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Behavioural responses to hypergravity in the CD-1 mouse

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Abstract

Male and female mice were subjected to rotationally-generated hypergravity of different duration (1 or 2 h) and linear acceleration (2 or 1.08 G). Pica behaviour and spontaneous activity were investigated before, during, and after rotation. Moreover, hole-board and plus-maze tests were performed 20 min and 24 h after the end of rotation. Pica behaviour arose in the post-rotation days and was more pronounced after 1 h of 2 G exposure. Spontaneous activity was almost or totally suppressed during rotation and failed to regain the pre-rotational levels in the group exposed to 2 G for 1 h. Exploratory behaviour in the hole-board was also impaired. A clear effect of hypergravity exposure emerged in the plus maze, with 2 G mice totalizing a minor number of arm entries than the other groups and also showing an altered emotional/anxiety profile. Generally, females were more susceptible than males to the changes in gravitational environment.
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1. Introduction

With the advent of long term interplanetary missions, involving increasing numbers of people, space biology is becoming an emerging area of research devoted to assess the effects of altered gravity conditions on human health. The necessity to develop reliable animal models to protect human health has led to a variety of animals studies concentrating on motion sickness (MS) syndrome (for reviews see [1,2]), which mimics a related disorder arising in astronauts during space missions (Space Adaptation Syndrome; [3–5]).

In laboratory rats and mice, species incapable of emetic response, pica behaviour (the consumption of non-nutritive substances such as kaolin), has been considered an appropriate index of MS [6–11].

The mouse, a much smaller-sized mammalian species than the rat, may be more suitable for space missions (standard habitat holding rack size in a shuttle or in the ISS is around 1000 in^3). In this species, besides inducing picaism, acute exposure for 1h to rotationally-generated 2 G hypergravity caused marked changes in the normal pattern of spontaneous activity [11] as well as a dramatic increase of neurotrophin levels in both frontal cortex and hypothalamus, two brain areas known to be involved in motion sickness syndrome [12].

The aim of the present study was to better characterise the behavioural responses of CD-1 mice after acute hypergravity exposure. MS was assessed both in male and female individuals since sex-dependent differences in mice pica behaviour has been previously found [11]. The short term effects of altered

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gravitational environment on mice explorative behaviour and emotional/anxiety responses were evaluated in the hole-board and in a plus-maze apparatus respectively 20 min and 24 h after the end of rotation.

Acute exposure experiments are generally aimed at gaining insight on the effects of short episodes of hypergravity that mimic what normally occurs at launch [13]. In this study, behavioural responses to hypergravity have been observed and used to have quantitative/qualitative measures of the response to the environmental challenge. Moreover, in order to evaluate early onset of habituation phenomena, an additional group of mice was exposed for 2 h to rotationally-generated hypergravity.

2. Materials and methods

2.1. Animals

Mice of an outbred Swiss-derived strain (CD-1) weighing 30–33 g (virgin males) or 28–30 g (virgin nulliparous females) were purchased from a commercial breeder (Charles River Italy, I-22050 Calco, Italy). Upon arrival at the laboratory, animals were kept in an air-conditioned room (temperature 21 ± 1 °C, relative humidity 60 ± 10%; lights on from 08:30 p.m. to 08:30 a.m.). Males and females were housed separately in groups of 5–7 in 42 × 27 × 14 cm Plexiglas boxes with sawdust as bedding. Pellet food and tap-water were continuously available. Ten days later, mice were randomly assigned to one of the following groups of 20 animals (10 males and 10 females): (1) stationary control (SC); (2) rotational control rotated at 1.08 G for 1 h (RC1); (3) rotational control rotated at 1.08 G for 2 h (RC2); (4) hypergravity rotated at 2 G for 1h (HG1); (5) hypergravity rotated at 2 G for 2 h (HG2).

2.2. Food and kaolin

Seven days before the rotation day (adaptation period) animals were singly housed in 33 × 13 × 14 cm in Plexiglas boxes with free access to pellet food (Enriched Standard Diet purchased from Mucedola, Settimo Milanese, I-20019, Italy) kaolin (Pharmaceutical grade kaolin hydrate aluminium silicate, Sigma, Milan, I-20151, Italy) and water. Kaolin pellets (prepared according to Mitchell et al. [7,8] and Santucci et al. [11]) were placed in the cages adjacent to food.

During the last 4 days of the adaptation period and for 5 days after rotation, food and kaolin were weighed daily at 11:30 a.m. to the nearest 0.1 g (to evaluate consumption rate) and refilled. Moreover, spilled food and kaolin pieces were collected, dried and weighed to obtain correct consumption values [6–8,14,15]. SC group for food and kaolin were placed close to the rotation apparatus during the rotation test (see below) where they were subjected to the noise and vibration of the turntable apparatus but not rotated.

2.3. Rotational device, procedures, and behavioural observations

The apparatus was a custom-made prototype designed and manufactured by Isolceram, Rocca Priora, I-00040, Italy, consisting of a turntable (radius = 50 cm) set in motion by a central rotor the number of turns of which could be adjusted by a digital switch. The turntable could hold up to six home cages and during the experiment it was rotated at the constant rate of 56 or 47.56 rpm, producing a resultant linear acceleration of 2 or 1.08 G, respectively.

After adaptation, HG and RC mice were rotated at 2 and 1.08 G, respectively for 1h (HG1 and RC1) or 2 h (HG2 and RC2), and their behaviours videorecorded both during, and for 1 h before and after rotation. After each rotational session, videotapes were scored by a highly trained observer using a dedicated ethological software [16]. The presence of the following behavioural items (see [11,17] for details) were recorded during 5 × 5 min-blocks (0–5, 10–15, 25–30, 40–45, 55–60) for each hour of videorecording.

Bar holding: grasping the metal top of cage holding itself above the level of the ground; *exploring*: moving around the cage, exploring the environment; *rearing*: standing on hind legs; *wall rearing*: standing on hind legs and placing forelimbs on the wall of the cage; *sniffing*: sniffing the environment; *resting* (either with *open* or *closed eyes*): no visible movements, eyes open or closed; *head moving*: moving the head up and down; *digging*: digging in the sawdust, pushing and kicking it around using the snout and/or both the forepaws and hindpaws, mostly moving around the cage and sometimes changing the whole arrangement of the substrate material; *grooming*: wiping, licking, combing any part of the body. Moreover, locomotor activity was evaluated: the cage was subdivided into four equal sections by lines placed on the video screen at the time of videorecording analysis, and the number of line crossings (with both forepaws) was counted.

2.4. Hole-board test

Twenty minutes after the end of post-rotation mice were tested in a hole-board apparatus. Testing was

carried out under red light between 14:00 a.m. and 16:00 p.m. The hole-board (Ugo Basile, Biological Research Apparatus, Varese, I-21025, Italy) consisted of a square unwalled platform (40 × 40 cm), raised 11.5 cm off the floor, containing 4 × 4 equally spaced (7 cm) holes, each 1.5 cm in diameter. The mice were placed individually on the platform and their behaviours videorecorded for 7 min using a Sony VO-5630 apparatus equipped with CH-1400 CE videocameras for red light. Locomotor activity was scored by determining the number of sub-areas crossed, while the number of holes explored was recorded by counting *head-dippings*: scored when the head entered the hole at least up to eye level. The latency to the first *head-dipping*, the number of different holes visited and the number of visits to the four central holes were also recorded. Moreover, an index of exploratory activity was calculated by dividing the total number of visits by the number of visits to the four central holes [18].

2.5. Plus-maze test

One day after the rotation mice were tested in a plus-maze apparatus. Tests were conducted under red light between 14:00 a.m. and 16:00 p.m. The elevated plus-maze comprised two open arms (30 × 5 × 0.25 cm) and two closed arms (30 × 5 × 15 cm) that extended from a common central platform (5 × 5 cm). The apparatus was constructed from Plexiglas (black floor, clear walls) and elevated to a height of 60 cm above the floor level. Mice were individually placed on the central platform facing an open arm and allowed to freely explore the maze for 6 min. All sessions were videorecorded by a camera linked to a monitor and VCR (see hole-board test) in the adjacent room and, to avoid unnecessary distractions, the experimenters retreated to this location during testing. Videotapes were scored using the same software adopted for behavioural scoring on the rotational apparatus [16]. Behavioural parameters included both conventional spatiotemporal and ethological measures (according to [19,20]). Conventional measures were the frequencies of total, open and closed entries (arm entry = all four paws into an arm), % open entries [(open/total) × 100], and % time spent in open and closed parts of the maze [e.g. (time open/session duration) × 100]. Ethological measures included frequency and duration scores for *rearing* (vertical movement against the side and/or end of the walls; note that mice very rarely exhibit unsupported rearing), *immobility*, *grooming* (licking, scratching and washing of the head and body), *head-dipping* (exploratory movement of head/shoulders over the side of maze) and *stretched-attend postures* (SAP: exploratory posture in which the body is stretched forward and then retracted to the original position without any forward locomotion) [19,20]. Moreover, in view of the importance of thigmotactic cues to rodent exploration in the plus-maze [21], *head-dipping* and SAP were also differentiated as a function of their occurrence in different parts of the maze. Thus, the closed arms and center platform were designated as "protected" areas (i.e. offering relative security) and the "percent protected" scores for *head-dipping* and SAP calculated as the percentage of these behaviours displayed in the protected areas (e.g. [(protected SAP/total SAP) × 100]). Moreover, as an additional index of animal hypergravity-induced anxiety, has been calculated by the latency to the first entry in the closed arm.

2.6. Statistical analyses

Kaolin and food consumption were analysed by a mixed-model ANOVA considering treatment and sex as grouping factors and pre and post-rotational days as repeated measures.

Kruskal–Wallis non-parametric ANOVA was performed on the behavioural parameters separately for pre-rotation, rotation 1st hour, rotation 2nd hour and post-rotation measures. The χ^2 partitioning was then applied to each variable to test the main effects and the interaction of rotation length (when appropriated), hypergravity level and sex (each with one degree of freedom).

Kruskal–Wallis non-parametric ANOVA was also applied to hole-board and plus-maze performances, considering the 10 groups of mice, that is the control (males and females) and the four rotated ones (males and females). The χ^2 partitioning was subsequently used to test the difference between control mice on one hand and the groups of rotated mice on the other hand. The main effects of the length of rotation and the hypergravity level and their interactions within the groups of rotated mice, and the main effect of sex and its interactions with the other factors have been also reported.

3. Results

3.1. Food and kaolin

Increase in kaolin consumption, but not in food, was evident in post-rotational days in rotated animals (treatment × repeated measures, $F_{(4,90)} = 2.34$; $p = 0.06$; Table 1). In particular, in the 1st day after rotation, all

Table 1
Mean (± SE.) consumption of kaolin in CD-1 mice pre- and post-exposure to 2 G

	Pre-rotation				Post-rotation			
	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
Stationary controls								
Females	0.14 ± 0.10	0.14 ± 0.10	0.03 ± 0.01	0.05 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00
Males	0.03 ± 0.01	0.12 ± 0.10	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00
1h rotational controls								
Females	0.02 ± 0.00	0.03 ± 0.00	0.05 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Males	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.03 ± 0.01	0.02 ± 0.00	0.01 ± 0.00
1h hypergravity								
Females	0.24 ± 0.14	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.26 ± 0.15	0.14 ± 0.11	0.37 ± 0.17	0.15 ± 0.11
Males	0.04 ± 0.01	0.04 ± 0.01	0.02 ± 0.00	0.02 ± 0.01	0.12 ± 0.09	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
2h rotational controls								
Females	0.12 ± 0.10	0.03 ± 0.01	0.12 ± 0.11	0.03 ± 0.01	0.23 ± 0.13	0.06 ± 0.01	0.05 ± 0.01	0.03 ± 0.01
Males	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.00	0.03 ± 0.01	0.02 ± 0.00
2h hypergravity								
Females	0.14 ± 0.10	0.23 ± 0.14	0.03 ± 0.01	0.13 ± 0.10	0.23 ± 0.13	0.25 ± 0.13	0.16 ± 0.10	0.04 ± 0.01
Males	0.03 ± 0.01	0.12 ± 0.10	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.01

Kaolin intake is shown as the difference between consecutive days. Rotational Controls, exposure to 1 G; Hypergravity, exposure to 2 G.

female groups, except SC and RC1, showed higher level of kaolin consumption, while only HG1 male showed a slight increase in kaolin consumption.

In the following days, HG females ate more kaolin than the other groups and on the last post-rotational day of observation only HG1 females were still eating more kaolin.

3.2. Behavioural observation (frequency of behavioural items are reported in Fig. 1A–B)

3.2.1. Bar holding

During the 1st hour of rotation a main effect of sex (S) emerged, females performing *bar holding* more often and longer than males (S (freq), $\chi^2_1 = 3.95$; S (dur), $\chi^2_1 = 6.22$; $p < 0.05$; Fig. 1A; data not shown for durations). Moreover, a $S\times$ level of gravity (G) interaction also emerged: HG females showing a dramatic reduction of *bar holding* when compared with RC females ($S \times G$ (freq), $\chi^2_1 = 4.87$; $S \times G$ (dur), $\chi^2_1 = 4.42$; $p < 0.05$); a trend toward this difference was also observed in post-rotation (S (freq), $\chi^2_1 = 3.31$; $p = 0.07$; S (dur), $\chi^2_1 = 3.11$; $p = 0.08$). In post-rotation, an interaction between time of exposure (T) and G was evident, with HG2 animals, but not HG1, recovering *bar holding* behaviour at the level of RC group ($T \times G$ (freq), $\chi^2_1 = 7.13$; $T \times G$ (dur), $\chi^2_1 = 4.8$; $p < 0.05$).

3.2.2. Exploring

Frequency of *exploring* behaviour was highly reduced in HG animals during the 1st hour of rotation (G (freq), $\chi^2_1 = 20.87$; $p < 0.05$). Furthermore, HG females spent less time *exploring* the environment than RC ones ($S \times G$ (dur), $\chi^2_1 = 6.07$; $p < 0.05$). In post-rotation, frequency of *exploring* recovered to pre-rotation levels in all groups, except in the HG1 one ($T \times G$ (freq), $\chi^2_1 = 11.38$; $p < 0.05$; $T \times G$ (dur), $\chi^2_1 = 2.93$; $p = 0.09$). Moreover, 2 h rotated females showed higher levels of this behaviour than males ($T \times S$ (dur), $\chi^2_1 = 4.18$; $p < 0.05$; $T \times S$ (freq), $\chi^2_1 = 2.8$; $p = 0.09$).

3.2.3. Rearing and wall rearing

Rearing and *wall rearing* behaviours were overall reduced during rotation. However, a G effect emerged, with HG group reducing these behaviours more consistently than RC during both the 1st hour (*rearing*: G (freq), $\chi^2_1 = 11.28$; G (dur), $\chi^2_1 = 5.93$; $p < 0.05$; *wall rearing*: G (freq), $\chi^2_1 = 29.82$, G (dur), $\chi^2_1 = 30.25$; $p < 0.05$) and the 2nd hour (statistically significant only for *wall rearing*: G (freq), $\chi^2_1 = 6.71$; G (dur), $\chi^2_1 = 6.99$; $p < 0.05$). A $T \times G$ effect was evident in post-rotation, with HG1 performing less *rearing* and *wall rearing* than HG2 group (*rearing*: $T \times G$ (freq), $\chi^2_1 = 13.02$; $T \times G$ (dur), $\chi^2_1 = 12.18$; $p < 0.05$; *wall rearing*: $T \times G$ (freq), $\chi^2_1 = 8.31$; $T \times G$ (dur), $\chi^2_1 = 4.28$; $p < 0.05$). A $T \times S$ trend for *rearing* ($T \times S$ (dur), $\chi^2_1 = 3.21$; $p = 0.07$) and a significant $T \times S$ interaction for *wall rearing* ($T \times S$ (freq), $\chi^2_1 = 3.74$; $p = 0.05$; $T \times S$ (dur), $\chi^2_1 = 4.14$; $p < 0.05$) emerged in post-rotation, females rotated for a longer time showing higher levels of vertical activity than the other rotated groups.

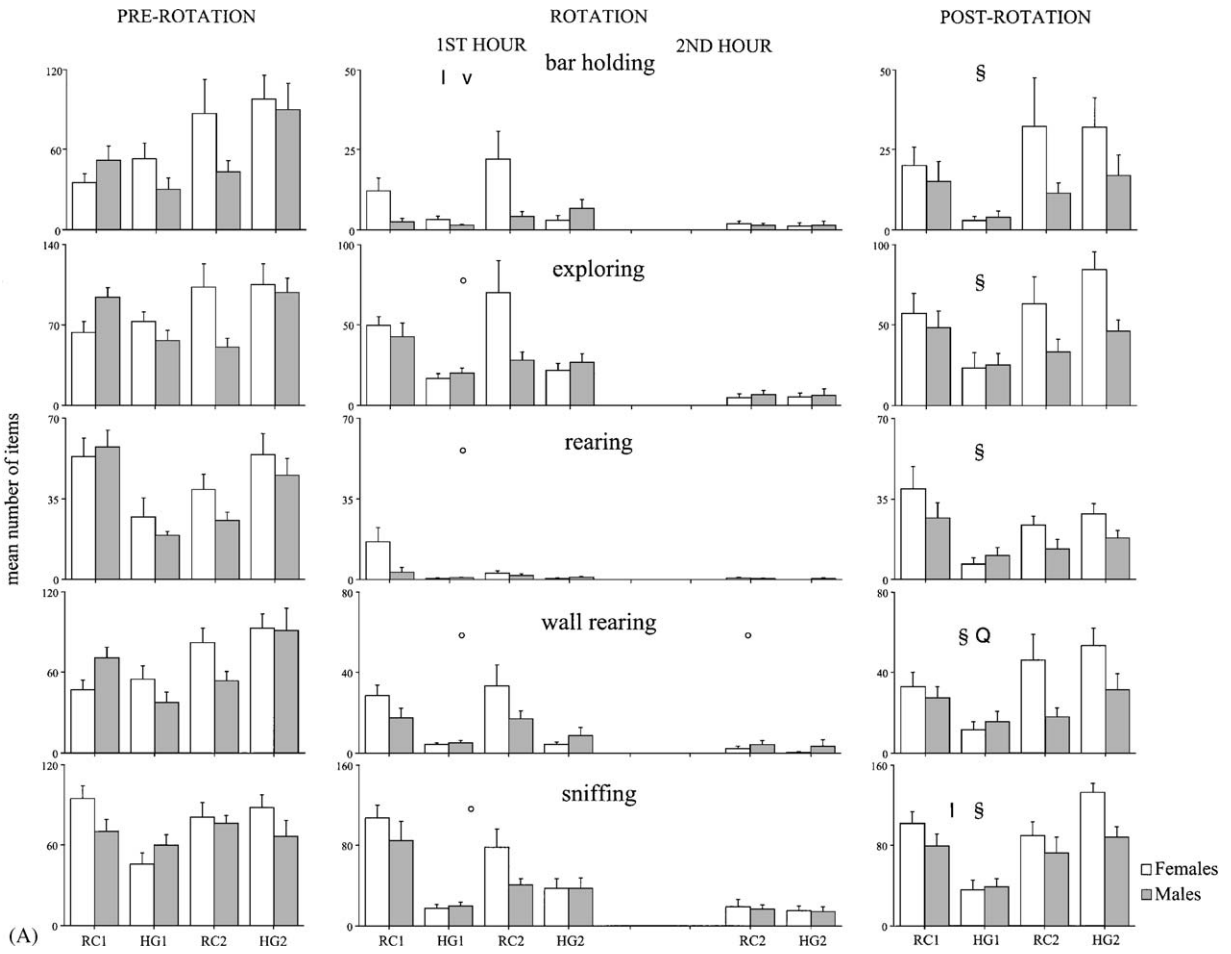

Fig. 1. Behavioural responses of male and female CD-1 mice occurring during 1 h pre-, 1 h or 2 h during and 1 h post-rotation periods. Significant effects ($p < 0.05$): I = main effect of S; ◯ = main effect of G; Δ = main effect of T; V = interaction $S \times G$; Q = interaction $T \times S$; § = interaction $T \times G$; S = sex; G = level of hypergravity (1 G; 2 G); T = exposure duration (1 h; 2 h).

3.2.4. Sniffing

Frequency and duration of *sniffing* behaviour were reduced during rotation in HG groups (G (freq), $\chi^2_1 = 28.64$; G (dur), $\chi^2_1 = 21.69$; $p < 0.05$). A $T \times G$ effect was evident in post-rotation, HG1 mice performing less and shorter and HG2 more and longer *sniffing* than the other groups ($T \times G$ (freq), $\chi^2_1 = 17.04$; $T \times G$ (dur), $\chi^2_1 = 6.26$; $p < 0.05$). Moreover, in post-rotation females sniffed the environment more often than males (S (freq), $\chi^2_1 = 4.61$; $p < 0.05$) and this effect was more evident in animals rotated for 1 h.

3.2.5. Open eyes resting

This behaviour appeared at the time of rotation and was more pronounced in HG mice than in RC ones (G (freq), $\chi^2_1 = 3.3$; $p = 0.07$; G (dur), $\chi^2_1 = 11.93$; $p < 0.05$; see Fig. 1B; data not shown for duration scores). Since the 1st hour of rotation a difference between males and females emerged; however, this difference became fully evident during the 2nd hour of rotation, when females showed higher values of both frequency and duration of *open eyes resting* than males (1st hour of rotation: S (freq), $\chi^2_1 = 4.88$; $p < 0.05$; S (dur), $\chi^2_1 = 3.89$; $p = 0.05$; 2nd hour of rotation: S (freq), $\chi^2_1 = 11.1$; S (dur), $\chi^2_1 = 11.8$; $p < 0.05$). Moreover, in post-rotation females rotated for 1h continued to rest more with eyes open ($T \times S$ (freq), $\chi^2_1 = 7.88$; $T \times S$ (dur), $\chi^2_1 = 5.24$; $p < 0.05$) than all the other experimental subjects.

3.2.6. Closed eyes resting

Overall, this behavioural endpoint considerably increased during rotation. In the course of the 1st hour of exposure RC groups performed *closed eyes resting* more and for a longer time than HG ones (G (freq), $\chi^2_1 = 5.17$;

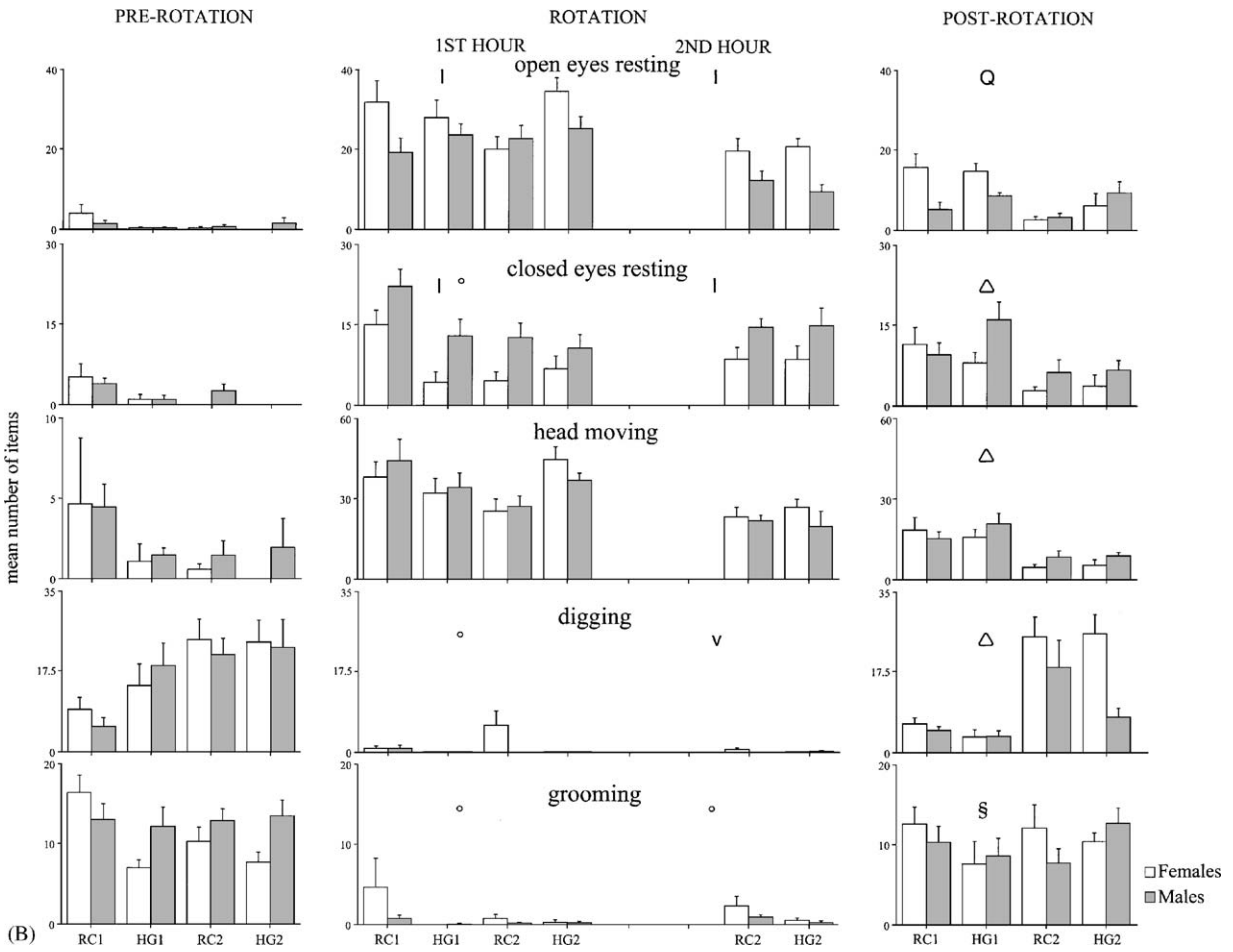

Fig. 1. (*continued*).

$p < 0.05$; G (dur), $\chi^2_1 = 3.22$; $p = 0.07$). Sex differences were fully evident since the 1st hour of rotation throughout the end of the post-rotation phase, male resting with closed eyes more than females (1st hour of rotation (S (freq), $\chi^2_1 = 11.54$; S (dur), $\chi^2_1 = 14.6$; $p < 0.05$; 2nd hour of rotation: S (freq), $\chi^2_1 = 5.19$; S (dur), $\chi^2_1 = 14.14$; $p < 0.05$; post-rotation: S (freq), $\chi^2_1 = 3.68$; $p = 0.05$; S (dur), $\chi^2_1 = 5.67$; $p < 0.05$). Moreover, in post-rotation animals rotated for 1 h rested longer and more often than those rotated for 2 h (T (freq), $\chi^2_1 = 12.77$; T (dur), $\chi^2_1 = 5.46$; $p < 0.05$).

3.2.7. Head moving

The frequency of this item steadily increased during rotation with HG showing higher level of this behaviour than RC (statistically significant only for the 1st hour of rotation: G (dur), $\chi^2_1 = 24.75$; $p < 0.05$). In post-rotation phase, a clear T effect emerged, mice rotated for 1 h performing *head moving* behaviour more often and for a longer time than mice rotated for 2 h (T (freq), $\chi^2_1 = 18.15$; T (dur), $\chi^2_1 = 13.16$; $p < 0.05$).

3.2.8. Digging

This behaviour dramatically decreased during rotation. In the course of the 1st hour of exposure, *digging* was totally suppressed only in the HG groups. (G (freq), $\chi^2_1 = 4.75$; $p < 0.05$; G (dur), $\chi^2_1 = 3.16$; $p = 0.08$). However, during the 2nd hour of rotation, a consistent reduction of this item was also observed in RC2 mice and particularly in males, which showed a complete suppression of this behaviour ($S \times G$ (freq), $\chi^2_1 = 4.36$; $S \times G$ (dur), $\chi^2_1 = 4.34$; $p < 0.05$). In post-rotation, a recover to pre-rotational levels was evident only in animals rotated for 2 h (T (freq), $\chi^2_1 = 26.3$; T (dur), $\chi^2_1 = 21.4$; $p < 0.05$). In particular, the trend was for females to exhibit this behaviour more often than males ($T \times S$ (freq), $\chi^2_1 = 3.63$; $p = 0.06$; $T \times S$ (dur), $\chi^2_1 = 3.04$; $p = 0.08$).

Table 2
Parameters of exploration of male and female CD-1 mice in a 7 min hole-board test

	Latency to the first head-dipping[a]	Number of holes visited[a]	Number of visits to the central holes	Total number of visits/Number of visits to the central holes	Number of sub-areas crossed[a]
Stationary controls					
Females	14.4 ± 4.3	15.4 ± 0.2	12.3 ± 1.6	3.5 ± 0.4	68.1 ± 4.3
Males	16.0 ± 4.4	15.1 ± 0.4	15.7 ± 2.5	2.7 ± 0.5	55.9 ± 6.2
1 h rotational controls					
Females	12.7 ± 1.7	14.8 ± 0.4	11.6 ± 0.1	2.8 ± 0.4	76.0 ± 4.6
Males	18.7 ± 5.0	12.3 ± 0.9	8.2 ± 1.3	3.4 ± 0.8	74.0 ± 7.8
1 h hypergravity					
Females	19.6 ± 5.0	14.6 ± 0.5	14.0 ± 2.5	3.7 ± 1.0	57.0 ± 4.6
Males	18.7 ± 4.9	14.0 ± 0.8	13.4 ± 2.4	2.9 ± 0.6	55.8 ± 3.2
2 h rotational controls					
Females	32.6 ± 5.9	14.0 ± 0.5	10.9 ± 2.0	3.3 ± 0.5	64.2 ± 6.2
Males	18.3 ± 3.8	15.0 ± 0.2	11.1 ± 1.5	4.0 ± 0.6	74.2 ± 6.0
2 h hypergravity					
Females	32.0 ± 6.6	14.2 ± 0.3	13.3 ± 1.8	2.6 ± 0.5	67.5 ± 6.3
Males	30.6 ± 8.6	14.1 ± 0.6	13.6 ± 2.8	3.3 ± 0.7	72.3 ± 6.1

[a]Rotated mice vs. stationary controls, $p < 0.05$. Rotational control, exposure to 1 G; hypergravity, exposure to 2 G.

3.2.9. Grooming

This behaviour was highly suppressed in both the 1st and 2nd hour of rotation in HG mice (1st hour of rotation: G (freq), $\chi^2_1 = 9.96$; G (dur), $\chi^2_1 = 10.52$; $p < 0.05$; 2nd hour of rotation: G (freq), $\chi^2_1 = 6.24$; G (dur), $\chi^2_1 = 6.93$; $p < 0.05$). In post-rotation phase, *grooming* reappeared reaching the pre-rotation levels in all groups, except in the HG1 one ($T \times G$ (freq), $\chi^2_1 = 4.08$; $p < 0.05$).

3.2.10. Locomotor activity

In pre-rotation female were more active than males (S, $\chi^2_1 = 7.16$; $p < 0.05$). During the 1st hour of rotation HG mice locomoted less than RC ones (G, $\chi^2_1 = 19.9$; $p < 0.05$); however, HG females continued to be more active than HG males ($G \times S$, $\chi^2_1 = 4.14$; $p < 0.05$). Locomotor activity was deeply depressed in the 2nd hour of rotation in RC and HG groups, while in post-rotation, with the exception of HG1 group ($T \times G$, $\chi^2_1 = 11.22$; $p < 0.05$), it quickly recovered with females being more active than males in all groups (data not shown).

3.3. Hole-board test

As a whole, rotated animals had longer latencies to the first *head-dipping* than SC ones ($\chi^2_1 = 0.29$; $p < 0.05$) and this was true in particular for RC2 females and both HG2 females and males ($T \times S$, $\chi^2_1 = 6.65$; $p < 0.05$; see Table 2). Rotation per se also affected the number of holes visited, RC mice visiting a minor number of holes than SC ones ($\chi^2_1 = 8.1$; $p < 0.05$). No differences were observed in the number of *head-dippings* in the four central holes and in the ratio total number of visits/number of visits to the four central holes. As for the locomotory activity, measured as number of sub-area crossed, a $T \times S$ effect was evident, females rotated for 2 h being less active than males ($T \times S$, $\chi^2_1 = 4.32$; $p < 0.05$).

3.4. Plus-maze test

HG mice entered less often than either RC or SC in both open and closed arms (open arms: G (freq), $\chi^2_1 = 3.74$; $p < 0.05$; closed arms: G (freq); $p < 0.05$). Moreover, with the exception of HG1 group, males were always more active than females, and entered the open arms more often than females ($T \times G \times S$ (freq), $\chi^2_1 = 5.03$; $p < 0.05$; see Fig. 2). A $T \times S$ interaction was evident for both frequency and duration of *rearing*, *head-dipping* and *SAP* behaviours (*Rearing*: $T \times S$ (freq), $\chi^2_1 = 22.05$; $T \times S$ (dur), $\chi^2_1 = 15.97$; $p < 0.05$; *Head-dipping:* $T \times S$ (freq), $\chi^2_1 = 10.44$; $T \times S$ (dur), $\chi^2_1 = 11.22$; $p < 0.05$; *SAP*: $T \times S$ (freq), $\chi^2_1 = 11.63$; $T \times S$ (dur), $\chi^2_1 = 6.75$; $p < 0.05$). In particular, RC2 and HG2 females showed reduced frequency and duration of *rearing* and *head-dipping* while consistently increased *SAP* (Fig. 2). A similar trend was also observed for the percentage of *SAP* performed in the protected areas ($T \times S$, $\chi^2_1 = 3.49$; $p = 0.06$). In addition, for this parameter a $T \times G$ trend also emerged ($T \times G$ (freq), $\chi^2_1 = 3.82$; $p = 0.05$) with HG mice performing *SAP* more than RC and SC.

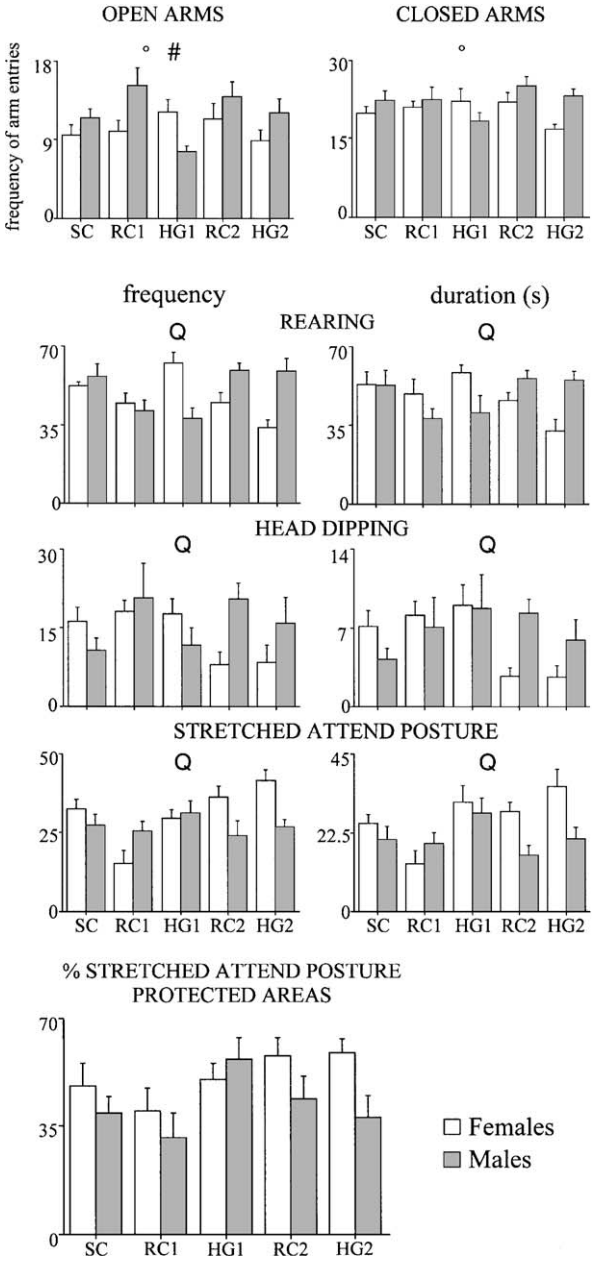

Fig. 2. Plus-maze performance of male and female CD-1 mice. Significant effects ($p < 0.05$): ◯ = main effect of G; # = interaction $T \times G \times S$; Q = interaction $T \times S$; S = sex; G = level of hypergravity (1 G; 2 G); T = exposure duration (1 h; 2 h).

4. Discussion

The present results clearly indicate that rotation-induced hypergravity influenced the behavioural responses of CD-1 mice, reducing their spontaneous activity and concomitantly increasing their resting behaviour. According to our previous research, this reduction of spontaneous activity may be suggestive of animals experiencing motion sickness syndrome as a consequence of being subjected to rotational stimuli [11,22]. In fact, in the present study pica behaviour, a motion sickness index measured through the consumption of a non-food substance such as kaolin, showed a tendency to increase upon rotation, although the effect was not supported by a statistical significance. Pica behaviour is reportedly difficult to assess due to large interindividual differences in MS susceptibility [23], and this may be the reason for the lack of a clear-cut effect of rotational exposure on kaolin consumption. Animals rotated at 2 G for 1 h seemed the most affected, HG1 females being the only experimental group still eating kaolin in the 4 day after rotation and HG1 males the only male group showing an increase in kaolin intake. These effects confirm that females are more vulnerable than males to the symptoms associated with motion sickness, [11,22]. Moreover in accordance with the effects on behavioural responses (see below) they are suggestive of HG1 mice being less able to recover than HG2 animals.

According to our previous experiments [11] mice spontaneous activity was deeply affected by single hypergravity exposure, and, in most cases, the effects were independent of the exposure duration. Specifically, during rotation, grooming, digging, exploring and vertical activity were almost or totally suppressed in HG mice, while open eyes resting—which in mice has been previously described as a specific index of motion sickness [11]—increased. Interestingly, males spent more time doing resting behaviour than females since the 1st hour of rotation, while in females such behaviour only increased during the second hour of rotation. Moreover, in post-rotation, HG1 females persisted in showing high levels of this behaviour. These data are in accordance with those on pica behaviour pointing out that females are more susceptible than males to the onset of motion sickness.

It should be noted, however, that in HG2 animals the majority of behaviours regained the pre-rotational levels soon after the rotation was terminated, while failed to recover in HG1 mice. In particular grooming behaviour reappeared reaching the pre-rotation levels in all groups except in the HG1 one. Grooming behaviour in rats is a classical measure of displacement activity but also coincides with the period after arousal and rather reflects the process of dearousal due to habituation to a stressful situation [24]. Therefore the reappearance of pre-rotation behavioural profile in the groups of mice rotated for the longest time could be reasonably a consequence of habituation processes taking place during the 2nd hour

of rotation, reducing motion sickness and leading to a recovery in the behavioural profile [12]. This is particularly evident in females which recover better than males, as indicated, in post-rotation, by their higher levels of several behavioural endpoints, such as exploring, vertical activity, digging, and locomotor activity.

Mice exploratory behaviour in the hole-board was impaired, as indicated by a minor number of holes visited and a higher latency to the first head dipping, and the changes appeared to be a consequence of the exposure to the rotational stimuli per se.

By contrast, a clear effect of gravitational environment emerged in the plus maze with HG mice more often avoiding both open and closed arms than the other groups. In addition, they showed a marked tendency to display SAP behaviour especially in protected areas. These results, pointing to alterations in locomotor activity [25], reflects a reduced motivation to explore as a consequence of increased anxiety. SAP behaviour in the elevated plus maze has been related to risk assessment and defensive behaviours [26,27]. Since it has also been shown to be particularly responsive to various anxiolytic drugs, high levels of this response have been considered a reliable index of increased anxiety [25,28,29].

Therefore it appears that rotation *per se* represents a stressful condition affecting the level of exploratory behaviour while hypergravity acts as an additional stimulus selectively affecting their emotional-anxiety profile.

Females appeared generally more susceptible than males to the distressful stimuli associated with rotational/gravitational environment, although they turned out to recover faster than males. They also appeared more anxious than males. In fact, in the plus maze, 2 h-rotated females showed lower levels of rearing and head dipping and higher levels of SAP than males. As documented by an extensive literature on the subject [30–32], sex differences in response to environmental variables are found across species in many behavioural aspects. Such differences can be attributed to proximate (e.g. endocrine, genetic and environmental factors acting on behaviour) as well as ultimate mechanisms such as different adaptive strategies in coping with stressful situations [28,33].

Therefore it appears that exposure to rotation can highlight sex differences in susceptibility and copying to rotational stimuli which, in turn, might be reflected in subsequent behavioural responses.

As a whole the present data confirm the mouse as a good model for future space biology research aimed at understanding the behavioural consequences of the adaptation to different gravity environments. Further studies need to be developed using a protocol of chronic exposure to hypergravity, which may prove useful to assess the consequences of a prolonged permanence in space and to provide important insights about the effects of long-term exposure to altered gravity on adaptive behavioural responses.

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