stress and survival pathways were affected, while the expression of VEGF111, known to be induced by genotoxic agents (1), might indicate the occurrence of DNA damages. Therefore we analyzed the phosphorylation of histone γH2AX (a double strand breaks tracer) (2), p53, Akt, JNK and the level of LC3 (II/I) (Fig.2) in cells experiencing the same temperature transitions as above. Maintaining cells at 25°C induced hyperphosphorylation of γH2AX, JNK and Akt by 6, 50 and 10 fold, respectively. The level of Pp53 was also increased at day 1 but returned close to basal level at day 5. The level of the protein LC3(II/I), a marker of autophagosomes, is 5 fold increased at 25°C. Phosphorylation of γH2AX, JNK and p53 was further increased, at least transiently, after re-warming of the cells at 37°C, while Akt was progressively dephosphorylated. Production of LC3 (II/I) is also further increased after warming up to 37°C. Interestingly the increased phosphorylation of γH2AX was higher in cells kept at 25°C for 5 days than for 1 day.

It is noteworthy that phosphorylation of γH2AX is also increased in other cell lines as in endothelial (hBME) or epithelial cells lines (HMEC) in these experimental conditions.

4. DISCUSSION

The data indicate that temperature transitions commonly occurring in time schedules in space biology experiments deeply affect the phenotype of the cells. They not only modulate the expression of a number of genes, but they also affect cell survival and genome integrity. The presence of DNA damages might be due to the production of ROS and/or a modification of the balance between damages production and repair.

Most effects were amplified when the cells were maintained at low temperature for longer periods. Hence we suggest that cells are kept at low temperature for the shorter time as possible before space flight experiments.

These regulations might blunt the effects of spaceflight conditions (microgravity, space radiations). Moreover they can either induce resistance or sensitize cells to this environment. Therefore they potentially provoke data misinterpretations and are worth to be considered during the design of the experiments.

ACKNOWLEDGMENT

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REFERENCES


DOES SIMULATED MICROGRAVITY ALTER THE DIFFERENTIATION PROCESS?

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Differentiation is the process by which cells become progressively more specialized and represents a normal way through which cells get final maturation. This process of specialization for the cell comes at the expense of its crude proliferative power. Together with apoptosis, differentiation contributes to cellular homeostasis. Adipogenesis is the process by which precursor cells differentiate into fat cells. This differentiation can be initiated during the entire lifespan of mammals by recruiting fibroblastic precursors. This process is dependent on an intricate interplay between external cues to activate key regulatory transcription factors, amongst which the so-called peroxisome proliferator-activated receptor γ (PPAR γ) is a master regulator. Adipocyte differentiation is an important process since the central role of adipose tissue in the obesity development, cardiovascular disease and type 2 diabetes (1).

To date, among other systems, pre-adipocytes, like 3T3-L1 cells, are the best characterized models for basic studies on cell differentiation. We took advantage of this study to study comparatively the effects of microgravity on the rate of differentiation.

This knowledge is certainly of the utmost importance in view of long journeys of man in deep space, to prevent health-threatening effects possibly caused by prolonged exposure to microgravity and cosmic radiation.

In this study we analyzed the effect of modeled microgravity (3D-RPM as ground based facility) on 3T3-L1 cells differentiation program. The adipogenic program is completed within a limited time span during which a series of coordinated and temporarily beaten molecular events take place.

Materials and Methods

Pre-adipocytes were obtained from ATCC. Cells were cultured in a medium containing 10% calf serum.

Differentiation. It was induced 25 cm² flasks on post confluent cells (4 days) in DMEM supplemented with 10% FBS, 3-isobutyl-1-methylxanthine, dexametha-sone, and insulin (DIM protocol). The complete program of differentiation has been conducted on two groups of 3T3-L1 cells, in modeled microgravity (RPM) and normal gravity conditions (NGC), respectively. After 4 days, the two groups of cells were incubated in RPM and NGC for additional 9 days in DMEM containing 10% FBS and insulin. Day by day, the differentiation process was monitored using different and independent criteria.

Staining and lipid content. The lipid vacuole formation in the induced cells at different, was analyzed using an Oil Red O staining. Quantitative assessment of accumulated lipids was performed by eluting the dye from the cells with isopropanol and measuring it spectrophotometrically (2).

Western blot analysis. Total cell protein preparations were obtained as described previously by Coinu R, Chiavello A, et al. (3). Proteins separated on the polyacrylamide gels were blotted onto nitrocellulose filters (Hybond-C pure, Amersham, Milan, Italy). Filters were washed and stained with specific primary antibodies and then with secondary antisera conjugated with horseradish peroxidase (Bio-Rad) diluted 1: 2,000. Filters were developed using an electro-chemiluminescent Western blotting detection reagent (Amersham Italia); profiles were acquired and grossly quantified by scanning with a Discover Pharmacia scanner equipped with a Sun Spark Classic Work-station. Specific monoclonal antibodies were used to identify and estimate the expression levels of c/EBP-β, c/EBP-α, PPAR-γ and Glut4.

Results and Discussion

Cell differentiation may be defined as a transition of a cell from one cell type to another involving a switch from one pattern of gene expression to another. One way to analyze this process is to divide it in early and late events. The early events include growth arrest at confluence, clonal expansion (that involves synchronous entry of all cells into S phase, leading to one or two rounds of mitosis), early expression of C/EBPδ and C/EBPβ. The late events include the expression of PPARγ and C/EBPα, the assumption of rounded morphology and biochemical changes related to the peculiar features of the terminal cell.

Preadipocyte differentiation is not diverse (4). It is, in fact, accompanied by dramatic alterations of cell shape, accumulation of lipid droplets (as consequence of increase in cell ability to synthesize lipids) and increase specific responsiveness to hormones (that is a specific function of the adipocyte in energy homeostasis).

In this report, we studied comparatively the occurrence of early and late events in preadipocytes compelled to differentiate in normogravitational conditions and in modeled gravity.

Figure 1
The two panels that compose Figures 1 show that formation of lipid droplets, characterizing the differentiated cells, is significantly favored in normogravitational conditions as compared to modeled gravity. Similar results are obtained when the cellular content of lipids is quantitatively estimated (Fig. 2).

![Figure 2](image1)

Consistently with the differentiation program, cytoskeleton proteins actin (Fig 3) and tubulin (not shown) were reduced in the two experimental conditions and C/EBPβ (Fig. 4) showed the same transient increase of its expression in the early stages both in RPM and in GC.

![Figure 3](image2)

As long as the late factors PPARγ and C/EBPα are concerned, it could be that, while the expression of C/EBPα and Glut4 is only weakly constrained by modeled microgravity (not shown), the expression of PPARγ seems greatly reduced.

![Figure 4](image3)

Our results indicated remarkable differences during the early events of differentiation program in 3T3-L1 cells exposed to modeled low g in comparison with ground controls. In addition, cultures grown in RPM showed a smaller number of differentiated cells; lipid accumulation was reduced as well.

The expression of early differentiation markers (C/EBPβ, actin and tubulin) was not definitely affected by modeled microgravity; conversely, the expressions of late differentiation markers PPARγ and, although to a minor extent, C/EBPα and GLUT4 were neatly reduced.

The reduced expression of PPARγ in RPM could be responsible of retarding or halting the differentiation process. Indeed, PPARγ is considered the master regulator of this process being necessary and sufficient to induce and sustain differentiation.

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ABSTRACT
The adaptation of sensory systems to their physical stimulus requires available sensory information at critical periods of the development. Critical periods were described in development of vision, audition or touch, and are supposedly required in the adaptation to gravity. The problem is complicated however because of the many structures and functions concerned by gravity. Recent studies reported the influence of gravity on the development of vestibular structures in several species, and on irreversible changes in the morphological and contractile properties according to the muscle type and function, that suggest the existence of critical periods in the affectation of functional properties in the muscles. These pieces of evidence are consistent with the idea that gravity is required for appropriate development of the projections from gravity receptors to the brain and spinal cord. The high plasticity of developing organisms masked the actual influence of gravity, and subtle changes were probably undetectable with the techniques previously used.

INTRODUCTION
The interplay of genetic and environmental influences on the acquisition of adult properties is a central question of developmental biology. An expanding set of data shows that the development of many functions needs the assistance of environmental information, during a critical period of their development, as was shown for audition [1], vision [2], and touch [3], and to some extent olfaction [4]. The question of possible critical periods in the adaptation to gravity was evoked many times [5-7] and was one of the key questions listed by the developmental biology workgroup [8]. The existence of a critical period in the development of the vestibular system was further reported in fish developed in microgravity [9] or hypergravity [10], as in amphibians [11], and could be a general rule in the development of the vestibular sensitivity. Meanwhile, the knowledge about molecular processes involved in critical periods has increased, and the pertinence of the concept for the adaptation to gravity deserves to be questioned. That is the aim of this short review.

WHAT IS A CRITICAL PERIOD?
A critical period is a time windows of the early postnatal life when the experience of external information is needed for the normal development of a structure or a function. The brain needs this external sensitivity period to tune the receptor with the source when precise information about the individual or the environment cannot be predicted and therefore cannot be genetically encoded. The critical period corresponds therefore to an interactive specialization in the functional organization of brain regions or cortical areas. The critical periods were initially characterized by a high level of plasticity confined to a short and sharply defined period of the life cycle, and a subsequent irreversibility of the acquisition in face of later experience. The time limits and temporal periods of the critical periods are indeed more or less adaptable, and consequences of sensory deprivation can be partly amendable. For this reason the terms or sensitive or optimal periods are sometimes used instead of critical periods.

During the critical period the response properties of cortical regions interact and compete each other to acquire their role in new abilities. The achievement of the critical periods involves three stages: 1) expansion of axonal branching and synapses formation in association with a high level of growth associated proteins and neurotrophic factors, particularly BDNF. With this process neurones invade narrow brain regions and elaborate new projection fields; 2) a further shaping of the circuit architectures is realized by pruning less solicited axons and synapses, on the basis of the competition between neural inputs on common targets. The structural consequences of the functional competition for brain regions was illustrated by the lateral dominance occurring in the cortical mapping of sensory entries after hemi deprivation of vision, audition or somatosensory system; 3) structural stabilization of potentiated synapses by the insertion of CAMS, change in the composition of NMA receptor and limitation of GABAergic large basket cells in an extracellular matrix. The consolidated synapses become invulnerable to further elimination and make further plasticity harder to occur.

Critical periods were first described for imprinting [12], then in various complex integrated functions such as social adaptation and language. They were also shown to occur during the development each sense. Basically, critical periods are involved in the adaptation of the cortical and brain regions to the sensory source through the detection, mapping and treatment of sensory inputs. Critical periods are not limited to brain structures however. The neuromuscular system was shown to be definitely modified by the motor experience during critical periods of the development. Durable change of the muscle fibre type distribution and in the locomotors kinematics was reported in rats [7]. More recently we demonstrated that mice exposed to hypergravity during the development of locomotors function (Postnatal day 10 to 30) showed changes in motor properties and muscle phenotype that persist even at the age of 9 months [13]. In the peripheral motor system of
developing mammals, axon branches of several motor neurones innervate the same muscles fibres. In rats this poly-innervation persists about 14 days after birth, then postnatal motor activity drives a process of synapse elimination that results in the strengthening of one and withdrawal of the others. Whether it concerns brain or muscle structures, the synaptic rearrangement during the critical period involves a territory expansion and a competitive process where synapse elimination is activity dependant [14].

A critical period can only open when the structure concerned has achieved its embryological development. Studies of molecular mechanisms of visual cortical plasticity showed that internal mechanisms related to the development of intra-cortical inhibitory circuitry mark the onset of critical period. Recent pieces of evidence showed the role of GABAergic neurones in this internal control of critical period timing. The onset and duration of visual critical period is advanced in mice over expressing BDNF, that accelerate maturation of GABAergic neurones [15]. Gad65 KO mice, that show poor GABA release, do not show critical period for ocular dominance until inhibition is restored with diazepam which acts as a GABA agonist [16]. On the other hand, relevant sensory information is required, and critical period can be delayed and prolonged when the information is not available. Dark rearing, for instance, delays the maturation of GABAergic transmission and the onset of visual critical period, but BDNF supplementation abolishes this delay [17].

The termination of the critical period is a consequence of the mechanisms by which the cortical regions become increasingly specialised and fine-tuned. Changes in the brain neurochemistry as for instance the composition of NMA receptor increase the rate of pruning of synapses and results in a freezing of the pattern of functional sensitivity. If the termination of plasticity is due to maturation, to the self termination of learning or to the stabilization of constraints remains an open question [18].

A critical period matures a particular function related to sensory information rather than the sensory information itself. The finalization can require multiple critical periods that develop in a logical sequence of time order ending earlier for functions dealt with at lower levels of the system. The organisation of visual cortex, for instance, shows successive critical periods for direction selectivity, ocular dominance and slow-wave sleep oscillations. On the other hand, some functions require information from structures that develop with different time schedules.

**ARE THERE CRITICAL PERIODS FOR GRAVITY ADAPTATION?**

As for the other sensory information, the nervous system probably needs postnatal experience to calibrate the gravity information. The question of the adaptation to gravity is complex, because of the multiple points of impact of gravity on the sensorimotor development, and because the information of vestibular organs is quickly mixed at the level of vestibular nuclei with information issued for other brain parts to converge with visual, cerebellar, and cortical information. The adaptation to gravity involves a set of cooperative structures including the management of verticality and accelerations, the control of gaze, head and body posture, with the tuning of muscle stiffness and a cerebellar motor control, that develop at different time during the second gestational week and the first postnatal month in mice and rats. The end organ receptor itself seems to be sensitive to the alteration of gravity during development. The relative size of otoconia is increased in animals developed in microgravity [19], while animals born in hypergravity, have smaller otoconia [20, 21] with increased cross-sectional areas of epithelial cells [5]. Under hypergravity, a delay was found in the postnatal development of connections between type I hair cells in the utricles and their afferent calyces [22], and a slower development of the potassium currents in type II hair cell has been reported [23]. The vestibular neurons develop early before birth. In mice and rats, they differentiate during the second week of gestation, and synaptic connections are almost complete at the end of the first postnatal week. Rats exposed to microgravity during the second half of the gestational development show a delay in the synaptogenesis of saccular neurones and an increased proportion of angular acceleration synapses [24]. These observations are consistent with delayed development and competition for neural branching, and therefore support the hypothesis of a critical period. The counter rotation of eye in response to body roll occurs early, and a critical period in this acquisition was shown in fish [9] and amphibians exposed to microgravity [11], and can be supposed in mammals. The vestibular nuclei of rats that developed in space showed a marked reduction in the size of neuronal cell bodies and a pronounced reduction in the growth and branching of their dendrites, with poor cerebellar projections [25]. At least two critical periods have been reported concerning the development of cerebellar structures in rat: the former occurs during the second week of pregnancy when purkinje cells develop, the latter during the third postnatal week (P15-P16) and corresponds to the elimination of polyinnervation of climbing fibres on purkinje cells [26]. Recent studies reported an alteration of cerebellum, in association with impaired motor behaviour, in rats exposed to hypergravity during these periods [27]. At the level of the spinal cord, hypergravity induced a delay in the development of descending pathways [28], with strong perturbations of monoaminergic projections that persisted in 8 months old rats [29].

Given the late maturation of neuromuscular junction, critical periods for motor adjustment probably occur during the development of locomotion beginning at about P10. Neonatal rats exposed to the absence of gravity from P9 to P25 during a space flight mission
showed a lower degree in synaptic innervations of motor neurons [30]. Experimental modifications of the motor stimuli during the development of neonatal rats induced modifications of the muscles properties. Rats born in hypergravity showed changes in the morphological and contractile properties of the soleus antigravity muscle [31], whereas hindlimb unloading induced muscle atrophy and blocked or delayed the transition of fast type 2 into type 1 [32, 33]. A critical period in the motor development of tail suspended rats was identified between postnatal day P13 and P31 [7]. The changes in muscle properties and motor output are paralleled with changes in the synaptic circuitry of hind limb neocortical representation[34]. These pieces of evidence suggest an altered morphological development, a reduced sensitivity, and a delayed development of the connectivity of the vestibular system. They demonstrate that the environment plays a critical role in fine-tuning of axons, and are consistent with the idea that gravity is required for appropriate development of the projections from graviceptors to the brain and spinal cord. They provide further evidence that the gravistatic sensory system has a genetically controlled phase of development for target finding and a stimulus-controlled phase for fine-tuning synaptic terminals.

HOW TO ANALYSE THE ADAPTATION TO GRAVITY ON EARTH ?

The adaptation to gravity requires that graviceptors interact with various levels of sensorimotor control, each with distinct critical periods. Even though pertinent information emerged from the meta-analysis of multiple studies, an integrative knowledge of the process of adaptation to gravity during development should encompass the whole pre- and postnatal stages of development, with the opportunity to produce targeted gravity perturbations at given ages. Such a strategy is hard to manage because of the difficulty to remove gravity vector from Earth environment. This question could be answered with long space flight duration as promised the ISS, but does not appear realistic. Experimental exposition to microgravity during mammal development concerned a limited set of space flight mission involving either prenatal flight (STS 66: E9-E20; STS70: E11-E20) or postnatal flight (STS-72: P15-P24; STS-90: P9-P25). Even though pertinent results, the conclusions of these experiments are minored by interrogations concerning the good fit of the flight opportunity with potential critical periods. An additional difficulty occurs because a critical period can be delayed until the sensory information is available, and quickly occurs after landing. Alternative strategies using ground-based experiments can take advantage of the recent development of targeted mutations in mice, and the use of chronic centrifugation. More than 25 mice lines with congenital vestibular mutations are available [35]. However, the definitive removal of graviception is susceptible to produce compensation mechanisms [36]. Thus the generation of conditioned KO is of prime importance and should be stimulated. Chronic centrifugation is an accessible tool to modify locally gravitational environment on Earth. From the point of view of critical period, hypergravity can be considered as a mirror situation to microgravity. Two main effects are expected from the development in microgravity: 1) a delayed maturation because the onset of critical period is triggered by the availability of the sensory information. This should leave immature characteristics in the system. 2) The colonisation of gravity related vestibular projections by competing afferences, e.g. the expansion of inputs from the angular acceleration detectors. At variance the development in hypergravity is supposed to produce: 1) no delay in maturation, but a possible faster maturation due to the hyperstimulation. 2) The expansion of gravity related pathways at the detriment of competing afferences. These assumptions disagree with the common idea that hypergravity delays the maturation. It is noteworthy however that a recent study [37] suggested that the various effects observed in rats developed in hypergravity may be associated with faster rates of maturation of embryos.

CONCLUSIONS

Accumulating evidences confirm that organisms are sensitive to gravity alteration during their development. Several critical periods for gravity adaptation are spread on the developmental process, in relation with the many structures concerned, and with variable incidence depending on the plasticity of the structures. The interactions between the various critical periods need to be explored in detail over the whole development, to evaluate the final incidence on the adult properties. Hopefully, the development of new techniques and a better knowledge of the plasticity process involved in critical periods allow studying these questions on Earth. Nevertheless the eventual validation of the hypothesis based on ground research by space flight experimentation is mandatory.

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ALONG THE GRAVITY CONTINUUM: 
PERINATAL STUDIES AND DEVELOPMENT AT HIGH G 

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ABSTRACT 
In the late 1950’s, interest in spaceflight and exposure to microgravity (<1xg) led scientists to study animals on Earth exposed to hypergravity or high g (>1xg) for sustained periods. Some of this work utilized immature mice, rats, chickens and other species yet, until recently, research specifying high g effects on developmental processes remained in its infancy. In this paper, I describe our series of studies of conception, pregnancy, and prenatal and postnatal development during chronic exposure to gravity loads spanning 1-2xg. Two conceptual perspectives are presented: (1) Gravity as a Continuum, and (2) Epigenetic Developmental Programming, to help facilitate interpretation of existing data and stimulate new research that will advance our understanding of gravity’s influence on the development of mammals. 

INTRODUCTION 
Gravity as a Continuum. Gravity operates as a continuum across a range of biological responses (Phillips, 2002; Wade, 2005). Many systems respond to sustained gravitational loading/unloading in a systematic dose-response manner, with high g producing effects opposite in directionality from low g. Body mass and composition, muscle (size/mass, fiber type shifts, function), red blood cell mass, body temperature, and vestibular (otoconial) morphology and/or mass are among the systematic changes reported in adult animals. Although similar relationships may exist for bone, cardiovascular, immune function, and central vestibular changes, the requisite studies have not yet been done. The clearest evidence for biological responses to gravity across a continuum comes from studies of mammary metabolic activity in pregnant rats exposed to either low (microgravity; 0xg) or high (hypergravity; 1.5, 1.75 or 2xg) for 11 days (days 9 to 20 of gestation). Upon Recovery (gestational day 20), glucose oxidation into carbon dioxide and incorporation into lipids in the mammary glands showed a strong negative correlation with gravity load. Approximately 99% of the variance in the measures could be accounted for by differences in gravity load (Plaut, Maple, Vyas, Munaim, Darling, Casey, Alberts, 2000; Plaut, Maple, Wade, Baer, Ronca, 2003), clearly demonstrating the utility of high g studies for predicting responses to low g. Given the expense and present limitations on spaceflight opportunities (Morey-Holton, Hill & Souza, 2007), much can be learned by establishing these dose-response relations in high g. 

Epigenetic Developmental Programming. Perinatal programming is a growing research area is focused on the developmental origins of health and disease (Barker, 1995). Beginning prenatally, the environment influences the mother and her developing offspring in ways that exert sustained effects on cellular function and physiology. These changes, in turn, form the basis for developmental origins of vulnerability to disease. For example, maternal under nutrition or stress resulting in low birth weight increases the risk of adult metabolic and cardiovascular disease. Environmentally induced, stable phenotypic modifications that are adaptive during early development are maladaptive in later life (Meaney, Szyf & Seckl, 2007). “Epigenetics” refers to the gene-environment interactions that bring about the phenotype of an individual by modulating gene expression. Epigenetic analysis (chromatin remodelling, DNA methylation, histone modifications) is increasing knowledge of how observable molecular changes result in stable, heritable changes in gene expression and later life phenotypes. Epigenetic developmental programming studies are ultimately needed to establish an understanding of lifespan and trans-generational changes of organisms that reproduce and develop in space. 

Herein I describe our systematic studies of perinatal rat development at high g that shed new light on responses across the gravity continuum and early epigenetic programming influences of gravity load. 

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EMPIRICAL FINDINGS

Centrifugation and High g. Investigators have used centrifugation to study acute responses and adaptation to high g. Our studies utilized the 24-ft dia centrifuge at the Center for Gravitational Biology Research, NASA Ames Research (Moffett Field, CA). This apparatus creates a high gravity environment spanning >1 to 3.5xg for small animal research. The centrifuge has ten radial arms with two opaque enclosures on each arm. Each enclosure holds four standard rat vivarium cages, and each arm has multiple enclosure mounting locations at different distances from a central vertical shaft spindle, thereby allowing various combinations of gravity levels to be introduced to different groups of rats in a single experiment (e.g., 1.5, 1.75, 2xg). In quadrupeds, gravitational forces are applied from the back through the feet. In order to accomplish this, the animal housing units on the centrifuge are gimbaled such that the resultant gravitational force is applied through the floor (Wade, 2005).

High g Studies of Pregnant and Perinatal Rats. We adopted a gravity continuum approach to our high g work based on existing spaceflight studies. Earlier, Jeff Alberts and I had the opportunity to study pregnant rats and their offspring flown during the second half of gestation (NIH.Rodent (R)1 and R2 space shuttle missions). We reported changes in the in-flight behavior of the dams, post-flight births, maternal care and perinatal vestibular function (Ronca, 2003; Ronca, Fritzsch, Bruce & Alberts, 2008). The high g studies at NASA ARC, in collaboration with Charles Wade and Lisa Baer, mimicked the design of the R1 spaceflight study by exposing rats to 1.5xg beginning on the ninth day of their 22-day pregnancies. Unhampered by flight constraints, we were able to continue high g exposure throughout birth and the postnatal period. These experiments have not yet been attempted in low g, but are vital to our ultimate understanding of gravity’s influence on mammalian development.

Response of Pregnant and Lactating Rats to High g. Acute responses of mid-pregnant rats to high g are similar to those observed in non-reproducing adult animals, viz., an initial decline in feeding, drinking, body mass, and physical activity (Ronca, Baer, & Wade, 2002). Following 4-6 days of acclimation, 2xg dams stabilize at 8-15% lower body mass and a near-term increase in physical activity relative to 1xg controls.

Perinatal Development in High g. Increased postnatal mortality has been observed in animals exposed prenatally then born at high g with a systematic decrease in pup survival associated with an increase in gravitational load (Baer, Ronca, & Wade, 2000; Figure 1).

![Figure 1. Survival of neonatal rats exposed prenatally to high g loads. (Adapted from Baer, Ronca, & Wade, 2000.)](image)

Body mass of newborn rats is also reduced following prenatal exposure to high g (Table 1).

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Pregnant No.</th>
<th>Litter Size (X +/- S.D.)</th>
<th>Birth Wgt (g) (X +/- S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5xg</td>
<td>30/32</td>
<td>12.3 +/- 2.8</td>
<td>6.0 +/- 0.6a</td>
</tr>
<tr>
<td>1xg-Y</td>
<td>30/32</td>
<td>12.6 +/- 2.5</td>
<td>6.9 +/- 0.4b</td>
</tr>
<tr>
<td>1.08xg-R</td>
<td>8/8</td>
<td>13.0 +/- 1.3</td>
<td>6.3 +/- 0.4a</td>
</tr>
<tr>
<td>1xg-N</td>
<td>16/16</td>
<td>13.0 +/- 2.3</td>
<td>6.9 +/- 0.4b</td>
</tr>
</tbody>
</table>

a,b Means with different superscripts are different (p< 0.05)

Maternal-Offspring Interactions. Development of a young mammal progresses within the context of an intact, integrated system comprised of a mother and her young (Alberts & Ronca, 2005). This family system is characterized by coordinated changes within and between a mother and her young. As the offspring are developing within the womb and nest, the maternal-offspring system follows its own ontogenetic trajectory. Mammalian development involves vital bi-directional linkages between mothers and infants that sustain and foster normal growth and development.
Gravitational Influences on the Maternal-Offspring System. The biological and behavioral interactions that exist between mothers and their young evolved under the constant force of the Earth's 1xg gravitational field. It is therefore reasonable to predict that maternal-offspring interactions will be altered in the weightlessness environment of space and the hyper-weighted environment of the centrifuge. The mother's role in development also changes during the transition from the first to the second pregnancy and lactation (i.e., direct vs. indirect). Finally, the young are dependent upon nursing, licking and retrieving by the dam, behavioral responses that may change in altered gravity environments. Thus, a major challenge in studies of the developing mammal is differentiating among 'direct' and 'indirect' effects of gravity on the young. For example, if mothers cannot stabilize their bodies in the nursing posture or if pups cannot retain metabolic heat because their huddling behavior is disrupted, the altered growth effect would clearly be an indirect consequence of weightlessness.

Reproductive Experience and High g. The transition from the first to the second pregnancy and lactation is accompanied by characteristic neural, endocrine and behavioral changes in mothers (Ronca, Baer, Daunton, & Wade, 2001). Since the maternal care system in mammals evolved within the confines of the Earth’s 1xg gravitational field, it is not surprising that altering gravity load leads to indirect changes in offspring survival induced by maternal factors. Reproductive experience, or parity, may be an important consideration in developmental space biology studies. We tested the hypothesis that maternal reproductive experience determines neonatal outcome following gestation and birth at high g. Primigravid (first pregnancy) and bigravid (second pregnancy) female rats were exposed to 1.5xg centrifugation from Gestational day [G] 11 throughout birth (G22/G23). At parturition, litter sizes were identical across gravity and parity conditions, although significantly fewer live neonates were observed among high g-reared litters born to primigravid dams as compared to bigravid dams (82% and 94%, respectively, p < 0.05). Centrifugation was continued throughout weaning (Postnatal day [P] 21). Neonatal mortality was observed in both parity conditions from the first to the seventh postnatal day, at which time litter sizes stabilized at approximately 75% of control values. Maternal reproductive experience ameliorated neonatal losses during the first 24 hrs after birth, but not on subsequent postnatal days. Maternal behavior patterns differed across parity conditions in the high g environment. Differential mortality of neonates born to primigravid versus bigravid dams denotes gravitational load as an environmental mechanism enabling the expression of parity-related variations in birth outcome, possibly enabled through changes in maternal behavior.

Developmental Programming of Adult Body Weight in High g. More recently, we tested the prenatal programming hypothesis that rat pups that underwent gestation at 2xg have significantly reduced birth weights and increased adult body weights as compared to 1xg controls. In these studies, young adult male and female rats were first adapted to 2xg centrifugation, then time-mated. Female rats conceived and underwent pregnancy at 2xg. At birth, offspring were fostered to non-manipulated dams at 1xg.

Conception, Pregnancy and Birth Outcome. Percentages of 1xg or 2xg exposed females that conceived did not differ (Table 1), however conception took significantly longer at 2xg. High g births occurred at the normal gestation length, i.e., on G22/23. Pup birth weights were significantly reduced in 2xg litters, whereas litter size and pup gender ratio was unaffected.

Table 1. Numbers of pregnancies and days to conception, % pups born, birth weights, and gender ratios following conception at 2x or 1xg.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. Pregnant</th>
<th>No. Days to Conception</th>
<th>Pups Born (%)</th>
<th>Pup Birth Wgts (gms)</th>
<th>Pup Gender Ratios (%F:M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2xg</td>
<td>8/9</td>
<td>11.0±6.54^a</td>
<td>100</td>
<td>6.30 ±0.11^a</td>
<td>(52:48)</td>
</tr>
<tr>
<td>1xg</td>
<td>8/9</td>
<td>4.14±3.02^b</td>
<td>100</td>
<td>7.49 ±0.22^b</td>
<td>(55:45)</td>
</tr>
</tbody>
</table>

^a,b\ Means with different superscripts are different (p< 0.05)

Body weights of pups conceived and gestated at 2xg were significantly reduced relative to 1xg pups until P12. Then, beginning on P63, body weights of the adult offspring conceived and gestated at 2xg were significantly elevated (approximately 7-10%) relative to 1xg controls. These differences persisted throughout the end of the study on P93. Thus, prenatal rearing at 2xg restricts neonatal growth and increases adult body weight (Figure 2).
Collectively, our longitudinal data support the hypothesis that gravity load alters the intrauterine milieu, thereby inducing persistent programming changes in phenotype across the lifespan.

Summary. High g studies have made important and unique contributions to our understanding of gravity’s effects on mammalian reproduction and development. Using this technique, fractional increments in gravity load from 1 to 2xg can be continuously applied to biological specimens for extended periods, enabling systematic dose-response relationships to be established. Future work is needed to establish the range and influence of epigenetic developmental programming influences and systematic exposure-response associations that will position high g studies in predicting outcomes in the microgravity of space. A major goal of space biology research is to broaden scientific knowledge of the Earth’s constant gravitational force (1g) on living organisms. To this end, studying the life cycles of mammals in space as well as in high g environments promises to uncover exciting new insights into how gravity shaped life on Earth.

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MICROGRAVITY SIMULATION COMPARISON AT GENOME LEVEL IN *DANIO RERIO* AND ROLE OF SOX4 TRANSCRIPTION FACTORS IN CRANIAL SKELETON DEVELOPMENT.


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ABSTRACT

Understanding of the effects of microgravity on the bone system remains unclear. We investigate the changes caused by microgravity and chemical treatments on the whole genome of zebrafish (*Danio rerio*). We analyze early gene expression modification by microarray and the long-term consequences by staining the bone structures. Our results reveal a number of genes regulated by the different treatments, but also indicate major differences between simulation devices. Furthermore, we explore the function of the Sox4 transcription factors during the cartilage and bone development. We show that both genes are expressed in the pharyngeal region, at different stages in development. Both genes appear to be important for the correct formation of the mandible and hyoid cartilage.

1. INTRODUCTION

Extensive bone loss is observed in astronauts after long-term space flight. Our aim is to investigate the changes caused by microgravity on the whole genome of the zebrafish [1] using two different approaches. First, we perform DNA microarray to analyze gene expression. Second, we analyze the long-term effect of simulated microgravity on bone development as revealed by staining the bone structures. We focus our attention on the first bone structures to be formed, the head skeleton and more precisely the pharyngeal structures. In addition, we investigate early gene expression and long-term effects on bone formation following chemical drug treatment.

The SOX (Sry-related HMG box containing) family of transcription factors contains, in mammals, at least 30 members classified into 8 groups according to their sequence similarity [2]. They are characterized by a conserved HMG-box containing DNA-binding domain and play important roles in various developmental processes [3]. The Sox4 gene, of the C group, is involved in development of the endocardial crests [4], the brain, the lung, teeth, gonads and lymphocytes. Heterozygous Sox4 KO mice present a decrease in bone mass and mineralization [5]. Sox4 is required for differentiation of osteoblasts in culture and its expression increases in differentiating chondrocytes [6]. In zebrafish, two homologs for the mammalian Sox4 are present, sox4a and sox4b. sox4b was shown to be required for formation of glucagon cells in the zebrafish pancreas [7].

2. MATERIAL AND METHODS

Total RNA extraction was performed by addition of trizol (Invitrogen) followed by purification (RNAeasy, Qiagen). We used Agilent microarrays harboring 44.000 zebrafish genes to analyze the effect of drug treatment and microgravity simulation on their expression. Anti-sense RNA probes were prepared by transcribing linearized cDNA clones with SP6, T7, or T3 polymerase using digoxigenin labeling mix (Roche) using the sox4a and sox4b clones previously described [6]. Whole-mount *in situ* hybridizations were performed as described [8, 9]. The morpholino oligonucleotides used were: sox4aMO, 5’-GGTCTGTGCCCATGCACTACAACAG-3’; sox4bMO, 5’-GACTCAGTCTGATTGCACACAG-TCC-3’ (Gene-Tools), that target the AUG initiation codon. 2-8 ng morpholino diluted in Danieau solution were injected into the yolk of wild-type embryos. Rhodamin dextran was added at 0.5% to the samples to check injection efficiency. Alcian blue and alizarin red staining was performed as described [10].

3. RESULTS

3.1 Microgravity simulators and chemical treatments.

We started the microgravity simulations and the chemical treatments at 5 days post-fertilization (dpf), because at that stage, endochondral bone begins to replace the existing cartilage. Microarrays were performed after one day treatment by clinorotation, Random Positioning Machine (RPM), Rotating Wall Vessel (RWV), ParaThyroid Hormone (PTH) and Vitamine D3 (VitD3). Analysis of the PTH treated animals revealed, among others, modifications in the PTH signaling pathway, such as decreased pth expression and increased...
expression of the pthr1. In contrast, VitD3 downregulated pthr1. Among the 206 genes significantly regulated by clinorotation, some are involved in muscle and skeleton development. RPM affected various genes for signaling molecules, such as notch and fgf. The RWV experiment revealed 160 significantly modified genes. Comparing the three micro-G simulation devices, among the 413 genes significantly modified genes. Comparing the three and various genes for signaling molecules, such as notch.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Gene modification</th>
</tr>
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<tr>
<td>Clinorotation</td>
<td>SLIT, LARGE</td>
</tr>
<tr>
<td>RPM</td>
<td>Netehl, FGFI4, Runxl</td>
</tr>
<tr>
<td>RWV</td>
<td>GSN, PPP1R13b, beta-estradiol</td>
</tr>
<tr>
<td>Clinorotation ∩ RWV</td>
<td>CORO1C, Jnk, JnkK, P38MAPK, TERT</td>
</tr>
<tr>
<td>Clinorotation ∩ RPM</td>
<td>CXCR3, DMBX1, IFNG, PAEP</td>
</tr>
<tr>
<td>RPM ∩ RWV</td>
<td>Histone H3, SMARCA4, TP53</td>
</tr>
<tr>
<td>Common genes</td>
<td>Akt, ERK, MAPK, NFKB, TGFB</td>
</tr>
</tbody>
</table>

Table1: example of genes modified by microgravity simulation.

Long-term effects on cartilage structures and bone development were analyzed after 5 days by alcan blue or alizarin red staining. PTH leads to a decrease in both cartilage and bone structures, while VitD3 increased formation of both. Five days clinorotation treatment induced a delay in bone formation, but no changes in the cartilage structures. No changes were observed in RWV simulation.

3.2 Expression pattern and function of Sox4 transcription factors.

We determined the precise location of sox4a and sox4b expression by whole mount in situ hybridization. sox4a is first expressed in brain and in the lateral part of the pharyngeal region. Double fluorescent in situ hybridization revealed that sox4a expression co-localized with migrating neural crest cell (CCN). At 24 hpf, sox4a presented an overlapping expression domain with sox9b, while it was present in cells intermingled with dlx2 expressing cells in the first cluster. sox4a is absent after 72 hpf.

sox4b is mainly expressed in brain at early stages and, from 40 hpf to 5 dpf, in the pharyngeal region [11]. Double in situ hybridization revealed that sox4b is not expressed in cartilage precursor cells, while co-localization with endodermal markers was observed in the dorsal part of the pharyngeal arches. Microinjection of 8 ng/egg specific morpholino against sox4a causes formation of a slightly shorter mandible and a wider angle in the hyoid (alcan blue staining at 4 dpf). Injection of 6 ng/egg MOsox4b generated morphological changes in the hyoid, a shorter mandible and straightened branchial arches. Finally, the head appears shorter than in the controls.

4. CONCLUSION.

We compare gene expression and bone formation using three techniques for microgravity simulation and chemical treatments. Each treatment resulted in specific effects on gene expression and bone formation, further analysis and experiments will be required to identify the genes involved in bone-specific microgravity effects.

Both Sox4 transcription factors are expressed at different stages in the pharyngeal arches, but not in the cartilage precursor cells. Microinjection experiments revealed that both genes are required for correct jaw formation. Further experiments will determine the exact tissue expressing the sox4 genes and their precise function.

5. REFERENCES.

THE FUNCTION OF THE EGR1 TRANSCRIPTION FACTOR IN CARTILAGE FORMATION AND ADAPTATION TO MICROGRAVITY IN DANIO RERIO.

M. Muller, J. Dalcq, V. Pasque(1), J. Aceto, P. Motte(2), J.A. Martial

ABSTRACT

In zebrafish, the cartilaginous elements that form the pharyngeal arches derive from cranial neural crest cells, whose proper patterning and morphogenesis require reciprocal interactions with other tissue types such as pharyngeal endoderm, ectoderm and mesoderm. The transcription factor Egr1 (Early growth response 1) is involved, among others, in pituitary development, wound healing and cancer. Our functional studies reveal that Egr1 is absolutely required for cranial cartilage morphogenesis and differentiation. Egr1 does not act on neural crest cell formation and migration, nor on endoderm formation. In contrast, Egr1 depletion abolishes expression of sox9b in endoderm and runx2b in cartilage. egr1 is expressed in the pharyngeal endoderm, where it activates expression of Sox9b which in turn activates runx2b expression via an extracellular signaling pathway. We also present a transgenic zebrafish line containing a Green Fluorescent Protein (GFP) cDNA controlled by the egr1 promoter, which expresses GFP in forming bones.

1. INTRODUCTION

The effect of microgravity on bone formation and homeostasis, as observed in astronauts after long-term spaceflight, is to date not completely understood. We use the zebrafish (Danio rerio) as model to investigate these processes in vertebrates [1]. This small fish model presents many advantages, such as external and rapid development, transgenesis and many technologies adapted for analysing bones on larvae. Finally, the genome has been sequenced and many genes present a high degree of homology compared to their human counterparts.

The zinc finger transcription factors Egr1 (Krox-24, NGFI-A, zif268) was first described as an early response factor upon stimulation of cell proliferation [2]. Since then, its expression in many adult tissues was described and it was shown to be involved in many processes such as blood cell differentiation, wound repair, angiogenesis, synaptic plasticity and reproduction [3, 4]. A role in bone homeostasis has also been suggested [5, 6]. We previously cloned the cDNA coding for zebrafish Egr1 and determined its expression pattern during fish embryogenesis [7]. Detailed studies revealed that Egr1 is first expressed during somitogenesis in the adaxial cells of the mesoderm, which will give rise to slow muscle cells in the somites, and in distinct rhombomeric in the hindbrain. At later stages, expression is restricted to midbrain, forebrain, heart and the pharyngeal region. Functional studies revealed that egr1 plays an crucial role in embryonic oculogenesis [8].

2. MATERIAL AND METHODS

Zebrafish were raised and cared for according to standard protocols [9]. Wild type embryos from the AB strain were used and staged according to Kimmel et al. [10]. Anti-sense RNA probes were prepared by transcribing linearized cDNA clones with SP6, T7, or T3 polymerase using digoxigenin labeling mix (Roche) using the clones previously described. Whole-mount in situ hybridization was performed as described [11].

The morpholino oligonucleotide used was: egr1Mo 5'-GGATTTAGTGCTTACCTCCAGCAAG-3' (GeneTools), that targets the splice donor site of the single egr1 intron [12]. Approximately 2-8 ng morpholino diluted in Danieau solution were injected into the yolk of wild-type embryos. Rhodamin dextran was added at 0.5% to the samples to check injection efficiency. Alcian blue staining was performed as described [13]. The egr1-eGFP reporter construct was obtained by inserting a fragment of the egr1 upstream regulatory region upstream of the cDNA coding for the enhanced Green Fluorescent Protein. The vector was micro-injected, along with the Sce1 meganuclease, into a one-cell fertilized zebrafish egg and the grown-up fish were used to generate transgenic offspring.

3. RESULTS

3.1 Loss of function experiments

We decided to characterize the involvement of Egr1 during embryogenesis in zebrafish. Our experimental approach is to block splicing of the egr1 RNA specifically by using antisense (morpholino) oligonucleotides injected into fertilized eggs. Consequently, a truncated inactive protein is produced, resulting in a loss of function phenotype in the injected embryos. First experiments revealed that depletion of egr1 in developing zebrafish embryos leads to severe defects in the patterning of the hindbrain and, at later stages, a complete absence of jaw cartilage (Fig. 1). In addition, the somites are disorganized and the trunk and tail are curved. These results indicate that egr1 plays an important role in the determination of the neural crest compartments at the level of the hindbrain, which will later form the jaw cartilage and bones. Additional experiments aiming to further investigate the function of
Egr1 in cartilage formation showed that dlx2, a marker for migrating neural crest cells leading to the formation of pharyngeal cartilage, or sox9a, a marker for the first differentiation of cartilage cells, are only marginally affected by egr1 morpholino injection after 24 or 48 hpf. In contrast, later differentiation markers such as flI1 or runx2b revealed a clear decrease of expression. We also looked at the effect of Egr1 depletion on markers for the other, neighbouring tissues that are known to be involved in cartilage formation. Indeed, we observe that, although the pharyngeal endoderm is present, an important gene for its function, sox9b, is no longer expressed in the pharyngeal region in egr1 morphants.

3.2 Expression pattern
When we analyzed more precisely the expression domain of egr1 in the pharyngeal region at various stages of development by double in situ fluorescent hybridization and analysis by confocal microscopy. These experiments allowed us to conclude that egr1 is not expressed in the presumptive cartilage cells directly, but rather primarily in the pharyngeal endodermal pouches surrounding the cartilages.

3.3 Transgenic line
We obtained a transgenic zebrafish line containing the Green Fluorescent Protein (GFP) reporter gene placed under the control of a fragment of the egr1 upstream regulatory sequence. Using these transgenic animals, we investigate the expression of the transgene (and the endogenous egr1) in living larvae and in microgravity simulation experiments. We observed a decrease of GFP expression in transgenic egr1-GFP larvae submitted to clinorotation between 5-7 dpf or 5-8 dpf (Fig. 2)

4. CONCLUSIONS
Our observations suggest that the function of Egr1 consists in controlling the signals that originate from the pharyngeal endoderm and that control the late differen-

Figure 2: Fluorescence of the egr1-GFP transgene in developing zebrafish embryos at 8 dpf in control (left, ventral view) and clinorotation-treated (right) larvae. Decrease of expression in the pharyngeal region is clearly visible upon clinorotation.

5. REFERENCES
DEVELOPMENT OF ROTIFER AND NEMATODE UNDER SIMULATED
HYPERGRAVITY AND MICROGRAVITY CONDITIONS

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ABSTRACT
The development of the bdelloid rotifer Macrotrachelcha quadricornifera and the nematode Panagrolaimus rigidus were subjected to microgravity using Random Positioning Machine and hypergravity using low speed centrifuge (20xg). Parameters investigated include cell morphology, rate of embryogenesis, and fitness-related traits. Results showed that no difference in cell architecture of the blastomeres of rotifer embryo. The number of nuclei in blastula and gastrula stages of both rotifer and nematode cultured in altered gravitational condition is higher than the control. Both species completed development and hatched at the same time as their respective controls, and reproduced normally. These results suggest that rotifer and nematode can develop normally under altered gravity, implicating that genetic make-up of an organism controls developmental processes and is not easily influenced by environmental perturbations.

1. INTRODUCTION
Gravity plays an important role in evolution of life on Earth and provides the required environment for life activities. Several studies have been undertaken to understand how multicellular organisms respond to gravitation perturbation, however, the results are often contradicting. This is mainly due to limited number of real space experiments. Two animals, the bdelloid rotifer Macrotrachelcha quadricornifera and the nematode Panagrolaimus rigidus, were proposed as models to study the role of cytoskeleton during cell division, as well as the consequences of cytoskeletal perturbation in animal morphology, were scheduled to fly on the International Space Station on 2010. In preparation to the flight, we investigated their responses to hyper- and microgravity under laboratory conditions. These animals were selected because of their small size, short life cycle, parthenogenetic mode of reproduction, determinate (spiral-like) cleavage, and eutely. Because of these characteristics, developmental patterns can easily be followed over a short period of time, and any damages occurred at any time of their development will be permanent.

2. MATERIAL AND METHODS

M. quadricornifera and P. rigidus used in this study were cultured in our laboratory for several years and their biology, ecology and development are well-studied, and published elsewhere.

2.1 Instruments used
Hypergravity was performed using a centrifuge constructed to simulate a gravity force up to 20g, and suitable for culturing small organisms. The working plate is an aluminum disk, that and can hold round glass tubes of up to 3ml, secured with a rubber belt to sample holders. An optical encoder provides the signal to detect speed, and the motor is driven by a constant voltage power supply. The value of gravity can vary continuously from 2.0 to 20.0 g (± 0.2). Two LCD displays show both speed and acceleration with a resolution of 1 rpm and 0.1 g. For microgravity, we used a desktop Random Positioning Machine (RPM, Dutch Space), that simulates Microgravity by continuous random change of orientation of the sample relative to the gravity vector and can reach forces as low as 0.0001g [1]. The setting used was ‘random mode scenario’ at 100Deg/sec.

2.2 Embryogenesis
Rotifer eggs laid within two hours were collected from the stock cultures and distributed randomly into: 1xg (control), 0.0001g (microgravity), and 20xg (hypergravity). Each treatment consisted of not less than 50 eggs. After 5h of treatment, the eggs were fixed in paraformaldehyde and processed for TRITC-Phalloidin and DAPI for actin and nuclei staining, respectively. The developmental stage corresponds to blastula. The same procedure was done in another batch of eggs, fixed after 8h of treatment which corresponds to gastrula stage. The embryos were viewed under confocal laser scanning microscope (CLSM). For nematode, not less than 50 newly laid eggs were also subjected to the three gravity conditions and the embryos were fixed after 3h and 6h which corresponds to the blastula and gastrula stage, respectively. The embryos were fixed in methanol and were stained with DAPI. The experiments were replicated three times. The eggs were examined under CLSM. Fifty rotifer and nematode eggs from each treatment were kept in a Petri dish and allowed to hatch to assess fitness-related traits (viability, duration of development, age at first reproduction)

3. RESULTS AND DISCUSSION

3.1 Cell architecture
The blastomeres of rotifer blastula and gastrula stages appear roundish. The shape of the blastomeres was
similar in all treatments. Gravitational perturbation did not affect cell architecture of rotifer blastomeres.

3.2 Embryogenesis

Under normal conditions the egg of *P. rigidus* is laid unsegmented and takes about 1 hour to undergo the first cleavage division. The three-cell-stage takes another hour. One hour later, 5 blastomeres are attained [2]. We defined this stage as ‘blastula’. The unsegmented eggs exposed to different gravities for 3h reached a cell number significantly higher in altered gravity conditions, denoting that mitoses occur faster (Table 1). The same trend was observed in embryos at gastrula stage (Table 1).

*Table 1. Number of *P. rigidus* nuclei attained under different gravitational conditions*

<table>
<thead>
<tr>
<th></th>
<th>1xg</th>
<th>µg</th>
<th>20xg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blastula</td>
<td>5.2 ± 1.4</td>
<td>6.0 ± 1.5*</td>
<td>6.7 ± 1.7*</td>
</tr>
<tr>
<td></td>
<td>(n=163)</td>
<td>(n=95)</td>
<td>(n=63)</td>
</tr>
<tr>
<td>Gastrula</td>
<td>24.0 ± 2.0</td>
<td>33.0 ± 6.0*</td>
<td>36.0 ± 6.0*</td>
</tr>
<tr>
<td></td>
<td>(n=16)</td>
<td>(n=26)</td>
<td>(n=25)</td>
</tr>
</tbody>
</table>

*aSignificantly different from 1xg (ANOVA, P<0.001)*

*Table 2. Number of *M. quadricornifera* nuclei under different gravitational conditions*

<table>
<thead>
<tr>
<th></th>
<th>1xg</th>
<th>µg</th>
<th>20xg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blastula</td>
<td>30.3 ± 7.2</td>
<td>33.4 ± 5.7</td>
<td>37.7 ± 8.2*</td>
</tr>
<tr>
<td></td>
<td>(n=42)</td>
<td>(n=24)</td>
<td>(n=30)</td>
</tr>
<tr>
<td>Gastrula</td>
<td>80.8 ± 16.9</td>
<td>126.0 ± 36.3*</td>
<td>144.8 ± 42.7*</td>
</tr>
<tr>
<td></td>
<td>(n=11)</td>
<td>(n=11)</td>
<td>(n=7)</td>
</tr>
</tbody>
</table>

*aSignificantly different from 1xg (ANOVA, P<0.05)*

The developmental pattern of *M. quadricornifera* has been described elsewhere [3]. Essentially, under normal conditions blastula stage is attained between 5-6h after the eggs were laid, and gastrulation follows, and continues until about 9h. The number of nuclei of rotifer blastula is higher in altered gravity conditions, although only hypergravity treatment resulted significantly different from the control. Moreover, embryos at ‘gastrula’ stage in altered gravities have more cells than the control (Table 2). From these results, there is enough evidence to claim that in both species gravity disturbances produce faster rate of mitotic divisions, and that, surprisingly, this effect is enhanced under hypergravity. As for fitness related traits, rotifers cultured under the three conditions hatched within 6 days and produced their first offspring 5 days after hatching (Fig 1A). Nematodes cultured under the three conditions also completed embryogenesis within 2 days and produced their first offspring on day 6 (Fig 1B). These results show that altered gravity did not affect either the whole developmental process or fitness related traits. In other words, while cell divisions are accelerated by altered gravity, cell differentiation seems to be unaffected. This result confirms previous findings [4].

4. CONCLUSION

RPM and hyperfuge are suitable instruments to investigate the effects of gravity in multicellular organisms. No cell damage or programmed cell death nor change in cell architecture was observed in rotifer and nematode cultured under altered gravity. However, altered gravities speeds up mitosis resulting in faster cell divisions in early stages of embryogenesis. In spite of rapid mitoses, both animals were able to recuperate these changes during differentiation and organogenesis resulting into a normal development, and no fitness related traits were affected. These results confirm previous data on faster mitoses and unaffected cell differentiation under microgravity, and evidence that hypergravity produce even faster mitoses. On the other hand, fitness related traits are unaffected, suggesting that the genetic program is strict enough to be not easily subjective to environmental perturbations like gravity.

5. REFERENCES


6. ACKNOWLEDGEMENT

Technical assistance of Elena Forasacco is highly appreciated. This study is financially supported by Italian Space Agency (MoMa-ASI) to C.R.
CARDBID FUNCTION AND CEREBRAL BLOOD FLOW FOLLOWING 60 DAYS HEAD-DOWN TILT WITH AND WITHOUT CHINESE HERB AND VIBRATION COUNTERMEASURES

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ABSTRACT
The weightless environment of space flight causes serious adaptive changes in cardiovascular function, and nearly all crew members develop cardiovascular deconditioning. Many different methods have been suggested to counteract orthostatic intolerance. The purpose of this study was to assess the effects of Chinese herb and vibration training on the cardiac function and cerebral blood flow velocity during 60 days of head-down tilt (HDT) bed rest. Twenty-one healthy male subjects were randomly divided into three groups: seven subjects experienced Taikong Yangxin Pill countermeasure (herb group), seven subjects underwent vibration training countermeasure (vibration group), and the seven others had no countermeasure (control group). We assessed cardiac pumping and systolic function using an impedance cardiography, and measured cerebral blood flow velocity by transcranial Doppler ultrasound during HDT. Stroke volume and cardiac output markedly decreased on day 60 of HDT in the control group and the vibration group compared with Pre-HDT, but had no significant change in the herb group. In the control group, end diastolic flow velocity (Vd) and mean flow velocity (Vm) in the cerebral middle artery decreased significantly on day 60 of HDT compared with the pre-HDT. In the vibration and the herb groups, peak systolic flow velocity, Vd and Vm showed no significant change throughout HDT. The results show that 60 days HDT bed rest induces cardiovascular dysfunction and a reduction of cerebral blood flow. Chinese herb is effective in preventing the effect of HDT on cardiac function and cerebral blood flow. Vibration training might partially improve cardiac functions.

1. INTRODUCTION
The weightless environment of space flight causes serious adaptive changes in cardiovascular function, and nearly all crew members develop cardiovascular deconditioning characterized by orthostatic intolerance and reduced exercise capacity, which appear on returning to Earth after space flight. Cardiac changes early in flight include altered blood volume distribution, impaired myocardial function, decreased stroke volume, and decreased peripheral vascular resistance [1]. Cardiac atrophy has also been reported after short-term spaceflight and during prolonged head-down tilt (HDT) bed rest, and this adaptive response likely contributes to the orthostatic intolerance [2]. Therefore, effective countermeasures during spaceflight are needed to maintain the cardiovascular system, and will be essential for the health, well-being and safety of the crew members during space flight and upon return to Earth. The purpose of this study was to assess the effects of Chinese herbal medicine and vibration training on the cardiac function and cerebral blood flow velocity during 60 days of HDT bed rest. The study here was a subset of a series of HDT studies in normal subjects to simulate the effects of weightlessness. We hypothesized that Chinese herbal medicine and vibration training would improve the cardiac function and meliorate cerebral blood flow. To test this hypothesis, we assessed cardiac pumping and systolic function using an impedance cardiography, and measured cerebral blood flow velocity by transcranial Doppler ultrasound during HDT.

2. MATERIALS AND METHODS
The present 60-day HDT was conducted at the China Astronaut Research and Training Center in Beijing, an organization with unique experience of long-term HDT, as a joint project of the French Space Agency and the Hong Kong Chinese University. The experimental protocol was approved by the China Astronaut Research and Training Center.

The subjects were twenty-one healthy male volunteers with a mean age of 30 yr (range 25-39 yr), a mean height of 168 cm (range 160-175 cm), and a mean body weight of 60 kg (range 54-68 kg). All subjects had undergone a comprehensive medical examination. Every subject signed an informed consent form meeting the requests of the Human Research Ethics and morality Committee at the China Astronaut Research and Training Center. The subjects were randomly divided into three groups: seven subjects experienced Chinese herbal medicine countermeasure (herb group), seven subjects underwent vibration training countermeasure (vibration group), and the seven others had no countermeasure (control group).

The experimental protocol of the bed rest group was the following: 15 days of ambulatory control period during which the subjects became adjusted to the standard diet; following by 60 days of bed rest in HDT at -6°; then 15 days of post-bed rest recovery. The subjects were strictly confined to bed for the entire bed-rest period and were not allowed to elevate their heads more than 30° above the horizontal level. The subjects remained head-down (-6°) without interruption for all daily activities including meals, bathing, urination, and physiological testing except defecation. The room temperature was maintained at 22°C~25°C.

The subjects in the herb group took the Chinese herbal medicine which named Taikong Yangxin Pill (TYP) 3 times one day for all the 60 days, and the dose is 200g every time. While the subjects in the control group just took the placebo of the same dose. The main ingredients of the TYP were extracted from Renshen (ginseng), Huangqi (milkvetch root), and Chuanxiong (Szechwan Lovage Rhizome), and it was manufactured by the China Astronaut Research and Training Center [3].

All vibration training was performed in the -6° HDT position. The subjects in the vibration group received vibration trainings for 20 min per day during 60 days HDT. A set of custom-crafted band had fixed the subject’s feet on the vibration platform before the vibration training began. One type of vibration transferred from feet to head; the frequency of 30 Hz and the amplitude of less than 100µm. The training protocol consisted of four sets of 5 min vibration and subjects rested for 1 min between sets. Certain load is important in order to get significant positive effects during vibration training, so we put a 1.5 times weight load on the vibrating platform.
The systolic and diastolic velocities of blood in the right middle cerebral artery were measured non-invasively by a transcranial Doppler sonography (TCD) (Mairui, China) and a 2-MHz transducer. Experiments were performed before HDT, on day 30 (D30) and on day 60 (D60) of HDT. The cardiac pumping and systolic functions were assessed by impedance cardiography (ZQ-Iv, Zhenqin) before and during (on days 30 and 60) the HDT. For each group, blood pressure, cardiac function and cerebral blood flow velocity were compared using a one-way ANOVA between Pre-HDT and the days of HDT. In addition, these values from the herb and the vibration subjects were compared with controls using two-way ANOVA and significant differences between groups and between measurement times (repeated measures) were determined using a post-hoc multiple comparison test (Tukey Honest Significant Difference).

3. RESULTS

3.1 Cardiac Pumping Function

The main results of cardiac pumping function are shown in Fig. 1, stroke volume (SV) showed a marked decrease during HDT (Fig. 1A). In the control group, SV decreased at D30 (20±4.1%, P<0.05), and showed a tendency to decrease further at D60 (31.4±5.3%, P<0.05), compared with Pre-HDT. In the vibration group, SV tended to be decreased at D30 (8.5±1.6%), and markedly decreased at D60 (25.1±2.4%, P<0.05), compared with Pre-HDT. In the herb group, SV had a tendency to decrease at D30 (8.1±1.8%) and D60 (18.1±18%) compared with Pre-HDT. Cardiac output (CO) and cardiac index (CI) showed decreases parallel to those of SV during HDT in the three groups (Fig. 1B and 1C). Fig. 1D showed the percent changes of total peripheral resistance (TPR) during HDT. TPR tended to be increased at D30 (control 25.1±6.1%; vibration 16.2±3.4%; herb 15.6±3.1%), and increased further at D60 (control 34.8±5.3%, P<0.05; vibration 37.4±5%, P<0.05; herb 27.2±6%) compared with Pre-HDT. There were no marked differences in these parameters among the three groups except for SV of the herb group heightened significantly compared with the control group at D60.

3.2 Cardiac Systolic Function

Left ventricular ejection time (LVET) tended to decrease during HDT in all conditions, with a marked decrease only in the control group at D60 (6.4±2.7%, P<0.01) compared with Pre-HDT. When compared to the control group, LVET was significantly higher at D60 in the herb groups (p<0.05). Prejection period (PEP) and PEP/LVET showed a marked increase during HDT. In the control group, PEP increased significantly at D30 (12.7±3.4%, P<0.05), and increased further at D60 (27.2±4.9%, P<0.01) compared with Pre-HDT. In the vibration and the herb groups, PEP tended to be increased at D30 (vibration 6.1±2.7%; herb 4±2.6%), and markedly increased at D60 (vibration 16.9±5.8%; herb 12.7±4%, P<0.01) compared with Pre-HDT. PEP/LVET showed increases parallel to those of PEP during HDT in the three groups. When compared to the control group, PEP/LVET was significantly lower at D60 in the vibration and herb groups (p<0.05), whereas PEP was significantly lower at D60 only in the herb group (p<0.05).

3.3 Cerebral Blood Flow Velocity

In the control group, the values for peak systolic flow velocity (Vd) and mean flow velocity (Vm) decreased significantly (14.2±5.4%, P<0.05 and 12.5±1.7%, P<0.05) at D60 compared with the pre-HDT value. In the vibration and the herb groups, Vs, Vm and end diastolic flow velocity (Vd) showed no significant change throughout HDT. There were no significant differences in these parameters among the three groups.

Fig.1. Percent change in cardiac pumping function before and during 60 days of head-down tilt (HDT) bed rest. Data are shown as means ± SE (n=7 in each group). Statistical analyses were performed on raw data are expressed here as percent change from pre-HDT. * P<0.05 vs. Pre-HDT. # P<0.05 vs. control group.

4. DISCUSSION

Our data show that 60 days HDT bed rest induces cardiovascular dysfunction and a reduction of cerebral blood flow. Chinese herbal medicine is effective in preventing the effect of HDT on cardiac function and cerebral blood flow. Vibration training might partially improve cardiac functions. These results suggest that Chinese herbal medicine that we used seems to provide an optimal cardiovascular countermeasure, but vibration training seems to have only potential beneficial effects on cardiovascular function. The suitable countermeasure program of vibration and Chinese herbal medicine should be studied further during prolonged spaceflight, and the mechanisms responsible for these countermeasures required an integrative perspective for a more complete understanding.

5. REFERENCES

ABSTRACT

Earth-Star-1 International Bed-Rest ExPeriment (ES-IBREP) is a 60 days head down bed-rest which was organized by the Astronaut Chinese Centre (ACC) in Beijing at the end of 2007. Subjects were twenty-one men, divided into 3 groups (control, whole body vibration and Chinese herbs). ACC invited French Space Agency (CNES) and French scientists (Tours and Angers universities) to participate. Six clinical protocols to study vascular, microcirculatory and autonomic impairments induced by weightlessness were implemented.

With respect to medical instrumentation, SEVE system developed by CNES was operated: SEVE is based on Cardiomed system, previously developed by CNES for use on-board ISS; some of SEVE instruments were developed initially for Cardiomed whose real-time software was re-used to implement SEVE medical protocols. Post-analysis tool, Physiopost, initially developed for Cardiomed was improved for analysing bed-rest data.

SEVE system is introduced in the first part of this paper together with the clinical protocols which were used during ES-IBREP bed-rest. The second part focuses on the off-line analysis of bed-rest data, using tool Physiopost. In the last part, future developments of SEVE system, aiming use in space, are introduced. The definition of this new system takes advantage of the lessons learnt using SEVE during ES-IBREP.

Keywords: Gravitational Physiology, Medical Instrumentation, ES-IBREP bed-rest, Physiological Data Analysis.

1. SEVE ARCHITECTURE

SEVE system is composed of: (1) Medical instruments, which acquire physiological parameters (heart rate, blood pressure, arterial flow velocity...). (2) A laptop computer with a real-time software to control/command instruments and implement medical protocols. (3) Software Physiopost for the off-line analysis of physiological data.

1.1 SEVE Instruments

Three SEVE instruments (Cardiopres, Doppler and BP holter) are also used in Cardiomed. LaserDoppler and Echograph are newly used instruments, but less integrated in SEVE system.

Cardiopres (BMEYE, Netherland): Continuous Blood Pressure acquisition at 200Hz using finger cuff photoplethysmography, surface ECG (1 to 12 leads derivations) at 1000Hz, changes in chest circumference at 50Hz.

Doppler (DMS, France): main arteries blood velocity measurements, up to three channels at a time, using different probes (cerebral and aortic at 2Mhz, femoral at 4Mhz and superficial at 8Mhz).

Blood Pressure Holter (PAR, Germany): Systolic, Diastolic and Mean Blood Pressure measurements with the oscillometric method.

Laser Doppler (Perimed, Sweden): laser probes were used to measure cutaneous blood flow; used probes included a iontophoresis chamber to deliver pharmacological agents using regulated current intensity.

Echographs: 3 different echographs (Vivid 7 and LogiqBook from G.E. and Aloka 500 from Terason) were used during ES-IBREP, depending on protocols.

1.2 SEVE ES-IBREP protocols

Six clinical protocols using SEVE were implemented during ES-IBREP bed-rest. Two protocols aimed at studying autonomous nervous system response using Cardiopres instrument; first during bicycle exercise and centrifuge, second during psychological stress (colour test) and breath patterns. As an active participation of subjects is required in this protocol, software user interface provides instructions in Chinese. Two other protocols focuses on vascular properties and vessel vasodilatation: (1) at macro-circulation level with protocol, FMD: echograph (Vivid 7) was used to measure brachial artery dilatation upon occlusion; (2) at micro-circulation level with protocol, iontophoresis. Arteriolar vasodilatation at the fore-arm level was measured with laser-doppler in response to Acetylcoline (Ach) and Sodium NitroPrusside (SNP). In the fifth protocol, cardiac structure changes through bed-rest were studied using echograph (LogiqBook). Tilt-test was performed before and at the end of bed-rest, to study orthostatic intolerance. This protocol was the most demanding one operationally: 21 subjects were submitted to tilt-test in 3 days. Each session lasted 45mn (15mn supine, 20mn tilt, 10mn supine). Four scientific teams operated simultaneously and limited time was available for subject preparation. Furthermore, Doppler...
was used to measure 3 artery velocities in parallel (femoral, temporal and cerebral arteries) and the temporal probe was to be hold manually by an operator, because not suitable harness was available at that time. Cardiopres and Doppler instruments were operated using a single real time software: physiological data (ECG, blood pressure, heart rate, doppler blood velocities) are transmitted from instruments to laptop, to be recorded and displayed on screen. For a better anticipation of syncope, Doppler indexes (femoral resistance Rf, cerebral and femoral flows Qc and Qf ) are computed and displayed in real time.

2. DATA ANALYSIS

For analysing measurements, a specific software (Physiopost) was developed with the following main functionalities: (1) precise synchronization of measurements provided by different instruments ; (2) extended beat per beat analysis ; (3) graphical visualization of measurements and export to Excel.

Analysis of those combined data presented some difficulties requiring to improve Physiopost tool: firstly the large amount of data (8GB) and number of sessions (21 subjects, 2 tilt sessions each) required a full automation of treatments for all sessions together . Secondly the quality of measurements was irregular due to noise and operational problems. To manage the poor quality episodes, algorithm robustness for analyzing ECG and Doppler flows was improved. Concerning the identification of R-peaks, two different algorithms are used enabling a cross-validation of results. Furthermore, Physiopost enables scientists to select graphically valid ranges of measurements (for instance, ranges at rest and other ones during tilt). These ranges are automatically used within Excel for computing statistics. Thirdly it was required to interface Physiopost to third-part software (Notocord, Matlab, Cardiview) to extend its functionalities/analysis tools and accommodate it to different scientific practices.

3. SEVE NEW GENERATION

A new generation of SEVE system is currently being studied in CNES with the objective to develop a system, useable not only on-ground but also in space and taking advantage of up-to-date technologies. SEVE NG should provide the same functions / measurements as the present SEVE system but with better integration, reliability, performances and operationability. Two types of use are foreseen: (1) Astronaut medical monitoring with improved diagnosis reliability, better control and countermeasure efficiency assessment; (2) Cardio-vascular studies, in particular in new fields of investigations (micro-circulation and cardiac structure).

Technical requirements and constraints for SEVE NG, and the corresponding solutions under study are the following: (1) SEVE on-board system should be optimized in terms of weight, size and energy consumption ; (2) SEVE NG should be upgradable to new instruments and medical protocols. The proposed system architecture includes a laptop and a main unit to integrate medical instruments of the last generation. Most instruments are permanently racked into main unit with servitude electronic boards (data transfer function, synchronization, power conversion). But this main unit provides also a slot for an extractable instrument. Fixed instruments provide the most often required measurements (ECG, blood pressure and respiration signal). Ultrasound Doppler and laser doppler are integrated as extractable instruments, using main unit slot, because their simultaneous use on-board is not foreseen. Echograph is integrated as a permanent module, because it should be useable together with ultra-sound doppler. Furthermore, the studied echograph module is of limited size (3cm large) and weight (~400g). This architecture enables to optimize instrument configuration with respect to used protocols but it is also opened to future instruments. Medical protocol evolutivity will be ensured at software level, enabling the user to define / configure its own protocols.

(3) Measurement acquired numerically from instruments should be precisely time-tagged and synchronized. To ensure time-stamping, an analog acquisition card is foreseen , taking instrument time counters as input.

(4) Operational ergonomy should also be improved. It requires to work with instrument manufacturers to develop new probes and harness. For instance, iontophoresis protocol requires to be simplified operationally to be useable on-board. Software User Interfaces are also very important and the Human Factor should be taken into-account.

(5) Real-time transmission from space station to ground, is also required to enable a real time follow up of experiments by the ground medical team.

4. CONCLUSION

ACC bed-rest was a very successful collaborative work, technically and scientifically but also operationally. Many lessons were learnt from the intensive use of SEVE system (six protocols, 21 subjects during 3 months) which are very useful to prepare SEVE next generation. Globally, the driving requirements for designing such a medical system are both the end to end scientific and medical use (and in particular, data post-analysis) and operationability. The technical and scientific objectives of SEVE NG are ambitious; the ongoing study shows that is possible to develop a modular system. The development of SEVE NG is part of the discussion between CNES and ACC about further cooperation.
FRENCH PARTICIPATION (CADMOS AND MEDES) IN PHYSIOLOGY EXPERIMENTS UNDER MICROGRAVITY CONDITIONS AND COUNTERMEASURES FOR COMPENSATING THE ADVERSE EFFECTS OF THE SPACE ENVIRONMENT

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ABSTRACT: The aim of this paper is to describe the participation of the French space agency (CNES) and its subsidiaries in Human spaceflight activities and more particularly research into human physiology in the Space environment.

CADMOS is a centre of the French Space Agency which manages all CNES monitoring operations for microgravity research during parabolic flight, the Foton mission and on the ISS.

MEDES is a private, non-profit organization, founded jointly by the French space agency, the Toulouse hospital and several French universities, to develop expertise in space medicine and health applications of space research.

1. CADMOS PURPOSE AND DESCRIPTION

The CADMOS\(^1\) centre was created by the French Space Agency, CNES\(^2\) in Toulouse in 1993 to prepare, organize and control French manned space missions. It is used for microgravity missions on the International Space Station, parabolic flights and on the Foton capsule.

CADMOS provides complete support from the start of feasibility studies through development of an experiment, to the monitoring of space operations and data collection, archiving, valorization and distribution.

Since 1998, CADMOS has been part of the ESA\(^3\) network of operational centres (USOCs\(^4\)) created to prepare and carry out the ESA scientific programme under microgravity conditions. In this context CADMOS is responsible for the EPM (European Physiology Modules facility) embarked with the Columbus laboratory in February 2008 and also for MARES (Muscle atrophy research and exercise system (2010), and ACES (Atomic clock ensemble in Space (2013). In addition, CADMOS prepares and undertakes national and bilateral research projects on nutrition (ENERGY experiment, SEM gastronomic Space food), Biology (Polca & Gravigen experiments), fluid physics (Déclic payload with NASA - August 2009) and physiology (CardioMed instrument with Russian cooperation for medical monitoring of the crew, slated for launch at the end of 2009).

The CADMOS centre includes laboratory zones for preparing the experiments and for housing the payload ground models as well as a control room linked to the Columbus centre in Germany and to NASA. About 34 people work at CADMOS. More than 50% of CADMOS activities concern physiology experiments.

2. CADMOS RESPONSIBILITY FOR PHYSIOLOGY RESEARCH

CADMOS is responsible for the main part of the ESA programme for physiology research in microgravity but shares the activity with the Danish USOC, the DAMEC. Since 2004, more than 12 different experiments have been carried out or supported from CADMOS, 8 are still in progress and 5 others are being prepared.

CADMOS manages stand-alone experiments with data which is downloaded later. Since the commissioning of the Columbus laboratory, it is able to use the EPM multidisciplinary research tool for combined experiments as well as the up-down data link. CADMOS can receive the scientific data on the ground in real time or later, which enables scientists to intervene if necessary.

EPM contains two scientific modules, one for cardiovascular studies (CARDIOLAB) and one for neuroscience (MEEMM) and also a biological sample collection kit (for blood, urine and saliva). These modules are equipped with several instruments or devices allowing for a wide range of measurements:

CARDIOLAB includes:

- CARDIOPRES for continual beat-by-beat recording of heart rate, arterial pressure and respiration,
- a portable DOPPLER device for simultaneous recording measurements of three arterial blood or surface vessels,
- HOLTER for recording arterial pressure over 24 hours,
- ECG Holter: for electrocardiogram recording over 24 hours,
- an air plethysmograph for measuring variations in limb volume during venous occlusion tests,
- a device for biochemical analysis of blood samples,
- an analyser for photometric measurement of hemoglobin concentration in blood,
• a mini centrifuge for taking hematocrit readings,
• stimulation equipment: arm and leg occlusion cuffs
  and a glove for creating hot and cold stimuli)

The MEEMM unit (Multi electrodes Electroencephalogram mapping Module) includes:
1) Integrated Equipment for recording electroencephalograms and for measuring evoked
   potentials, 2) A portable device for use in ambulatory mode, 3) A signal amplifier unit and 4) A Cap system
   for correctly positioning electrodes (up to 64).

**PHYSIOLOGY EXPERIMENTS IN THE FRAME OF ESA SUPPORTED BY CADMOS**

- **CARDIOCOG**: Cardiovascular –ESA- From increment 12 to 15 - End: PI: Aubert (B)
- **ETD**: Neurosciences – ESA - From increment 9 to 16 – End - PI: Clarke (D)
- **3D SPACE**: Neurosciences – ESA - From increment 17, still on going - Pls: G. Clement (F), C.
  Lathan (USA)
- **CARD**: Cardiovascular – ESA - From increment 14, still on going – PI: P. Norsk (DK)
- **EDO**: Physiology - ESA- From increment 15, still on going – PI: L. Vico (F)
- **IMMUNO**: Immunology – ESA - From increment 12, still on going - PI: A. Chouker (D)
- **MOP**: Physiology - ESA- From increment 16 to 18 - End - PI: Gröen (NL)
- **MUS**: (LOW BACK PAIN / -MUSCLE) - Physiology- ESA- From increment 14 to 19 - PI:
  Pool-Goud-zwaard (NL)
- **NEUROSPAT**: Neurosciences – ESA - From increment 19, still on going - PI: L. Balazs (H), G.
  Cheron (B)
- **SOLO**: Physiology- ESA - From increment 17, still on going – PI: M. Heer (D).
- **SPIN**: Physiology- ESA - From increment 16, still on going - PI F. Wuyts (B).
- **PASAGE**: Neurosciences – ESA - From increment 22 – Pls: M. Luayat(F) and J. McIntyre (F)
- **ENERGY**: (potential experiment) : Physiology-ESA- From increment 24 - PI : S. Blanc (F)

The CADMOS website (http://cadmos.cnes.fr) offers further information on its activities.
Email : cadmos@cnes.fr

**3. MEDES ACTIVITIES**

**3.1. SPACE-RELATED AND CLINICAL RESEARCH**

MEDES does extensive clinical research, with the objective of evaluating scientifically based
countermeasures, of determining relationships between the space environment and health, and of opening up
space research to the greatest number of health professionals, thus facilitating space research. MEDES
relies on a dedicated clinical research centre, the Space
Clinic, located in the premises of the Toulouse
Rangueil hospital. This facility can host up to twenty
volunteers who take part in biomedical studies in a
controlled environment (with adjustable acoustic
levels, temperature, natural or artificial lighting).
MEDES studies mainly concern fields related to space
research, such as physiology (balance problems, decreased effort capacity, osteoporosis...),
pharmacology and assessment of biomedical systems.
As far as space research is concerned, MEDES
develops experimental procedures and investigates the
effects of isolation and confinement on sleep and
circadian rhythms by simulating microgravity-induced
effects... Since its creation, MEDES has undertaken
more than twenty studies of this type, that lasted from
a few days to several months. A major study was the
international WISE long-term (2 months) bed-rest
study with 24 female subjects that involved the French,
European, US and Canadian Space Agencies (i.e.
respectively CNES, ESA, NASA, CSA).

**3.2. HEALTH APPLICATIONS**

Space is a unique environment to which bodies and
organisms have to adapt, that can be considered as a
model of a hyper-sedentary way of life or accelerated
ageing for several physiological processes. In the
framework of the ERISTO (European Research In
Space and Terrestrial Osteoporosis) and ADOQ
(Advanced Detection Of bone Quality) projects,
MEDES has studied the role of mechanical forces in
bone remodelling and has contributed to the clinical
validation of a system for measuring bone quality.
MEDES is also involved in educational projects to
increase young people’s awareness of science and
technology and the importance of protecting their
health, and is a partner of the European “Ulisse”
project involving the USOCs.

Finally, MEDES helps develop satellite applications
for health-related services.

The MEDES website (http://www.medes.fr) offers
more detailed information on its activities.

**4. CADMOS/MEDES RELATIONSHIPS**

MEDES has been involved in CNES and ESA’s
manned missions since 1990 and has gained significant
expertise in human physiology and space medicine.

Drawing on this expertise, MEDES provides technical
assistance to the European Astronaut Centre (EAC) and
takes part in the screening, training, medical
certification and follow-up of astronauts during space
flights and crew rehabilitation.

MEDES also co-operates closely with CADMOS by
offering CADMOS its expertise in physiology and
clinical research.

(*) joint scientific experiments with Russia
ASSOCIATION BETWEEN PSYCHOLOGICAL AND CARDIAC FUNCTIONING IN A CONFINED POPULATION: MARS500 STUDY.

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ABSTRACT

In this study we aim to evaluate possible effects of long duration confinement and isolation on changes in mood and affect of a crew and to correlate these fluctuations with changes in cardiac functioning using: 1) psychological questionnaires, 2) 24-hour Holter recordings, 3) methods of heart rate and blood pressure variability, 4) methods of telemedicine/cardiology and 5) neurohumoral assessments.

1. INTRODUCTION

Besides microgravity, inactivity is likely to play a role in cardiovascular deconditioning and alterations in autonomic cardiovascular control. Moreover, confinement for a long period of a crew into narrow quarters, as for the Mars simulation (105 and 520 days), could elicit emotional stress disturbing autonomic balance, which can further alter cardiovascular function and undermine well-being.

Because of the long-lasting isolation in a small habitat, the confinement may be considered similar to a simulated Mars mission, except for the effects of microgravity and radiation. Therefore, isolation studies represent a unique opportunity to evaluate the net effect of psychological influences on physiological variables, avoiding the net effect of confounding factors such as real microgravity and radiation hazards in space.

The study consists of different experimental protocols: 1. questionnaires to assess mood alterations; 2. 24 hour Holter recordings that will be correlated with psychological data, spectral analysis will be performed to assess circadian rhythm and sleep alterations; 3. short duration ECG and noninvasive blood pressure recordings. Heart rate variability and blood pressure variability will be obtained from spectral analysis and baroreflex and synchronization of cardiorespiratory coupling determined; 4. tele-echocardiography and tele-auscultation allow a performant, non invasive follow-up of cardiac function during the mission and give some insight in heart mechanics (echo) and hemodynamics (heart sounds).

The objective of the Mars simulation experiment is as follows:

- obtaining experimental data about health state and work capability of humans staying for a long time in conditions of
- isolation in hermetically closed environment and confined volume during simulation of the main peculiarities of the Martian flight (duration, functional autonomy, changed conditions of communication with the Earth - signal delay, limitation of the consumable resources).

In summary, this project is aimed to assess, through several complementary approaches, the modifications in psychological and physiological functioning induced by living in closed quarters and its effects on short- and long term cardiovascular autonomic control.

2. METHODS.

2.1 FACILITIES

The Mars confinement facility at the IMBP installed in Moscow, Russia, consists of 4 modules: Module 1: Laboratory; Module 2: Habitable module; Module 3: Mars lander; Module 4: Storage module (Fig. 1). A crew consisting of 6 volunteers will live in these facilities first for a pilot study during 105 days and later (planned end of 2009) for a real simulated Mars voyage, with landing, for a total of 520 days. Out of 5680 candidates a crew of 6 subjects (4 Russians, 1 French 1 German) was finally selected after thorough medical and psychological testing and final interview by a DLR psychologist and an IMBP head flight surgeon.
2.2 MEASUREMENTS

2.2.1 Psychophysiology: mood/affect

Extreme living conditions, simulating a Mars based environment, influences psychological state and sleep. This issue will be addressed by self-report measures, such as validated questionnaires and diary methodology, during the confinement of 105 or 520 days.

The participants will be triggered to rate their affective state on a set of 16 items (Fig.2). These are selected on the basis of the Positive Affect/Negative Affect Circumplex Model. This model posits that most affective states can be arranged in a two-dimensional space, constituted by a dimension of arousal and valence.

![Figure 2 The positive affect/negative affect circumplex model](image)

2.2.2 Cardiovascular autonomic neural control

Changes in psychological state and sleep, induced by living in a simulated Mars mission and Mars based environment, will elicit changes in control of the autonomic cardiovascular system (ANS), sleep and cardiopulmonary function.

Variations of cardiovascular autonomic regulatory mechanisms reflect adaptive mechanisms of the ANS [1]. The pivotal role of the ANS in regulating instantaneous cardiovascular responses to intermittent stressors justifies the interest of investigating cardiovascular variability.

To address this issue heart rate variability (HRV), blood pressure variability (BPV) and baroreflex (BRS) will be determined as obtained from ECG, continuous blood pressure and respiration [2]. Spectral analysis of Holter recordings will be performed and related to sleep diary data and assessment results of psychological state. HRV is expressed in the frequency domain and low frequency (LF: 0.04-0.15Hz) and high frequency (HF: 0.15-0.4 Hz) determined.

2.2.3 Telemedicine

Telecardiology and telemedicine in general, will be of absolute necessity during long duration space missions to Mars and therefore this aspect will also be implied in the Mars confinement study. Also delays in transmission from the Mars unit to the exterior world up to 20 min will be included, to simulate a real interplanetary mission. Cardiovascular function will be determined from tele-echocardiography and teleauscultation. Eventual cardiac deconditioning will be observed from monitoring of echo (dimensions) and heart sounds (hemodynamics).

The importance of telemedicine is not only as a follow up tool in case of any cardiovascular or respiratory disease by a crew member, but also as general health indicator. The flight surgeon and external medical specialists can be available to give adequate advice concerning the road map to follow.

3 CONCLUSIONS

Some results from related studies have been published. Bhattacharyya et al [3] found depressed affect associated with a rise in HF and depressed LF. During a 4 day confinement study, LeTraon et al [4] found a decrease in baroreflex sensitivity. It can be concluded that a relationship seems to exist between stress/mood alterations as induced by confinement on cardiovascular function.

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HAND FREE ECHOGRAPHIC IMAGE CAPTURE AND DELAYED 3D POST PROCESSING (Application to Tele echographic diagnostic during MARS 500).

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Abstract: Objective: to test a delayed tele-echography mode using a volumic echographic capture and delayed processing. Method: A dedicated motorized probe holder (TILTER) was used for tilting a 2D probe from −45 to +45°. In parallel a similar tilting movement was performed by a non sonographer operator holding the probe in his hand (Hand Free TILT) and tilting it from −45 to +45° manually. The serie of 2D images captured during the tilt was sent at the expert center by Telephone line, or Internet. This volume was investigated by the expert using a soft developed in our lab, that allow to navigate inside this volume with a virtual plan. Results: The delayed echography using the TILTER and the Hand Free TILT, were tested on 40 patients. For each organ approximately 50 to 100 images were captured in 3-4 seconds by both mode. The organs were adequately visualized and the diagnostic in agreement with direct echography by a sonographer in 90% of the cases after 2 to 3 capture per organ. The average time from the first capture until the diagnostic was delivered for these 6 organs was 40+/−10 minutes. Conclusion: Delayed tele-echography based on the capture of a volume of 50 to 100 2D echographic images covering the organ provided similar information as direct examination by a sonographer at least in 90% of the cases. No false diagnostic was reported, in the remaining 10% cases only one of the 6 organs was not adequately sonicated and thus not completely inside the volume scanned during the tilt. Keywords: Echography, isolated site, Remote, 3 dimension

INTRODUCTION:
Small secondary hospitals 20 to 100 km from a main hospital, dispensaries in Africa, Amazonia and polar areas, or moving units such as rescue vehicles, boats, airplanes.. and in the near future space vehicle and human colonies, are examples of sites with limited medical facilities. Various pathologies such as abnormal heart rate, pericardial collection, renal lithiasis, cholecystitis, abnormal pregnancy, ovarian cyst, acute appendicitis and phlebitis… may affect subjects without any medical history. Ultrasound echography and Doppler are currently used in hospital emergency departments for evaluating the degree of emergency for the patient. Unfortunately, many small medical centers and isolated sites do not have an appropriate well-trained sonographer to perform the initial evaluation 24h a day. On the other hand a non trained person cannot capture the appropriate echographic views requested for a safe diagnostic on current patients, even if guided by voice information (personal data). In case of high flow data transmission the echography of the patient at the isolated site can be performed by tele-operating a robotic arm that reproduce the movements of the expert hand (1-2). If such link does not exist or if the link is of low quality, or when the robotic equipment is not available we propose to perform a delayed Tele-echography using a volumic echographic capture mode and a delayed processing. It is assumed that there would be no sonographer at the patient site and that the operator at the isolated site assisting for capturing the images have been only familiarized (1 to 3 hours) with the echographic capture process but is not trained at all to get the images by himself. The objective is to test a delayed tele-echography method using a volumic echographic capture and delayed processing.

MATERIAL & METHOD:
For the first step of the project a dedicated motorized probe holder (TILTER) was used for tilting a 2D probe from −45 to +45°. In parallel a similar tilting movement was performed by a non sonographer operator holding the probe in his hand (Hand Free TILT) and tilting it from −45 to +45° manually. The serie of 2D images captured during the tilt was sent at the expert center by Telephone ISDN line, or Internet (ADSL). This volume was processed by the expert using a soft developed in our laboratories, that allow to navigate inside this volume with a virtual plan. Thus the expert could make his own echography inside the volume.

The location where to put the probe for the image capture sequence was indicated by the Expert under audio and video control. A body mapping of the acoustic windows (AC) of the main abdominal organ was designed, on a population of 300 patient, in order to identify skin areas where to find the acoustic window of each organ with a probability higher than 85% (Arbeille et al personal data). Moreover the acoustic windows mapping serve in case there was not realtime video transmission between the 2 sites or a video link of insufficient quality for transferring echographic data.

Thus the non sonographer subject was asked to locate the probe on top of the acoustic window by translating the probe along to the costal
border or using the body mapping. When he found the acoustic window (partial view of the organ) he placed the probe perpendicular to the skin and then Tilted it from $-45^\circ$ to $+45^\circ$ keeping the probe head motionless and captured the images generated during that Tilt and send them directly to the expert site.

RESULTS:
The delayed echography using the TILTER and the Hand Free TILT, were tested on 40 patients. For each organ approximately 50 to 100 images were captured in 3-4 seconds by both mode. The organs were adequately visualized and the diagnostic in agreement with direct echography by a sonographer in 90% of the cases after 2 to 3 capture per organ. In each patient 6 organ were explored: Liver, 2 kidney, Spleen, Bladder, Aorta. The average time from the first capture until the diagnostic was delivered for these 6 organs was 40+/−10 minutes. The diagnostic delivered by both modes (TILTER and Hand Free TILT) were not different.

CONCLUSION:
Delayed tele-echography based on the capture of a volume of 50 to 100 "2D echographic" image covering whole the organ provided similar information as direct examination by a sonographer at least in 90% of the cases. No false diagnostic was reported in the remaining 10% cases, only one of the 6 organs was not adequately insonated in these cases and thus not totally present inside the volume scanned during the tilt. The contribution of the non sonographer operator by the side of the patient consisted of locating the probe (or the Tilter) on top of the organ acoustic window and, (a) keep the Tilter motionless during the motorized tilting movement or (b) Tilt manually the probe from $-45$ to $+45^\circ$ from the vertical to the skin. Then he had to send the volume of image captured to the Expert center by Internet or ISDN Telephone. Both the “Tilter” and “Hand free tilt” provided similar image capture set and view of the organs after processing. The present method will be used for the ESA Vessel-Imaging experiment between the ISS and the ground with a realtime link between these 2 sites, and during the MARS 500 experiment with a 20min delay in the transmission of the information between the expert and isolated sites.

(Work supported by CNES and ESA)

REFERENCES
AUGMENTED HUMAN DESIGN FOR HUMAN SPACE LIFE AND ACTIVITIES: AN INTEGRATIVE PHYSIOLOGICAL THEORETICAL AND EXPERIMENTAL APPROACH.

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ABSTRACT

How to assist human space life activities during short or long term space flights? Especially how to integrate virtual environment technologies to monitor and assist technical gesture performed during extra-vehicular activities taking into account physiological requirements for such behavioral technological design and engineering and their assessment?

In this paper we propose to explore how to ground technological gesture assistance design and systems engineering rules and its experimental assessment method on a theoretical integrative physiology and its general principals.

1. GENERAL SPECIFICATIONS

Long term human space flights lead dramatic physiological modifications as well as for basal function, e.g. cardiovascular, than for sensorimotor, e.g. posture or motion, and cognitive functions, e.g. spatial cognition or memory. Thus, astronaut’s relative cognitive impairments and lost of sensorimotor skills needs a long and hard training on earth first to perform the mission in weightlessness.

Augmented human design aims to enhance human capabilities and performance using noninvasive wearable interactive technologies such as virtual reality and augmented reality. It needs both an integrative theory that takes into account the specificity of the biological organization of living systems, according to principles of physics, and a coherent way to organize and integrate structural and functional artificial elements. To ensure global performance, robustness, safety and reliability of human system integration for improving human performance [1], we propose to ground augmented human design first on an integrative physiological theoretical framework: Chauvet’s mathematical theory of integrative physiology (MTIP).

2. MTIP: A THEORETICAL FRAMEWORK

The mathematical theory of integrative physiology, developed by Gilbert Chauvet [2] [3], examines the hierarchical organization of structures (i.e., anatomy) and functions (i.e., physiology) of a living system as well as its behaviour. MTIP introduces principles of a functional hierarchy based on structural organization within spaces scales, functional organization within time scales and structural units that are the anatomical elements in the physical space. It copes with the problem of structural discontinuity by introducing functional interaction, for physiological function coupling, and structural interaction, for anatomical structural coupling. Unlike interaction in physics, at each level of organization, functional interactions are non-symmetrical, non-local, and augment the system stability of a living system by coupling two structural elements.

The human (Ω) [figure 1] is represented as the combination of the hierarchical structural (z) and functional (Y) organizations. The (x) axis corresponds to the ordinary physical space. Each physiological function \( \psi \) is represented in the x\( \psi \)y plane by a set of structural units hierarchically organised according to paces scales. Two organizational levels are shown: \( \psi_1 \) and \( \psi_2 \). The different time scales are on the (y) axis, while space scales, which characterize the structure of the system, are on the (z) axis. The role of space and time clearly appears. \( \psi_{1ij} \) is the non-local and non-symmetrical functional interaction.

Units at upper levels of the physiological system represent the whole or a part of sensorial and motor organs. HSI (Ω’) [figure 2] consists in creating an artificially extended sensorimotor loop by coupling two artifactual structural units I’ and J’. Their integration into the physiological system is achieved by the functional interactions they generate. From sensors outputs to effectors inputs, the synchronized computerized process S’ controls and adapts the integration of the functional interactions artificially.

Figure 1: 3D representation of a biological system based on the Chauvet’s MTIP

Figure 2: 3D representation of a dysfunctional system based on the Chauvet’s MTIP
created into the dynamics of the global and coherent system.

Figure 2: representation of an human in the loop system coupling the biological system with an artefactual system on the left to an artificial sensorimotor loop.

It is a new theoretical paradigm for human system integration modelling.

3. EXPERIMENTAL PROTOCOL

To assess that integrative physiological design approach we developed a neurophysiological and gesture-based experimental protocol using or not a wearable interactive system made up of virtual environment technologies for gesture assistance. To confirm the effectiveness of the integrative physiological modeling, our design prototype was validated on ground and evaluated during parabolic flights (9th CNES NOVESPACE) in both hypergravity and weightlessness. In order to achieve this goal, we set up a protocol based on graphical gesture analysis, more specifically of the drawing of ellipses within 3D-spaces, inspired by neurophysiology of movement, as described in [5]. Using analysis of three-dimensional hand movements, we compare the dynamical sensorimotor integration and motor performances (orientation, shape, figural and kinematics features) with or without the assistance of virtual environments [figure 3 and 4]. Using this gesture-based method we evaluate physiological effects and integration of both change of gravity and artifactual environment on performance.

4. CONCLUSION

Further research and development remain to achieve an effective sensorimotor countermeasure system for human space activities. Particularly, sensorial multimodality has to be explored and used to improve accuracy of gesture.

However, we demonstrate how artificial visual information dynamically generated by a wearable virtual environment, may help gesture in the three-dimensional space parabolic flight, according to the MTIP principles. These experiments highlight the pertinence and practicability of the developed integrative approach of human modelling for augmented human design and human system integration by showing improved motor skills and gesture performance. Therefore, integrative physiological design is a framework for future developments. As virtual environments or wearable technologies, integrative artifacts will found the next assisting and countermeasures systems and smart environments for human space activities.

Figure 3: Og, example of ellipse drawing in vertical sagittal orientation without assistance. We observe a total loss of shape and orientation accuracy.

Figure 4: Og, example of ellipse drawing in vertical sagittal orientation with assistance in vertical sagittal orientation. Even if the shape is not precise, orientation of movement is very accurate and stable (taking into account the magnetic field distortion) despite that loss of the gravitational referential and vestibular perturbations. Artificial visuomotor functional interaction coupling by virtual environment enhance stability according the Chauvet’s MTIP theory and its principles of auto-associative stabilization [3].

5. REFERENCES


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