FOTON M-3
Russian - US Collaborative "Receptor II"
Research Proposal by Richard Boyle

Descriptive Title: "Receptor II: Structure and Function of the Gravi-sensing Statocyst System following an Earth's orbital mission."

NASA/USA Investigators

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Objectives: The "Receptor" project for Foton M-2 was modified by replacing the crayfish experimental model with the snail *(Helix lucorum)* due to biocompatibility problems late in the payload preparation period. The basic hypotheses were not modified, only expanded. This modification had a very significant and positive impact on the success of the project.

During the periods of adaptation to μG and re-adaptation upon return to Earth, it is evident that changes occur within the neural system responsible for transducing and processing the gravity information. We have studied the neural response to transitions between gravity levels with two specific aims:

1. Determine the regulation of expression of the preproHPep gene (gene that is expressed in the primary statocyst receptor cells) as a consequence of space flight and during the re-adaptation to Earth's gravity. Regulation of this gene might signal how the statocyst receptor is ""tuned" by the gravity vector.

2. Specify the cellular response and time-dependent $|Ca^2|$ signaling of the statolith receptors and their influence on the ganglion cells to which they target during the re-adaptation process to Earth's gravity. This combined approach of conventional electrophysiology and optical imaging of somatic Ca^2 measures will provide direct evidence of the cellular events occurring during the readaptation process.

Introduction: After return to Earth many astronauts developed sensations that can be attributed to gravireceptor dysfunction, e.g., illusionary feelings, vertigo, nausea and vomiting, gaze fixation disorder and ocular nystagmus. Our ability to understand the basic mechanisms underlying such dysfunction is limited by our knowledge of the adaptive and re-adaptive responses of the equilibrium organ at the cellular and molecular levels. Adaptation within the gravito-inertial sensing organs can occur rapidly in an organism in direct response to a change in gravitational force (Boyle et al. Neural readaptation to Ig following return from space. *J. Neurophysiol.* 86: 2118-2122. 2001). Our hypothesis is that microgravity challenges the gravireceptors and causes changes in their cellular function, that these changes are manifested in the regulation of specific gene expression and in the electrical behavior of the receptors, and that re-adaptation to 1 g is a time process that can be tracked by intracellular measurements.

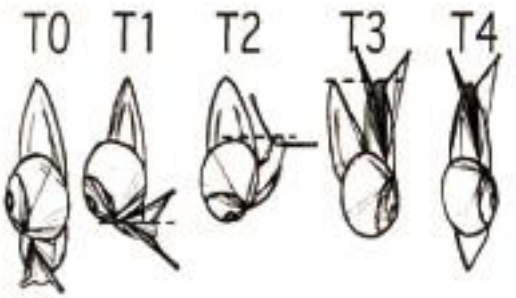
We propose to again use the land snail *Helix lucorum* Linnaeus (Pulmonata, Gastropoda) as experimental specimens. These snails are small, strong and can survive unattended with for weeks on a moistened substrate. In addition to Foton M-2. snails have flown on Shuttle. MIR and ISS missions in the past. We continue the M-2 approach for the M-3 opportunity, i.e., we will address our original and extended aims using techniques such as molecular gene expression, intersensory neuronal interactions between the gravi- and photo-sensors, electrophysiology of the voltage and current responses to reorientation with respect to gravity, and optical imaging of the real-time intracellular calcium and voltage signals.

Specify linkage to the NASA Mission:

The basic mechanisms of how living systems sense gravity, transduce the signal and process the information are at the root of understanding how to mitigate problems of orientation, locomotion, and performance both on the Earth, in microgravity and in the fractional gravity environments of the moon and Mars. Behavioral, molecular and electrophysiological measures will be used pre- and post-flight to track changes in organism and afferent/receptor sensitivity.

Hypothesis: The snail statocyst contains calcium carbonate crystals - statoconia - similar to the weight lending otoconia in mammalian otoliths, and the statoreceptors like their otolith counterparts in vertebrates, are activated by changes in animal orientation with respect to gravity. Our hypothesis is that the gravi-sensing receptors are not static sensors but undergo significant structural and functional changes as a result of transitions from one gravity state into another. These changes might be compensatory, but nevertheless maladaptive to the organism that moves into a new gravity state or intermittently along gravity gradients. The initial or adaptive phase could be monitored as the organism transitions into microgravity during flight, but this is technically challenging. The recovery or readaptation of the gravi-sensing statocyst can be experimentally interrogated upon return to Earth's gravity following an orbital mission, and this is our strategy.

Summary of M-2 Findings: The "Receptor I" project evolved into 4 complementary experiments that investigated the influence of microgravity exposure on the structure and function of the gravi-sensing statocyst organ of the snail. The adult snails were studied using two electrophysiology paradigms, and each snail was first behaviorally tested to its response to a sudden 90° reorientation from horizontal to the vertical. These results are designed to put the physiologically measured findings into a behavioral perspective. In the initial test the behavioral "negative gravitaxis" responses of the snails showed that the flight snails in general responded faster than their control counterparts. Figure 1 shows the test procedure: phases 1-4 represent the epochs of behavioral response following a horizontal to vertical pitch of the snail. Figure 2 plots of the time of the behavioral responses at the separate 4 phases for the negative gravitaxis response for the 15 flight and 8 control snails. Flight snails were faster (shorter latency) in their response to pitch stimulation at each phase, and this became significant ($p < 0.05$: nonparametric. two-tailed, unpaired Mann-Whitney test) at phases 3 and 4.



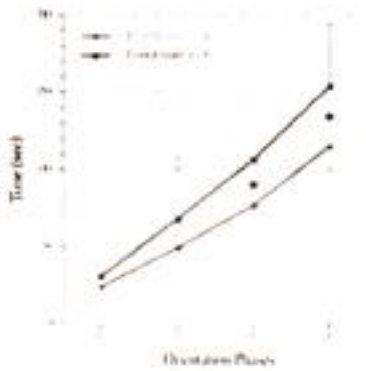


Figure 1 Figure 2

In the morphology study 20 juvenile snails were studied at two time periods following landing. Examination of the regulation of expression of the preproHPep gene was performed on post-flight and control snails using mRN'A expression techniques. This pedal peptide is found in the primary statocyst receptor cells, and the regulation of this gene might signal how the statocyst receptor is "tuned" by the gravity vector. Compared to controls, flight snails have a higher level of expression in statocyst ganglia but not in other ganglia.

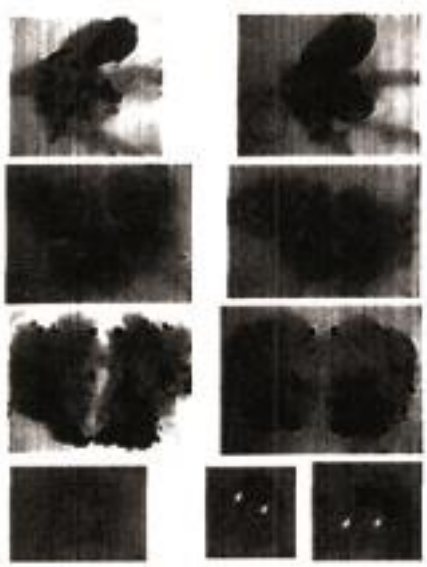


Fig. 3: Pedal peptide gene expression in snail nervous system.

Figure 3 shows light microscopic images of the preproHPep gene expression in 4 separate regions of the snail nervous system. From top to bottom are: cerebral ganglia subesophageal ganglia complex, pedal ganglia, and the *statocysts.* The control tissue is on the left and the flight sample in on the right. *Note the 2 labeled siatoreceptors labeled in the flight snails (arrows).* No significant difference was observed in the other tissue regions between the control and flight samples. suggesting that the upregulation of the gene expression to the pedal peptide was specific to the statocyst. Results of in situ hybridization with probes to the pedal peptide snail gene are given in Table I.

Table 1:

Conditions	# snails	# statocysis	# statocysts with stained neurons
24 h post-flight	6	12	6(4L. 2 R) stained. 2 neurons in each. Rostral part of statocyst
36 h post-flight	6	12	4 stained (2 L. 2 R). 2 neurons in each. Rosiral part
Control	12	24	No stained neurons
Naive snails	10	20	No stained neurons
Naive 24 h after foot cut	4	8	In 1 statocyst onl) 2 stained neurons
Naive 24 li after centrifuge	7	14	No stained statocysts

The two separate electrophysiology experiments were conducted to study the readaptation of the statoreceptor and orientation systems following exposure to microgravity and return to lg. A tremendous amount of data was collected from the whole nerve preparation and individual statoreceptors in these experiments, and we are still analyzing the data and compiling the results. At this moment we can state the following findings. 1) Intersensory interaction between the photosensors and the gravisensors is altered. A significant difference was observed in the effect of light stimulation on the background firing rate of statoreceptors in control and post-flight snails (Fig. 4). In addition, extracellularly recorded neural responses of the statocyst nerve to adequate motion stimulation in the post-flight snails were independent of the motion direction while in the control animal significant differences in responses to different directions were observed. Further, a high significant difference in whole nerve activity responses before and after rise and fall of the test platform was seen. The simplest explanation of these results is that the snail loses preferential direction (i.e. orientation) during flight, and this is reflected in the measured responses in the intersensorv interaction tests.

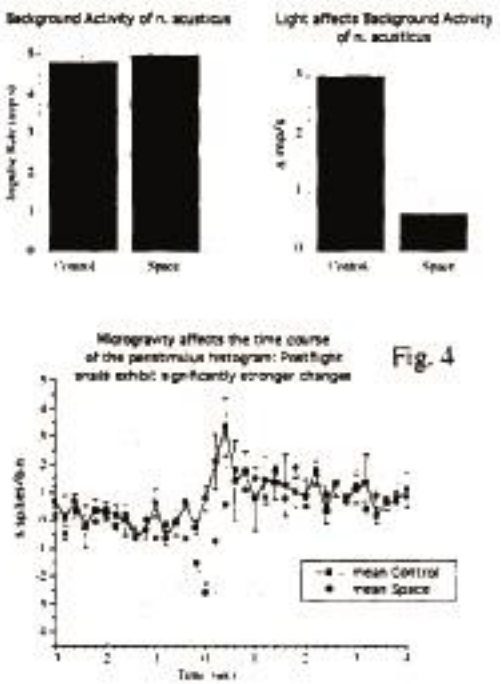


Fig. 4

2) The intracellular response sensitivity of individual gravi-receptors to natural tilt/pitch stimulation was measured using electrophysiology (bridge and clamp amplifier: npi Germany) and real-time voltage- and calcium-sensitive dyes imaging techniques (high-speed fluorescent microscopy and RedShirtlmaging software). Salt-free solutions were used in some experiments to query the role of sodium and calcium ions in shaping the voltage and current responses of the statoreceptors to tilt in post-flight and control snails. The inward sodium current drives the action potential, but the results are still being evaluated and discussed. Although the sample is small, the results support the hypothesis that the statoreceptor sensitivity is altered -an

upregulation - by the exposure to microgravity. This agrees with the evidence in vertebrate species (fish, rats, monkeys and humans) that an initial phase of hypersensitivity occurs in the otolith organ. The results are presented in Figs. 5 and 6. Figure 5 shows the current response under voltage clamp conditions of the statoreceptors. The membrane potential was held near rest in control (red trace at -57 mV) and in post-flight (blue trace at -50 mV) statoreceptor. The inward current (downward movement of the current trace) in response to a 10° tilt of the animal in its preferred plane was more pronounced and showed less recovery or adaptation during the stimulus in the post-flight snail. Figure 6 shows the results obtained using calcium imaging techniques. Two individual statoreceptors were intracellular^ labeled with Oregon Green indicator dye (enhanced image on right side) and the measured calcium signals are shown for one cell on the left. In response to a 10° tilt the cell showed a 3 fold increase in voltage activated calcium spikes (associated with the action potential) and an enhance calcium conductance.

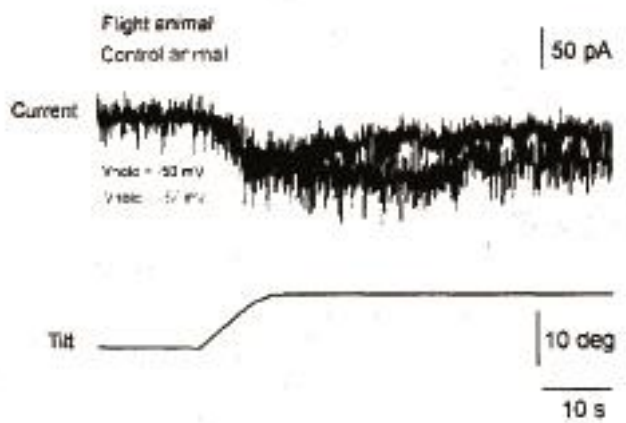


Fig. 5: statoreceptor current responses

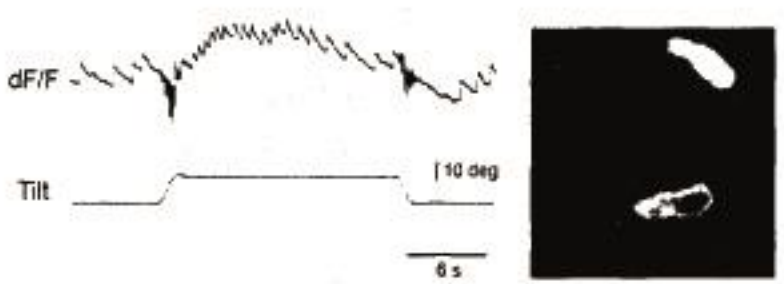


Fig. 6: calcium signaling and imaging
Abstract of Foton M-2 Report:

27lh Annual International Gravitational Physiology Meeting 23-28 April, 2006 Osaka University, Osaka, Japan

STRUCTURE AND FUNCTION OF THE SNAIL STATOCYST SYSTEM AFTER A 16-DAY FLIGHT ON FOTON-M-2. P. M. BALABAN, A. Y. MALYSHEV, I. S. ZAKHAROV, N. A. ASEEV, N. I. BRAVARENKO, V. N. lERUSALIMSKY. A. I. SAMAROVA, D. VORONTZOV, Y. POPOVA*, R. BOYLE* Institute of Higher Nervous Activity and Neurophysiology. Russian Academy of Sciences, Butlerova 5A, 117485. Moscow, Russia and *NASA BioVIS Center, Ames Research Center, USA.

In terrestrial gastropod snail *Helix lucorum L.* we studied the changes after a 16-day exposure to microgravity in: behavior, neural responses to adequate motion stimulation. intersensory interactions between the photosensory pathways and the statocyst receptors, and in expression of the HPeP gene in the primary statocyst receptor cells. In behavioral experiments it was found that the latency of body position change to sudden orientation change (flip from horizontal to downwards position) was significantly reduced in the postflight snails. Extracellularly recorded neural responses of the statocyst nerve to adequate motion stimulation in the postflight snails were independent of the motion direction while in the control animals significant differences in responses to different directions were observed. It was possible to distinguish responses of up to 11 primary receptor neurons from the 13 that comprise the statocyst in the snail. No effect of light (intersensory interactions) was observed in postflight snails in the statocyst receptor responses, while in control animals the presence of light significantly modified the responses to different directions of motion. The HPeP gene peptide product is known to be involved in cilia beating control in the snail. Significant differences in the HPeP gene mRNA expression pattern in the statocyst receptor neurons were observed in postflight (24h) and control snails, thus supporting the observed postflight changes in receptor responses to gravitational stimuli. Obtained results confirm the possibility to elucidate the influence of microgravity exposure on subcellular structural mechanisms and function of gravireceptors using this simple model animal.

Plans for Foton M-3 Mission "Receptor II"

The basic experimental design developed for M-2 will be followed for M-3 experiments. A major obstacle that all space biologists face in publishing microgravity results obtained from orbital missions in the best journals is the fact that confirmation of findings is often not possible - the study essentially represents an "n" of one. The M-3 project will be an important contribution to the scientific conclusions reached from M-2, and will allow not only a confirmation of the M-2 results, but also an improvement of techniques and expansion of the study. The overall goal is to understand the influence of altered gravity, such as microgravity, and transitions between low to higher level, on the gravi-sensors.. The M-3 studies are important steps in reaching this goal. The snail has proven to be a robust model, easy to maintain, and possesses a nervous system and gravi-sensing statocyst organs that are responsive to the space environment. The Russian members are experts with this model organism, particularly in the field of learning and memory. The animal habitats were successfully flown on M-2 and provided an adequate environment for both juvenile and adult snails despite the limitations of the Foton satellite. Importantly, the snails were active during the mission, and only one adult snail went into a hibernating state. Thus, a successful experimental study on M-3 is not in doubt. We also assume that the return of the animals from M-3 to the host labs will be significantly improved by the modification of the M-3 for a controlled (thrust capabilities) re-entry and a landing at a Russian site. This would be in itself

a *significant* and *an important* enhancement of the projects. Neural readaptation in vertebrates begins immediately upon return to the atmosphere and is well underway if not virtually complete in about 30-48 hours post-flight. In snails it appears to be delayed as evident from our M-2 results. The early return of the animals to the lab would permit more thorough and extensive experimentation, and the examination of the specimens in the more critical hours after landing.

All experiments are conducted at the Institute of Higher Nervous Activity and Neurophysiology. Preflight control experiments are performed to verify the experimental protocol such as sequencing of tests, correct if necessary technical or instrumentation issues, and sharpen the skills. Experiments continue during the flight as needed to establish baselines and benchmarks. Post-flight experiments are conducted, followed by the delayed (2-3 days) flight temperature control experiments. Preliminary survey of the data is made with the entire science team in Moscow, and the thorough data analysis will be performed in Moscow and at NASA Ames.

M-3 snails will be separated as before into two groups: juvenile snails for the antibody and gene expression labeling studies and the adult snails for the behavioral and electrophysiological experiments. The gene expression techniques have worked as expected in the Russian lab, and they now have the antibody staining techniques perfected (the antibody labeling was unsuccessful at the time of the M-2 landing). The M-3 experiments will be performed to improve the reliability (see Table 1) of the M-2 results and extend the antibody labeling for functional verification of the upregulation in petal peptide gene expression. We are currently exploring the option to look for other peptides expressed to reveal the specificity of different statocyst neurons. It is thought that each of the 13 neurons has a specific intracellular signature, as in a mosaic arrangement of different neurotransmitters. In this case immunocytochemistry techniques will be used on one of the pair of statocysts. Along the same lines we will determine whether this specificity can be linked to intracellular interconnections between individual cells before/after the flight using intracellular recording or using real-time intracellular calcium imaging techniques. The additional behavioral experiments from M-3 are needed to quantitate the changes in negative gravitaxis response (see Figs. 1 and 2). In addition these tests take little time and provide a behavioral reference from which to evaluate the other results. We are discussing adding another paradigm to measure both the speed of behavioral response to negative gravitaxis with a positive response to chemical stimuli (acetone/carrot). Differences here might reflect functional disturbances of the spatial orientation system. The results of inter-sensory interaction between gravity- and photo sensor showed that there is significant difference in effect of light on background firing rate in control and post-flight snails, but differences in the effects of light on normalized responses in control and post-flight animals are absent! The M-3 data will validate these observations. We will repeat the voltage recordings and calcium signaling in individual receptors to tilt stimulation. It is essential that we obtain a larger sample of statoreceptors recorded simultaneously. We are currently testing the non-toxic delivery mechanism by Gene Tools (Endo-Porter) to transport the dye into the entire population. If successful, then the entire statoreceptor pool can be tested simultaneously to the natural tilt stimuli shortly after return. If the delivery mechanism proves less than reliable in control studies, we will intracellularly label using the glass microelectrode (proven technique, see Fig. 6) in quick succession up to 6-8 individual statoreceptors in the M-3 snails.

FOTON M-2 EXPERIMENT MANAGEMENT PLAN

A. EXPERIMENT TITLE; "RECEPTOR" Neural Preadaptation of Crayfish Statolith

Receptors to Earth's Gravity Following Return from Space.

B. INVESTIGATORS/RESPONSIBILITIES:

Russian: Glvl I. Gorgiladze, Dr. Scl. (Biol.), RF SRC-Institute of Biomedical Problems, Russian Academy of Sciences

USA: Richard Boyle, Ph.D., NASA Ames Research Center

C. OBJECTIVE AND HYPOTHESIS:

In-flight recordings would be highly desired but the post-flight study of readaptation is of particular merit, and would be complementary to our past Shuttle missions. Once the crayfish are retrieved, we will Identify the presumed changes that occurred In the statocyst organ as a result of the exposure to mlcrogravity, and following the readaptation of the neural system to the normal 1 g environment.

Only recently did we learn that adaptation of physiological response in the gravltolnertial sensing organs can occur rapidly In an organism In direct response to a change In gravitational force (Boyle R, Menslnger AF, Yoshlda K, Usui S, Intravaia A, Tricas T, and Highsteln SM. Neural readaptation to 1G following return from space. *J. Neurophyslol.,* 86: 2118-2122, 2001). Within the first day after STS-90 and STS-95 shuttle landing, a hypersensitivity was observed. The magnitude of response of toadflsh utricular afferents to applied translations was enhanced on average three-times greater than for controls. The reduced gravity in orbit apparently resulted In an upregulation of afferent sensitivity. The time course of return to normal afferent sensitivity parallels the reported decrease In vestibular disorientation in astronauts after return from space. While our data do not pinpoint a mechanism, the observed changes are assumed limited to a few possibilities: a) an increase In the sensitivity of the transducer, b) a temporary structural alteration affecting the mechanoreceptlon of the otolith, or otolith-stereociliary coupling that causes an enhanced bundle deflection for a given movement, or c) a pre- or post-synaptic alteration in the strength of synaptic transmission. Since the number of synaptic ribbons in certain type II hair cells In rodent is labile, increasing following exposure to microgravlty (Ross MD. Changes In ribbon synapses and rough endoplasmic reticulum of rat utricular macular hair cells in weightlessness. *Acta Otolaryngol,* 120: 490-499, 2000), an increase In number of synaptic ribbons In toadflsh otolith hair cells following exposure to microgravity could potentially explain the present results.

Aim: The electrophysiological recordings taken at different Intervals during the re-adaptation following space exposure will be used to track changes In afferent/receptor

sensitivity. Hypothesis: The re-adaptatlon during the transition from uG to 1G conditions changes the gravltoinertial sensitivity of utricular otolith afferents.

D. FLIGHT EXPERIMENT:

1. Overview

Experimental Protocol: Upon landing the crayfish will be prepared for electrophysiological experiments. The electrical activity of individual afferent fibers supplying the statocyst on one or both sides will be recorded using routine techniques. The afferent response modulation to controlled Inertlal acceleration along the z-axls parallel to the earth's vertical will be examined In both the amplitude and frequency domains at progressive time intervals following landing. A sufficient sample of afferent fibers will be examined In each crayfish (e.g. 20-30 fibers) over a roughly 3-hour period for each recording session, A separate experiment will begin, followed progressively by each crayfish. The recording sessions will then be repeated In each crayfish to provide another sample of afferents at a new time. Tissue will be extracted at the end of the experiment where possible for evaluation of the mechanoreceptor to afferent synaptic junction at both the scanning and transmission electron microscopic levels.

Our team has the expertise from the STS-90 and -95 missions, we published our results in the Journal of Neurophysiology (see above), and we have all the necessary hardware and software In place, operational, and available for the project.

2. Animal/Specimen Requirements:

3. Data Requirements:

In-flight recordings would be high desired but unlikely

4. Equipment Requirements:

Electrophysiological equipment for single fiber recordings, acceleration platform, arbitrary function generator, data acquisition system, computer. Preferably: 110V, 60 Hz line. Transformer 220/110 needed, with 50 to 60 Hz conversion desired. - this needs to be determined

General supplies associated with crayfish, Including crayfish.

We recently constructed an acceleration platform to study the neural responses of fish lagenar afferents to control vertical accelerations and vibrations. The device is ideal for the study of readaptation of crayfish statocyst afferents to 1 g following the exposure to a space mission. The acceleration system consists of a 40 pounds peak sine force permanent magnet shaker with a 2.5 cm stroke (displacement), with a frequency range of DC to 6.5 kHz, attached to base plate; the base plate is supported by four linear bearings and pneumatic lifts. An air-cooled, direct-coupled audio amplifier drives the shaker. Multi-axis micromanipulators are attached by posts to the base plate to allow glass (preferably) or metal electrodes to penetrate the nerve for responding the electrical activity of Individual nerve afferent fibers. All neurophyslologlcal equipment Is available, including amplifiers, buffer amplifiers, oscilloscopes, mlcropipette pullers, etc. Afferent activity and signals from

accelerometers attached to the base plate are digitized using an external computer interface (CED 1401 Plus) and recorded using Spike2 acquisition software on a Pentium PC. The data are analyzed using routines already written In WaveMetrics Igor.

The electrophysiological equipment and data acquisition system run under 120V (60Hz). The most sensitive Instrument is the Intracellular amplifier and It can operate on 220 V (50 Hz). It is recommended to have a 220-110 V transformer and preferably a 50-60 Hz converted. All equipment is commercially available worldwide, the lab-defined software Is written in C-llke language and has no commercial or proprietary value.

5. Preflight Procedures:

Conduct separate control experiments to establish the population response of statocyst afferents to inertlal accelerations. Control experiments can be performed both In the Ames lab in California, In the host laboratory In Moscow, or both. It Is important that the Pis team early in the process, and conduct experiments together to ensure the scientific objectives are met.

6. Flight procedures.

To be coordinated by Russian Pis.

7. Postflight Procedures.

Conduct experiments. See Overview above.

E. CONTROL EXPERIMENT(S):

The experiments are conducted on non-flight animals following same protocols - see Overview.

F. PRELAUNCH EXPERIMENTAL VERIFICATION TESTING:

1. U.S. Tests

Currently, there are 2 aquaria in use for the toadflsh projects. One could be converted for freshwater crayfish use; however, It is best to obtain a simple, self-contained freshwater system. We will need to do preliminary experiments to Identify the recording requirements In this Invertebrate to ensure all is appropriate for the recording sessions after M~2 landing. At Ames we can perform the necessary control experiments before and after the launch to establish the required benchmarks upon which to correlate the results obtained from the space crayfish.

2. Russian Tests

The Russian team led by Dr. Gorglladze Is skilled in the dissection to visualize the statolith nerve In the crayfish. It would be extremely Important to spend some time with the Russian team to finalize the dissection procedures and the experimental protocol. With that information the US PI can determine the equipment needs and analytic procedures to enhance the experimental results.

3. Integrated Tests & Baseline Data Collection

It is desired to conduct experimsnts on designated equipment before shipping to Russia to verify performance and confirm system Integration.

G. SPECIMEN COLLECTION AND LABELING PROCEDURES: To be coordinated by Russian Pis.

H. ANIMAL PREPARATION/TEST PROCEDURES:

1. Chemicals or Drugs used: State dosage, route of administration, timeline, hazardous (I.e., radioactive etc.)

To be coordinated by Russian Pis. This will be minimal.

2. Incompatibilities for experiment: (I.e., steroids given prior to Immunological assays will affect experiment results)

To be coordinated by Russian Pis. This should not be an Issue. I. DATA SHEET AND/OR FLOW SHEET; if applicable

J. DATA TRANSFER & ANALYSIS REQUIREMENTS/PROCEDURES:

1. Data Recording: To be determined after site visit, US PI has the necessary data acquisition system.

2. On-site Data Analysis: To be determined after site visit. US PI has the necessary data analysis tools.

K. PHOTOS/DIAGRAMS: As needed for publication.

ATTACHMENT 4

FOTON-M-2 EXPERIMENT MANAGEMENT PLAN

1. **EXPERIMENT TITLE: "RECEPTOR-F2"**
   Structure and Function of the Snail Statocyst System after a 16-Day Flight on Foton-**M-2**

2. **PRINCIPAL INVESTIGATORS:**
   From Russia: Dr. Pavel M. Balaban, Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences
   From US: Dr. Richard Boyle, NASA Bio VIS Center, Ames Research Center

3. **CO-INVESTIGATORS:**
   From Russia: Dr. Alexei Y. Malyshev, Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences
   Dr. Igor S. Zakharov, Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences

4. **OBJECTIVES:**
   During the periods of adaptation to μG and re-adaptation upon return to Earth, it is evident that changes occur in the neural systems responsible for processing the

gravitational information. These neural elements provide input to motor pathways and also interact with other sensory pathways, forming a basis for sensorimotor coordination. We will fill critical gaps in our understanding of the neural response to transitions between gravity levels with two specific aims.

1. Determine the regulation of expression of the preproHPep gene (gene that is expressed in the primary statocyst receptor cells) as a consequence of space flight and during the re-adaptation to Earth's gravity. Regulation of this gene might signal how the statocyst receptor is "tuned" by the gravity vector.
2. Specify the changes in excitability of the gravireceptors of the statocyst organ during the readaptation period following space flight. Dorsal and ventral receptors cells exist in the snail statocyst organ, and it has been shown that these cells differ in their response properties. We will use control versus flight animals (inter-group) and dorsal versus ventral receptor cells (intra-group) and apply electrophysiological techniques and optical imaging of time-dependent [Ca^{2+}] signaling to study:

a) the potential changes in intersensory interaction between the photosensory and olfactory pathways and the statocyst receptors. It is known that strong intersensory interactions exist in the snail nervous system. In humans intersensory interactions provide the basis for sensorimotor coordination, and it is known that the microgravity environment can adversely modify sensorimotor coordination in humans.

b) determine the mechanical and electrical excitability of the statocyst receptor during the readaptation period. We will record the response of the statocyst receptor to adequate motion stimulation and measure the same cell's membrane excitability to electrical depolarization pulses. These tests will help determine the underlying mechanism(s) responsibility for a change in excitability of the gravireceptors.

c) We will measure the changes in the internal Ca^{2+} concentration in the statocyst receptors in response to mechanical stimulation. The cell's response to intracellularly-induced action potentials will be used as a calibration tool. This combined approach of conventional electrophysiology and optical imaging of somatic Ca^{2+}measures will provide direct evidence of the cellular event occurring during the readaptation process.

5. BACKGROUND/HYPOTHESES:

After return to Earth many astronauts/cosmonauts developed sensations that can be attributed to gravireceptor dysfunction, e.g., illusionary feelings, vertigo, nausea and vomiting, gaze fixation disorder and ocular nystagmus. In addition to adverse sensations it is well known that human sensorimotor capabilities are adversely influenced during the microgravity exposure, and efficient terrestrial locomotion is impaired immediately following space flight (Bloomberg et al., 1998). Our hypothesis is that space flight in microgravity challenges the gravireceptors and causes changes in their cellular function, that these changes are manifested in the regulation of specific gene expression, in the electrical behavior of the receptors and in the integration of multi-sensory events, and that re-adaptation to 1 g is a time process that can be tracked by intracellular measurements.

We propose to use land snail *Helix lucorum* Linnaeus (Pulmonata, Gastropoda) as experimental specimens. These snails are small enough to allow an adequate sample population within the available habitat space and they have demonstrated their reliability as a model organism - snails have flown on shuttle, MIR and ISS missions in the past. Exposure to microgravity has been linked to an increased growth of the statoconia in Helix (Gorgiladze 2002), similar to the reported results in another species of snail and mollusks (Wiederhold et al., 1997), xenopus (Lychakov 1991) and the swordtail fish (Wiederhold et al., 2000). In Helix following a 163 day flight on Mir station the background discharge activity of the statocyst cell increased and its responses to a step-like static stimulation changed (Gorgiladze et al., 2002).

In order to reach the goal, we have to investigate the statocyst receptor function as a consequence of spaceflight, in particular the re-adaptation phases. We will address this question using different techniques such as molecular gene expression, electrophysiology and optical imaging of intracellular calcium signal.

Pedal peptide was first identified by Lloyd in sea slug *Aplysia californica* (Lloyd and Connolly, 1989). This peptide was purified and sequenced from extracts of pedal ganglia and later was found to be widely distributed throughout the nervous system of this animal (Pearson and Lloyd, 1989). Later a similar peptide was found in the nudibranch mollusk *Tritonia diomedea* (Lloyd et al., 1996) and the pteropod mollusk *Clione limacinta* (Malyshevet al., 1999). The precise role of this peptide in the nervous system is still unknown. However, it was been shown that pedal peptide modulated ciliary beat frequency of epithelial cells in *Tritonia* (Willows et al., 1997), modulated foot muscle contractions in *Aplysia* (Hall and Lloyd, 1990) and accelerated heart contractions in *Clione* (Malyshev et al., 1999). In 1997 the gene encoding pedal peptide was identified in terrestrial snail *Helix lucorum* in our laboratory (Poteryaev et al., 1997). According to *in-situ* hybridization data this gene is widely expressed in the nervous system of *Helix* and as well in some statoreceptor hair cells. Taking into account known effect of pedal peptide on cilia beating and muscle contraction we can assume that it might play an important role in regulation of functioning of hair cells of statocyst (for instance, pedal peptide could be involved in regulation of actin-myosin complex of the hair cells's cilia) and expression of gene encoding this peptide may be changed after microgravity conditions.

Sensorimotor integration is fundamental to posture and movement control, spatial orientation, locomotion, object manipulation, and vestibular sensation. Critical tasks associated with multi-sensory and multi-system interactions and integration include: control of landing and docking, unaided egress, and extravehicular activity. It is of particular relevance to determine how intersensory integration is affected by microgravity conditions and to determine the conditions for adaptive recovery. Our ability to design specific and targeted countermeasures, e.g. altered or artificial gravity paradigms or other external interventions, to help the crew live safely and perform tasks in space and to transition effectively back into a gravity environment is aided by basic science rationale and an increased understanding of the cellular mechanisms underlying the adaptive and re-adaptive responses of the equilibrium organ. Intersensory interactions in the CNS of the Helix has been extensively studied in Moscow partner laboratory by A. Ovchinnikov, and particularly the chemosensory, photic and vestibular pathways (Morphologic features of the chemosensory, visual and vestibular pathways of Helix lucorum. Neirofiziologiia.18: 7-16, 1986; Intersensory interactions in the CNS of Helix lucorum. [Intersensory interactions in the CNS of Helix lucorum. Neirofiziologiia. 18:17-26, 1986; Hair cell interactions in the statocyst of the snail.

Neirofiziologiia. 17:230-9, 1985). Polymodal sensory input to command neurons in Helix has also been studied by Balaban and Zakharov (1982). Recently, a clear demonstration was given by Sakakibara and coworkers of intersensory integration between the statocyst and photic systems in the pond snail Lymnaea stagnalis (Neurosci. Lett. 337: 46-50, 2003; J. Neurophysiol. 93: 493-507, 2005). Lymnaea can be classically conditioned by paring light pulses with a rotational stimulus, and demonstrated that sensory information for associative learning converges on the statocyst hair cell; further, a difference in response, depolarization or hyperpolarization, is observed for caudal or rostral hair cells, respectively. The statocyst neurons in Helix also respond to photic stimulation (A. Malyshev. unpublished observations). We will use the model and plan to study the intersensory integration between the staotcyst, photovisual and the olfactory systems during the postflight readaptation process.

Only recently did we learn that adaptation of physiological response in the gravito-inertial sensing organs can occur rapidly in an organism in direct response to a change in gravitational force (Boyle R, Mensinger AF, Yoshida K, Usui S, Intravaia A, Tricas T, and Highstein SM. Neural readaptation to 1G following return from space. *J. Neurophysiol*, 86: 2118-2122, 2001). Within the first day after STS-90 and STS-95 shuttle landing, hypersensitivity was observed. The magnitude of response of toadfish *(Opsanus tau)* utricular afferents to applied translations was enhanced on average three-times greater than for controls. The reduced gravity in orbit apparently resulted in an up-regulation of afferent sensitivity. The time course of return to normal afferent sensitivity parallels the reported decrease in vestibular disorientation in astronauts after return from space. While our data do not pinpoint a mechanism, the observed changes are assumed limited to a few possibilities: a) an increase in the sensitivity of the transducer, b) a temporary structural alteration affecting the mechanoreception of the otolith, or otolith-stereociliary coupling that causes an enhanced bundle deflection for a given movement, or c) a pre- or post-synaptic alteration in the strength of synaptic transmission. Since the number of synaptic ribbons in certain type II hair cells in rodent is labile, increasing following exposure to microgravity (Ross MD. Changes in ribbon synapses and rough endoplasmic reticulum of rat utricular macular hair cells in weightlessness. *Acta Otolaryngol.* 120: 490-499, 2000), an increase in number of synaptic ribbons in toadfish otolith hair cells following exposure to microgravity could potentially explain the present results.

We will evaluate the re-adaptation process in the *Helix* from microgravity to 1 g conditions with respect to that observed in the vertebrate nervous system. The question is then: “Is there an upregulation of the receptor sensitivity in the invertebrate *Helix* as a result of exposure to the microgravity environment?” If this question is validated, then both invertebrates and vertebrates might use a common mechanism to compensate for transitions between gravity states.

6. FLIGHT EXPERIMENT:

1. Overview:

Twenty-four (24) snails, *H lucorum,* will be used to evaluate the re-adaptation of the statocyst system to lg. The objectives are to determine the gene expression of the preproHelPep gene (gene that is expressed in the primary statocyst receptor cells) and to specify the function of the statolith receptor and their target cells over the re-adaptation process. The cellular function of the statocyst receptors will be studied using three techniques: 1) Intracellular recording from the statocyst receptor itself in response to mechanical stimulation; 2) extracellular population response of the target cells from the statocyst receptors to mechanical

stimulation; and 3) time-dependent [Ca2+] signals from identified statocyst somas using optical recording techniques.

There will be three (3) separate population of 8 snails:

Group 1: Gene Expression will be done with four (4) snails at two (2) time points (total 8 snails). The time points are: recovery +10 hours and recovery +72 hours.

Group 2: Electrophysiology and intersensory integration studies will be conducted on four (4) snails at 2 time points (total 8 snails). The time points are: recovery +10 hours and recovery +48 hours.

Group 2: Electrophysiology and time-dependent [Ca2+] measurements will be conducted on four (4) snails at 2 time points (total 8 snails). The time points are: recovery +10 hours and recovery +72 hours.

Group 1: Whole-mount in-situ hybridization.

Animals will be anesthetized by injection of isotonic MgCb (about 15% of the animal weight). The central ganglionic ring will be removed from the animal and pinned to a Sylgard-coated dish. After fixation for 2 h in 4% paraformaldehyde at room temperature (15-20°C) connective tissue sheath will be removed from preparation using fine forceps and scissors. Preparations will be dehydrated and then rehydrated (to increase tissue permeability) by sequential incubation in 3:1 1:1 and 1:3 PTW/methanol solutions. The preparations will be treated next with 10 μg/ml proteinase K solution, post-fixed in 4% paraformaldehyde, washed in glycine and PTW, treated with 1% hydroxylammoniumchloride and prehybridized 6-8 h in hybridization buffer. Tissues will be then moved to hybridization buffer containing digoxigenin-labeled RNA probe and hybridized for 12-14 h. Preparation will be washed thoroughly, incubated in 10% heat-inactivated sheep serum and then incubated in anti-digoxigenin antibodies conjugated to alkaline phosphatase for 12-14 h. Then tissues will be washed and incubated in substrate for alkaline phosphatase in the dark. The alkaline phosphatase substrate gives a deep-purple reaction product. Reaction will be stopped after visual inspection with Tris-EDTA buffer. Preparations will be then cleared in xylene, mounted in Permount and viewed under the light microscope.

Group 2: Electrophysiology studies on intersensory interaction.

Animals will be anesthetized by injection of isotonic $MgCl_2$ (about 15% of the animal weight). The central ganglionic ring and upper tentacles (carrying photo- and chemoreceptors) with connecting nerves will be removed from the animal and placed into a Sylgard-coated two-compartment recording chamber. Ganglia and tentacles will be separated by a wall with holes for the nerves. Connective tissue sheath from the ganglia will be partially removed using fine forceps and scissors. In order to facilitate further desheathing, ganglia will be treated with Protease (1 mg/ml) (Type XIV, SIGMA, USA) for 10 min at room temperature, washed out and then the fine sheath will be completely removed. The CNS will be bathed in a solution containing (in mM): 100 NaCl, 4 KC1, 7 $CaCl_2$, 5 $MgCl_2$, and 10 Tris-HCl buffer (pH 7.8). Extracellular recording from the vestibular nerve will be made by placing the loop of the nerve into small isolated chamber with AgCl electrode separated from the main compartment with

Vaseline wall. Intracellular recording from the hair cells of statocyst will be made using standard electrophysiological techniques. Individual hair cells will be penetrated with glass microelectrodes filled with 2M potassium acetate (tip resistance 50-80 MOhm). Signals will be amplified (BRAMP-01R for intracellular and EXT 10-2F for extracellular, both from NPI, Germany), digitized and stored on computer (Digidata 1200A A/D converter and Axoscope 8.0 software, both from Axon Instruments, USA).

Responses of statoreceptors will be recorded intra- and extracellulary to the stimulation of tentacles by light and application of various chemicals (carrot juice and quinine solution). In control animals stimulation of both photo- and chemoreceptors induced depolarization and increasing of the spontaneous firing rate in statocyst hair cells.

Group 3: Electrophysiology and optical imaging of intracellular calcium signaling.

Snails will be cooled, injected with an isotonic solution of MgCl2 to minimize pain and dissected to expose the statocysts bilaterally and the central nervous system. One to two specific statocyst receptor cells will partially desheathed and injected with a calcium sensitive dye. The dye consists of 5% Oregon Green 488 BAPTA 1 (Molecular Probes, USA) dissolved in 3 ul of 20% Pluronic F127 in DMSO and in 0.3 ml of 0.1M KC1, and filtered through a 0.22 urn membrane. The filled neurons will be used for simultaneous intracellular recording and optical recording. Conventional intracellular techniques using KCl-filled glass microelectrodes will be used to record the background and induced electrical activity of the statocyst receptor cells. Multi-axis micromanipulators are mounted on the microscope stage and controlled remotely or manually. The fluorecsent Olvmpus BX1W51 microscope is equipped with a charge-coupled device camera (NeuroCCD-SM camera and operated with RedShirtlmaging, USA). Typical readout rates are 40 frames per second (can be increased to 1000 f/s). Recordings of membrane potential changes of the statocyst receptor cells and the fluorescence intensity changes are synchronized and stored as a single file. Fluorescence changes of Oregon Green are measured with single wavelength excitation (470 ± 20 nm) and emission >510 nm. Ca^{2+} concentration changes are expressed as deltaF/F, where F is the fluorescence intensity when the cell is at rest, and deltaF is the change in fluorescence during activity. The time course of responses will be corrected for bleaching using a linear regression computed through the mean values 60 sec before the stimulation and by subtracting the extrapolated values. Mechanical stimulation of the statocyst organ will be made using computer-controlled oscillations of the recording optical bench along the Earth's horizontal. The optical/electrophysiology setup is positioned atop a thrust bearing and thus the oscillations can be varied to range from pitch to roll by manual rotation of the device. Stimuli will be static displacements of ±20-30° and dynamic displacements centered about a static level. Mechanical stimuli will be delivered at supra-threshold intensity to activate the statocyst receptors, for a duration of 3-5 minutes, and followed by intracellular optical imaging. Therefore, we will apply electrophysiological recording and optical imaging techniques to track the statocyst response to intermittent mechanical stimulation at different periods following return to earth (during the readaptation process). A similar paradigm will be used to record the population response from the target neurons of the statocyst receptors. The neurons will be identified by their synaptic response to electrical pulses applied to the statocyst nerve.

7. CONTROL EXPERIMENTS:

A delayed synchronous control experiment will be performed on snails confined in a replicant snail habitat and exposed to a temperature comparable to that onboard the spacecraft. Laboratory controls will serve as the benchmark measures for the flight and the delayed synchronous controls.

8. PRELAUNCH EXPERIMENTAL VERIFICATION TESTING:

1. Russian Tests: Perform preliminary experiments to validate the statocyst receptor response to acceleration and photic stimulation.

2. U.S. Tests:
US PI will modify the existing equipment as needed, including software routines.

3. Integrated Russian and US Tests:
A verification test will be performed in Moscow including U.S. supplied equipment such as an amplifier, data acquisition system and mechanical accuator with the participation of the US Investigator to establish the threshold level of acceleration that the snails can detect. Final procedures will be modified as needed after the verification test. The US investigator will also participate in the experiment in Moscow peri- and post-flight.

9. SPECIMEN COLLECTION AND LABELING PROCEDURES:
1) After euthanasia the snails of group 1 will be dissected, tissue extracted and protocols performed to evaluation gene expression. These procedures will be performed in Moscow.
2) The electrophysiology and imaging studies of snails of groups 2-3 will be performed on the postflight snails at the Institute oCHNA.

10. SPECIMEN PREPARATION/TEST PROCEDURES:
Young specimens *ofH. lucorum* will be supplied by the IHNA laboratory. Specimens will be selected and separated into 2 groups based on size.

11. DATA SHEET AND/OR FLOW SHEET:
TBD

12. DATA TRANSFER AND ANALYSIS REQUIREMENT/PROCEDURES:
1. Data Recording: To be determined after site visit. Russian and US Pis have the necessary data acquisition systems.
2. On-site Data Analysis: To be determined after site visit. Russian and US Pis have the necessary data analysis tools, the Russian PI has the imaging software, and the US PI has mechanical stimulation software.

13. PHOTOS/DIAGRAMS:
not available

For the US side: For the Russian side:

Dr. Richard D. Boyle Dr. Pavel M. Balaban

2. Specimen Requirements:

Specimens *of Helix lucorum* Linnaeus (Pulmonata, Gastropoda) obtained from the Institute of Higher Nervous Activity and Neurophysiology will be used. Twenty-four (24) snails will be selected for flight and from the same pool of candidates one additional group of 24 will be selected for delayed synchronous control. Sixteen (16) snails of groups 2-3 will be large (4-5 cm in diameter; approximately 5 years old) for the electrophysiology studies and 8 smaller snails (3 cm in diameter approximately 3-4 years old) will be used for anatomical studies of group 1 snails. A comparable number of laboratory control snails will be used to provide benchmark measures.

3. Data Requirements:

No in-flight data requirements. Temperature measures are expected to serve as input to ground delayed synchronous control experiments.

4. Equipment Requirements:

- Snail habitat. (Russian provided)
- Biopotential amplifier with a power supply (US provided)
- Software for data processing (US provided)
- PC-compatible computer system with interface to CED peripheral system (US provided)
- Data acquisition system (e.g. CED 1401 Plus). (US provided)
- Optical imaging of intracellular calcium signal, including fluorescent microscope equipped with a charge-coupled device camera. (Russian provided)
- Electrophysiological instruments for single cell recordings. (US and Russian provided)
- Data acquisition software (Spike2) that is easily configured to sample analog signals and digital event inputs. Data analysis is performed using WaveMetrics Igor and user-defined analysis routines are already written. (US provided)
- An acceleration system consisting of a 40 pounds peak sine force permanent magnet shaker with a 2.5 cm stroke (displacement), with a frequency range of DC to 6.5 kHz, attached to base plate; the base plate is supported by four linear bearings. An air-cooled, direct-coupled audio amplifier drives the shaker. (US provided)

5. Pre-flight Procedures:

None. The snails are group housed in the Russian snail habitat.

6. In-flight Procedures:

The air temperature to be telemetrically monitored is expected to be in the range 20-30° C.

7. Post-flight Procedures:

ПЛАН ПРОВЕДЕНИЯ ЭКСПЕРИМЕНТА "РЕЦЕПТОР" ПО ПРОЕКТУ "ФОТОН"-М-2

1. НАЗВАНИЕ ЭКСПЕРИМЕНТА: "Рецептор"; Реадаптация рецепторов статоциста пресноводных раков к земной гравитации после космического полета

2. ОТВЕТСТВЕННЫЙ ИСПОЛНИТЕЛЬ:

От России: Д-р Г. И. Горгиладзе, ГНЦ РФ Институт медико-биологических проблем РАН

От США: Д-р Ричард Бойль, Эймсский исследовательский центр НАСА

3. ЗАДАЧИ И ГИПОТЕЗЫ:

Регистрация нервных импульсов в полете была бы крайне желательной, однако особое значение имеет послеполетное исследование процесса реадаптации, результаты которого явятся дополнением к данным, полученным нами ранее в полетах КК Шаттл. После возвращения биообъектов мы хотели бы исследовать предполагаемые изменения в статоцистах, возникшие в результате пребывания в условиях невесомости, и реакции, развивающиеся в процессе реадаптации нервной системы к земной гравитации.

Совсем недавно мы установили, что адаптация физиологических реакций в гравитоинерциальных анализаторах может проявляться достаточно быстро как прямой ответ на изменение гравитационных сил (Boyle R, Mensinger AF, Yoshida K, Usui S, Intravaia A, Tricas T, and Highstein SM. Neural readaptation to 1G following return from space. *J, Neurophysiol,* 86: 2118-2122, 2001). В течение первых суток после приземления КК СТС-90 и СТС-95 мы наблюдали гиперчувствительность. Величина ответной реакции афферентов утрикулюса рыбы-жабы (Opsanus tau)На вертикальные колебания возрастала в среднем в три раза по сравнению с контролем. По всей видимости, пребывание в условиях пониженной гравитации в орбитальном полете приводило к усилению афферентной чувствительности. Динамика восстановления нормальной чувствительности афферентов соответствует наблюдаемому у астронавтов ослаблению вестибулярных нарушений после космического полета. Хотя полученные нами данные не позволяют точно установить механизм описанных изменений, они свидетельствуют об ограниченном числе возможных причин: а) усиление чувствительности трансдуктора, б) преходящее структурное изменение, оказывающее влияние на механорецепцию отолитового органа или на отолит-стереоцилиарное взаимодействие, которое обусловливает более выраженное отклонение пучка при конкретном движении, и в) пред- и пост-синаптическое изменение силы синаптической передачи. Известно, что число синаптических полосок в определенных волосковых клетках II типа у грызунов легко изменяется, а после воздействия невесомости увеличивается (Ross MD. Changes in ribbon synapses and rough endoplasmic reticulum of rat utricular macular hair cells in weightlessness. *Acta Otolaryngol.* 120: 490-499, 2000). Можно поэтому предположить, что указанные выше изменения объясняются увеличением числа синаптических полосок в волосковых клетках отолитового органа рыбы-жабы после пребывания в условиях невесомости.

Цель: Изучить путем регистрации электрофизиологических сигналов на разных этапах реадаптации после космического полета изменения чувствительности системы афферент/рецептор.

Гипотеза: В процессе реадаптации к земной гравитации после пребывания в условиях микрогравитации изменяется гравитоинерциальная чувствительность афферентов утрикулюса отолитового органа.

4. ПОЛЕТНЫЙ ЭКСПЕРИМЕНТ:

1. Общее описание

Протокол эксперимента: После посадки биоматериал обрабатывают для электрофизиологических исследований. С помощью стандартных методик регистрируют электрическую активность идущих к статоцисту афферентных волокон с одной или двух сторон. В определенные временные интервалы определяют амплитуду и частоту афферентных ответов на дозируемые инерциальные ускорения по оси Z, ориентированной параллельно земной вертикали. Для каждого сеанса регистрации исследуют достаточное количество афферентных волокон (например, по 20-30), Затем выполняют новый эксперимент, повторяя регистрацию электрофизиологических сигналов с тем, чтобы получить еще одну выборку афферентных ответов в другой отрезок времени. По окончании регистрации выделяют ткани для исследования соединения механорецептора с синапсом афферента в сканирующем и трансмиссионном микроскопах,

Наша лаборатория накопила опыт работы в экспериментах, выполненных в полетах СТС-90 и СТС-95, и опубликовала полученные результаты в упомянутой выше статье *(Journal of Neurophysioly).* Лаборатория оснащена всем необходимым оборудованием, включая программное обеспечение, которое мы готовы предоставить для реализации предлагаемого эксперимента.

2. Требования к биообъектам:
3. Требования к регистрируемым данным:

Было бы желательно провести регистрацию электрической активности в полете, но на данном этапе это представляется маловероятным.

4. Требования к научной аппаратуре:

Электрофизиологическая аппаратура для регистрации электрической активности отдельных волокон, стенд для создания вертикальных колебаний, генератор, система регистрации и хранения информации, компьютер. (Наши предпочтения: 110 В, 60 Гц, трансформатор 220/110 с преобразователем 50/60 Гц). Подробные требования к аппаратуре подлежат согласованию.

Недавно мы сконструировали стенд вертикальных колбаний для изучения нейрональных ответов лагенарных афферентов рыб на контролируемые вертикальные ускорения и вибрации. Такой стенд идеально подходит для исследования реадаптации афферентов статоциста раков к земной гравитации после космического полета. Стенд

включает магнитный шейкер, создающий синусоидальные перемещения силой 40 фунтов, амплитудой 25 мм и частотой постоянного тока в пределах до 6, 5 кГц; шейкер соединен с платформой, которая устанавливается на четыре линейных подшипника и пневматические подъемники. Шейкер приводится в действие аудио усилителем с прямым подсоединением и воздушным охлаждением. Мультиаксиальные микроманипуляторы подсоединяются к штырям платформы, что дает возможность проводить стеклянные (что предпочтительно) или металлические электроды в нерв и таким образом регистрировать электрическую активность отдельных нервных волокон афферента. Мы располагаем всем необходимым электрофизиологическим оборудованием, включая усилители, буферные усилители, осциллографы, микропипетки и т.п. Сигналы афферентной активности и акселерометра, подсоединенного к платформе, оцифровываются с помощью внешнего компьютерного интерфейса (CED 1401 Plus) и регистрируются компьютером Pentium PC с использованием программы Spike2. Анализ данных выполняется стандартными методами, описанными в WaveMetrics Igor. Электрофизиологическая аппаратура и система накопления информации работают при 120 В (60 Гц). Наиболее деликатным инструментом является внутриклеточный усилитель, который может работать при 220 В (50 Гц). Желательно иметь трансформатор на 220/110 В и частотный преобразователь 50-60 Гц. Все оборудование имеется в продаже на мировом рынке; специальная программа написана на языке типа С; она не имеет коммерческой ценности и не охраняется правом собственности.

5. Предполетные процедуры:

Контрольные эксперименты проводятся с целью определения популяционных ответов афферентов статоциста на инерциальные ускорения. Такие эксперименты можно провести в нашей лаборатории в Эймсском исследовательском центре, в лаборатории в Москве или в обеих лабораториях. Очень важно, чтобы соисполнители заранее договорились о программе эксперимента и проводили его совместно, стремясь обеспечить максимальную научную отдачу.

6. Полетные процедуры: Подлежат согласованию с российским PI.

7. Послеполетные процедуры: См. Общее описание.

5. КОНТРОЛЬНЫЕ ЭКСПЕРИМЕНТЫ: Контрольные эксперименты проводятся на биообъектах по протоколу, который используется в опытах с полетными раками. См. Общее описание.

6. ПРЕДПОЛЕТНЫЕ ВЕРИФИКАЦИОННЫЕ ИСПЫТАНИЯ:

1. Американские испытания:

В настоящее время мы имеем 2 аквариума, которые мы используем в своих опытах на рыбах-жабах. Один из них можно использовать для экспериментов с пресноводными раками; однако оптимальным решением было бы приобретение простой автономной пресноводной системы. Нам необходимо провести специальные исследования для определения требований по регистрации электрофизиологических сигналов у данного

вида беспозвоночных с тем, чтобы гарантировать успешную регистрацию по окончании полета КК "Фотон"-М-2. В своей лаборатории на базе Эймсского исследовательского центра мы можем выполнить контрольные эксперименты до и после полета с тем, чтобы получить исходные данные для сравнения с данными, полученными у полетных биообъектов.

2. Российские испытания:

Группа российских специалистов» возглавляемая д-ром Горгиладзе, обладает опытом визуализации статолитовых нервных волокон у пресноводных раков. Было бы чрезвычайно полезно поработать некоторое время вместе с российскими специалистами с тем, чтобы окончательно согласовать методы выделения биоматериала и протокол эксперимента. С учетом согласованных решений американский PI сможет определить научную аппаратуру и методы анализа, которые будут способствовать оптимизации результатов эксперимента.

3. Комплексные испытания и регистрация исходных данных:

Желательно провести предварительные эксперименты на предлагаемой научной аппаратуре до отправки в Россию, чтобы проверить ее надежность и возможность интегрирования с российскими системами.

8. ВЗЯТИЕ БИОМАТЕРИАЛА И СПОСОБЫ МАРКИРОВКИ:

Подлежат согласованию с российским PI.

9. ФОРМА РЕГИСТРАЦИИ ДАННЫХ: Подлежит согласованию с российским PI.

10. ТРЕБОВАНИЯ ПО ОБМЕНУ ДАННЫМИ И ИХ АНАЛИЗУ/ МЕТОДЫ:

1. Регистрация данных:

Подлежит согласованию с российским PI. Американские специалисты располагают необходимой системой регистрации и хранения данных.

2. Анализ данных на месте запуска/ посадки:

Подлежит согласованию с российским PI. Американские специалисты располагают необходимыми средствами и методами анализа данных.

11. ФОТОГРАФИРОВАНИЕ/ДИАГРАММЫ: В случае необходимости для публикаций.

ПРЕДЛОЖЕНИЕ К ПРОЕКТУ («БИОН»)

ИССЛЕДОВАНИЕ ИМПУЛЬСНОЙ АКТИВНОСТИ РЕЦЕПТОРОВ СТАТОЦИСТА ПРЕСНОВОДНЫХ РАКОВ В УСЛОВИЯХ НЕВЕСОМОСТИ

Шифр эксперимента « РЕЦЕПТОР»

Цель проекта: Оценка функционального состояния гравирецепторов в невесомости и в периоде реадаптации.

Задачи:

- изучение импульсной активности рецепторов статоциста в покое;
- изучение импульсной активности рецепторов статоциста на вертикальные синусоидальные затухающие колебания;

Вышеуказанные задачи будут решаться в экспериментах, проводимых в лабораторных условиях и в невесомости.

Объем исследований:

- в предполетном периоде: за 3-4 суток до полета в статоцисты пресноводных раков вживляются микроэлектроды для хронической регистрации импульсной электрической активности гравирецепторов.
- в полете: ежедневно три раза в сутки производится регистрация импульсной активности рецепторов статоциста в покое и на вертикальные синусоидальные затухающие колебания
- -в послеполетном периоде: аналогичные полетным исследования проводятся непосредственно после возвращения животных на Землю и далее с определенными интервалами времени в течение 2 месяцев.

ТРЕБОВАНИЯ К АППАРАТУРЕ

1. Для проведения эксперимента «РЕЦЕПТОР» используются:
   - 5 контейнеров, поставляемых в составе, указанном в таблице 1.

Габаритные размеры каждого контейнера не более, мм:
- 80x50x40.

Масса каждого контейнера не более, кг:
- 0,4 кг.
- усилитель биопотенциалов; -накопитель информации;

– механический стенд вертикальных синусоидальных затухающих колебаний амплитудой до 100 мм и частотой 0,1 и 0,5 Гц;

Таблица

Наименование	Количество
1. Контейнер в составе: - контейнер с мембраной - защитная крышка - разъем	5
2. Пресноводный рак	5

НАУЧНОЕ ОБОСНОВАНИЕ ЭКСПЕРИМЕНТА

У многих членов экипажей космических летательных аппаратов наблюдались явления, приписываемые гравирецепторной дисфункции: различного рода иллюзорные ощущения, головокружение, тошнота и рвота, нарушение фиксации взора и нистагм глаз. Не вызывает сомнения, что для понимания причин возникновения указанных расстройств необходимы сведения о функциональном состоянии органа равновесия и в первую очередь ее рецепторной части в условиях невесомости. В доступных информационных источниках отсутствуют сведения об исследованиях, предлагаемых в настоящем техническом задании, а также об аналогичных проектах. Новизна эксперимента состоит в изучении фоновой и вызванной афферентной импульсации и количественных характеристик «стимул-реакция» гравирецепторов в статоцистах ракообразных. Согласно литературным данным статоцист беспозвоночных животных является аналогом вестибулярной системы позвоночных животных и его чувствительные клетки реагируют на изменение положения тела и/или головы в пространстве (J. J. Geuze, 1968; H. Wolff, 1970; Я.А.Винников и др., 1971; В.А.Соколов, С.Н. Ковалев, 1979; С.Н. Ковалев и др., 1981; Г.И.Горгиладзе, 2002, Г.И.Горгиладзе и др., 2001, 2002.).

Объектом исследования выбраны пресноводные раки Procambarus cubensis (т.н. кубинский голубой рак). Они имеют небольшие размеры, чрезвычайно выносливы, хорошо переносят длительное (многомесячное) отсутствие корма. В 1990-91 гг. на станции «Мир» (ЭО-5) в течение 2 месяцев находился аквариум с двумя раками этого

вида. Газообмен обеспечивался наличием на одной из стенок аквариума воздухопроницаемой фторопластовой мембраны, и этот эксперимент был успешно завершен (см. научный отчет МН-9669, ИМБП 1997 г.).

Планируемые исследования предусматривают изучение импульсной активности рецепторов статоциста в покое и на вертикальных синусоидальные затухающие колебания.

Сущность используемых в эксперименте методов состоит в регистрации электрофизиологических реакций гравирецепторов с помощью вживленных в статбцисты микроэлектродов. В качестве критерия оценки используется импульсная электрическая активность рецепторных клеток.

Подготовка эксперимента. Рак наркотизируется в парах эфира. Через небольшое отверстие, проделанное в стенке антеннулы, вводится вольфрамовый микроэлектрод толщиной кончика 10-15 мкм, и с помощью микроманипулятора осторожно продвигается в сторону статоциста, расположенного в углублении ее базального членика. При появлении характерной электрической активности на экране осциллографа в области введения электрода в антеннулу наносится небольшое количество биологического клея «цеакрин», который прочно фиксирует электрод к хитину. К электроду через переходник присоединен электрический провод в водонепроницаемой изоляции. В стенке аквариума вмонтирован разъем, через который сигнал подается на усилитель биопотенциалов. Таким способом имплантированный электрод позволяет в течение длительного времени регистрировать импульсную активность рецепторов статоциста раков в покое и при вертикальных синусоидальных перемещениях. Настоящая методика была отработана нами ранее (см. научный отчет МН-9769, ИМБП **1**997 г.)

Ожидаемые результаты. Результаты исследований позволят понять особенности функционирования гравирецепторов в условиях невесомости.

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Усилитель биопотенциалов

Предназначен для усиления внеклеточной импульсной активности рецепторов статоциста. Технические характеристики усилителя:

- входное сопротивление не менее 100 мОм;
- постоянная времени - 15 мс;
- полоса пропускания - 30 Гц - 1 кГц;
- коэффициент усиления - до 40 000;
- минимальный входной сигнал - 20 мкВ;
- собственный шум усилителя - не более 15 мкВ. Усилитель имеет автономное питание.