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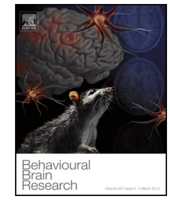

Research report

The long-term consequences of the exposure to increasing gravity levels on the muscular, vestibular and cognitive functions in adult mice

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HIGHLIGHTS

- The long-term response to altered gravity does not fit the principle of continuity.
- The duration of exposition is more relevant than the level of hypergravity.
- The functions sensitive to gravitational force react to different ranges of gravity.

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ABSTRACT

Adult male mice C57Bl6/J were exposed to gravity levels between 1G and 4G during three weeks, and the long-term consequences on muscular, vestibular, emotional, and cognitive abilities were evaluated at the functional level to test the hypothesis of a continuum in the response to the increasing gravitational force. In agreement with the hypothesis, the growth of body mass slowed down in relation with the gravity level during the centrifugation, and weight recovery was inversely proportional. On the other hand, the long-term consequences on muscular, vestibular, emotional, and cognitive abilities did not fit the hypothesis of a continuum in the response to the gravity level. The hypergravity acted as endurance training on muscle force until 3G, then became deleterious at 4G. The vestibular reactions were not affected until 4G. Persistent emotional reactions appeared at 3G, and particularly 4G. The mice centrifuged at 3G and 4G showed an impaired spatial learning, probably in relation with the increased level of anxiety, but a greater difficulty was also observed in mice exposed at 2G, suggesting another cause for the impairment of spatial memory. The long-term response to the hypergravity was shown to depend on both the level of gravity and the duration of exposition, with different importance depending on the function considered.

1. Introduction

The constant physical constraint of the gravitational force shaped many biological functions during the evolution of life on Earth. The progresses in space research have stimulated a new field of research about the processes underlying this adaptation to gravity, and the perspective of the exploration of deep space has opened the question of the adaptation to various gravity environments. Given the physical impossibility to subtract from gravity on Earth, and the limited opportunities for space flight, many experimenters used the paradigm of hypergravity to analyse the adaptation to altered gravity. The paradigm consists in adding a centrifuge force to the gravity. This is done by housing animals in a carousel whose gondolas are gimballed in such a way that the resultant force is perpendicular to the cage floor. The use of hypergravity environments to study the alteration of gravity rests on the principle that alteration of gravity acts as a continuum. The hypothesis of a continuum was formulated early in gravitational biology [1–5]. It assumes that variations on either side of the homeostatic Earth gravity level elicit opposite dose-dependent effects. This paradigm is fruitful as it supposes that studies on hypergravity could be extrapolated to microgravity [5]. The meta-analysis of the literature on microgravity and hypergravity studies involving various animal species reported dose-dependent effects that supported the hypothesis of a continuum in the response to the variations of gravity [6]. However the hypothesis was never formerly tested except

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for mammary metabolism [7]. The argumentations based on the comparison between experiments are hindered by the difficulty to conciliate the effects specifically related to gravity because of uncontrollable changes in the experimental protocols and conditions. In addition the confinement in the centrifuge is susceptible to induce side effects [8], no specifically related to gravity, and with variable amplitude depending on the animal model. In a view to evaluate the hypothesis of a continuum in the response to gravitational change, we analysed the effects of the exposure of a single species to various gravity levels in identical experimental conditions. For this purpose, C57BL/6j mice were subjected to chronic centrifugation at values ranging between 1 and 4G, and the consequences were evaluated in various functions that are known to be sensitive to the gravity. The evaluation concerned the muscular efficiency, the vestibular reaction, the motor properties, and the emotional and cognitive responses. This study was carried out in the frame of a wider program that analysed also the change in the muscle morphology [9], vascularisation, bones [10] and humoral immunity response [11] immediately after the centrifugation. In the present study, the mice were left 15 days to recover. This study focused therefore on the long-term consequences of the chronic centrifugation and did not account for the short term reactions that are corrected by the system plasticity within hours or days immediately after return to Earth gravity. The effects of the variable gravity levels are discussed in relation with the previous set of knowledge on the effects of the exposition to hypergravity or microgravity.

2. Material and Methods

2.1. Animals

Eight-week-old C57BL/6J male mice were purchased from Charles River (Les Oncins, 69210 Saint Germain sur l'Arbesle, France). They were housed by groups of four mice in standard cages (36 x 20 x 14 cm) with bedding material, food and water ad libitum, in a quiet room with constant temperature (22 °C), 50% relative humidity, and a 12/12 h light-dark cycle (dark period 20:00 h–08:00 h). After a week of habituation period, the cages were transferred into the gondolas of a centrifuge that consisted in a large carousel (3.7 m in diameter during rotation) with rotational speeds producing available gravity vectors until 5G [12]. The cages were supplied with enough food and water to allow the centrifuge to operate continuously for duration of 21 days, without interruption. Infrared videos fixed on the gondolas allowed a day and night remote control of the mice in their cages. All the environmental conditions, but gravity level, in the centrifuge were similar to standard housing. Three groups of mice were subjected to the chronic centrifugation with rotational speeds producing a gravity level of 2G, 3G or 4G respectively. In addition, a control group was housed in the same room, and in the same gondolas as the centrifuged mice, but in a static position (1G). The sample and initial weight of the various groups consisted of: 20 1G mice (20.38 ± 0.53 g); 16 2G mice (2G: 21.81 ± 0.28 g); 12 3G mice (21.77 ± 0.27 g); 16 4G mice (22.22 ± 0.3 g). After 21 days, the mice returned to standard housing for 15 days, to prevent the responses to the tests to be biased by behavioural impairment related to the re-adaptation to normal gravity. After the recovery period, the mice were subjected to a set of behavioural tests.

All experimental procedures were carried out in conformity with the European Community Council directive of November 24, 1986, and were approved by our local ethical committee ("Comité Régional d'Ethique pour l'Expérimentation Animale de la Région Provence").

2.2. Behavioural tests

2.2.1. Grip test

The muscle strength was measured using a commercial grip strength meter for mice (Bio-GT3, Bioseb, France) consisting of a grid connected to a digital dynamometer. Mice were placed in front of the grid, which they usually grabbed spontaneously, and gently pulled backwards until they released the grid. In a first series, the mice were allowed to grasp the grid with the forelimbs only. In a second series, they grasped the grid with the four limbs. In each series, the individual performance was the highest peak force among ten trials. The force was normalized with reference to the body weight.

2.2.2. Testing vestibular reactions

Negative geotaxis. The set-up consisted of a metal grid fixed to a column with a joint that allowed free inclination. The grid was set horizontally, and a system of pulley and counterweight allowed the grid to tilt at a speed of 20°/s when a mass of 20 g. The mouse was gently posed on the grid in such a way that it was in a head-down position when the grid tilted due to its weight. The delay that the mouse needed to orient its head–body axis upwards was recorded. Each mouse performed a single trial.

Air righting. The set-up consisted of an electrified metal grid fixed to a 45 cm high column above a soft pad. White curtains around the setup masked the external visual landmarks. The mouse was gently deposited on the grid that was then turned upside down so that the mouse hung with four legs. A shock dispenser simultaneously sent an electric shock to the grid and lit a Light-Emitting Diode that provided the time 0 signal to the video recorder. When the mouse received the shock, it dropped the grid. A high-speed video facing the set-up recorded the fall at a rate of 250 frames per second. The righting movement during the free-fall was analysed frame by frame, and the time when the head axis crossed the vertical plane was recorded with a time accuracy of 4 ms.

Splay test. The mouse was suspended by the neck at a 15-cm height and dropped onto a plate consisting of glass lit from the side by means of a 18-W neon light [13] such that, when the feet touched the glass support, the pads and fingers appeared as bright spots. A high-speed video below the glass recorded the footprints at the exact time of landing, and the distance between the palms of the hind feet was measured. The mean distance was computed for each mouse from 10 falls. In addition, the distance between the fore and hind feet was measured once the mouse stood on the four legs to compute the polygon of sustentation.

Rotarod test. The motor coordination and balance were assessed using a commercial accelerating rotarod (LETICA-LE-8200, Bioseb). The mice were placed on the rod rotating at an initial speed of 4 rotations per minutes (r.p.m.); the speed gradually increased to 40 r.p.m. over a 5-min session, and the latency to fall was recorded. The mice were given three successive trials per day for two consecutive days. The rotarod score was established as the best performance over three trials for each day. In addition, the evolution of the performance in each group was evaluated from the means of the regression curve of the distribution of scores during the six sessions.

2.2.3. Locomotion

The endurance was assessed using a one-lane commercial treadmill (LETICA-LE-8700, Bioseb, France), made of a continuous belt (40 cm long and 14 cm wide), operating at adjustable speed (ranging from 4 to 50 cm/s), and slope (from 0° to 25°). An electrified grid at the end of the belt delivered a slight intensity foot shock to force walking. The slope was set to 10° and the belt speed was successively set to 10 cm/s for 1mn (habituation stage), 33 cm/s for 15 min (training stage), and 50 cm/for 5 min (endurance stage). The relative time that mice spent walking on