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Research report

Behavioural consequences of hypergravity in developing rats

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Abstract

Gravity represents a stable reference for the nervous system. When the individual is increasing in size and weight, gravity may influence several aspects of the sensory and motor developments. To clarify this role, we studied age-dependent modifications of several exteroceptive and proprioceptive reflexes in five groups of rats conceived, born and reared in hypergravity (2 g). Rats were transferred to normal gravity (1 g) at P5 (post-natal day 5), P10, P15, P21, and P27. Aspects of neural development and adaptation to 1 g were assessed until P40. Hypergravity induced a delay in growth and a retardation in the development of contact-righting, air-righting, and negative geotaxis. However, we found an advance in eye opening by about 2–3 days in HG-P5 and HG-P10 rats and an increase in grip-time. No differences were found in tail and grasp reflexes. Our results show that hypergravity leads to a retarded development of motor aspects which are mainly dependent upon the vestibular system.

Theme: Motor systems and sensorimotor integration
Topic: Control of posture and movement

Keywords: Gravity; Development; Reflex; Vestibular system; Postural control; Motor system

1. Introduction

Motor development is dependent upon maturation of the sensory organs, muscles as well as on central processing in CNS regions involved in motor control. In rats, most sensory systems are functioning at birth with the exception of the visual and auditory systems but all of these undergo drastic developmental changes in the first postnatal weeks. The same holds true for muscles and central areas of which the development of the cerebellum, largely taking place in the postnatal period, is the most obvious example. The CNS has the greatest adaptive capacities at early stages of development and changes in sensory information in this period therefore will lead to the most pronounced behavioural adjustments.

Changes in gravity induce modifications of vestibular, proprioceptive and cutaneous inputs. It is known that astronauts readily adapt to a new gravitational field, although experiencing disorientation and nausea (also called Space Adaptation Syndrome). This adaptation is probably based on a recalibration of sensory inputs and this leads to an update of motor commands [12,18,30,53]. The sensory inputs which are the least affected by the changes in gravity and vision in particular, seem to play the most important role in these adaptational processes, as Clément et al. [12] suggested in their study on postural control in humans experiencing weightlessness. After landing on earth, another perturbing period occurs, during which visual, proprioceptive and cutaneous information have again a major role in the readaptation [39].

In young individuals, such well-established frames of reference obviously do not yet exist. Only a few studies aimed at the development of the CNS during or after a

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modification of the gravitational vector, and mainly a reduction of gravity. The development of the vestibular system proceeds normally in weightlessness [16,42], as far as the expression of the genetic program is concerned, although the fine tuning of neuronal connections is delayed [42], and perhaps even modified [44]. Moreover, the size of otoconiae is increased in animals subjected to microgravity during development [54]. Prenatal exposure to microgravity does not interfere with the development of walking in rats [55], although the vestibular reflexes are temporarily altered [41]. On the other hand, exposure to spaceflight during 16 days either from postnatal day 8 (P8) or from P14 does not interfere with spatial learning and memory [49].

Several studies into the effects of hypergravity (HG) in developing rats showed changes in the vestibular system development which are opposite to those in rats which are bred in microgravity conditions. Indeed, animals conceived, born and reared in HG, have thinner utricular membranes [31] with increased cross-sectional areas of epithelial cells [58], smaller otoconiae (in rats: Refs. [27,31] and in hamsters, Ref. [48]) and their formation is delayed [2,24]. Additionally, a retardation in the development of neuronal circuitries in the vestibulum seems to occur, as Gaboyard et al. [20] showed a delay in the development of connections between type-I hair cells in the utriculus and their afferent calyx in rats which remained in 2 g from gestation until P6. Besides, Chabbert et al. [10] recently observed a slower development of K+ currents in utricular type-II hair cells. As to central nervous structures, Krasnov et al. [28] showed a hyperactivity of lumbar motoneurones in rats born under 2 g and Gimenez y Ribotta et al. [21] found a delay in the development of monoaminergic pathways in the spinal cord. However, it is still unknown what the effects of HG are on the development of motor behaviour. In the present study, we investigated the consequences of hypergravity (2 g) on the development of reflexes in rats. The main questions are: what are the effects of the modification of the gravito-inertial force on the sensorimotor development of young rats and how long the adaptation process to normal gravity lasts?

2. Material and methods

All the procedures in the present study have been reviewed and approved by the Ethics Committee of the Medical Faculty of the University of Amsterdam (DEC Number: DKN19).

2.1. Hypergravity

Animals were exposed to hypergravity (HG) in a centrifuge [56] with two horizontal arms (length: 1.10 m) and ventilated free-swinging gondolas (length: 1.10 m, width: 0.45 m, height: 0.725 m) situated at the end of the arms. Each gondola contained four cages ($0.36\times 0.44\times 0.30$). A camera in each gondola allowed observation of the animals. The HG vector was perpendicular to the bottom of the cages and had a magnitude of 2 g (angular velocity: 3.18 rad/s; tilt of the gondolas: 57°). Cages were cleaned twice a week and rotation was then alternated from clockwise to anti-clockwise or back to prevent the development of unilateral compensation for Coriolis forces during locomotion. Ambient noise level for HG and CONT rats was approximately the same (53 dBA) and the main source of this noise was caused by ventilation.

2.2. Experimental animals

Eight females and four males of the black and white Hooded Lister strain were placed in the centrifuge and allowed to adapt to 2 g during 1 week. After this week, one male was housed together with two females. Rats were weighed twice a week. Females being pregnant (as indicated by their increase in weight) were separated from the males. When parturition was imminent, the centrifuge was stopped once a day for inspection. The day of birth was indicated as P1.

Six groups of rats were investigated. Five groups were exposed to HG from conception until varying periods after birth. From birth, the pups remained in the centrifuge for the following periods: HG-P5 until P5, N=7; HG-P10 until P10, N=7; HG-P15 until P15, N=6; HG-P21 until P21, N=7; and HG-P27 until P27, N=7. After these periods, their behaviour was tested at 1 g and their physical development was recorded. In addition, we studied a group of control rats (CONT; N=7). The control rats remained in the same room to ensure that they would be exposed to the same general conditions, as noise, humidity and temperature.

2.3. Testing schedule, physical characteristics

In the HG rats, behavioural testing was performed immediately after they left the centrifuge, and then daily for 10 days (15 days for HG-P5) and thereafter with 5-day intervals until P40. The control rats were tested from P5 and daily until P20 and then with 5-day intervals until P40. At these days, the rats were weighed and the age at which they opened their eyes was noted.

2.4. Behavioural tests

Half an hour before testing began, the cages were placed in the experimental room and 5 min before testing, the pups were separated from the dam. The individual rats were identified by the black and white patterning on their backs. Motor performance was recorded at 50 Hz and the temporal resolution was therefore 20 ms.

2.5. Contact-righting

Contact-righting was assessed twice on each testing day until P15. To this, the rat was placed on a table in a supine position, with the back in contact with the support. A positive response was turning into a prone position with the four paws in contact with the ground within 15 s. This value appeared to conveniently differentiate between immature and mature responses (see also Ref. [22]).The reaction was videotaped. Analysis of the frames permitted to analyse the performances for each rat and the mean time they needed to right.

2.6. Air-righting

Air-righting was tested from P16 in normal lighting conditions. Each rat was dropped in a supine position from a height of 50 cm above a soft pad. The rat's reaction, tested twice on each testing day, was videotaped. From these recordings, we analysed the performances of each individual rat as well as the time necessary for each rat to reach a prone position in the air.

2.7. Negative geotaxis

Rats until P20 were placed once per testing day in a position with the nose pointing downwards on a ramp tilted at 45° and covered with glass paper to avoid them sliding down. The reflex was considered positive when they were able to reach a nose-up position within 30 s. This arbitrary chosen value differentiated between immature and mature responses. The time they needed to reach the nose-up position on the board was noted as well.

2.8. Grasp reflex

To assess this reflex, rats (until P20) were held by the experimenter, and a thin rod (diameter: 1 mm) was touching the palmar surface of fore or hind paws. Flexion of the digits indicated a positive reflex.

2.9. Forepaw grip time

Grip time was used to evaluate the force and fatigability in the forelimbs in rats. The rat was suspended when grasping the rod (diameter, 2 mm). The time the rat kept grasping the rod before falling was recorded. This test was applied from P6 to P20.

2.10. Postural tail reflex

Rats were held gently by the tail, suspended one meter above the floor, and forelimb and hindlimb extension was observed. Normal adult rats extend neck as well as fore- and hindlimbs. Rats showing such response were assigned grade 1 but rats with no extension of either fore or hindlimbs or both, were assigned grade 0.

2.11. Analysis of the data

Transitions in reflexes were characterised by the age at which the cumulative distribution in the group of rats with the changed reactions reached the 50% level (see also Ref. [22]). We also calculated the 25% and the 75% levels of these distributions. The data were triangularly smoothed (Bartlett filtering) in order to avoid incidental increases or decreases in performance. To this, twice the value at a particular age, the value on the preceding day and that on the following day were summed and divided by 4. In formula $P'_n=(P_{n-1}+2P_n+P_{n+1})/4$, P is the reaction of the group on a particular day, and n is the age. A similar calculation characterised the age at eye opening. In addition, for some reflexes we recorded the time needed to accomplish the reaction.

Comparisons between each HG group (HG-P5, HG-P10, HG-P15, HG-P21, HG-P27) and the control group were performed for each of the tests. We applied ANOVA testing to compare differences between the groups of rats in regard to ages of transition. The differences were considered to be significant at $p\leq0.05$.

3. Results

3.1. Weights

The weight increases in control rats were similar to data collected over the years in our Institute in the same strain of rats (Fig. 1). Rats which were subjected to HG showed decreases in their weights, relative to control rats, which varied with the periods the pups spent in HG. The groups which remained in HG until P5 weighed 7.22 g (±0.79) at P5, whereas control rats at that age weighed 8.57 g (±1.13; p=0.025) (Fig. 1). The groups which remained in the centrifuge until P10 weighed 13.32 g (±1.59) at P10 which is about 3 g less than control rats at that age 16.12 g (±2.84) but this decrease was not significantly different (p=0.065). HG-P15 group weighed on average 4.55 g less than control rats at P15 (p=0.037). Rats of the HG-P21 and HG-P27 groups when they left the centrifuge weighed about 10 g less than controls at P21 (p=0.011 at P25 for HG-P21) and P27 (p=0.014 at P30 for HG-P27), respectively (Fig. 1). A remarkable finding was that after having left the centrifuge, rats of the HG-P5 group increased more in weight than control rats, such that from P18 their weights surpassed significantly those of controls (p=0.042 at P18).

3.2. Eye opening

Fifty percent of the control rats had opened their eyes at P15.7 (25–75% range: P15.1–16.2). In the groups of rats

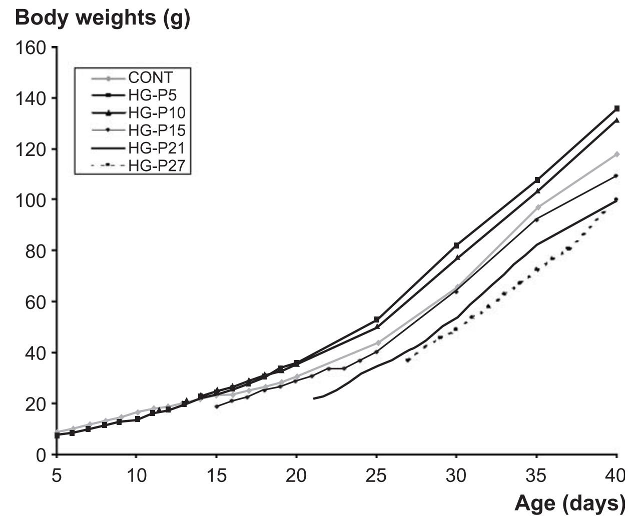

Fig. 1. Mean body weights (in grams) of hypergravity and control rats as a function of age. CONT: control group; HG: Hypergravity rats; P5, P10, P15, P21, P27: age of transfer from 2 to 1 g.

remaining in HG until P5, we observed the eyes to open at P13.0 (range: P12.9–P14.4) and those in HG until P10 even at P12.9 (range: P12.5–P14.0). The advance in eye opening in these two HG groups was 2.75 days on the average. The differences between control rats and those in HG were significant (p<0.05). The differences between the groups HG-P5 and HG-P10 did not reach significance.

3.3. Grasp reflex and tail reflex

The grasp reflex was positive in control rats and in rats of the HG-P5, HG-P10 and HG-P15 groups from the first day of test and we did not detect any abnormality. Also in the tail reflex, we did not observe any difference between the groups. All rats whatever their group, extended their four limbs and their neck when suspended by the tail.

3.4. Forepaw grip time

Control rats at P6 were able to remain hanging on a stable rod for 20 s (±17 s) and thereafter they fell. This period increased till P9 when they could keep hanging on the rod for 49.2 s (±22.9 s) (Fig. 2). Rats kept in HG, could hang for increased periods on the rod. Rats of the HG-P5 group on P6 hung for 78 s (±40.2 s) on the rod, which is significantly more than controls (p=0.032). At P7, they remained during 58.21 s (±34.17 s) while control rats kept hanging for 21.07 s (±6.82 s) (p=0.015). HG-P5 showed again a longer grip time on P19 with 20.5 s (±9.48 s) versus 8 s (±3.2 s) in control rats (p=0.006). In rats from the HG-P10, the grip time was 22.71 s (±14.56 s) at P10, and this was 37.57 s (±19.1 s) for controls at the same age. In HG-P15 rats, the grip time was 23.08 s (±10.3 s) at P15 and 18.14 s (±4.90 s) in controls (see Fig. 2). The differences at P10 and P15, however, did not reach significance.

3.5. Contact-righting

Fifty percent of the control rats at the age of P6.1 were able to right within 15 s (range: P4.4–P6.3) while HG-P5 rats reached this stage at P6.5 (25–75% range: P4.4–P7.3). This indicated a delay in development of about 0.5 day (for the percentages of positive reactions, see Fig. 3A). When considering the actual time needed for righting, it appeared

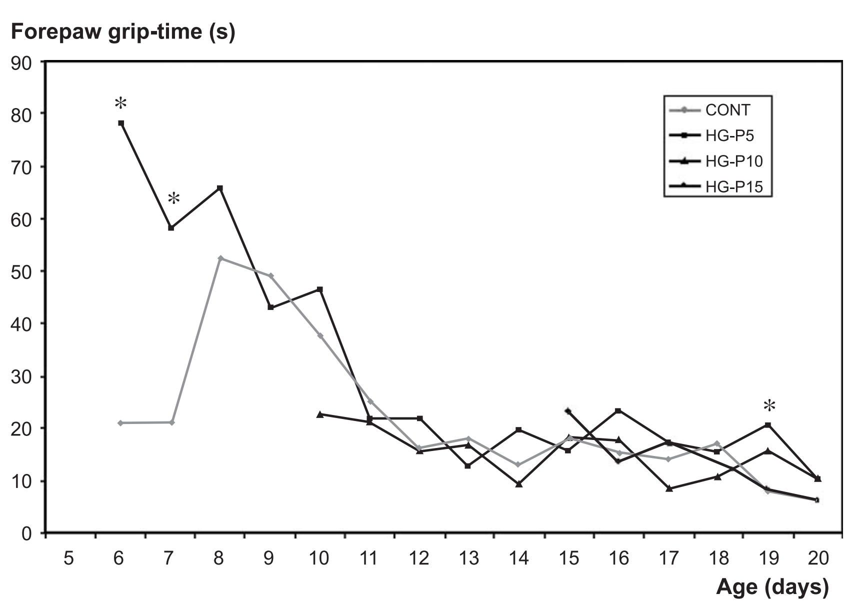

Fig. 2. Forepaw grip time in seconds in hypergravity and control rats from P6 to P20. Asterisks represent a significant difference (p<0.05). For further abbreviations, see Fig. 1.

that HG-P5, HG-P10 and HG-P15 rats needed more time than controls in reaching a prone position (Fig. 3B). These delays had recovered by P9 for rats of the HG-P5 group and by P11 for the HG-P10 rats.

3.6. Negative geotaxis (only tested until P20)

The time allowed to turn into a nose-upward position was set at 30 s and with this criterion we observed a delay in HG rats. Fifty percent of control rats accomplished to turn within 30 s at P8.0 (range: P6.4–P9.0) and in the HG-P5 rats this was at P8.4 (range: P6.2–P9.1) (Fig. 4A, please note that we applied a triangularly shaped smoothing window). These results indicate that the HG-P5 rats, kept in HG conditions are delayed in their performance by about 0.5 days. Remarkably, all groups of rats, irrespective of their treatment had reached a positive response in 100% at P12. In addition, we calculated the actual time needed for turning. As expected from the previous set of data, the HG groups of rats needed more time to accomplish the negative geotaxis reflex, particularly on the first testing days. With some delay, they reached values of the control rats (Fig. 4B).

3.7. Air-righting reflex

Fifty percent of the rats in the control group landed on their four paws at P17.3 (range: P 16.3–P18.6) (Fig. 5A). The rats kept under HG conditions for varying periods of time reached this level at later ages. In the HG-P5 rats, the age was, P18.4 (range: P17.2–P18.6); in HG-P10 rats, P18.2 (range: P16.63–P18.6); in HG-P15, P19.4 (range: P17.3–P19.7); HG-P21 at P22.6 (range: 21.9–23.7) and in HG-P27 at P27.0 (75% at P28.6). This implies that HG-P5 and HG-P10 reached control values 1 day later, HG-P15 2

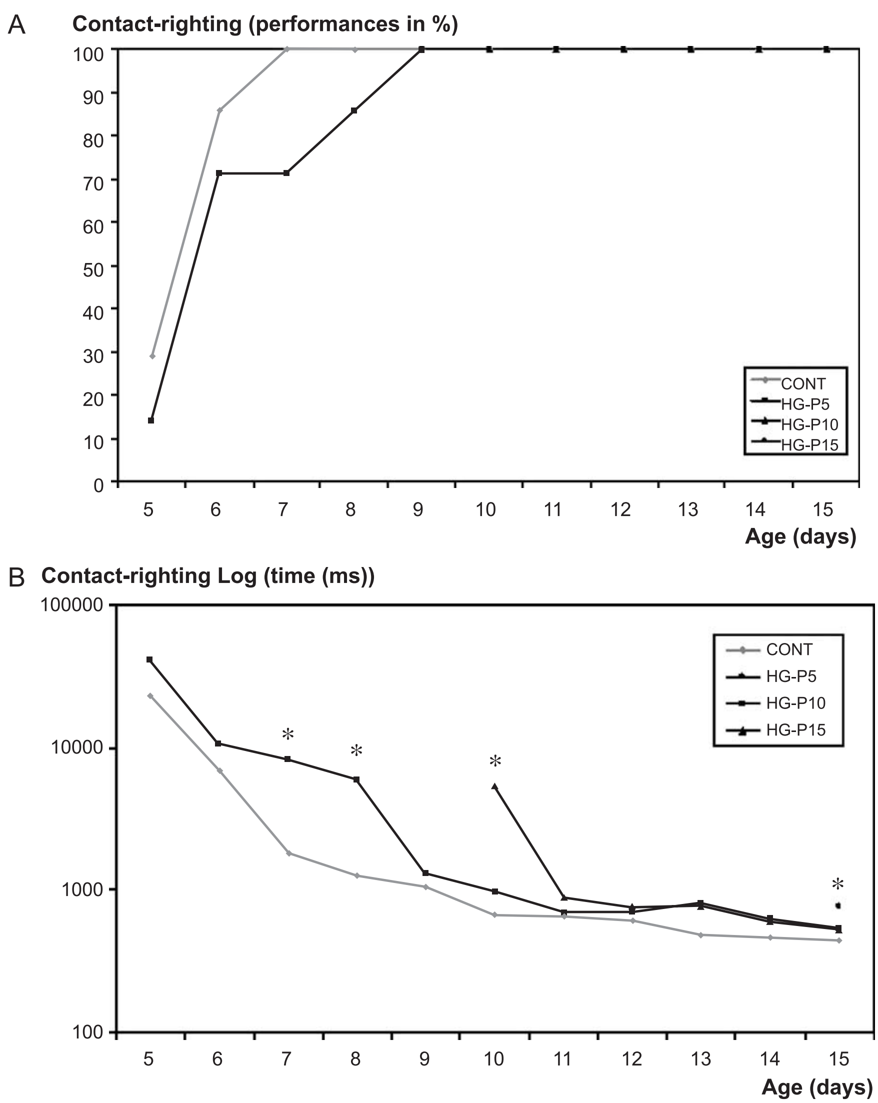

Fig. 3. Contact righting in hypergravity and control rats as a function of age. (A) Performances of HG and control rats, expressed as the percentage of animals which turned from supine to prone position within 15 s. Note that HG-P5 group performances increased with age but slower than control rats. (B) Time for righting in milliseconds plotted logarithmically. For further abbreviations, see legends in Fig. 1. Asterisks represent a significant difference with a probability $p<0.05$.

days later and HG-P21 about 4 days later. Remarkably, in the rats of the HG-P27 group, performances decreased again after P30, and only by P38 they had recovered. We also calculated the time needed to right (with 20 ms [the yield time of a video frame] as a unity of time). This indicated that HG rats of all groups were slower to right in the air than controls, but this reached significance only for some of the testing days (Fig. 5B).

4. Discussion

Our results demonstrate that hypergravity (2 g) during the foetal period and varying periods of time thereafter, induces a delay in particular aspects of motor development, particularly in those reflexes dependent on vestibular inputs (contact- and air-righting, negative geotaxis). However, other reflexes as the tail reflex and the grasp reflex showed a normal development. In addition, we observed a retardation in weight increase. Remarkably, however, the exposure to normal gravity induced a catch-up in weights and at P40, the average weight of the rats kept in HG until P5 and P10 even surpassed that of control rats. We also observed an advance in some of the developmental parameters, HG-P5 and HG-P10 rats opened their eyes before controls (2–3 days) and HG-P5 rats initially displayed an increased grip time. However, after some time spent in normal gravity, the performances of HG rats normalised and no differences between the animals were observed anymore at P40. Several physiological changes induced by hypergravity could account for these differences.

A lower weight at birth in rats raised in hypergravity has been observed previously [1,3,7]. To prevent an effect of stress mediated by corticosterone, whose level increased only during the first 4 days of centrifugation [36], in our experiments, the mothers of pups had been allowed an adaptation period of 1 week before introducing the male into the cage. However, other hormones may have influenced mother's behaviours and indirectly pups growth. For instance, Megory and Oyama [35] observed decreases in plasmatic prolactin concentrations which could have induced some disturbances in maternal behaviour and which

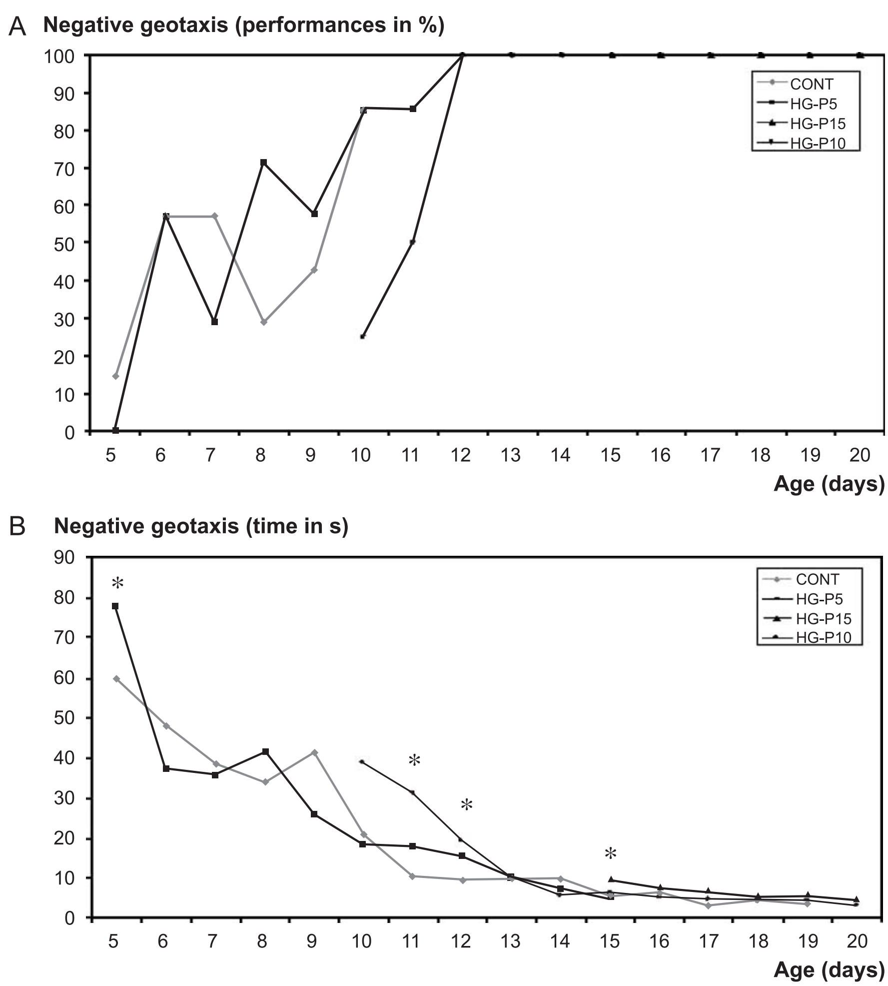

Fig. 4. Negative geotaxis reaction from P5 to P40 in hypergravity and control rats. (A) Percentages of rats turning into a position with the nose pointing downward towards their nose pointing upwards within 30 s. (B) Average times needed to accomplish a positive reaction. For further abbreviations, see legends in Fig. 1. Asterisks represent significant differences (p<0.05).

have previously been observed in HG [35,43]. In our experiments, although we did not videotaped all the events happening from mating till transfer to 1 g, mothers seemed normally nursed their pups. In low-intensity HG, like we used, other studies also reported normal maternal cares [4,38]. In addition, it has to be considered that the hypergravity condition induces a high-energy expenditure, as most motor activities require more energy. These increased energetic demands are probably responsible for a loss in body fat reserves [19,26], which might explain the weight decreases we observed. Although HG rats displayed decreased weights, the rats in the HG-P5 and HG-P10 groups caught up in weight when returning into a 1-g environment and after sometime they even surpassed in their growth curves those of controls at P40. This result might be explained by an increased food intake of HG rats during the period in the centrifuge [17,37]. A similar catch-up might have been observed in the other groups (HG-P15, HG-P21 and HG-P27) in case of a longer observation period (see also Refs. [17,51]), but in the present study these trends have not been studied. Moreover, we cannot exclude hormonal alterations, notably in hormones linked with the energy metabolism and the distribution of energy supplies. Thyroid functioning, for instance, seems sensitive to the level of gravity [29,32,45] and this might also have effects on body weight in all rat groups.

Given the numerous elements involved in the vestibular reflexes (the afferents to the CNS, the several stages of central processing, and the effectors), the cause of the delay we observed in the maturation of vestibular reflexes is not easy to identify. First of all, the sensory epithelium of the vestibular system which detects linear accelerations might have changed. When a rat is placed in a position with the nose pointing downwards (the negative geotaxis test) or in a supine position (the contact- and air-righting reflexes), the CNS is informed about the position of the head relative to

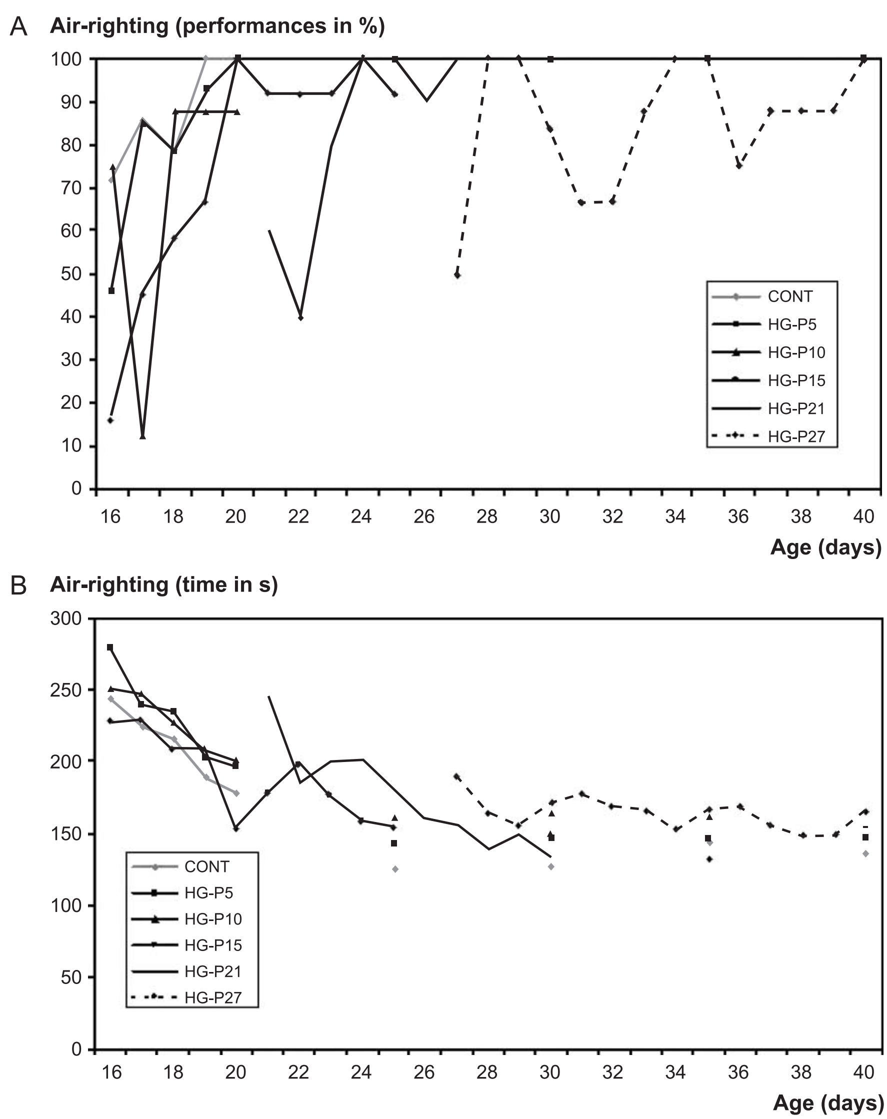

Fig. 5. Air-righting in hypergravity and control rats from P16 to P40. (A) Performances expressed in percentages of rats landing on their forepaws. (B) Air-righting time expressed in ms. For further abbreviations, see legends in Fig. 1.

the vertical axis, i.e. the gravity vector. In these tests, the vestibular system is involved in the detection of the head position and in triggering the reaction aiming at repositioning the body and the head. The retardation in these reactions in HG rats, previously observed in young rats after exposure to microgravity [52] might be due to a delay in vestibular development. The vestibular system starts developing during the gestational period but its full maturation continues until the end of the first month of life [11,13,14]. Under hypergravity conditions, a delayed growth has been observed in the otoconiae in chick embryos [24], and in fish [2], and probably as well as in hamsters [48]. Moreover, recent results showed a delay in the postnatal development of connections between type I hair cells in the utriculus, and their afferent calyces [20]. Additionally, a slower development of the potassium currents in type II hair cell has been reported in rat utriculi under hypergravity conditions [10]. The development of synaptic connections in the vestibular system is affected as well by hypergravity. Ross and Tomko [44], for instance, observed a 40% decrease in the number of synapses with type II hair cells. Interestingly, Bruce and Fritzsch [8] observed a delay in synaptogenesis in the medial vestibular nuclei after pre- and post-natal exposure to microgravity. When summarising this evidence, an altered morphological development, a reduced sensitivity of the vestibular system (see also Refs. [25,47,57]), and a retarded development of the connectivity of the vestibular system could well explain the delayed development in the behavioural reflexes we observed.

Although the development of the vestibulo-spinal pathway has not been extensively studied in HG, two studies suggest that the development of certain projections from the CNS to the spinal motoneurons is sensitive to hypergravity. The first [21] showed a delay in the development of monoaminergic projections to the spinal cord in rats exposed from day 11 of gestation (E11) to birth. These diffuse projections play a role in gain-setting of motor responses [3]. Secondly, Brocard et al. [7] recently observed a reduction in the development of descending pathways, including reticulo- and vestibulo-spinal tracts, in rats exposed to 1.8 g from E11 to the end of the first post-natal week. These two observations could account for the delay both in motor responses and in the righting reflexes.

Another aspect to be taken into account is the muscles which are involved in the motor reactions. We observed an increased grip-time in HG-P5 rats and also in HG-P10 and in HG-P15 during the first days of recording. This probably has to be explained by increased muscle force and fatigue resistance in hypergravity. Indeed, Burton and Smith [9] showed that chickens, adapted to hypergravity had increased their muscle force and muscle endurance and Picquet et al. [40] reported an increased maximal tension in the soleus muscle in rats conceived, born and reared at 2 g. The increased grip-time also might be due to a modification in muscle development and its contractile properties. Unfortunately, although Martrette et al. [34] demonstrated that antigravity muscles develop faster in hypergravity, not much is known about the development of muscle force in hypergravity conditions. For the control rats, the grip time increased between P8 and P10 and thereafter decreased, but we cannot yet explain this finding. However, as observed by Thullier et al. [50], after time spent in 1 g, rats early exposed to hypergravity do not display anymore a longer grip time.

The adaptations we observed when the animals were transferred to 1 g might have taken place in central structures. Indeed, Gustave Dit Duflo et al. [23] showed that hypergravity (2 g) induced an increased Fos-activity in the vestibular nuclei in rats. Moreover, Krasnov [27] reported that in rats, developing in 2 g, a strong activity occurred in the granular layer of nodular cerebellar cortex, a region receiving afferents from the vestibular nuclei. At the cortical level, D'Amelio et al. [15] observed a decrease in the GABA-immunoreactiviy in cells and terminals in the sensorimotor cortex and also in the cerebellum in rats adapted to 2 g. These changes in activity might well play a role in the central adaptation to the modifications in gravity. In young animals, such adaptations rapidly occur since in our experiments the HG-P5 rats reached the control values in about 2 days while older animals, HG-P27, had not even completely recovered at P40. The latter results are in accordance with the results of Bouët et al. [5,6] showing a period of 3 weeks for a complete behavioural adaptation in adult rats.

Strikingly, we observed an advanced opening of the eyes by 2–3 days in HG-P5 and HG-P10 rats. To our knowledge, this is the first report of such a precocious development. Duration of gestation may account for the earlier eye-opening. In our experiments, it was not possible to determine the exact duration of gestation. However, previous studies showed that duration of gestation is largely preserved in such intensities of hypergravity exposure [35,43]. Another possibility is that the change from 2 to 1 g which is perceived by the vestibular system as a decreased stimulation at the macular level of the vestibulum leads to a take-over by visual inputs. Several studies have shown that in case of vestibular deprivation by neurectomy, or mechanical destruction of the labyrinth, visual inputs take a predominant role in the afferent influences on motor activities [33,59]. Similarly, the visual system also plays an important role in adaptations to modified gravity [12]. We speculate that the decrease in vestibular stimulation following the transfer from 2 g to 1 g might induce an intersensory compensation in developing rats, leading to an earlier eye opening and thus to an increased influence of the visual system. Interestingly, Schönfelder and Schwartze [46] observed analogously an earlier opening of the eyes after destruction of the olfactory bulb at P1 in the rat.

In conclusion, we showed that early exposure to hypergravity induced a delay in motor development in rats and particularly in reflexes dependent upon vestibular information. This retardation recovered rapidly in rats transferred to 1 g before P15 and slower for older rats, transferred after that age. Besides, we observed an advance in the eye opening as well as a temporary increase in muscle force. In this perspective, it is of great interest to know the consequences of these modifications on the development of locomotion. This development, after a situation of hypergravity, which is strongly dependent upon postural development as well as visual inputs, is currently the topic of our investigations.

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