

Vestibular-induced behaviour of rats born and raised in hypergravity

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ABSTRACT: One group of rats were bred and kept under hypergravity (HG) conditions (2.5 g) in a centrifuge. Another group were bred and kept under normal gravity conditions (1 g). Rats from both groups were dropped from a supine position into a water basin under infrared illumination leaving only gravity (1 g for both groups) for orientation. The airrighting reflex and reappearance at the water surface were examined. The success rate for airrighting of HG rats is 47% versus 45% for controls, and is performed about equally fast by both groups. The success rate of HG and control hamsters is $\leq 25\%$ and $\geq 80\%$, respectively [22]. This interspecific difference does not appear to support the conjecture that altered behaviour is caused by a structural change of vestibular end organs during ontogenetic development under HG. The success rate for surfacing of control rats is 100%. Surfacing of young HG rats is less successful (36% at age 6 weeks) and requires more time. On average, surfacing of adult rats of both groups is about the same. Apparently, the repeated stay of centrifuge-bred rats at 1 g for experiments and daily care suffices to recalibrate and improve their orientation, which is essential for surfacing. © 2000 Elsevier Science Inc.

KEY WORDS: Gravity, Centrifuge, Airrighting, Surfacing, Vestibular system, Otolith organs.

INTRODUCTION

Traveling by car, boat or plane can disturb the sense of equilibrium of people to such an extent that normal functioning becomes impossible. This phenomenon is generally called 'motion sickness'. A related disorder can occur with people in space at zero gravity: the Space Adaptation Syndrome (SAS). A gravity level change from 1 g to prolonged weightlessness, but also from prolonged hypergravity (HG) to normal gravity (NG), can cause symptoms of motion sickness [1,12]. The cause for SAS or motion sickness in general is thought to originate from conflicting information between vestibular input and input that relates to body posture from other sensory systems (like the visual system and the muscle spindles).

The vestibular sensory organ provides information about the acceleration of the head, which includes angular acceleration (semicircular canals) and linear acceleration (otolith organs). The gravity vector is a constant factor in our normal environment and

the assessment of its direction by the vestibular system contributes to the process of spatial orientation. Because the exact relationship between a prolonged change of gravity and either motion sickness or SAS in man is still not understood, we devised an experiment in which animals could be exposed to a decreased level of gravity. This type of experiments has its limitations because (1) animal studies cannot explain all motion sickness-related symptoms (subjectively) experienced by humans, and (2) the otolith sense organs will always be stimulated while prolonged zero gravity cannot be simulated on earth. Nevertheless, our experimental set-up allows investigation of the relation between vestibular functioning and orientation-dependent behaviour after a gravity change.

For a wider research project focussing on the effects of altered gravity on animal physiology, rats were bred under conditions of 2.5 times normal gravity. For these animals, normal gravity (1 g) can be considered as a hypogravity environment. It has been shown that vestibular-induced behaviour of hamsters born and raised at 2.5 g is less adequate than that of control animals, and it was conjectured that this was caused by structural changes in the vestibular end organs [22]. If this hypothesis is correct, one might expect a similar reduction of vestibular function in other species. The centrifuge-bred rats of the present study were utilized to investigate how their vestibular system is functioning under hypogravity (1 g) conditions. Two types of vestibular-induced behaviour have been studied, i.e., the airrighting reflex (turning from a supine to a prone position during fall) and the reappearance at the surface after a fall into a basin filled with water. Because these experiments are performed under infrared illumination, both types of behaviour only depend on the orientation capability of the animal relative to the direction of gravity. The question is whether this behaviour is affected by the ontogenetic development of the vestibular system under HG conditions.

MATERIALS AND METHODS

Long-Evans rats were bred in our laboratory. One group lived under normal gravity conditions at 1 g (NG; 15 animals). Another group were conceived and born, and thereafter lived, at increased gravity (hypergravity) at 2.5 g in a centrifuge (HG; 14 animals). For a detailed description of the acceleration animals experience, see the Appendix. Weaning was at the age of 3 weeks for both groups. Housing of the rats consisted of acrylate boxes (0.36 ×

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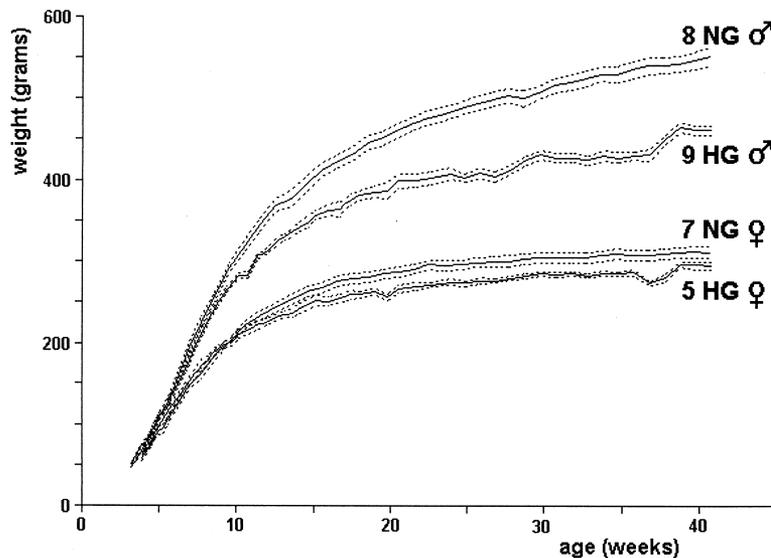


FIG 1. Growth of hypergravity (HG) males ($n = 9$) and females ($n = 5$), and normal gravity (NG) males ($n = 8$) and females ($n = 7$). Weighing, at least once a week (but more frequently when younger), started after weaning. The curves (solid lines) represent the average body weight of each group as a function of age. The standard error of the mean (SEM) is indicated by the dotted lines. Body weight is 6% less for HG females and 18% less for HG males relative to the NG animals. Note the increase of growth rate of HG animals in week 38 when the centrifuge was not operational.

0.44 × 0.30 m). Lighting was switched on during 12 h per day. Food and water were always available *ad libitum*.

The centrifuge comprises two horizontal arms (length: 1.10 m) with aerated free-swinging gondolas (length: 1.10 m, width: 0.45 m, height: 0.725 m). The centrifuge constantly rotates at 34.3 cycles/min resulting in a hypergravity level at the bottom of the gondola of $|Z| = 2.5 g$, with the Z-vector always perpendicular to the bottom. Daily, the centrifuge was stopped during 20–60 min for animal care and experiments. Rotation was alternated from clockwise to anti-clockwise (or vice versa) every day. During the first 10 days after birth, gravity level in the centrifuge was set at 1.8 g, thus increasing the probability of newborns being nursed by their mothers successfully [16]. Animals inside the gondolas could be observed by means of cameras. Animal treatment was in accordance with the Dutch law and the European Communities Council Directive (86/609/EEC; 24 November 1986) on the use of animals in scientific research.

Experiments started 5–7 days after weaning (at the age of 4 weeks) and were performed at 1 g for all animals. Every experimental session, animals were successively dropped three times from a supine position (height: 0.65 m) into a water basin (temperature: 33°C). This test was initially performed every 2 weeks and afterwards, when the animals had reached the age of 12 weeks, with intervals of 4 weeks. Although the airrighting reflex of rats is initiated by vestibular input and is only modulated by visual input [19], infrared illumination was used to eliminate the latter. In order to minimize the distress for animals remaining under water, the light was switched on 3 s after the rat hit the water surface, and it was rescued. The time required for three trials per animal is, generally, less than 1 min. The animal's activity was recorded on videocassette recorder with a temporal resolution of the video images of 20 ms.

Afterwards, the number of correct airrighting reflexes and the time interval necessary to perform this reflex (from release to

attaining a prone position) were determined. Also, the time intervals between hitting the water surface and the subsequent resurfacing were measured. Data from both experimental groups were statistically examined (significance: $p < 0.05$).

RESULTS

During the first few days after the transfer of non-adapted rats (e.g., the parents of our HG rats) from NG to HG conditions the animals lose weight (10–15%). The adaptation to the new situation takes approximately 1 week, after which the weight curves resume their course and the animals appear to resume their normal life including procreation. There appears to be no acute distress among centrifuge-bred or centrifuge-adapted rats when the centrifuge is stopped. Immediately when the centrifuge is being decelerated all animals become very active (they start running, romping, climbing, and stand on their hind legs). The growth rate of HG and NG animals involved in the present behavioural study is shown in Fig. 1. Animals in the centrifuge have a lower body weight than their counterparts at normal gravity (females: –6%, males: –18%). A lower body weight of animals that live in a centrifuge is characteristic for the HG condition [18,22]. Due to a technical failure the centrifuge was not operational for 5 days in week number 38. In that period the growth rate for the HG animals suddenly increased (Fig. 1).

When a rat was released from a supine position it always reacted by realigning body posture. In almost all cases this realignment was ended before the water surface was reached. Besides attaining a prone position (a correct airrighting reflex), the most frequently observed touch-down was with the rat's long axis in vertical direction (head up). We did not notice any difference between HG and NG rats with respect to the sequence of body posture change while performing either a correct or an incorrect airrighting reflex. The percentages of correct airrighting reflexes

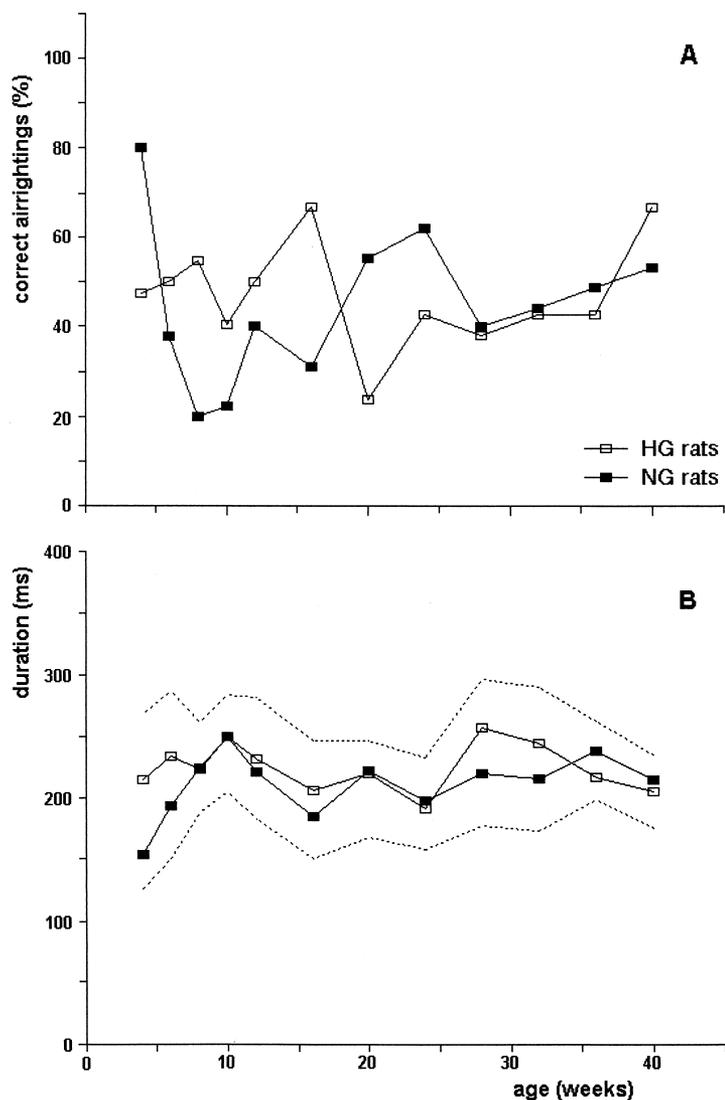


FIG. 2. Airrighting reflex of 14 hypergravity (HG) rats and 15 normal gravity (NG) rats as function of age. Experiments (three trials per animal) started at age 4 weeks. Initially, experiments were repeated every 2 weeks but from age 12 weeks onward every 4 weeks. (A) Percentage of correct airrightings; the overall success rate is 47% for the HG and 45% for the NG rats. (B) Mean duration of the airrighting; upper dotted line connects the values of the mean + SD of HG rats; lower dotted line indicates the values of the NGs mean - SD.

are shown in Fig. 2A. The score for the HG group varies considerably (between 24–67%), but does not change much with age. The airrighting of the NG group was very successful in the first experimental session only (week 4), but thereafter dropped to a level also shown by the HG group (Fig. 2A).

The duration of the airrighting reflex (mean \pm SD) for NG rats and HG rats is shown in Fig. 2B. The analysis of variance of the data was performed by means of a two-way classification for unweighted means [7]. The airrighting reflex of NG animals is a little faster than that of HG animals [Group effect, $F(1,455) = 11.3, p < 0.001$].

The success rate for reappearing at the water surface after hitting the water, within the limit of 3 s, is shown in Fig. 3A. At

the age of 6 weeks, only a minority of the surfacings of HG rats (36%) occurred within 3 s after hitting the water. The success rate for surfacing for HG animals of 8 weeks and older was either 100% or a little less. The few unsuccessful surfacings of the older HG animals (>8 weeks) were all due to a single individual (see below). Surfacings of the NG group were performed within 3 s without any exception at any age.

The time interval (mean \pm SD) between hitting the water and reappearing at the surface (nose up) is shown in Fig. 3B. Analysis of the data was the same as for the duration of the airrighting reflex [7]. In addition to the higher success rate of 4 and 6 weeks old NG animals (Fig. 3A), it is shown in Fig. 3B that 4–10-week-old NG animals complete their surfacing faster than HG rats of the same

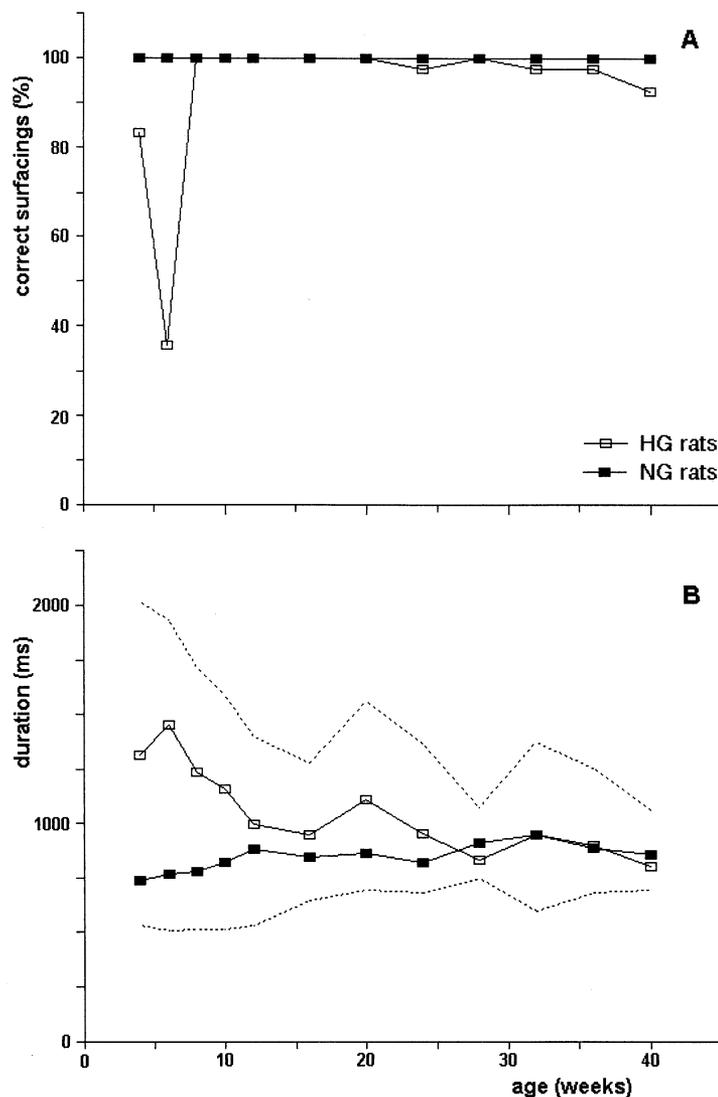


FIG. 3. Surfacing performed within 3 s. (A) Percentage of correct surfacings; the success rate for surfacing for hypergravity (HG) animals of 8 weeks and older was 100% or a little less. All 'failures' occurred with the same animal. The score for 4 and 6 weeks old HG rats was (much) less than 100%. For normal gravity (NG) rats surfacings were always successful irrespective of their age. (B) Mean duration of surfacings; upper dotted line connects the values of the mean + SD of HG rats; lower dotted line indicates the values of the NGs mean - SD. Note that the young HG rats need more time to find the water surface.

age [Group effect, $F(1,979) = 95.0$, $p < 0.001$]. For NG animals the time necessary for surfacing increases with age, while for HG animals the opposite happens [Interaction effect, $F(11,979) = 11.2$, $p < 0.001$].

Because in our experimental design three consecutive airrighting reflexes and surfacings are measured, it is possible that some kind of correlation exists in this sequence of occurrences. The relation between a surfacing and the preceding airrighting reflex was inspected. It turned out that on average a successful airrighting reflex is followed by a faster surfacing (Fig. 4). The time necessary to reappear at the water surface after a successful airrighting was 808 ± 223 ms for NG animals and 900 ± 396 ms for HG animals. When the airrighting was not performed correctly surfacing times

were 872 ± 259 ms and 1155 ± 463 ms, respectively. So, after an unsuccessful airrighting the HG group needs relatively more time to find the surface again (cf. Fig. 4A and B).

Another possible correlation we analyzed was that between the three consecutive surfacings which, for HG rats, lasted 872 ± 397 , 1045 ± 450 and 1176 ± 451 ms (mean \pm SD), respectively. In Fig. 5A, the distribution of duration differences between the second and the first surfacing, and between the third and the first surfacing of HG animals, are shown. For comparison, the normal distributions around the respective means and around 0 are also shown. The latter distribution would be expected when the duration of a surfacing was independent of a preceding trial. Thus, in our experiment the second surfacing of HG animals takes signif-

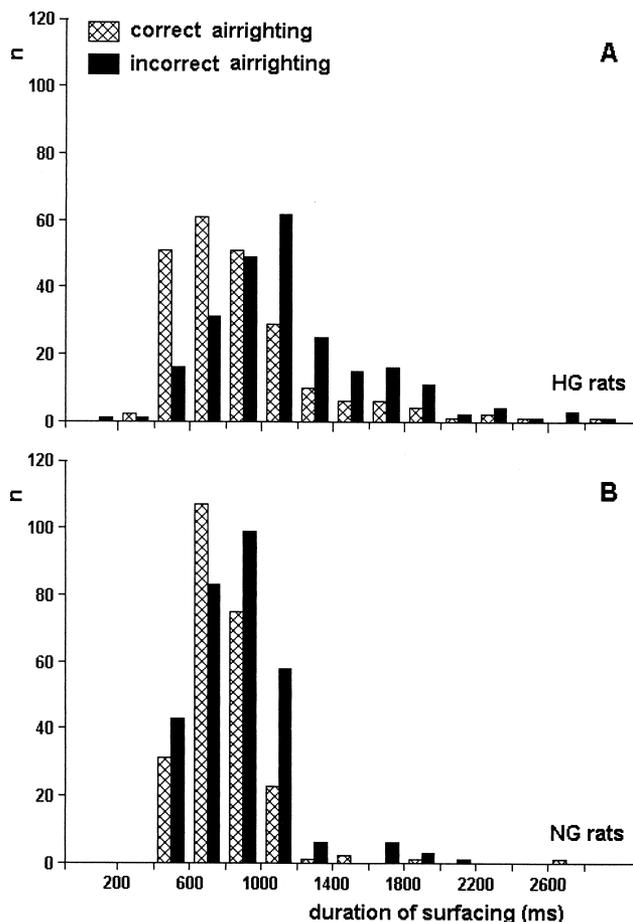


FIG. 4. Duration of surfacings subsequent to a correct airrighting (cross-hatched bars) and of surfacings preceded by an incorrect airrighting (black bars) of hypergravity (HG) rats (A) and normal gravity (NG) rats (B). A correct airrighting reflex is usually followed by a faster reappearance at the water surface; an incorrect airrighting reflex generally delays surfacing, especially for HG animals.

icantly longer than the first (t -test, $p < 0.001$), and the third surfacing again takes longer than the second ($p < 0.001$). This effect was present during the entire period in which these experiments were performed. Consecutive surfacings of young HG rats (4–16 weeks) lasted 997 ± 466 , 1150 ± 542 and 1292 ± 450 ms; for older HG rats (20–40 weeks) this was 764 ± 287 , 950 ± 347 and 1072 ± 423 ms.

For NG animals, the distribution of duration differences between the second and first surfacing and between the third and first surfacing are shown in Fig. 5B. Consecutive surfacings lasted 806 ± 253 , 850 ± 250 and 873 ± 229 ms, respectively. The second surfacing of NG animals takes a little longer than the first ($p < 0.05$), but the third surfacing does not take significantly longer than the second. In other words, the duration of a surfacing of a NG rat hardly depends on a preceding trial while a surfacing of a HG rat tends to take longer when it has been preceded by one surfacing and even longer when preceded by two.

Between the three consecutive airrighting reflexes of neither the HG group nor the NG group a similar correlation was observed. All NG rats and most of the HG rats showed normal swimming behaviour. One HG rat (the one of the few unsuccessful

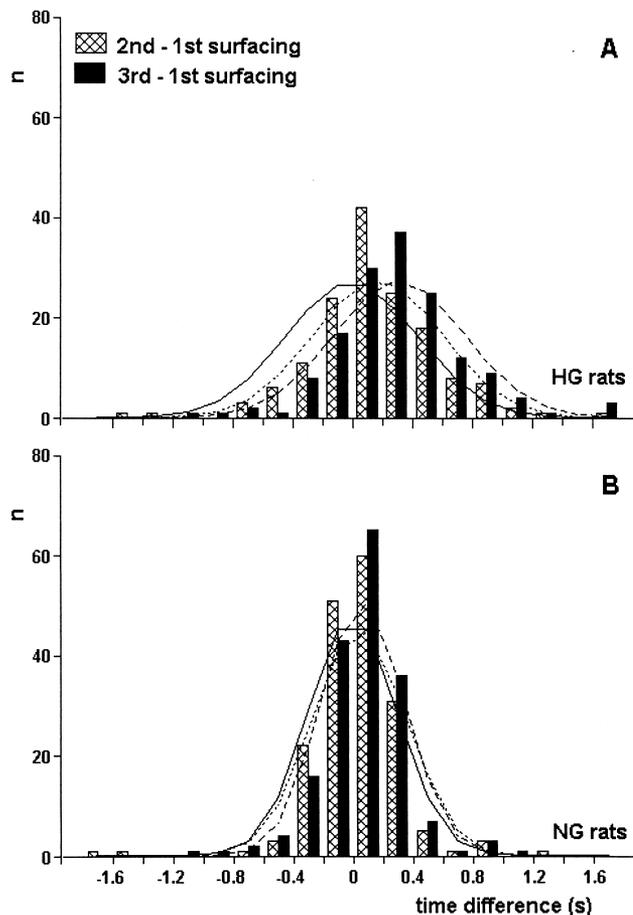


FIG. 5. Difference between the duration of consecutive surfacings of hypergravity (HG) (A) and normal gravity (NG) (B) animals. The differences between the second and first surfacing (cross-hatched bars), and between the third and the first surfacing (black bars) have been calculated for both groups. From the respective means and standard deviations, the matching normal distributions were plotted. The solid curve shows the normal distribution with zero mean, which is the distribution one would expect when subsequent surfacings were independent. Dotted curve: normal distribution for the measured mean and SD of the second minus the first surfacing. Dashed curve: normal distribution for the measured mean and SD of the third minus the first surfacing.

surfacings at age >20 weeks) systematically started spinning around its body axis (even before reappearing at the surface) at one turn per second or faster. A few more HG animals showed similar behaviour but to a lesser degree, i.e., spinning occurred only occasionally and much less vehemently.

DISCUSSION

Adaptation of human subjects to much slower rotation (and negligible gravity increase) takes days [10] and also NG rats need several days to adapt to a hypergravity (and rotating) environment. The HG rats' behaviour inside the centrifuge suggests that they do not suffer from 'sickness induced by long duration centrifugation' experienced by many (unadapted) human subjects [1,10].

In a previous study on hamsters, a relation was found between a disturbance of vestibular controlled behaviour (like swimming and equilibrium maintenance) and malformations of the otoconia

of the saccule and/or utricle [23]. It appears to be very likely that the HG rats of the present study which showed spinning in water, especially the animal that developed this behaviour in an extreme way, also suffered from a more or less impaired peripheral vestibular organ. Probably, this has to be attributed to one or more defective otolith organs [6,9,13,15,23]. Animals with impaired labyrinths also show typical spinning behaviour in conditions of zero gravity during a parabolic flight on board an aeroplane [17]. Our spinning HG rat displayed the same behaviour as soon as it was released to float freely during a zero gravity period on such a parabolic trajectory of the aeroplane, while other HG rats and NG rats did not show any spinning during zero gravity at all (unpublished results). Perhaps, the probability of peripheral deficiencies increases with hypergravity, although the effect that hypergravity has on, for instance, otoconial development appears to be limited [14,21].

In our centrifuge, animals are bred and live under hypergravity conditions. For a detailed description of the acceleration animals experience, see the Appendix. Every day, rotation of the centrifuge has been reversed in order to prevent the possible development of a unilateral compensation for the Coriolis force during locomotion. It is important to realize, however, that each time an animal moves it will experience a different acceleration depending on the chosen direction of movement (with the Coriolis component reversed on the next day). It is conceivable that under these circumstances the development of motor reflexes which depend on vestibular sensory input is not optimal. Unfortunately, this complicates the interpretation of behavioural changes that are observed because they may not be exclusively the result of hypergravity. When simulating a hypergravity habitat, however, this cannot be avoided.

None of the rats in the airrighting test reached the water surface in the original supine position. This means that, if a rat's life-long stay at 2.5 g has any effect on the sensitivity of the otolith system, its threshold for linear acceleration is not raised above the level of 1 g. In experiments by Daunton et al. [5] the otolith-spinal reflex was measured from rats exposed to hypergravity (2.0 g or 2.8 g) for a relatively short time (7–14 days). The authors conjecture that the decreased amplitude of the otolith-spinal reflex of HG exposed rats which they observed is due to a sensitivity decrease of the otolith system. Given the small differences between the airrighting performance of the HG and NG rats of our study, it would be interesting to repeat the experiment of Daunton et al. [5] with rats that have spent all their lives in a hypergravity environment.

The results presented here are in sharp contrast with those obtained for hamsters, for which it was shown that airrighting is less successfully performed by HG-bred animals (25% or less correct airrighting reflexes for HG animals vs. more than 80% for NG animals [22]). The lower score of NG rats relative to NG hamsters may be attributed to learning that a water basin was awaiting them (our experiments were every 2 weeks initially vs. every 4 weeks for the hamsters). The better performance of HG rats relative to HG hamsters, however, indicates a better functioning of the HG rats' vestibular system in this experimental situation. Our results do not show remarkable differences between the duration of the airrighting reflex of HG and NG rats, although at ages 4 and 6 weeks NG animals appear to be somewhat faster. It is possible that this type of experiment is not the most conclusive for discriminating between vestibular functioning of HG and NG rats.

With respect to surfacing, HG rats seem to experience some trouble in the first few weeks after weaning. Video recordings show animals floating at the water surface, but with their heads submerged. Young (4–10 weeks) HG rats take more time for a successful surfacing than young NGs (Fig. 3B). From the age of 8 weeks onward, HG rats almost always find their way to the surface. The data of three more HG rats (data not shown) are

consistent with the results shown here. It has been reported that HG hamsters have considerable problems with swimming [22,24], a problem which HG rats seldom have. NG rats have no problem in finding the water surface at any age.

The simulation of increased gravity during (and after) the ontogenetic development alters the vestibular-induced behaviour that has been investigated (airrighting and surfacing). Apparently, however, the degree of behavioural change and the characteristics of this change also depend on the species (e.g., rat vs. hamster) that is investigated. Moreover, with rats age or, more likely, adaptation to normal gravity (see below) also plays a role. The most conspicuous differences between young HG rats and controls (success rate and duration of surfacing, see Fig. 3) disappear with age. At first glance, adult HG animals perform equally well as NG adults. This indicates that adequate vestibular input is available, and it seems, therefore, that the ontogenetic development of the peripheral vestibular system can not have been disturbed dramatically by a hypergravity level of 2.5 g.

A closer analysis of the chronological order of occurrences in our experimental set-up indicates that the orientation of HG animals relative to the direction of gravity is much more affected by preceding events than for NG rats (Figs. 4 and 5). This also suggests that the alteration of vestibular-induced behaviour of HG rats is of neuronal origin and is not caused by dysfunction of the vestibular sensory epithelia. This conjecture appears to be supported by studies demonstrating the plasticity of vestibular innervation. The number of synapses in the otolith organs of adult rats adapts to the level of gravity [20], and the activity of vestibular primary afferents is modified to a new gravity level [2,3]. The amount of data on this subject, however, is scarce and ambiguous [4].

It has been demonstrated that sensory functions can be recalibrated to new situations. Localizing a sound source while wearing artificial external ears can be learned without interfering with the system's original capability [11]. Although, the otolith organs and semicircular canals detect linear and angular acceleration explicitly (unlike sound localization which results from processing implicit acoustic cues), the close integration of the vestibular system with other sensory and motor control systems also necessitates a permanent readjustment to changing conditions [8]. At the age of 10 weeks, when resurfacing is about equally successful and equally fast for both groups, the HG rats have spent more than 24 h under 1 g conditions. Apparently, the repeated stay of centrifuge-bred rats at 1 g for experiments and/or daily care suffices to recalibrate the vestibular system to this new situation and thereby improve orientation. But, although recalibrated to a new level of gravity, their vestibular induced behaviour does not attain the same efficiency as was observed for NG rats. Underwater orientation of HG rats becomes somewhat deteriorated with repeated surfacing (Fig. 5). Sustained conditions in which the relation between the animal's motion and the detected acceleration is (partially) unpredictable may hamper the stability and/or fine-adjustment of motor reflexes.

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APPENDIX

Hypergravity is defined as the acceleration that is directed perpendicular to the bottom of the gondola (and the bottom of the box in which the rats live). Because the distance to the axis of rotation of the centrifuge differs with position inside the gondola the magnitude of this HG acceleration will be a gradient rather than a constant value. Furthermore, the centre of mass of the gondola will generally not coincide with the position of the animals and, thus, a shearing acceleration, perpendicular to the HG vector, will act upon the rats inside the centrifuge. To calculate the acceleration as a function of position inside the gondola, the swing-out angle of the gondola (α) has to be calculated first. For this, we have to estimate the location of the centre of mass of the gondola (plus its contents). Because we put the boxes, with the rats inside, at the bottom of the gondola the centre of mass is located somewhere between the bottom and halfway the gondola’s height, i.e., between 0.725 and 0.725/2 m. Figure A1 illustrates how we calculated α .

Calculations show what effect the exact position of the centre of mass of the gondola has on the acceleration that occurs at, for instance, the centre of the bottom of the gondola (Table A1). Apparently, the effect on α and on HG is small. For the shearing acceleration’s magnitude the effect is more prominent. Because the animals inside the centrifuge are free to move around in their housing, it is relevant to know how these forces change with the animal’s position. For $\alpha = 63.6^\circ$, we calculated the gradients of both the HG and the shearing acceleration (Fig. A2). It is shown that on the floor of the boxes where the rats live, HG varies from 2.4 g to 2.6 g and shearing acceleration from 0.1 g to 0.5 g. Actual measurements of the acceleration, in three directions and at different positions inside the gondola, are in agreement with the model presented here.

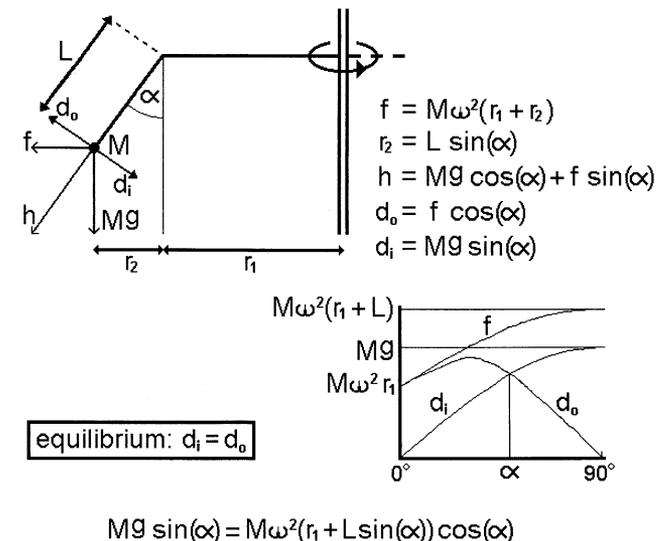


FIG. A1. A mass (M) is suspended on a free-swinging arm (L) which itself is attached to a horizontal arm (r_1). In this system, rotating at $\omega = 3.6$ radians/s (34.3 cycles/min), equilibrium is achieved when the opposite forces d_i and d_o have equal magnitudes. The equation with the single unknown variable α cannot be solved analytically, but α has to be somewhere between 0° and 90° . Assuming the centre of mass of the gondola with its contents at 0.483 m, numerical analysis shows that $\alpha = 63.6^\circ$ (cf. Table A1).

TABLE A1

SWING-OUT ANGLE (α) HYPERGRAVITY (HG) AND SHEARING FORCE AT THE CENTRE OF THE BOTTOM OF THE GONDOLA AS A FUNCTION OF THE POSITION OF THE CENTRE OF MASS OF THE GONDOLA AND ITS CONTENTS

Centre of Mass Position	α	HG	Shear
0.50 L (0.363 m)	61.8°	2.49 g	0.20 g
0.67 L (0.483 m)	63.6°	2.51 g	0.13 g
0.75 L (0.544 m)	64.5°	2.51 g	0.09 g
1.00 L (0.725 m)	66.7°	2.53 g	0.00 g

When the animals move around in their boxes they experience another force which is typical for a rotating system, i.e., the Coriolis force. Its strength depends on the velocity (v) of the animal and on the angle of the animal's motion vector with the plane of rotation (angular velocity $\omega = 3.6$ radians/s). The Coriolis acceleration can be expressed as the vector product $2\omega \times v$. At a velocity of 1 m/s (a realistic velocity for rats running or jumping) the maximum Coriolis force that can be experienced in our centrifuge is about 0.7 g. The direction of the Coriolis force depends on the direction of rotation. When simulating a HG environment (of particular magnitude), a large centrifuge radius is preferable because the acceleration gradients of hypergravity and shear, and the strength of the Coriolis force will be less. Nevertheless, as a consequence of the remaining Coriolis force, space inside the

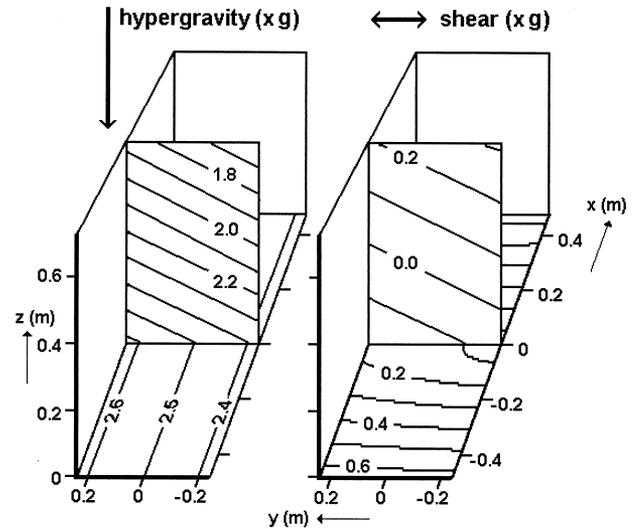


FIG. A2. Acceleration gradients inside the gondola of hypergravity (HG) (left) and shearing. Swing-out angle was chosen at $\alpha = 63.6^\circ$ (cf. Table A1) which is a realistic estimate of the conditions in which our HG rats live.

centrifuge housing is inherently anisotropic. Each time animals move they will experience a different acceleration depending on the chosen direction of their movement (with acceleration reversed on the next day).