THE EFFECTS OF HYPERGRAVITY ON VESTIBULAR EPITHELIA AND BEHAVIOUR OF THE RAT

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INTRODUCTION In order to assess the effects of gravity, miscellaneous physiological systems of several species have been investigated. The main reason for this research has been the wide-scale consequences of weightlessness in space for human physiology. Available methods to manipulate the level of gravity for physiological research are 1: parabolic flight, 2: orbital flight, and 3: a centrifuge. The only possibility to breed (mammalian) species successfully at an altered level of gravity is inside a centrifuge at hypergravity (HG).

The duration of this HG period can be adjusted to the investigator's design. For rodents, it has been shown that this HG period can start at conception and can be extended to the animal's entire life-span. In our laboratory, hamsters and rats have been bred inside our centrifuge (Fig. 1) at 2.5g (6,7,9,10).

Gravity conditions that deviate from the normal level on earth (i.e. both weightlessness and hypergravity) may have a significant impact on growth, body composition, metabolism and sensory functioning. One obvious cause for these physiological changes is the altered mechanical load (and/or energy requirements) of a particular system (bone, cardiovascular, renal/fluid, muscle, blood). For instance, adult rats living at 2.5g have a lower body mass than normal gravity (NG) animals (9). When NG animals are transferred to HG conditions they lose weight (10-15%) and remain relatively inactive during a period of approximately 1 week. After that period they appear to be adapted to the new situation: animals start growing again (although when compared to NG animals, their weight remains reduced) and they 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).

A change of gravity will affect the vestibular input which, in turn, may have a wide range of serious effects (on posture, locomotion, ocular reflexes, righting reflexes) and which can cause motion sickness.

The entire scope of effects of a prolonged change of gravity level is far from clear yet. It is to be expected, however, that possible effects will depend on the developmental stage during which the animal is subjected to altered gravity, and that the nature of these effects will probably be diverse. For these reasons, our research has been focussed on different topics that are directly related to the vestibular system: 1: the histology of the peripheral sensory system, 2: vestibular induced ocular reflexes, and 3: vestibular-controlled behaviour. Elsewhere in this volume results from a study on vestibulo-ocular reflexes are presented. Here, we will show some results of the histological and behavioural studies.

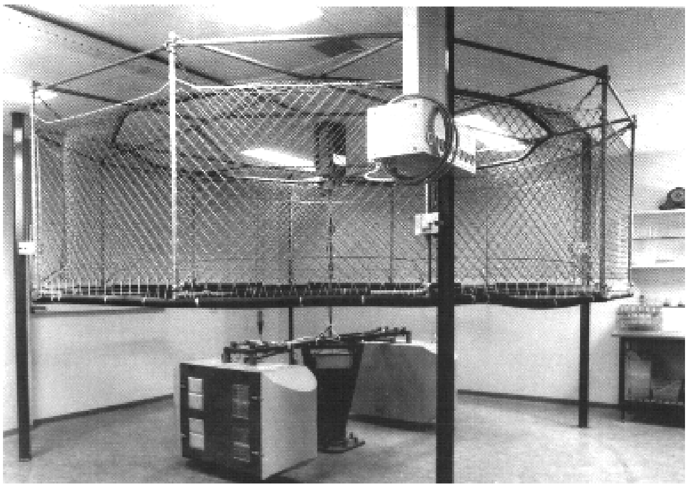

Figure 1: The centrifuge comprises two horizontal arms with free-swinging gondolas (1.10?0.45?0.73 m) in which experimental animals can be housed. Angular velocity was set at 34.3 cycle/minute to achieve a hypergravity level of 2.5g at the bottom of the animal housing. For daily care and experiments, the centrifuge was stopped during 20-60 minutes. During the first 10 days after birth, gravity level in the centrifuge was set at 1.8g, thus increasing survival of newborns. Animals inside the gondolas could be observed by means of cameras.

HISTOLOGY The vestibular epithelia of the rat were immuno-histochemically labeled for the cytoskeletal proteins actin and tubulin. Four weeks old rats were transferred to the centrifuge and stayed there for 9 months. Scans of the actin- and tubulin-labeled structures of the utricle and the saccule were obtained with a confocal microscope and analyzed.

An example of a utricle from a HG and from a NG rat is shown in Fig. 2, in which the actin-labeled structures are visible.

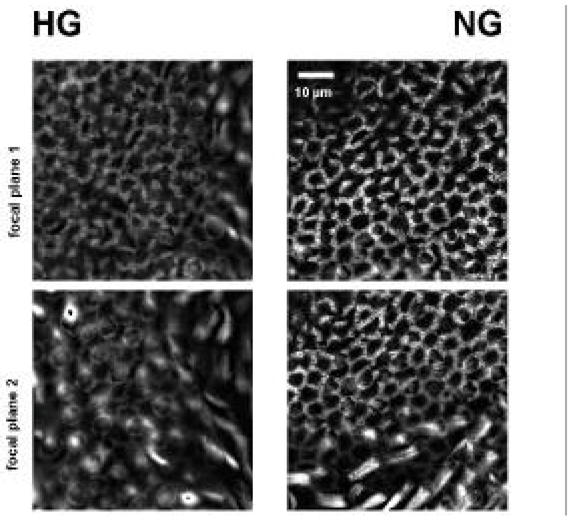

Figure 2: Actin-labeled structures of the utricle of a HG and a NG rat. Visible are the bundles of stereocilia and the actin belt of tight junctions between hair cells and supporting cells. Visibility of structural details in a particular image depends on the focal plane in which a scan was obtained. From each utricle two samples are shown which were 2 ?m apart (focal plane II is apical of focal plane I).

Depending on the focal plane of the scan, several details of the hair cells can be discerned. In general, the maculae of HG rats appear to be intact. The honeycomb-like structure formed by the tight junctions' actin belts, was analyzed with respect to area and shape. For the utricles of rats that had lived inside the centrifuge, the area enclosed by the actin belts is a little smaller (5%) and their circumference appears to be somewhat more irregular. For the saccule no significant differences were found. In Fig. 3 the tubulin-labeled structures of the same utricles (and focal planes) as in Fig. 2 are shown.

The preliminary results of our immuno-histochemical study suggest that the effect of prolonged HG exposure on mature vestibular sensory epithelia is small. When 3 weeks old hamsters were transferred from normal gravity to the 2.5g environment inside the centrifuge, and subsequently lived there for 6 months, no difference between the otoconia of these animals and control animals was observed (5). Calcium content, shape and size of the otoconia were identical. Therefore, it might be concluded that HG exposure only has small effects on the mature peripheral vestibular system. Other studies have shown, however, that within a few days the innervation of the vestibular sensory organs of adult animals can be adapted by either increasing or decreasing the number of synapses terminating on hair cells (4).

When hamster embryos were developed under HG conditions the otolith area with large otoconia decreased and the area with smaller otoconia increased (6). However, this doesn't seem to imply a major dysfunctioning of the otolith system. The analysis of immuno-histochemically labeled epithelia of rats, which were gestated under 2.5g conditions, is in progress.

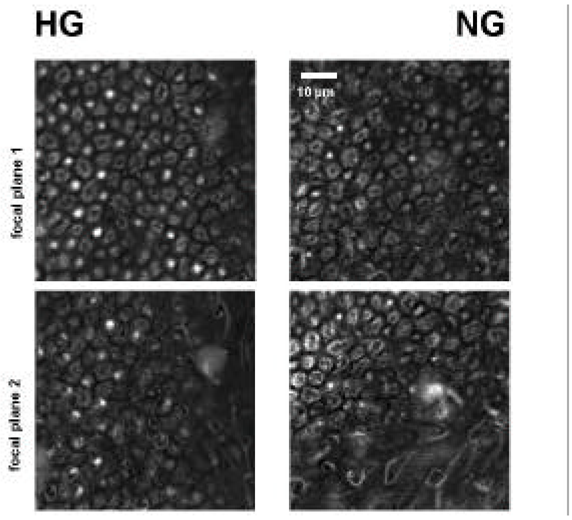

Figure 3: Tubulin-labeled structures of the same utricles as in Fig. 2. Structural details are the kinocilia and the tightly packed microtubules in the 'neck' region of type-I hair cells (bright white spots).

BEHAVIOUR The air-righting reflex (turning from a supine to prone position during fall) has been recorded for the hamster (7) and for the rat (9). In both studies, exposure of HG animals to 2.5g included gestation. Because the behavioural experiments were performed under infrared illumination orientation of the animals depended solely on the functioning of their vestibular system. Results from both studies are shown in Fig. 4. For NG hamsters, 80-100% of the air-rightings is performed successfully against 20-25% for HG hamsters. This difference doesn't change with age. Only during the first experimental session NG rats reach a score of 80% correct air-rightings. Thereafter, the score from both groups is about the same.

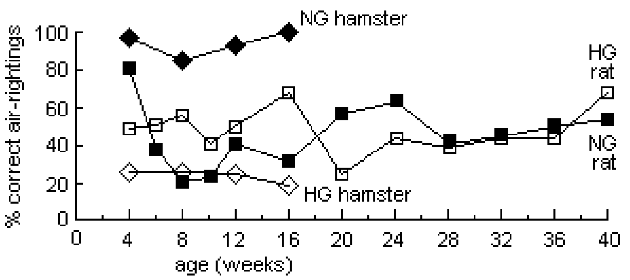

Figure 4: Air-righting of HG hamsters and NG hamsters (adapted from ref. 7), and of HG and NG rats (adapted from ref. 9) as a function of age.

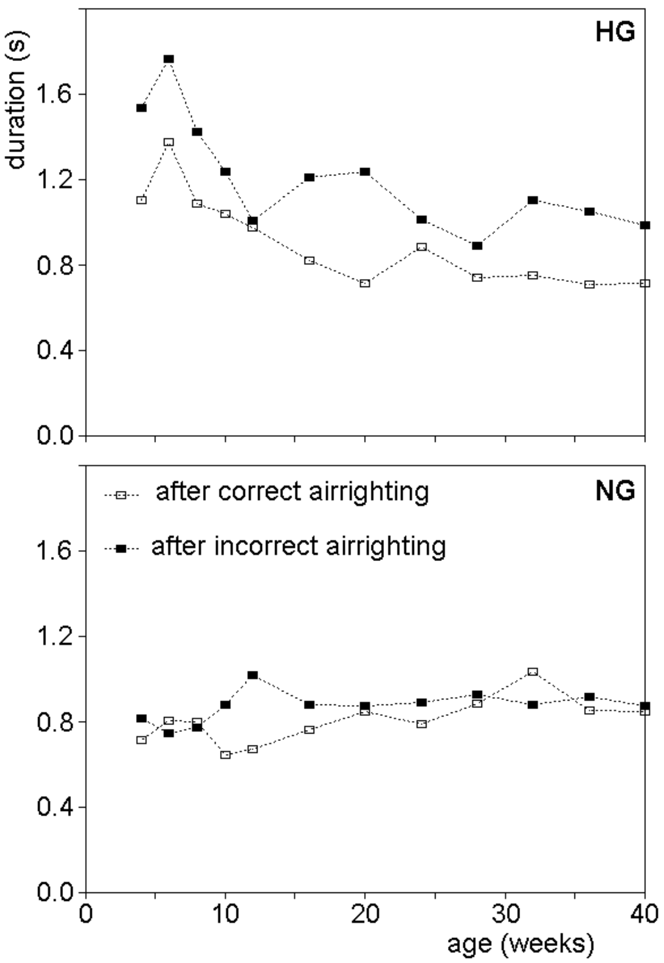

Figure 5: Mean duration of surfacings of HG (upper panel) and NG rats after a correct and an incorrect air-righting respectively. Surfacings of HG rats take more time after an incorrect air-righting reflex. This behaviour is characteristic for HG rats of all ages.

Under water (i.e. after each air-righting), the rats can only use vestibular cues for orientation in order to swim towards the water surface. Young HG rats are unable to find the water surface sometimes, and if they succeed they take significantly more time for it. NG rats of all ages and older HG rats have no problems performing this task (9). This result suggests that older HG rats have adapted to the gravity level of 1g. (It is estimated that 10 weeks old HG rats have spent about 24 h at 1g because of daily care and experiments.) For HG hamsters, swimming remains a more strenuous task during their entire life-span (7).

The combination of air-righting and reappearing at the surface, after falling into the water, was performed three times in a row within about one minute. A detailed analysis of this sequence of vestibular induced behaviour shows that vestibular functioning of HG rats deviates from that of NG rats (9). Briefly, the behaviour of NG rats appears to be independent of previous events while the behaviour of HG rats shows a tendency to deteriorate during the experimental sequence of events (Figs. 5 and 6). This effect lasts the entire life of HG rats.

Apparently, vestibular induced behaviour is species dependent. Although this conclusion may seem obvious, it is important to keep in mind when using animals as a model for human (sensory) physiology.

DISCUSSION An increase from 1g to 2.5g (8 dB) is, a priori, not expected to pose a serious problem to the vestibular sensory system. Also, the ontogenetic development of the vestibular system at 2.5g does not result in the loss of vestibular functioning altogether. Apparently, even the effects of development under 0g conditions (potentially, a fundamentally different situation) are moderate and do not lead to serious defects of the peripheral vestibular sensory organs (2).

In a previous study, a relation has been reported between vestibular controlled behaviour and malformations of the otoconia of hamsters (8). When they grew older, a few of the HG rats from our behavioural study, sometimes, started spinning around their body axis in the water (9). Usually, this aberrant behaviour is attributed to a defective otolith organ (1). Possibly, the probability of peripheral deficiencies increases with hypergravity.

As for vestibulo-ocular reflexes (10) the changes in behavioural reflexes of HG rats are probably due to neurological alterations.

Some behaviour can be recalibrated to new stimulus conditions, as has also been demonstrated by experiments in which embryonic development (partly) occurred under 0g conditions during spaceflight (3). Our results suggest, however, that ontogenetic development under altered gravity conditions also has a permanent effect on the vestibular system.

The possibility to alter gravity, by means of a centrifuge, provides a unique tool to investigate the critical periods of an animal's development, during which it is most sensitive to environmental changes. Also, the plasticity of the vestibular system and of physiological processes for which vestibular input is essential can be studied by transferring animals from one gravity level to another.

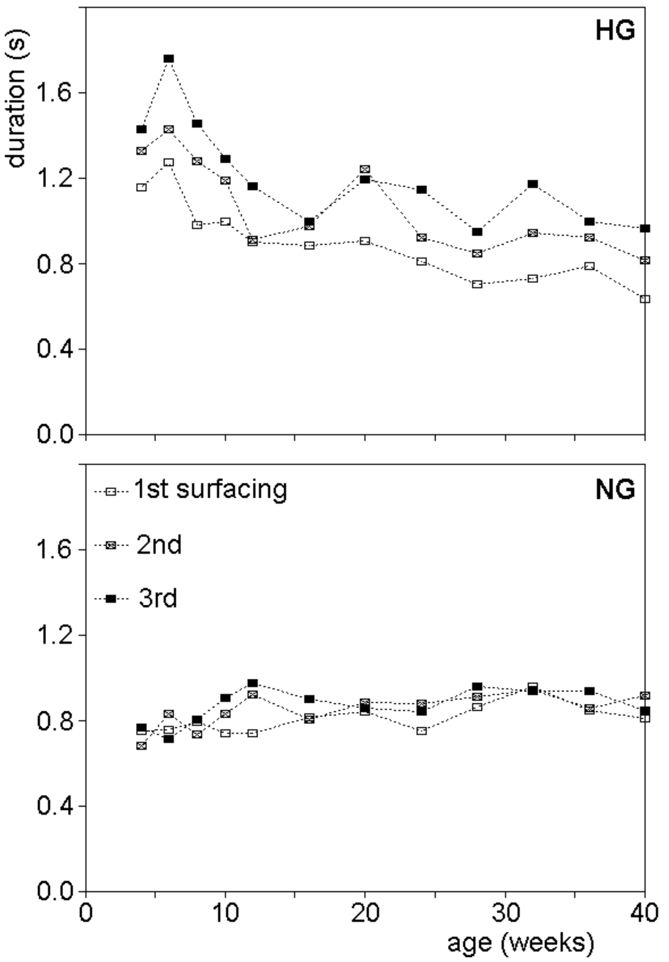

Figure 6: Mean duration of surfacings of HG (upper panel) and NG rats. For NG rats, no difference between subsequent surfacings was observed. The surfacings of HG rats tend to take longer one after the other. This behaviour is characteristic for HG rats of all ages.

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