PHOTON-M-2 EXPERIMENT MANAGEMENT PLAN

1. EXPERIMENT TITLE: "GECKO"

The Effect of Microgravity on the Morphology and Function of the Nervous System, Skeleton and Endocrine Organs of the Gecko

2. PRINCIPAL INVESTIGATORS:

From Russia: Dr. Sergei V. Savelyev, Institute of Human Morphology, Russian Academy of Medical Sciences

From US: Dr. Eduardo A.C Almeida, University of California, San Francisco, NASA Ames Research Center

3. CO-INVESTIGATORS:

From Russia:

From US: Dr. Ruth Globus, NASA Ames Research Center Dr. Wenonah Vercoutere, NASA Ames Research Center

4. OBJECTIVES:

To perform histological examinations of the central nervous system, peripheral organs of senses (visual, auditory, vestibular, olfactory and vomero-nasal systems), musculoskeletal system (bones, tendons, ligaments), endocrine and reproductive systems of geckos in order to detect cell growth and morphological tissue changes.

5. BACKGROUND/HYPOTHESES:

The experiment provides an opportunity to use amniotes for a comprehensive investigation of the effects of microgravity on morphology and cell proliferation in the the central nervous system, peripheral organs of senses (visual, auditory, vestibular, olfactory and vomero-nasal systems), musculoskeletal system (bones, tendons, ligaments), endocrine and reproductive systems. The flight duration and profile will allow allow us to model the effects of the space environment on mammals using the biological specificities of geckos.

One of our working hypothesis is that a comprehensive examination of the nervous system, skeletal elements and hormonal mechanisms regulating calcium metabolism of geckos will help reconstruct neurological and metabolic changes allowing amniotes to adapt to microgravity.

In addition we propose to examine the proliferation of cells in intact live geckos during the flight. Cell proliferation can be upregulated by increased gravity in-vitro (Almeida et al.). Conversely, microgravity-induced bone and muscle-loss suggest that lack of mechanical stimulation may down-regulate the proliferative rates of osteoprogenitor as well as other somatic stem cells that are responsible for tissue regeneration and maintenance. An additional working hypothesis is that *microgravity mayy decrease the proliferative rates of various somatic stem cells* .

The specific question we ask, is whether or not microgravity and spaceflight slow down the growth of cells that regenerate various human tissues such as bone, muscle, blood, *et cetera.* An understanding of this issue is key to determining how to design countermeasures for microgravity and spaceflight tissue loss. Although drugs that prevent bone-loss may be sufficient to alleviate this specific problem we may have serious additional body-wide tissue regeneration problems still undetected. We hope this study will tell us if 16-day exposure to microgravity affects the growth rates of somatic stem cells in the gecko and will pave the way to design human studies.

6. FLIGHT EXPERIMENT:

A Overview:

We plan to launch 5-7 geckos of the *Hemidactylis tursicus* for a 16-day flight. The animals are on the average 10-12 cm long (with the tail), weighing 3.6 grams each. We expect to use females but it is also possible to have 5 females and 1 male. It is not possible to house more than 1 male in a small-size unit (2.2 liter) because of territoriality. The housing unit contains a 60 ml water bowl equipped with a wick. The humidity level in the unit is also high to reduce water requirements of the enclosed animals. No food is provided. No specific lighting requirements, except that the illumination, if any, should be dim because geckos are nocturnal animals and bright light can be stressful. In the natural environment geckos are active at 30-32 deg C in the daytime and 24 deg C at night. During flight the temperature range of 25-28 deg C is acceptable. It is important to note that a long exposure of geckos to a temperature below 15 deg C or above 35 deg C may be lethal. We required that the hole in the housing unit bottom be covered with a small-mesh net to protect the animals.

Our experimental procedure involves the administration of an immunodetectable nucleotide analog bromodeoxyuridine (brdU) using a time-programmable Alzet osmotic pump inside the water reservoir, The pump will be filled with a brdU solution and a oil "delay" layer of 5 days to accommodate pre-launch loading of the experiment. The osmotic pump will deliver brdU into the drinking water from day 2 of flight through day 16. After landing and recovery we will perform dissection and paraformaldehyde fixation tissue samples for histomorphological studies and measurement of brdU incorporation in specific cell types.

B Animal Requirements:

Geckos have been selected as experimental objects due to the following factors:

- Reptiles are an oldest group of terrestrial vertebrates that are perfectly well adapted to adverse environments. Their dry skin covered with horny scales is essentially devoid of glands, thus providing an excellent mechanical protection and minimal moisture loss. In addition to these advantageous features, geckos are characterized by noticeable metabolic changes, which can be detected even after a short-term 16-day flight.
- Geckos can long survive without food and in a low moisture environment. Another important advantage is that they produce a very small amount of wastes.
- The lower surface of lizard's toes is covered with so-called ampillary rows of enlarged scales ending in microscopic outgrowths (one toe can carry as many as 200 mln of them). These structures allow geckos to grasp miniscule protuberances of off-vertical or vertical surfaces and move along them. It can be assumed that these structures will also work in microgravity and allow geckos to remain attached to the walls of the housing unit.
- Parathyroid calcitonin-secreting glands are similar to those of mammalians but morphologically separated from the thyroid gland. This allows their qualitative and quantitative measurements.

C Data Requirements -

We require that the temperature, humidity and illumination levels be maintained within a constant and optimal range. Tissues to be examined histologically should be isolated and fixed immediately after animal dissection: any delay will impact the quality of immunohistochemical and ultrastructural data. The temperature profile of the flight, g-level, and radiation exposure should be measured and recorded.

D Equipment Requirements: -

At the landing site, upon removal from the capsule, animals need to be photographed to record their condition (photographic recording equipment). After transport to Moscow and within 24hr of recovery we will require dissection microscopes as well as dissection equipment including several sets of precision surgical tools. After flight, for morphological and brdU incorporation studies we will need a microtome, histology equipment and an epifluorescence microscope with digital imaging and recording/data storage capabilities.

E Pre-flight Procedures:

Preliminary studies:

1) Control experiment on cell proliferation rates with imitation of temporal spaceflight parameters. Specifically we will test brdU delivery via the drinking water using osmotic pumps, and brdU as well as PCNA immunostaining of tissues. (Experiment in Russia and in USA with animals provided by Russia). Reagents and supplies provided by USA).

2) Study of organ tissue morphology in control animals (Russia)

3) Study of enriched diet prior to launch to ensure survival for the flight period, specifically we will test several versions of diet to choose the most adequate for a 20 day fast period.

F In-flight Procedures: -

We required that the environmental parameters are maintained within comfortable levels (oxygen, water, temperature, illumination requirements are specified above in experiment overview).

G Post-flight Procedures:

Photo/video to record animal activity upon return to the ground
Animal dissection, biosample isolation and fixation
Histology
Immunohistochemistry of brdU and PCNA
Confocal microscopy of tissue three-dimensional structure
Electron microscopy
Computer-aided data processing, statistical analysis, and review
Isolation of mRNA from selected tissues

After the landing of spacecraft the animals are brought to Moscow alive and are euthanized at the Institute of Human Morphology RAMS. Samples for research are extracted both by NASA scientist(s) and Russian scientists. For each part of the project samples are isolated and fixed by the interested parties. Transportation to USA of tissue samples will be performed by NASA scientists or staff.

7 CONTROL EXPERIMENTS:

Laboratory assyncronous control
Vibration and g lever control (using an IMBP facility)
Hypergravity control (using an IMBP facility)

8 PRELAUNCH EXPERIMENTAL VERIFICATION TESTING:

US/Russian Integrated Testing: Determine optimal amount of cell proliferation/DNA synthesis brdU label in drinking water to use to obtain measurable results after 16 days experiment. Test the use of miniature osmotic pumps to deliver brdU, in the drinking water for rate optimization. Test dissection procedures needed to expedite isolation and fixation of bone and multiple tissue samples. The animal experiment portion of these studies will be performed by Russian scientists. The tissue staining for brdU will be performed by NASA scienists

Russian Testing

We will verify whether the above number of geckos can survive for as long as 20 days in a flight-like environment. We plan to house geckos of the species selected in a flight-type unit with no food and with water supplied by a flight-like system. This experiment will demonstrate whether females only can be used in-flight and whether animals can tolerate a 20-day enclosure without detrimental changes in their behavior and well-being.

US Testing

Optimize type and concentrations of somatic stem cell marker antibodies as well as brdU, and PCNA antibodies labels to use. Tissue labeling testing will be carried out at NASA Ames Research Center using conventional and scanning confocal microscopy and immunofluorescence techniques.

9 SPECIMEN COLLECTION AND LABELING PROCEDURES:

Russian/US Common Procedures

Animal are injected intraperitoneally with Nembutal anhestetic prior to euthanasia
Euthanasia is performed by decapitation
Following euthanasia, carcass kept at 4°C until tissues dissected
Tissues dissected as soon as possible after euthanasia (no later than 1-hr post death)
Tissue type, animal identification number, and group labeled on each tube.

Russian Procedures

Heads isolated and fixed
Limbs are isolated and fixed
Central nervous system, peripheral sense organs (visual, auditory, vestibular, olfactory and vomero-nasal organs), musculoskeletal (bones, tendons, ligaments), endocrine and reproductive systems are isolated and fixed
We will use Bouin's fixative for histology, neutral buffered paraformaldehyde for electron microscopy, and immunochemistry samples. Each biosample is placed in an individual vial and labeled inside and outside.

NASA Procedures

Dissection and partitioning of tissue samples should be done at 4°C.
After excision, we will rinse tissue in PBS and add tissues to tubes of 4% neutral buffered formaldehyde (NBF) fixative at 4°C.
NASA scientists will use the balance of tissue samples not required by Russian scientists (described above in Russian Procedures). This include a portion of all tissues containing pluri and totipotent cells with emphasis on load-bearing long bones and non-load bearing skull bones, skeletal and, heart muscle, testes/ovaries, intestinal wall, liver, kidney, and blood. Samples will be taken from each animal of each condition, including space flight, and ground asynchronous control. Transport back to NASA Ames Research Center in NBF at 4°C (on ice without freezing). Alternatively, histochemistry proliferation samples may be fixed and embedded in paraffin in Russia and carried as embedded tissue blocks to NASA.

10 ANIMAL PREPARATION/ TEST PROCEDURES:

- Flight and control animals are tested for infections and parasites (routine veterinary check)
- For a month prior to launch the animals are housed in a comfortable environment and provided with an enriched diet

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

Delivery of brdU in drinking water. Dosage will be determined to optimize results for a 16-day exposure in geckos. No radioactive labeling will be used.

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

Delay of tissue recovery beyond 1 week post-landing.
Delay of dissection or heating of carcass above room temperature prior to recovering tissues.

9. DATA SHEET AND/OR FLOW SHEET:

- Descriptions
- Photos
- Videos
- Light microscopy images
- Processed data
- Plots

10. DATA TRANSFER AND ANALYSIS REQUIREMENTS:

1. Data Recording

- Photo, video
- Experimental protocols
- Histological and immunohistochemical preparations
- Results of preparation analysis together with video
- Processed metabolic data

2. On-site Data Analysis

- Animal visual examination and description of their status and behavior
- Photo/video

11. PHOTO/ DIAGRAMS:

To be performed after all investigations.

FOTON-M-2 EXPERIMENT MANAGEMENT PLAN

1. EXPERIMENT TITLE: "GECKO-F2"

The Effect of Microgravity on the Morphology and Function of the Nervous System, Skeleton and Endocrine Organs of the Gecko

2. PRINCIPAL INVESTIGATORS:

From Russia: Dr. Sergei V. Savelyev, Institute of Human Morphology, Russian Academy of Medical Sciences

From US: Dr. Eduardo A.C Almeida, NASA Ames Research Center

3. CO-INVESTIGATORS:

From Russia: Dr. Victoria I. Gulimova, Institute of Human Morphology, Russian Academy of Medical Sciences

From US: Dr. Ruth Globus, NASA Ames Research Center

Dr. Wenonah Vercoutere, NASA Ames Research Center

4. OBJECTIVES:

To perform histological examinations of the central nervous system, peripheral organs of senses (visual, auditory, vestibular, olfactory and vomero-nasal systems), musculoskeletal system (bones, tendons, ligaments), endocrine and reproductive systems of geckos in order to detect cell growth and morphological tissue changes.

5. BACKGROUND/HYPOTHESES:

The experiment provides an opportunity to use amniotes for a comprehensive investigation of the effects of microgravity on morphology and cell proliferation in the central nervous system, peripheral organs of senses (visual, auditory, vestibular, olfactory and vomero-nasal systems), musculo-skeletal system (bones, tendons, ligaments), endocrine and reproductive systems. The flight duration and profile will allow us to model the effects of the space environment on vertebrates using the biological specificities of geckos.

One of the Russian PI's working hypotheses is that a comprehensive examination of the nervous system, skeletal elements and hormonal mechanisms regulating calcium metabolism of geckos will help reconstruct neurological and metabolic changes allowing amniotes to adapt to microgravity.

The US PIs propose to examine the proliferation of cells in intact live geckos during the flight. Cell proliferation can be upregulated by increased gravity in-vitro (W. A. Vercoutere, M. Parra, C Roden, M. DaCosta, A. Wing, C Damsky, E. Holton, N. Searby, R. Globus, and E. Almeida. Constant Applied Force Stimulates Osteoblast

Proliferation Via Matrix-Integrin-Signaling Pathways. *Molecular Biology of the Cell*, 2003, Vol. 14, 205a-206a). Conversely, microgravity-induced bone and muscle-loss suggest that lack of mechanical stimulation may down-regulate the proliferative rates of osteoprogenitor as well as other somatic stem cells that are responsible for tissue regeneration and maintenance. An additional working hypothesis is that microgravity may decrease the proliferative rates of various somatic stem cells.

The specific question the US PIs ask, is whether or not microgravity and spaceflight slow down the growth of cells that regenerate various human tissues such as bone, muscle, and blood. An understanding of this issue is key to determining how to design countermeasures for microgravity and spaceflight tissue loss. Although drugs that prevent bone-loss may be sufficient to alleviate this specific problem, we may have serious additional body-wide tissue regeneration problems still undetected. We hope this study will tell us if 16-day exposure to microgravity affects the growth rates

of somatic stem cells in the gecko and will pave the way to design human studies.

6. FLIGHT EXPERIMENT:

1.Overview:

The Russian PIs plan to launch 5-7 geckos of the *Hemidactylis turcicus* for a 16-day flight. The animals are on the average 10-12 cm long (with the tail), weighing 3.6 grams each. We expect to use females but it is also possible to have 5 females and 1 male. It is not possible to house more than 1 male in a small-size unit (2.2 liter) because of territoriality. The housing unit contains a 60 ml water reservoir equipped with a wick. The humidity level in the unit is also high to reduce water requirements of the enclosed animals. No food is provided. No specific lighting requirements, except that the illumination, if any, should be dim because geckos are nocturnal animals and bright light can be stressful. In the natural environment geckos are active at 30-32 deg C in the daytime and 24 deg C at night. During flight the temperature range of 25-28 deg C is acceptable. It is important to note that a long exposure of geckos to a temperature below 15 deg C or above 35 deg C may be lethal. We required that the hole in the housing unit bottom be covered with a small-mesh net to protect the animals.

The US PI's experimental procedure involves the administration of an immunodetectable nucleotide analog bromodeoxyuridine (BrdU) using a time-programmable Alzet osmotic pump inside the water reservoir, The pump will be filled with a BrdU solution and a oil "delay" layer of 5 days to accommodate pre-launch loading of the experiment. The osmotic pump will deliver BrdU into the drinking water from day 2 of flight through day 16. After landing and recovery, dissection and paraformaldehyde fixation tissue samples for histomorphological studies and measurement of BrdU incorporation in specific cell types will be performed.

2.Specimen Requirements:

Geckos have been selected as experimental objects due to the following factors:

- Reptiles are an oldest group of terrestrial vertebrates that are perfectly well adapted to adverse environments. Their dry skin covered with horny scales is essentially devoid of glands, thus providing an excellent mechanical protection and minimal moisture loss. In addition to these advantageous features, geckos are characterized by noticeable metabolic changes, which can be detected even after a short-term 16-day flight.
- Geckos can long survive without food and in a low moisture environment. Another important advantage is that they produce a very small amount of wastes.
- The lower surface of lizard's toes is covered with so-called ampillary rows of enlarged scales ending in microscopic outgrowths (one toe can carry as many as 200 mln of them). These structures allow geckos to grasp miniscule protuberances of off-vertical or vertical surfaces and move along them. It can be assumed that these structures will also work in microgravity and allow geckos to remain attached to the walls of the housing unit.
- Parathyroid calcitonin-secreting glands are similar to those of mammalians but morphologically separated from the thyroid gland. This allows their qualitative and quantitative measurements.

3.Data Requirements:

It is required that the temperature, humidity and illumination levels be maintained within a constant and optimal range. Tissues to be examined histologically should be isolated and fixed immediately after animal dissection: any delay will impact the quality of immunohistochemical and ultrastructural data. The temperature profile of the flight, g-level, and radiation exposure should be measured and recorded.

4.Equipment Requirements:

Russian Responsibility

• At the landing site, upon removal from the capsule, animals need to be photographed to record their condition (photographic recording equipment).

• After transport to Moscow and within 24hr of recovery dissection microscopes will be required.

US Responsibility

• After transport to Moscow and within 24hr of recovery dissection equipment including several sets of precision surgical tools will be required.

• After flight, for morphological and BrdU incorporation studies a microtome, histology equipment and an epifluorescence microscope with digital imaging and recording/data storage capabilities will be required.

5. Pre-flight Procedures:

Preliminary studies:

1) Control experiment on cell proliferation rates with imitation of temporal spaceflight parameters. It is required that the BrdU delivery via the drinking water using osmotic pumps, and BrdU as well as PCNA immunostaining of tissues be tested. Russian and US experiments will be conducted on tissues provided by the Russian PI. Some reagents and supplies will be provided by USA.

2) Study of organ tissue morphology in control animals (Russia).

3) Study of enriched diet prior to launch to ensure survival for the flight period, specifically we will test several versions of diet to choose the most adequate for a 20 day fast period. (Russia)

6.In-flight Procedures: -

It is required that the environmental parameters are maintained within comfortable levels (oxygen, water, temperature, illumination requirements are specified above in experiment overview).

7.Post-flight Procedures:

Russian Responsibility

• Photo/video to record animal activity upon return to the ground
• Animal dissection, biosample isolation and fixation of selected tissues
• Histology of selected tissues
• Electron microscopy
• Computer-aided data processing, statistical analysis, and review of selected tissues
• Isolation of mRNA from selected tissues

US Responsibility

• Animal dissection, biosample isolation and fixation of selected tissues
• Histology of selected tissues
• Immunohistochemistry of BrdU and PCNA
• Confocal microscopy of tissue three-dimensional structure
• Computer-aided data processing, statistical analysis, and review of selected tissues

After the landing of spacecraft the animals are brought to Moscow alive and are euthanized at the Institute of Human Morphology RAMS. Samples for research are extracted both by the US PI and Russian scientists. For each part of the project samples are isolated and fixed by the interested parties. Transportation to USA of tissue samples will be performed by US personnel.

7. CONTROL EXPERIMENTS:

• Laboratory delayed synchronous control
• Vibration and g level control (using an IMBP facility)
• Hypergravity control (using an IMBP facility)
•

8. PRELAUNCH EXPERIMENTAL VERIFICATION TESTING:

1.0 Russian Tests:

It is required to verify whether the above number of geckos can survive for as long as 20 days in a flight-like environment. We plan to house geckos of the species selected in a flight-type unit with no food and with water supplied by a flight-like system. This experiment will demonstrate whether females only can be used in-flight and whether animals can tolerate a 20-day enclosure without detrimental changes in their behavior and well-being.

2.0 US Tests:

Optimize type and concentrations of somatic stem cell marker antibodies as well as BrdU, and PCNA antibodies labels to use. Tissue labeling testing will be carried out at NASA Ames Research Center using conventional and scanning confocal microscopy and immunofluorescence techniques.

3.0 Intergrated Russian and US Tests:

Determine optimal amount of cell proliferation/DNA synthesis BrdU label in drinking water to use to obtain measurable results after 16 days experiment. Test the use of miniature osmotic pumps to deliver BrdU, in the drinking water for rate optimization. Test dissection procedures needed to expedite isolation and fixation of bone and multiple tissue samples. The animal experiment portion of these studies will be performed by Russian scientists. The tissue staining for BrdU will be performed by the US PI in the US.

The US will provide BrdU, osmotic pumps, dissection equipment, reagents, and antibodies. The US investigator will participate in the post-flight studies in Moscow.

9. SPECIMEN COLLECTION AND LABELING PROCEDURES:

Russian/US Common Procedures

- Animal are injected intraperitoneally with Nembutal anesthetic prior to euthanasia
- Euthanasia is performed by decapitation
- Following euthanasia, carcass kept at 4°C until tissues dissected
- Tissues dissected as soon as possible after euthanasia (no later than 1-hr post death)
- Tissue type, animal identification number, and group labeled on each vial.

Russian Procedures

- Heads isolated and fixed
- Limbs are isolated and fixed
- Central nervous system, peripheral sense organs (visual, auditory, vestibular, olfactory and vomero-nasal organs), musculo-skeletal (bones, tendons, ligaments), endocrine and reproductive systems are isolated and fixed
- Bouin's fixative will be used for histology, neutral buffered paraformaldehyde for electron microscopy, and immunochemistry samples. Each biosample is placed in an individual vial and labeled inside and outside.

US Procedures

- Dissection and partitioning of tissue samples should be done at 4°C.
- After excision, we will rinse tissue in PBS and add tissues to vials of 4% neutral buffered formaldehyde (NBF) fixative at 4°C.
- NASA scientists will use the balance of tissue samples not required by Russian scientists (described above in Russian Procedures). This include a portion of all tissues containing pluri and totipotent cells with emphasis on load-bearing long bones and non-load bearing skull bones, skeletal and, heart muscle, testes/ovaries, intestinal wall, liver, kidney, and blood. Samples will be taken from each animal of each condition, including space flight, and ground delayed synchronous control.

Transport back to NASA Ames Research Center in NBF at 4°C (on ice without freezing). Alternatively, histochemistry proliferation samples may be fixed and embedded in paraffin in Russia and carried as embedded tissue blocks to NASA.

10. SPECIMEN PREPARATION/ TEST PROCEDURES:

- Flight and control animals are tested for infections and parasites (routine veterinary check)
- For a month prior to launch the animals are housed in a comfortable environment and provided with an enriched diet

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

Delivery of BrdU in drinking water is planned. Dosage will be determined to optimize results for a 16-day exposure in geckos. No radioactive labeling will be used.

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

- Delay of tissue recovery beyond 1 week post-landing.
- Delay of dissection or heating of carcass above room temperature prior to recovering tissues.

11. DATA SHEET AND/OR FLOW SHEET:

- Descriptions
- Photos
- Videos
- Light microscopy images
- Processed data
- Plots

12. DATA TRANSFER AND ANALYSIS REQUIREMENTS/PROCEDURES:

1. Data Recording

- Photo, video
- Experimental protocols
- Histological and immunohistochemical preparations
- Results of preparation analysis together with video
- Processed metabolic data

2. On-site Data Analysis

- Animal visual examination and description of their status and behavior
- Photo/video

13. PHOTO/DIAGRAMS:

To be performed after all investigations.

14. EQUIPMENT LIST:

To be provided to labs by US.

No.	Equipment	Pkg	No of pkg	LOANED / CONSUMABLE
1	Avidin from egg white	25 mg	2	CONSUMABLE
2	Biotin	1g	2	CONSUMABLE
3	Anti-Mouse IgG (whole molecule)-Biotin from rabbit	1 ml	1	CONSUMABLE
4	ExtrAvidin-Peroxidase Conjugate (Buffered	1ml	1	CONSUMABLE

	aqueous solution)			
5	Trypsin from Bovine Pancreas	1 g.	1	CONSUMABLE
6	Glycerol minimum 99% for molecular biology	500 ml	1	CONSUMABLE
7	Eppendorf adjustable volume digital pipettes Series 2100			
	Size:0.5-10µl	1each	1	CONSUMABLE
	Size:2-20µl	1each	1	CONSUMABLE
	Size: 10-100µl	1each	1	CONSUMABLE
	Size:20-200µl	1each	1	CONSUMABLE
	Size: 100-1 000µl	1each	1	CONSUMABLE
8	PLASTIBRAND pipette tips, racked, sterile			
	Tip:20µl	1pkg	1	CONSUMABLE
	Tip:200µl	1pkg	1	CONSUMABLE
	Tip:1000µl	1pkg	1	CONSUMABLE
9	Electrophoresis gel loading pipet			CONSUMABLE
8	Tips	10 each	5	CONSUMABLE
9	Hamilton Microliter 700 series syringe, Model 701RN with removable needle (1 µl capacity).	1 each	2	CONSUMABLE
10	Replacement needle	1 pcg	2	CONSUMABLE
11	Tape, label, Sigmaware			
	blue Label Tape	1 each	2	CONSUMABLE
	green Label Tape	1 each	2	CONSUMABLE
	white Label Tape	1 each	2	CONSUMABLE
	red Label Tape	1 each	2	CONSUMABLE
12	Parafilm M			
	Roll 4in x 125 ft	1 each	2	CONSUMABLE
13	Scissors, micro-dissecting, Size: 4 in, curved sharp point	1 each	4	CONSUMABLE
14	Scissors, micro-dissecting, Size: 4-1/4 in, straight sharp point	1 each	4	CONSUMABLE
15	3-Amino-9-ethylcarbasole powder	10g	1	CONSUMABLE
16	N,N-Dimethylformamide	500ml	1	CONSUMABLE
17	4-Chloro-1-napthol, crystalline	2.5g	1	CONSUMABLE
18	Triethanolamine	11	1	CONSUMABLE
19	Dioxane	11	2	CONSUMABLE
20	Teflon-coated termometer, temperature -20-+100°C	leach	2	CONSUMABLE
21	Timer, ModelT-590	leach	2	CONSUMABLE
22	Forcepts, jewelers Dumont No. 5	leach	2	CONSUMABLE
23	Forcepts, micro-dissecting (Curved)	leach	2	CONSUMABLE
24	Forcepts, micro-dissecting (Straight)	leach	2	CONSUMABLE
25	Forcepts, micro-dissecting (Very fine point)	leach	2	CONSUMABLE
26	Scalpel handles No.3	leach	3	CONSUMABLE
27	Scalpel handles No.3L	leach	2	CONSUMABLE
28	Scalpel blades No. 10	50each	1	CONSUMABLE
29	Scalpel blades No.11	50each	1	CONSUMABLE
30	Bottles style 2105			
	30ml	12each	3	CONSUMABLE
	60ml	12each	3	CONSUMABLE
	125ml	12each	3	CONSUMABLE
31	Bottles style 2120			

	2000ml	leach	3	CONSUMABLE
	4000ml	leach	2	CONSUMABLE
32	Bottles style 2114			
	175ml	12each	2	CONSUMABLE
	250ml	12each	2	CONSUMABLE
	500ml	12each	2	CONSUMABLE
	1000ml	6each	3	CONSUMABLE
33	Bottle, wide mouth, PLASTIBRAND, LDPE	Pack Contents - 100	1	CONSUMABLE
34	Centrifuge Tubes Graduated Conical Tubes, Corning			
	15ml	100each	1	CONSUMABLE
	50ml	100each	1	CONSUMABLE
35	Tubes, microcentrifuge, Eppendorf Safe-Lock			
	Size: 2.0ml, Assorted colors	500each	1	CONSUMABLE
36	Parafilm M Roll:4in.x125ft	leach	2	CONSUMABLE
37	Ashless Filter Paper			
	Diameter: 11.0cm	1pkg	1	CONSUMABLE
	Diameter: 1 5.0cm	1pkg	1	CONSUMABLE
38	Sigmaware lab markers			
	black, Fine tip	10each	1	CONSUMABLE
	black, Broad tip	10each	1	CONSUMABLE
	green, Fine tip	10each	1	CONSUMABLE
	green, Broad tip	10each	1	CONSUMABLE
39	Embedding molds (Color - clear)	leach	1	CONSUMABLE
40	Cover glasses			
	Size:24mm x 50mm	lease	1	CONSUMABLE
41	Slide box, hinged	leach	5	CONSUMABLE
42	Slides, Microscope, Plain	lease	1	CONSUMABLE
43	Tweezers			
	Style#5, needle-sharp	leach	2	CONSUMABLE
	Style#7, sharp, hooked	leach	2	CONSUMABLE
44	Spinbar magnetic stir bars			
	Octagonal 1 in x 5/16in	leach	3	CONSUMABLE
	Octagonal 1-1 /2in x 5/1 6in	leach	2	CONSUMABLE
	Polygonal 25mm x 8mm	leach	2	CONSUMABLE
	Polygonal 30mm x 8mm	leach	2	CONSUMABLE
	Polygonal 40mm x 8mm	leach	2	CONSUMABLE
	Polygonal 60mm x 8mm	leach	2	CONSUMABLE
1	Monoclonal antibody to PCNA, clone PC10	1ml	2	CONSUMABLE
2	Immuno-peroxidase labeled streptavidin-biotin kit (LSAB)	1 kit	1	CONSUMABLE
3	Antibody Diluent	125ml	2	CONSUMABLE
4	Liquid-repellent Slide Marker Pen	leach	1	CONSUMABLE
5	Peroxidase-Conjugated Rabbit Anti-Mouse Immunoglobulin	2ml	1	CONSUMABLE
1	Rabbit Antibody to PCNA	1ml	2	CONSUMABLE

BRDU	CONSUMABLE
osmotic pumps	CONSUMABLE

For the US side:

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Signatures affixed on 6/22/04

For the Russian side:

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1. EXPERIMENT TITLE: "GECK0-F2"

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2. PRINCIPAL INVESTIGATORS:

From Russia: Dr. Sergei V. Savelyev, Institute of Human Morphology, Russian Academy of Medical Sciences
From US: Dr. Eduardo A.C. Almeida, NASA Ames Research Center

3. CO-INVESTIGATORS:

From Russia: Dr. Victoria I. Gulimova, Institute of Human Morphology, Russian Academy of Medical Sciences
From US: Dr. Ruth Globus, NASA Ames Research Center
Dr. Wenonah Vercoutere, NASA Ames Research Center

4. OBJECTIVES:

To perform histological examinations of the central nervous system, peripheral organs of senses (visual, auditory, vestibular, olfactory and vomero-nasal systems), musculoskeletal system (bones, tendons, ligaments), endocrine and reproductive systems of geckos in order to detect cell growth and morphological tissue changes.

5. BACKGROUND/HYPOTHESES:

The experiment provides an opportunity to use amniotes for a comprehensive investigation of the effects of microgravity on morphology and cell proliferation in the central nervous system, peripheral organs of senses (visual, auditory, vestibular, olfactory and vomero-nasal systems), musculoskeletal system (bones, tendons, ligaments), endocrine and reproductive systems. The flight duration and profile will allow us to model the effects of the space environment on mammals using the biological specificities of geckos.

One of our working hypothesis is that a comprehensive examination of the nervous system, skeletal elements and hormonal mechanisms regulating calcium metabolism of geckos will help reconstruct neurological and metabolic changes allowing amniotes to adapt to microgravity.

In addition we propose to examine the proliferation of cells in intact live geckos during the flight. Cell proliferation can be upregulated by increased gravity in-vitro (Almeida et al.). Conversely, microgravity-induced bone and muscle-loss suggest that lack of mechanical stimulation may down-regulate the proliferative rates of osteoprogenitor as well as other somatic stem cells that are responsible for tissue regeneration and maintenance. An additional working hypothesis is that microgravity may decrease the proliferative rates of various somatic stem cells.

The specific question we ask, is whether or not microgravity and spaceflight slow down the growth of cells that regenerate various human tissues such as bone, muscle, and blood. An understanding of this issue is key to determining how to design countermeasures for microgravity and spaceflight tissue loss. Although drugs that prevent bone-loss may be sufficient to alleviate this specific problem, we may have serious additional body-wide tissue regeneration problems still undetected. We hope this study will tell us if 16-day exposure to microgravity affects the growth rates of somatic stem cells in the gecko and will pave the way to design human studies.

6. FLIGHT EXPERIMENT:

1. Overview:

We plan to launch 5-7 geckos of the *Hemidactylis tursicus* for a 16-day flight. The animals are on the average 10-12 cm long (with the tail), weighing 3.6 grams each. We expect to use females but it is also possible to have 5 females and 1 male. It is not possible to house more than 1 male in a small-size unit (2.2 liter) because of territoriality. The housing unit contains a 60 ml water bowl equipped with a wick. The humidity level in the unit is also high to reduce water requirements of the enclosed animals. No food is provided. No specific lighting requirements, except that the illumination, if any, should be dim because geckos are nocturnal animals and bright light can be stressful. In the natural environment geckos are active at 30-32 deg C in the daytime and 24 deg C at night. During flight the temperature range of 25-28 deg C is acceptable. It is important to note that a long exposure of geckos to a temperature below 15 deg C or above 35 deg C may be lethal. We required that the hole in the housing unit bottom be covered with a small-mesh net to protect the animals.

Our experimental procedure involves the administration of an immunodetectable nucleotide analog bromodeoxyuridine (brdU) using a time-programmable Alzet osmotic pump inside the water reservoir, The pump will be filled with a brdU solution and a oil "delay" layer of 5 days to accommodate pre-launch loading of the experiment. The osmotic pump will deliver brdU into the drinking water from day 2 of flight through day 16. After landing and recovery we will perform dissection and paraformaldehyde fixation tissue samples for histomorphological studies and measurement of brdU incorporation in specific cell types.

2. Animal Requirements:

Geckos have been selected as experimental objects due to the following factors:

- Reptiles are an oldest group of terrestrial vertebrates that are perfectly well adapted to adverse environments. Their dry skin covered with horny scales is essentially devoid of glands, thus providing an excellent mechanical protection and minimal moisture loss. In addition to these advantageous features, geckos are characterized by noticeable metabolic changes, which can be detected even after a short-term 16-day flight.
- Geckos can long survive without food and in a low moisture environment. Another important advantage is that they produce a very small amount of wastes.
- The lower surface of lizard's toes is covered with so-called ampillary rows of enlarged scales ending in microscopic outgrowths (one toe can carry as many as 200 mln of them). These structures allow geckos to grasp miniscule protuberances of off-vertical or vertical surfaces and move along them. It can be assumed that these structures will also work in microgravity and allow geckos to remain attached to the walls of the housing unit.
- Parathyroid calcitonin-secreting glands are similar to those of mammalians but morphologically separated from the thyroid gland. This allows their qualitative and quantitative measurements.

3. Data Requirements:

We require that the temperature, humidity and illumination levels be maintained within a constant and optimal range. Tissues to be examined histologically should be isolated and fixed immediately after animal dissection: any delay will impact the quality of immunohistochemical and ultrastructural data. The temperature profile of the flight, g-level, and radiation exposure should be measured and recorded.

4. Equipment Requirements:

At the landing site, upon removal from the capsule, animals need to be photographed to record their condition (photographic recording equipment). After transport to Moscow and within 24hr of recovery we will require dissection microscopes as well as dissection equipment including several sets of precision surgical tools. After flight, for morphological and brdU incorporation studies we will need a microtome, histology equipment and an epifluorescence microscope with digital imaging and recording/data storage capabilities.

5. Pre-flight Procedures:

Preliminary studies:

1) Control experiment on cell proliferation rates with imitation of temporal spaceflight parameters. Specifically we will test brdU delivery via the drinking water using osmotic pumps, and brdU as well as PCNA immunostaining of tissues. Experiment in Russia and in USA with animal tissue provided by Russia. Some reagents and supplies will be provided by USA.

2) Study of organ tissue morphology in control animals (Russia).

3) Study of enriched diet prior to launch to ensure survival for the flight period, specifically we will test several versions of diet to choose the most adequate for a 20 day fast period.

6. In-flight Procedures: -

We required that the environmental parameters are maintained within comfortable levels (oxygen, water, temperature, illumination requirements are specified above in experiment overview).

7. Post-flight Procedures:

- Photo/video to record animal activity upon return to the ground
- Animal dissection, biosample isolation and fixation
- Histology
- Immunohistochemistry of brdU and PCNA
- Confocal microscopy of tissue three-dimensional structure
- Electron microscopy
- Computer-aided data processing, statistical analysis, and review
- Isolation of mRNA from selected tissues

After the landing of spacecraft the animals are brought to Moscow alive and are euthanized at the Institute of Human Morphology RAMS. Samples for research are extracted both by NASA scientist(s) and Russian scientists. For each part of the project samples are isolated and fixed by the interested parties. Transportation to USA of tissue samples will be performed by NASA personnel.

7. CONTROL EXPERIMENTS:

- Laboratory asynchronous control
- Vibration and g level control (using an IMBP facility)
- Hypergravity control (using an IMBP facility)

8. PRELAUNCH EXPERIMENTAL VERIFICATION TESTING:

US/Russian Integrated Testing:

Determine optimal amount of cell proliferation/DNA synthesis brdU label in drinking water to use to obtain measurable results after 16 days experiment. Test the use of miniature osmotic pumps to deliver brdU,in the drinking water for rate optimization. Test dissection procedures needed to expedite isolation and fixation of bone and multiple tissue samples. The animal experiment portion of these studies will be performed by Russian scientists. The tissue staining for brdU will be performed by NASA scientists

The US will provide brdU, osmotic pumps, dissection equipment, reagents, and antibodies. The US investigator will participate in the experiment in Moscow post flight.

Russian Testing

We will verify whether the above number of geckos can survive for as long as 20 days in a flight-like environment. We plan to house geckos of the species selected in a flight-type unit with no food and with water supplied by a flight-like system. This experiment will demonstrate whether females only can be used in-flight and whether animals can tolerate a 20-day enclosure without detrimental changes in their behavior and well-being.

US Testing

Optimize type and concentrations of somatic stem cell marker antibodies as well as brdU, and PCNA antibodies labels to use. Tissue labeling testing will be carried out at NASA Ames Research Center using conventional and scanning confocal microscopy and immunofluorescence techniques.

9. SPECIMEN COLLECTION AND LABELING PROCEDURES:

Russian/US Common Procedures

- Animal are injected intraperitoneally with Nembutal anesthetic prior to euthanasia
- Euthanasia is performed by decapitation
- Following euthanasia, carcass kept at 4°C until tissues dissected
- Tissues dissected as soon as possible after euthanasia (no later than 1-hr post death)
- Tissue type, animal identification number, and group labeled on each tube.

Russian Procedures

- Heads isolated and fixed
- Limbs are isolated and fixed
- Central nervous system, peripheral sense organs (visual, auditory, vestibular, olfactory and vomero-nasal organs), musculoskeletal (bones, tendons, ligaments), endocrine and reproductive systems are isolated and fixed
- We will use Bourn's fixative for histology, neutral buffered paraformaldehyde for electron microscopy, and immunochemistry samples. Each biosample is placed in an individual vial and labeled inside and outside.

NASA Procedures

- Dissection and partitioning of tissue samples should be done at 4°C.
- After excision, we will rinse tissue in PBS and add tissues to tubes of 4% neutral buffered formaldehyde (NBF) fixative at 4°C.
- NASA scientists will use the balance of tissue samples not required by Russian scientists (described above in Russian Procedures). This include a portion of all tissues containing pluri and totipotent cells with emphasis on load-bearing long bones and non-load bearing skull bones, skeletal and, heart muscle, testes/ovaries, intestinal wall, liver, kidney, and blood. Samples will be taken from each animal of each condition, including space flight, and ground asynchronous control. Transport back to NASA Ames Research Center in NBF at 4°C (on ice without freezing). Alternatively, histochemistry proliferation samples may be fixed and embedded in paraffin in Russia and carried as embedded tissue blocks to NASA.

10. ANIMAL PREPARATION/TEST PROCEDURES:

- Flight and control animals are tested for infections and parasites (routine veterinary check)
- For a month prior to launch the animals are housed in a comfortable environment and provided with an enriched diet

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

Delivery of brdU in drinking water is planned. Dosage will be determined to optimize results for a 16-day exposure in geckos. No radioactive labeling will be used.

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

- Delay of tissue recovery beyond 1 week post-landing.
- Delay of dissection or heating of carcass above room temperature prior to recovering tissues.

11. DATA SHEET AND/OR FLOW SHEET:

- Descriptions

- Photos
- Videos
- Light microscopy images
- Processed data
- Plots

12. DATA TRANSFER AND ANALYSIS REQUIREMENTS:

1. Data Recording
    - Photo, video
    - Experimental protocols
    - Histological and immunohistochemical preparations
    - Results of preparation analysis together with video
    - Processed metabolic data
2. On-site Data Analysis
    - Animal visual examination and description of their status and behavior
    - Photo/video

13. PHOTO/DIAGRAMS:

To be performed after all investigations.

For the US side:

Signatures affixed on 6/22/04

For the Russian side:

Тел. 120-00-50
Fax. 403-03-43
Савельев С.В.
1
Утвер

ПЛАН ПРОВЕДЕНИЯ ЭКСПЕРИМЕНТА.

1. НАЗВАНИЕ ЭКСПЕРИМЕНТА.

«ИССЛЕДОВАНИЕ ВЛИЯНИЯ МИКРОГРАВИТАЦИИ НА МОРФОФУНКЦИОНАЛЬНЫЕ ПАРАМЕТРЫ НЕРВНОЙ СИСТЕМЫ, СКЕЛЕТА И ЭНДОКРИННЫХ ОРГАНОВ ГЕККОНА»

2. ОТВЕТСТВЕННЫЙ ИСПОЛНИТЕЛЬ (ФИО, должность, учреждение)

От России - *Савельев Сергей Вячеславович*, д.б.н., профессор, заведующий отделом эмбриологии НИИ морфологии человека РАМН.

От США

3.СОИСПОЛНИТЕЛИ (ФИО, должность, учреждение)

От России - 1.*Гупимова Виктория Игоревна*, к.б.н., старший научный сотрудник лаборатории развития нервной системы НИИ морфологии человека РАМН.

2. - Макаров Алексей *Николаевич* - старший научный сотрудник НИИ морфологии человека РАМН.

3 - *Андреева Елена Владимировна* - младший научный сотрудник НИИ морфологии человека РАМН.

4 - *Ерофеева Елена Александровна* - младший научный сотрудник НИИ морфологии человека РАМН.

От США

4. ОСНОВНЫЕ ЦЕЛИ ЭКСПЕРИМЕНТА:

Гистологическое исследование послеполётных изменений в тканях центральной нервной системы, периферических органов чувств (зрительная, слуховая, вестибулярный аппарат, обонятельная и вомероназальная системы), опорно-двигательный аппарат (кости, хрящи, связки), эндокринная и репродуктивная системы.

5. ОБОСНОВАНИЕ ЭКСПЕРИМЕНТА/ГИПОТЕЗЫ

- Основой для проведения эксперимента является возможность использования амниот для комплексного анализа влияния условий невесомости на центральной нервной системы, периферических органов чувств (зрительная, слуховая, вестибулярный аппарат, обонятельная и вомероназальная системы), опорно-двигательный аппарат (кости, хрящи, связки), эндокринная и репродуктивная системы. Продолжительность и условия эксперимента позволят использовать биологические особенности гекконов для адекватной оценки влияния полётных условий на позвоночных.

-

- В качестве рабочей гипотезы предполагается, что комплексный анализ нервной системы, скелетных элементов и гормональных механизмов регуляции кальциевого обмена позволит реконструировать как неврологические, так и ключевые метаболические адаптационные изменения организма амниот к условиям невесомости.

6. ПОЛЁТНЫЙ ЭКСПЕРИМЕНТ:

1. **Общее описание:** планируется экспериментальный запуск на биоспутнике 6-и

гекконов Hemidactylus sp. (возможный вариант объекта - Cosymbotus sp.) на срок 20 дней. Средний размер животных - 10-12 см. (с хвостом), средний вес-3.6 грамма. Пол животных - самки (возможный вариант - 5 самок, 1 самец). Отправка большего количества самцов в одном контейнере запланированного объёма (2.2 л) невозможна в силу выраженного территориального поведения гекконов. В контейнере предусматривается поилка объёмом 60 мл в виде ёмкости с фитилём, увлажняющимся за счёт диффузии в продолжение полёта, а также высокая влажность, что будет способствовать уменьшению потребностей животных в воде. В запасах пищи на период полёта нет необходимости. Специальных требований к освещению не предъявляется, за исключением одного: при наличии света в контейнере он не должен быть ярким, поскольку предлагаемые для эксперимента гекконы - ночные животные и яркий свет для них является фактором стресса. Температурный режим, подразумевающий активность гекконов в естественных условиях составляет +30-32°С днём и +24°С ночью. В условиях полёта приемлемая средняя температура - +25-28°С. Необходимо предусматривать то обстоятельство, что длительное пребывание животных при температуре ниже +15°С или выше +35°С может привести к летальному исходу. Отверстия в днище контейнера должны быть затянуты мелкоячеистой сеткой во избежание попадания туда животных.

2. Требования к биообъектам - выбор геккона в качестве биообъекта обусловлен следующими факторами:

- рептилии являются древнейшей группой наземных позвоночных и оптимально приспособлены к неблагоприятным внешним воздействиям. Сухая кожа, покрытая роговыми чешуями и практически не имеющая желёз, обеспечивает максимальную механическую защиту и минимум потери влаги. Из рептилий предпочтение отдано именно гекконам, которые обладают всеми преимуществами, характерными для данной группы, но имеют достаточно интенсивный метаболизм, что позволит наблюдать динамику даже после относительно кратковременного 20-дневного полёта.

- гекконы могут подолгу обходиться без пищи, существенно также минимальное количество выделяемых экскрементов по сравнению с другими животными. На нижней поверхности пальцев геккона имеются специальные приспособления -ампилярные ряды, состоящие из расширенных чешуек, которые заканчиваются микроскопическими выростами (до 200 млн. на одном пальце), позволяющими охватывать ничтожно-малые неровности наклонных или вертикальных поверхностей и легко по ним передвигаться. Можно предположить, что данные структуры окажутся эффективны и в условиях микрогравитации, что позволит гекконам сохранять естественное для них прикреплённое положение.

- Паращитовидные и кальцитонинсекретирующие железы сходны с аналогичными органами млекопитающих, но морфологически обособлены от щитовидной железы. Это даёт возможность для их объективного сравнительного исследования как в количественном, так и в качественном отношении.

3. Требования к регистрируемым данным - нет.

Температура, влажность и освещённость должны сохраняться на постоянном и оптимальном уровне в течение всего полёта. Органы и ткани, которые планируется исследовать на гистологическом уровне должны быть отпрепарированы и подвергнуты фиксации сразу же после умерщвления животных (любая задержка неизбежно скажется на качестве иммуногистохимических и ультраструктурных исследований).

4. Требования к научной аппаратуре - нет.

Необходимо зафиксировать на плёнке кинематику движений животных непосредственно

после полёта, для чего требуется присутствие квалифицированных специалистов непосредственно при вскрытии контейнера после приземления. Для послеполётных исследований было бы желательно обновить парк имеющегося оборудования на 10-30%.

5. Предполётные процедуры: содержание контрольных и экспериментальных животных на обогащенном рационе с целью приведения их в оптимальное для полёта физическое состояние.

6. Полётные процедуры -нет.

Обеспечение животных влагой и кислородом. Поддержание необходимой температуры. Соблюдение режима освещённости (см. основное описание эксперимента).

7. Послеполётные процедуры:

-кино/фото/видеосъёмка кинематики движений животных после приземления;
-препаровка животных, забор и фиксация материала;
-гистологические исследования;
-иммуногистохимические исследования (при наличии дополнительного финансирования);
-электронно-микроскопические исследования (при наличии дополнительного финансирования);
-видеозахват изображений, полученных с помощью светового микроскопа и построение графических реконструкций;
-компьютерная обработка материала, статистический анализ и обобщение полученных результатов.

7 . КОНТРОЛЬНЫЕ ЭКСПЕРИМЕНТЫ.

-лабораторный контроль;
-вибрационный контроль (при возможности использования установок ИМБП);
-гипергравитация (при возможности использования установок ИМБП).

8. ПРЕДПОЛЁТНЫЕ ВЕРИФИКАЦИОННЫЕ ИСПЫТАНИЯ.

1. <u>Американские испытания.</u>

2. <u>Российские испытания:</u>

- необходимо проверить возможность успешного выживания указанного количества животных в условиях, максимально приближенных к полётным. С этой целью предполагается 20-дневное содержание гекконов выбранного вида в контейнере, по объёму соответствующему полётному - без пищи, но при соответствующей влажности и с поилкой выбранной конструкции. Проведя данное испытание с группами животных, различными по половому составу, можно будет оценить степень необходимости отправки исключительно самок, и оценить, насколько в принципе содержание в столь ограниченном объёме может сказаться на физическом состоянии и поведении животных.

- необходимо разработать методику фото и видео съёмки, которая позволит в кратчайшее время и наиболее эффективным образом оценить изменения в кинематике движений экспериментальных животных.

3. <u>Комплексные испытания и регистрация исходных данных.</u>

Сохранение данных предварительных экспериментов в электронном виде.

9. ВЗЯТИЕ БИОМАТЕРИАЛА И СПОСОБЫ МАРКИРОВКИ.

-умерщвление животных введением в перитонеальную полость раствора нембутала.
-декапитация с частичной препаровкой головы и помещение её в фиксатор;
-препаровка конечностей с последующим помещением в фиксатор;

-выделение и фиксация центральной нервной системы, периферических органов чувств (зрительная, слуховая, вестибулярный аппарат, обонятельная и вомероназальная системы), опорно-двигательного аппарата (кости, хрящи, связки), эндокринной и репродуктивной систем.

В качестве фиксатора для гистологии предполагается использовать жидкость Буэна, для электронной микроскопии - забуференный раствор параформальдегида, для иммуногистохимии - замораживание в жидком азоте. Каждый образец помещается в отдельный контейнер, который этикитуруется снаружи и внутри.

10. ПОДГОТОВКА БИООБЪЕКТОВ/МЕТОДЫ ТЕСТИРОВАНИЯ.

-биообъекты должны быть проверены на отсутствие инфекционных заболеваний и паразитов (стандартное ветеринарное обследование);

-на протяжении месяца перед запуском необходимо содержание животных на специальном рационе в максимально благоприятных условиях для подготовки к полёту.

11. ФОРМА РЕГИСТРАЦИИ ДАННЫХ.

-описания
-фотографии
-видеоматериалы.
-изображения, полученные в результате видеозахвата через световой микроскоп.
-результаты статистической обработки данных.
-графические реконструкции.

12. ТРЕБОВАНИЯ ПО ОБМЕНУ ДАННЫМИ И ИХ АНАЛИЗУ/МЕТОДЫ.

1. Регистрация данных.

- фото, видеосъёмка животных;
- протоколы экспериментов;
- гистологические и иммуногистохимические препараты;
- описания с анализом препаратов, прилагаемые к видеоматериалам;
- статистическая обработка данных по минеральному обмену;

2. Анализ данных на месте запуска/посадки.

-визуальный осмотр и описание поведения животных; -фото/видеосъёмка;

13. ФОТО/ДИАГРАММЫ.

После проведения исследовательских работ.

Примечание: мы не располагаем данными ни о каких идеях наших коллег из США. Было бы адекватным шагом с их стороны выслать нам свою заявку.