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Facilities to Mimic Micro-Gravity Effects

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Animals: Unloading, Casting

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12.1 Introduction

The aim of this section is to provide description of techniques of on-ground microgravity simulation based on animal models such as hindlimb unloading, casting, and denervation.

It is well known that exposure to microgravity leads to notable restrictions in general movement and mechanical loading in astronauts. Conditions of spaceflight together with spacecraft environment, confinement, altered diet and altered ambient atmosphere, and relatively high radiation result in significant alterations in normal physiological processes. Existing countermeasures, based on physical exercises, are not able to completely substitute normal Earth gravity loading. It is undoubtedly true that the development of new countermeasures is a crucial step on a way to the long-term space missions.

One of the problems with spaceflight experiments is that opportunities to carry them out are expensive and rare. That is where animal ground-based models come into play. With ground-based models, there are no limitations related to number of animals. What is also important is that there is no ideal imitation of all conditions of long-term spaceflight. Even techniques such as head-down-tilt bed-rest studies and water immersion which are generally accepted as the gold standard imitate only some of the spaceflight conditions [see also Chapter 13]. At the same time, parabolic flights and drop towers provide weightlessness, but only for very short periods of time [see also Chapter 6 and Chapter 7].

In the past, varieties of mammalian species, including monkeys, dogs, and rabbits, were used for research purposes. Nowadays, rodents have become one of the most used animals in all areas of scientific studies. There are many reasons in favor of using them instead of primates or rabbits: mice and rats grow fast and reproduce quickly, and it is easy to house and maintain them. Also, with rats and especially mice, it is possible to conduct uniform studies with genetically identical animals. As it is easier for the mice to be genetically modified, it is also easier to breed either transgenic or knockout animals.

In order to develop an acceptable ground-based model for the simulation and study of spaceflight aspects, NASA-Ames Research Center has formulated the following requirements: experimental animals should demonstrate physiological response similar to that during spaceflight; the model should provide thoraco-cephalic fluid shift; the model should unload limbs without motion restriction or paralysis, and provide ability to recover; and the model should not be stressful for animals. Such technique would be valuable for predicting the effects of spaceflight, studying possible mechanisms of these effects, and developing countermeasures [1].

Nowadays, different immobilization techniques are widely used for the simulation of mechanical unloading. Immobilization itself can be combined with dietary or pharmaceutical intervention. Generally, methods can be merged into two groups: conservative (bandaging, casting, hindlimb unloading, and confinement) and surgical (nerve resection, denervation with botulin toxin, spinal cord resection, and tendon resection). Immobilization provided by casting, denervation, and tendon resection is widely used for the quick development of disuse osteopenia or muscle atrophy. Therefore, these models are useful for studying different countermeasures against bone or muscle loss. However, this approach has serious limitations: surgical intervention or casting does not mimic effects of spaceflight on cardiovascular system, nervous system, and immune system. In addition, with the existing surgical models, recovery from disuse is impossible or difficult. Such surgical models may also result in inflammation, altered trophic, perfusion, and innervation of immobilized limb [2].

Among microgravity simulation models, hindlimb unloading fits most of the NASA requirements. It induces muscle atrophy and alterations in bone structure similar to physiological consequences observed in humans after spaceflight or bed rest. Other physiological changes similar to spaceflight such as synaptic plasticity changes [3] and immune system suppression have also been reported in this model [4]. In cardiovascular functions, rodent head-down-tilt simulates cephalic fluid redistribution and hypovolemia. It also leads to vessel's structural and functional adaptations and alters baroreflex function [5]. Putting all these factors together, it is clear why the use of tail traction in the hindlimb unloading model has become the technique of choice for studying spaceflight-like changes in rats and mice.

12.2 Hindlimb Unloading Methodology

Emily R. Morey-Holton has done significant work on the development and standardization of hindlimb unloading. The review of technical aspects of the method produced in 2002 has been widely used as a base for microgravity simulation studies [2]. Here we provide the description of the method based on this review. Before the experiment, animals are acclimated to their cages for at least two days prior to the hindlimb unloading. First, a strip of traction tape, pre-attached to the plastic tab, is attached to the pre-cleaned tail just above the hair line. Then, the traction tape is fixed by two strips of filament tape placed around the base of the tail and on about half-way up the traction tape. To protect the traction tape, gauze bandage can be wrapped around the tail. The gauze bandage should not cover the whole tail, because the tail plays an important role in thermoregulation. Daily health checks confirmed that the exposed tip of the tail remained pink, indicating adequate blood flow [6]. The animal is then attached to the top of the cage. Such way of harnessing aims to distribute the load along the length of the tail and avoid excessive tension on a small area.

The body of the animal makes about a 30° angle with the floor of a cage, and thus the animal does not touch the grid floor with its back feet (Figure 12.1).

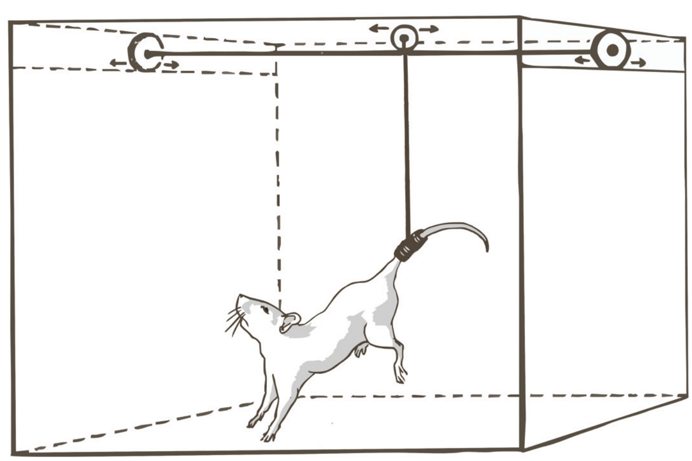

Figure 12.1 Hindlimb unloading model.

At this position, 50% of rat's body weight applies to its forelimbs. A 30° angle of unloading is recommended because it provides normal weight bearing on the forelimbs, unloads the lumbar vertebrae but not the cervical vertebrae, and induces a cephalic fluid shift [2]. The angle and height of the animals are checked, and then adjusted if necessary, on a daily basis. In order to use the system on animals with different behavioral pattern and smaller size of the body, such as mice, some adjustments to the hindlimb unloading system are needed. These include the use of smaller cages, and inclusion of a device to prevent mice from climbing the harness and chewing it. As the mice are generally weaker than rats and have smaller body weight in relation to the total weight of the unloading system, they find it difficult to freely move around the cage and have access to food and water. Therefore, a significant adjustment to the friction is needed between the roller and the wire.

There are some disadvantages of the method: tail examination can be difficult due to the size of gauze bandage; animals can chew the traction tape and release themselves; and tail can undergo inflammation or necrosis [7]. Therefore, in the literature, several modified techniques are suggested to harness fixation based on minimally invasive surgery. As one of the methods, the authors have made a harness for hindlimb unloading by inserting a surgical steel wire through intervertebral disc space of the tail. After that, the wire was ring shaped and used for suspension. Detailed step-by-step video description is publicly available on the Internet [7].

Similar method was proposed for long-term studies on adult rats. Continuous hindlimb unloading for longer than 3 weeks can be complicated in rats with high body weight (350 g or more). Animals come down from suspension because of sloughing of tail skin. Even very short periods of reloading induce changes in muscle physiology. Therefore, frequent release of animals from suspension apparatus can compromise a study [8]. In such case, after passing a steel cable through rat's tail skin and wrapping it loosely with gauze, authors used a 5-ml syringe cut in half longitudinally together with orthopedic casting to provide strong structural integrity. This was performed to ensure that the tail remained in a straight line with respect to the body once the animal was hindlimb suspended [8].

These methods are principally close to the "classical" tail suspension. Interestingly, there is a new method that uses a different unloading model. Partial weight suspension was described in Erica B. Wagner's paper in 2010 [9]. The main difference is that this system allows the distribution of gravitation loading among hindlimbs and forelimbs in a desired proportion between 10 and 80% of total weight bearing, with an accuracy of ±5%.

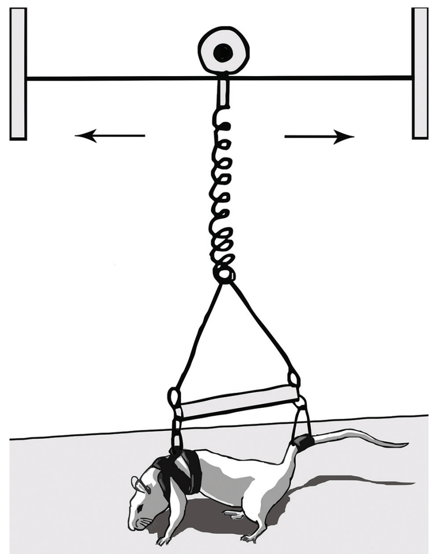

Figure 12.2 Partial weight suspension model.

With such a technique, animals have linear freedom of motions; feeding and cleaning are easy; and animals can be exposed to quadrupedal unloading for at least three weeks Figure 12.2. Also, this system allows for a full recovery of animals after an experiment [9].

To fix an animal in the desired position, two harnesses are used. One is a ring of bandage put around the tail's base. The second one is a flexible, breathable "jacket" secured around the chest cage. The tail's and the chest's harnesses are connected to adjustable chains and hollow metal rod. With this model, researchers performed a fascinating study where they imitated gravitational conditions of Mars planet [9]. Possible disadvantages of this model of microgravity simulation include an absence of head-down-tilt and physiological changes related to it.

12.3 Recommendations for Conducting Hindlimb Unloading Study

While conducting the research on animal models, it is important to remember that any interference of normal life activity is a stress for animals. Hindlimb unloading, restraint stress, and social isolation cause significant perturbations in blood pressure, heart rate levels, [5] and plasma corticosterone level [10].

Therefore, it is recommended to use minimal restraints and avoid unnecessary manipulations during preparatory period and period of tail suspension. Physiological and environmental parameters, including body weight, room temperature, and angle of unloading, should be monitored on a daily basis [11].

Animals from a control group should be kept in identical cages. Behavioral or physiological modifications produced by environmental variables can cause false results or give wrong hypothesis [11]. Another important factor related to control group is feeding. Unloaded animals lose weight during the experiment despite easy access to food and water because of the alterations in energy balance. The difference in weight between experimental and control groups, both fed ad libitum, can be from 5 to 20% in adult rats [2]. Hence, it is recommended to either feed control group with average amount of food consumed by suspended group or reduce caloric intake for control group. However, the latter can result in physiological and behavioral alterations [2]. Although some authors argue that forelimbs can be used as an internal control [2], we would advise considering possible systemic effects of hindlimb unloading and being careful when applying it. It is also relevant to other immobilization techniques where one of the limbs remains "unaffected" and could be used as an internal control.

12.4 Casting, Bandaging, and Denervation

Different surgical techniques such as nerve or tendon ectomies have been used in the past for the reproduction of microgravity effects by induction of localized extremities disuse. For instance, commonly used sciatic neurectomy is a visually confirmed resection of 3–4 mm of sciatic nerve that leads to efficient denervation of all regions of the hindlimb [12]. These techniques lead to not only irreversible immobilization and significant bone loss and muscle atrophy but also multiple side effects.

Non-invasive methods such as casting, bandaging, and injection of Clostridium botulinum toxin have become more popular in the recent years. Clostridium botulinum toxin type A is a bacterial metalloprotease causing muscle paralysis and therefore limb disuse by the inhibition of neurotransmitter release. The injection is done into the posterior lower limb musculature. The major advantage of this technique compared to neurectomy is non-invasiveness and possibility of complete recovery within several months [13].

Bandaging and casting are methods of immobilization based on fixation of extremities in constant position by applying either elastic tape (bandaging) or hard orthopedic plaster (casting). During bandaging procedure, a hindlimb of anesthetized animal is immobilized against the abdomen with few layers of elastic bandages. Ankle joints and the knee are placed in extension, and the hip joint is placed in flexion [14]. The immobilized limb should not touch the floor of the cage during animal's movement. The gravitational loading, normally distributed between both hindlimbs, rests on the free limb. Animals are free to move and can easily reach food and water. The bandage should be examined daily and replaced twice a week [14].

Casting is very similar to bandaging but it allows to fix animal's extremities in desirable positions with precise adjustment of joint angles and muscle straitening. This feature of casting techniques allows to get either plantarflexion or dorsiflexion cast immobilization. Dorsiflexion of the ankle joint at an angle of 35° by casting of a limb was used by Nemirovskaya [15] in her study of adaptation mechanisms to microgravity in combination with tail suspension model. Casting can be not only unilateral but also bilateral. This model was recommended as a reliable cast immobilization particularly for mice because small size of animals is a technical challenge [16]. Casting is performed on anesthetized animals. The cast covers both hindlimbs and the caudal fourth of the body. A thin layer of padding is recommended to be placed underneath the cast to prevent abrasions. To minimize freedom of movement of limbs, slight pressure should be applied when wrapping the casting tape. To resist the cast against chewing on, fiberglass material can be applied over the cast. The animals can move using their forelimbs to reach food and water. The mice should be monitored daily for abrasions, chewed plaster, venous occlusion, and problems with ambulation [16].

12.5 Conclusions

Nowadays, hindlimb unloading is the only technique which imitates more physiological alterations relevant to spaceflight than any other on-ground model. Social isolation is not a common case for experiments conducted in space but it is a significant source of stress for animals subjected to unloading. This aspect should be taken into account when comparing results from spaceflights to results from on-ground models. Future development of hindlimb unloading could help tackle this issue.

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