Altered Behaviour in Hamsters Conceived and Born in Hypergravity

H. N. P. M. SONDAG,† H. A. A. DE JONG AND W. J. OOSTERVELD

Vestibular Department ENT, Room D2.218, Academic Medical Center, University of Amsterdam, Meibergdreef 9, NL-1105 AZ Amsterdam, The Netherlands

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ABSTRACT: We studied vestibular function in 37 hamsters (1 month old) conceived and born in either hypergravity (n = 21) or normal gravity (n = 16). Four groups were made: (1) HL group: 20 weeks in 2.5 G and 14 weeks in 1 G; (2) HS group: 4 weeks in 2.5 G and 30 weeks in 1 G; (3) CON group: 34 weeks in 1 G; and (4) ROT group: 4 weeks in 1 G, 16 weeks in rotation in 1 G, at the centre of the centrifuge and 14 weeks 1 G. When the hamsters were 4 weeks old, their locomotor activity, swimming ability, and air-righting was assessed. We found that HL and HS hamsters had no disturbances during locomotion in 1 G but their swimming ability was disturbed (swimming underwater, circling, and decreased speed of swimming). The HL hamsters showed less activity during 2.5 G and showed fewer correct air-rightings than the other groups. Differences between groups in swimming ability and the number of correct air-righting responses remained even after 3 months of normal gravity. Based on these findings, we suggest that the persistent behavioural disturbances are caused by the embryonal development of the hamsters in a hypergravity environment. Furthermore, hypergravity and rotation each have a different effect on behaviour. © 1997 Elsevier Science Inc.

KEY WORDS: Centrifugation, Locomotion, Balancing, Air-righting, Swimming.

INTRODUCTION

During missions in space, conflicting sensory input from the vestibular and visual perception systems seem to cause symptoms of motion sickness (Space Adaptation Syndrome) that decreases the performance of the astronauts [11,24,27]. After exposure to increased gravity (hypergravity or HG), astronauts reported the same kind of sensations as those after a space flight [2,20]. The sensory input from the peripheral vestibular organs seems to have been changed during exposure to an altered gravity. The question remains if long-term exposure to an altered gravity state leads to irreversible alterations in the peripheral vestibular system and its function. Animal research is necessary to study the mechanisms involved in adaptation of this system to different G-levels.

In a centrifuge, it is possible to breed and keep animals in hypergravity for long periods without severe effects on their anatomy [8,23]. In this experiment we bred hamsters in a centrifuge in 2.5 times normal gravity (HG hamsters). Earlier, we reported a structural alteration in the distribution of the utricular otoconia of these hamsters [32]. Here, we report the effects of hypergravity on their behaviour. During hypergravity, the animals’ locomotor activity might be changed because of the increased G-load. We assessed their locomotor activity by means of an activity wheel in the centrifuge.

Living in hypergravity could have affected motor programs and muscle function, thus affecting the locomotion of these HG-bred hamsters in normal gravity. Therefore, the placing of the paws (distance between paws) and dynamics (swing and stance time) of normal locomotion in 1 G was studied.

The disruption in spatial orientation, which occurs in humans subjected to an altered gravity, seems to have a vestibular background [2]. Animals postnatally exposed to hypergravity also showed disruption of behaviour in tasks that require normal vestibular functioning [4,5,9,31]. Swimming and air-righting are tasks that depend on correct peripheral vestibular information for normal performance [10,12,21,33]. Pharmacological studies have shown that the peripheral vestibular system is involved in swimming [10,14,25]. Furthermore, the swimming performance proves to be a sensitive index for measuring the degree of vestibular dysfunction, ranging from inability to swim or having problems to orientate in the water [12,16,29]. The air-righting reflex is highly dependent on proper otolithic function. Rats postnatally exposed to hypergravity showed disruption of this behaviour during normal gravity [6,9]. Both swimming and air-righting will be assessed in our experiment.

MATERIALS AND METHODS

The study was done in a group of 37 subadult Golden hamsters (Mesocricetus auratus). The hamsters were conceived and born at our laboratory. Of this group, 21 hamsters were conceived and born under conditions of 2.5 G (HG hamsters) in the centrifuge, while the remaining 16 hamsters were conceived and born in normal gravity (CON hamsters). After weaning, eight animals of the HG group were placed in normal gravity (HS for Short Hypergravity period) to investigate whether age is an important factor concerning adaptation to an altered gravity. The other HG animals were placed in normal gravity after 20 weeks of hypergravity (HL for Long Hypergravity period). During the first part of the experiment (part 1: weeks 4 to 20) the HL lived in hypergravity while the ROT hamsters were exposed to rotu-
tion. In the second part (part 2: weeks 21 to 34), all groups lived in normal gravity.

Animals were distributed into four groups: (a) HG hamsters living in 2.5 G for 20 weeks and 14 weeks in 1 G (HL group, n = 13). (b) HG hamsters living in 2.5 G for 4 weeks and 30 weeks in 1 G (HS group, n = 8). (c) CON hamsters living in 1 G for 34 weeks (CON group, n = 9). (d) CON hamsters living in 1 G for 4 weeks, then in rotation (1 G, 34.3 rpm) for 16 weeks and 14 weeks months 1 G (ROT group, n = 7).

The hamsters of all four groups were 4 weeks old with a mean weight of 32.5 g for the HS and HL hamsters and 41.8 g for the CON and ROT hamsters at the start of the experiment. They lived in acrylate boxes (22 × 37 cm; three or four animals per box), inside the centrifuge-gondola (HL group), in the centre of the centrifuge (ROT group), or in similar housings under normal gravity (CON and HS hamsters). Food and water were available ad lib and the day–night cycle was reversed (lights on 1900–0700 h) to study their behaviour during their activity period. During a centrifuge stop of 30 min, the hamsters were tested in a laboratory room with dimmed lights.

After testing for 30 weeks (age of animals = 34 weeks), the hamsters were killed for histological and morphological examination of the otoconia. Our findings on these were presented elsewhere [32]. The body weight was measured twice a week. The experiments were performed following the Principles of Laboratory Animal Care (NIH publication No. 86-23, revised 1985) and with the recommendations provided in a special licence as required by the Dutch Law on the Use of Animals in Scientific Research.

The animal centrifuge consisted of a centrally placed 3.5-kW DC motor drive and two horizontally mounted arms (length = 115 cm) with aerated and darkened free-swinging gondolas (length: 110 cm, width: 45 cm, height: 80 cm, length arm + gondola: 194 cm). During centrifugation, the Z-vector was constantly directed towards the floor of the gondola. At a rotation speed of 34.3 rpm a 2.5 G-value was reached at the bottom of the boxes inside the gondola. A video camera was installed inside the gondola to allow animal observation. The ROT group was placed in a box located exactly on the rotation axis of the centrifuge. The centrifuge was stopped daily for animal care and testing.

**Tasks**

**Activity wheel**

To observe locomotor activity of HL hamsters during 2.5 G and of CON and HS hamsters during 1 G, an activity wheel was placed in one of the boxes. Each day, the locomotor activity of one hamster of the CON, HL, and HS groups was recorded by means of a bicycle computer (CC-MT200, CatEye, Osaka, Japan). The locomotor activity of the hamsters was measured by the average speed and the distance covered on the wheel in one day. The activity of each hamster was assessed once in 2 weeks. Because of limited space in the centre of the centrifuge this test was not assessed for the ROT hamsters.

**Gait and Stride Task**

The front and hind paws of the hamsters were dipped in a tray containing film developer (Agfa). They had to walk in a small Plexiglas walkway (length 43 cm, width 9.5 cm) in which an undeveloped x-ray film (Curix) was laid. The film with the paw prints was dipped in rapid fix (Agfa) for 5 min, washed for 10 min, and dried. This method was earlier described by de Medici et al. [19]. For evaluation of gait and stride we used the method of Hruska et al. [13]. We measured the distance between consecutive left hind paw prints (stride length) and the distance of the interposed right hind paw perpendicular to the line connecting the two consecutive left hind paws (stride width). Simultaneously, the dynamic aspects of the walking behaviour were recorded on tape (Panasonic, NV-FS90). We measured (intervals of 20 ms) the swing time (elevation and forward movement of the foot) and the stance time (placement of the foot upon the floor until the next swing) and calculated the percentage of swing time during one stride (swing time/stance time + swing time). This task was assessed once in 4 weeks.

**Rail Task**

The rail (acrylate tube: length 100 cm, diameter 20 mm), placed 20 cm above ground level was fixed to standards. The tube was covered with tape to give the hamster a better grip. A platform was placed at the end of the rail where the hamster could collect sunflower seeds for 5 s. One training day preceded testing, in which the hamsters were trained to walk the full length of the rail. Once a week, each hamster had to cross the rail three times.

**Swimming in a Lane**

We used the lane (140 × 10 cm, height of the walls 30 cm, water-depth 25 cm, water temperature approximately 30°C) to test swimming ability and speed. The hamsters had to swim to the end of the lane where they could climb an escape ladder. One extra day was used to train the hamsters for 5 min to swim to the ladder. On the testing days, the animals swam at maximum three trials in the lane and three in the water maze. The crossing time for the middle part of the lane (length 100 cm) was measured. These tests were assessed once a week.

**Orientation ability during swimming in a maze (140 × 70 cm).** The shape of the maze was similar to our earlier experiments [30,31]. If the hamsters were capable of finding the ladder in the lane, their orientation ability in the maze was assessed. Here, they had to find the ladder at the opposite end of the maze. The crossing time was measured and the orientation strategy (swimming along the walls or straight through the centre of the basin) was scored.

**Air-righting reflex and resurfacing.** The hamsters were dropped from a supine position (height 80 cm) into a water basin. To avoid visual input, this test was performed under infrared light conditions (870 nm) in which hamsters are unable to use visual information [34]. The hamsters’ behaviour was recorded on tape. Afterwards, we measured the number of correct air-righting responses (landing with feet parallel to the surface of the water) and the time needed to resurface after hitting the water during three trials. This task was assessed once in 4 weeks.

**Statistics**

For the crossing time and the falling frequency in the rail test, the crossing times in the swimming tests, the number of correct air-righting responses and for the time needed to resurface the mean of the three trials per animal was calculated. Data of the activity wheel, the stride test, swimming and air-righting were statistically assessed with repeated analysis of variance (ANOVA). The rail test was analysed with repeated analysis of variance with weight as covariates (ANCOVA). Post hoc analysis was done with the Tukey’s honestly significant difference test. We used the statistical software SPSS PC+ 5.0 for our analysis (significance: p < 0.05).
RESULTS

Body Weight

Repeated measurements showed group, $F(3, 31) = 17.91, p < 0.000$, time, $F(27, 744) = 985.59, p < 0.000$, and group $\times$ time interaction effects, $F(72, 744) = 12.82, p < 0.000$, during the first part of the experiment. Post hoc analysis revealed that all hamsters increased in weight but the HL and the HS groups weighed less than the CON and the ROT group ($p < 0.01$). Group, $F(3, 29) = 14.05, p < 0.000$, time, $F(12, 348) = 37.65, p < 0.000$, and group $\times$ time interaction effects, $F(36, 348) = 4.05, p < 0.05$, and post hoc analysis showed similar results for the second part of the experiment when all four groups were living in normal gravity (Fig. 1).

Activity Wheel

During part 1, repeated measurements revealed group, $F(2, 27) = 76.97, p < 0.000$, time, $F(8, 216) = 33.58, p < 0.000$, and group $\times$ time interaction effects, $F(16, 216) = 13.16, p < 0.000$, for average speed on the activity wheel and group, $F(2, 27) = 71.97, p < 0.000$, time, $F(8, 216) = 22.86, p < 0.000$, and group $\times$ time interaction effects, $F(16, 216) = 7.87, p < 0.000$, for the time spent on the wheel. Post hoc analysis ($p < 0.01$) revealed that the HL hamsters had a lower average speed and used the treadmill less often than HS and CON hamsters. The results stayed constant for the HL group, while speed and the time of using the treadmill decreased in the HS and CON groups. During the second part of the experiment all groups decreased their average speed on the wheel [time effect, $F(5, 115) = 3.88, p = 0.003$] and the time spent on the wheel [time effect, $F(5, 115) = 2.70, p = 0.024$; Fig. 2].

Gait and Stride Test

During the first part of the experiment, repeated measurements and post hoc analysis revealed that stride length [time effect, $F(5, 165) = 10.79, p < 0.000$, and group $\times$ time interaction effect, $F(15, 165) = 3.08, p < 0.000$] increased in all groups, but faster in the ROT and CON groups than in the HL and HS groups. Stride width was larger for the ROT group [group effect, $F(3, 30) = 11.96, p < 0.000$] than for the other groups. Stride width increased for all groups during part 1 [time effect, $F(5, 165) = 27.69, p < 0.000$]. In the second part, no differences were found in stride length. Stride width was the same for all groups at the end of the experiment [group $\times$ time interaction effect, $F(9, 87) = 2.82, p = 0.006$]. During part 1, no differences were found in the percent of stance or swing time between the groups. In the second part of the experiment, the ROT group showed larger stance times [group effect, $F(3, 29) = 5.18, p = 0.005$, group $\times$ time interaction effect, $F(9, 87) = 4.08, p < 0.000$] than the other groups. Stance times increased for all groups [time effect, $F(3, 87) = 2.97, p = 0.036$] during this part of the experiment. The differences in stance time responses for the ROT group also affected the percent of swing time, (group effect $F(3, 29) = 3.37, p = 0.032$, time effect, $F(3, 87) = 2.89, p = 0.040$, and group $\times$ time interaction effect, $F(9, 87) = 2.62, p = 0.010$).

Rail Task

Three of 13 HL hamsters and 2 of 7 ROT hamsters were not able to walk on the rail during the first and second testing day, whereas all 9 CON hamsters and all 8 HS hamsters could walk on the rail. Concerning crossing time, repeated measurement revealed time, $F(12, 312) = 14.17, p < 0.000$, and group $\times$ time interaction effects, $F(36, 312) = 5.26, p < 0.000$. Post hoc testing ($p < 0.01$) showed that the ROT hamsters needed more time (increased crossing time) than the other groups during the end of the first part. Time, $F(12, 312) = 7.75, p < 0.000$, and group $\times$ time interaction effects, $F(36, 312) = 4.73, p < 0.000$, were found for the fall frequency. Post hoc testing ($p < 0.01$) showed that the ROT and HL hamsters fell more from the rail than the CON or HS hamsters and their number of falls increased more than in the other two groups (Fig. 3). During the second part, repeated measurements revealed time, $F(10, 260) = 3.14, p = 0.001$, and group $\times$ time interaction effects, $F(30, 260) = 5.12, p = 0.000$, for the crossing time and a time effect, $F(10, 260) = 3.37, p = 0.000$, for the fall frequency. Post hoc testing ($p < 0.01$) showed that the ROT hamsters needed more time than the other groups, and this time increased more for the CON.
group than for the other groups at the end of the experiment. The fall frequency decreased for all groups (Fig. 3).

Swimming Behaviour in Lane and Maze

In the lane: the HL hamsters showed disturbances in swimming such as circling under water (half of the group had to be saved from drowning), swimming in an almost vertical position and trying to cling on to the walls of the lane. At the end of the first part, 7 of 13 HL hamsters (54%) were able to swim to the stair in the lane during the three trials (Fig. 4). Repeated measurement analysis for this group was impossible because of missing trials during the whole experiment (the hamsters were not able to finish the trials every week, due to swimming problems). Therefore, the data of this group were excluded from the statistical analysis. Of the HS hamsters, five of eight hamsters (63%) found the stair after 6 weeks of testing. The CON and ROT hamsters swam straight to the stair. During week 7 to the end of part 1, the HS hamsters that were able to find the stair needed more time to swim to the stair than the CON or ROT hamsters [group effect, $F(2, 18) = 7.73, p < 0.05$].

The crossing time decreased during this period for all groups [time effect, $F(7, 126) = 2.65, p = 0.014$]. In the second part, 5 of 11 HL hamsters (45%) and 5 of 8 HS hamsters (63%) finished the trials in the lane after 3 months of normal gravity (Fig. 4). The crossing time of the HS hamsters remained higher than for the CON and ROT groups [group effect, $F(2, 18) = 8.03, p = 0.003$].

In the maze, 4 of 13 HL hamsters (31%), 5 of 8 HS hamsters (63%), 6 of 7 ROT hamsters (86%), and all 9 CON hamsters were able to find the stair during the three successive trials in the first and second part. The data of the HL group was excluded from statistical analysis. During the first part, the CON and ROT hamsters needed less time to find the ladder (crossing time) than the HS hamsters [group effect, $F(2, 17) = 5.19, p = 0.017$]. The crossing time decreased for all three groups [time effect, $F(6, 102) = 1.51, p = 0.003$]. During the second part, the HS group needed more time to find the stair than the CON and ROT groups [group effect, $F(2, 16) = 6.53, p = 0.008$]. For all three groups the crossing time decreased [time effect, $F(10, 160) = 2.02, p = 0.034$].

Air-Righting Reflex

During both parts of the experiment, the HL hamsters made less correct air-righting responses than the other groups [group effect part 1, $F(3, 33) = 110.55, p < 0.000$, group effect part 2, $F(3, 29) = 44.86, p < 0.000$]. At the end of part 2, the number of correct responses increased for the HL hamsters [group × time interaction effect, $F(9, 87) = 6.56, p < 0.000$] (Fig. 5).

DISCUSSION

To our knowledge, this study is the first one that investigated the effects of prenatal development in hypergravity on behaviour. Other studies have reported the behavioral disturbances in animals postnatally exposed to hypergravity [4–6,9,30,31]. The re-
Hypergravity hamsters weighed less than hamsters conceived and born in normal gravity (HL and HS hamsters vs. CON and ROT hamsters), which is in accordance with earlier studies concerning the effect of hypergravity on body weight in rodents [3,22]. Rotation in the centre of the centrifuge (ROT group) did not result in a body weight decrease, a finding also reported by Martin [18]. The HL and HS hamsters remained low in body weight and looked smaller even after a long period in normal gravity. More evidence that HG animals are smaller is found in the work of Antman and Oyama [1] who found a repression of linear growth in the overall size of rats exposed to 2.76 G. The persistent body weight decrease was also found in hamsters postnatally exposed to hypergravity [31]. This alteration occurred in both pre- and postnatally hypergravity exposed animals and thus proved to be independent of the gravity level in which the embryonal development took place. Pitts et al. [26] showed that, after a decrease in food and water consumption in the first days of centrifugation, the daily food intake of the rats living in hypergravity increased and exceeded that of controls. The authors suggested that sustained body weight decrease was the result of a physiological regulation to adjust the animal to hypergravity. Hamsters born in hypergravity and hamsters postnatally exposed to hypergravity weighed less than the CON hamsters even after months of normal gravity showing a persistent alteration of the body weight [31].

The performance of the HL hamsters on the activity wheel (wheel usage and average speed) in hypergravity was lower than for the HS and CON groups but resembled the level of those groups when living in 1 G (second part). While the increased G-load fatigued the hamsters, it did not affect their motivation to use the wheel.

We found that stride length for the CON and ROT hamsters increased faster than for the HL and HS hamsters. We assume that the step size is smaller in hypergravity than in normal gravity due to the increased pressure on the limbs. There is also evidence that animals subjected to long-term hypergravity are smaller than control animals [1]. Both the acquired normal locomotion in a hypergravity condition and their smaller body length could cause the decrease in step size as was observed in HG bred animals. We observed that the ROT hamsters were more often standing still than the other hamsters, causing an increase in stance time. We assume that this difference cannot be attributed to a rotation effect.

During the first month of the experiment, balancing on the rail was more difficult for the HL and ROT groups than for the HS and CON groups, causing increases in the crossing time and/or the number of falls. Balance problems, among others, can arise due to body weight increases (altering the centre of gravity) or to a sensory conflict between visual, vestibular, and proprioceptive information in the central nervous system (CNS). We suggest that this sensory conflict is due to altered input from the vestibular and proprioceptive systems to the CNS, while the visual information has not changed after adaptation to rotation or hypergravity. The HL hamsters had balance difficulties in 1 G from a sensory conflict in the CNS, while the CON hamsters had more balance problems when their body weight increased. The ROT hamsters had more balance problems than the other groups because of the combination of the body weight increase and this sensory conflict.

A persistent disturbance was found in the swimming ability of HG bred hamsters (both the HL and the HS group). Even after 8 months of normal gravity, the HS group swam worse than hamsters born in normal gravity. Rodents postnatally subjected to hypergravity showed less severe swimming disturbances than our HG developed hamsters and the disruption in this behaviour recovered after several days [9,30,31]. The air-righting reflex was also severely disturbed in the HL group but not in the HS or the ROT groups. We assume that the disturbed air-righting reflex in the HL animals was caused by the continuous exposure to an increased G-load. The HS hamsters did not show these disturbances suggesting an adaptation to normal gravity. The number of correct responses in HL animals never reached the level of the other groups, showing a persistent disturbance in this behaviour.

The disturbances in swimming behaviour and air-righting reflect a dysfunction of the otothalic organs [7,10,14,16,17]. A number of alterations in the otothalic organs were reported after a
hypergravity exposure including a decline in the number of synapses of type II hair cells, a decrease in the size of the otoconia, and an alteration in the otocytology distribution of the utricular otoconial layers [15,28,32]. Based on these findings, we hypothesize that the persistent swimming disturbances of hamsters born in hypergravity were caused by an alteration in peripheral vestibular input to the CNS. The better performance of the HS hamsters when compared with the animals that stayed in hypergravity for a longer period (HL group) points to a critical period for adaptation to an altered gravity level.

We conclude that development in hypergravity causes the disturbances in balancing, swimming, and air-righting reflex ability probably by a structural alteration in the vestibular end organs. These disturbances in behaviour become persistent after a critical period has been passed. Furthermore, hypergravity and rotation each have a different effect on vestibular evoked behaviour.

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