Acta Otolaryngol (Stockh) 1998; 118: 86–89

Behavioural Changes in Hamsters with Otoconial Malformations

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Sondag HNPM, de Jong HAA, van Marle J, Oosterveld WJ. *Behavioural changes in hamsters with otoconial malformations.* Acta Otolaryngol (Stockh) 1998; 118: 86–89.

For a period of 10 months, the perceptive–motor skills of golden hamsters were tested as part of an experiment to investigate vestibular controlled behaviour. We found that four out of 40 hamsters had more difficulties with swimming and equilibrium maintenance than the rest of the group. These disturbances either were apparent during the first months of testing or developed at a later period. In three hamsters the disturbances persisted over time while in one hamster performance in perceptive–motor skills increased. Histological examination with scanning electron microscopy revealed otoconial abnormalities in the saccule and/or the utricle. The otoconia were either malformed or replaced by spherulites. We conclude that the observed behavioural disturbances were caused by a defective peripheral vestibular organ. The results show similarities with data from pathology in other animals as well as in the human inner ear. *Key words*: *scanning electron microscopy*, *labyrinths*, *otolith organs*, *utricle*, *saccule*.

INTRODUCTION

The utricle and saccule comprise the otolith organs, which are part of the peripheral vestibular system, and which play a role in posture and movement. The otoconia, crystals of calcite located on the macula of the otolith organs, are necessary for normal vestibular-controlled behaviour such as equilibrium maintenance and swimming behaviour. Disturbances in swimming behaviour have been reported in animals with otoconial defects, such as otoconial malformations or total absence of otoconia (1, 2). Most of these otoconial alterations were found in animals treated with ototoxic agents or genetically mutant animals. However, alterations in the otoconial layer were also reported in the normal guinea-pig (3). Until now nothing was known about otoconial alterations in the golden hamster.

As part of an experiment on the effects of hypergravity on vestibular behaviour, the perceptive–motor skills of golden hamsters were tested during 10 months (4, 5). We found that four out of 40 hamsters had behavioural disturbances and otoconial malformations on the utricular and/or saccular otolithic membrane. The structural alterations in the morphology of the otoconia and their consequences to vestibular-controlled behaviour in animals and humans will be discussed in this article.

MATERIALS AND METHODS

Animals

The 40 male hamsters (*Mesocricetus auratus*, Harlan, Zeist, The Netherlands) were 4 weeks old (mean body weight 47 g) at the start of the experiment. They were housed in acrylate boxes (22 × 37 cm) and food and water were available ad libitum. The day–night cycle was reversed (light on 19:00–07:00 h). The hamsters were tested in a laboratory room with dimmed lights. Swimming and balancing on tubes were registered weekly and treadmill activity once every 2 weeks. The experiments were performed in accordance with 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.

Behaviour

Swimming in a lane. Swimming in a lane (140 × 10 cm, water-depth 25 cm, water temperature 30°C) was used to test the animals' swimming ability and speed. The hamsters had to swim to the end of the lane where they could climb an escape ladder. One session, preceding the testing days, was used to train the hamsters for 5 min to swim to the ladder. On the testing days, the animals swam three trials. The crossing time for the middle part of the lane (length 100 cm) was measured.

Balancing on tubes. The acrylate tubes (length 100 cm, diameter 2 cm), placed 20 cm above ground level, were either fixed to standards (fixed tube task) or movable, connected by elastic cords which were attached to the standards (mobile tube task). The testing procedure is described in detail in Sondag et al. (5). Each hamster had to cross the tube three times once a week.

Treadmill activity. A treadmill was placed in one of the boxes. The treadmill activity of one hamster was recorded daily by means of a cyclocomputer (CC-MT200, Cateye, Osaka, Japan), providing the average speed, the running time and the distance covered in 1 day.

Histology

After 10 months, the hamsters were killed and the temporal bones were dissected. The patches of utricle and saccule were fixed in 2.5% gluteraldehyde + 0.5% paraformaldehyde in phosphate buffer solution (0.1 M, pH 7.4) for 20 min. After rinsing in distilled water and air-drying, the specimens were mounted on aluminium stubs and coated with gold for electron microscopical scanning (ISI SS40).

For crossing times in the swimming tests and crossing times and falling frequency in the tube tests, the mean of three trials per day per animal was calculated. Data from the swimming tests were statistically assessed with repeated analysis of variance (ANOVA) and from the tube tests with repeated ANOVA with body weight as covariates (ANCOVA). The statistical software SPSS PC + 5.0 was used for the analysis (significant at $p < 0.05$).

RESULTS

The four hamsters showed normal locomotion, and treadmill activity (ambulation, average speed and time spent on the treadmill) did not differ significantly from the other 36 hamsters.

The results for the remaining 36 hamsters were as follows. *Swimming in a lane*: Within 4 weeks, all 36 hamsters were able to swim to the other side of the lane. The hamsters swam in a doggy-paddle style using both forelimbs and hindlimbs. Mean swimming speed was 0.22 m/sec (range 0.2–0.26 m/sec). *Tube tasks*: Within 5 weeks all 36 hamsters managed to walk on both tubes. The mean crossing time for the fixed tube was 16.4 sec (range 12–26 sec) and the mean number of falls was 0.53 falls per crossing (range 0.05–1.8 falls). For the mobile tube the crossing time was 24 sec (range 17–40 sec) and the mean number of falls was 1.1 falls per crossing (range 0.1–2.3 falls). *Histology*: All 36 hamsters showed normal otoconia on both the utricle and saccule, and no abnormalities were found in the size or shape of the otoconia (Fig. 1).

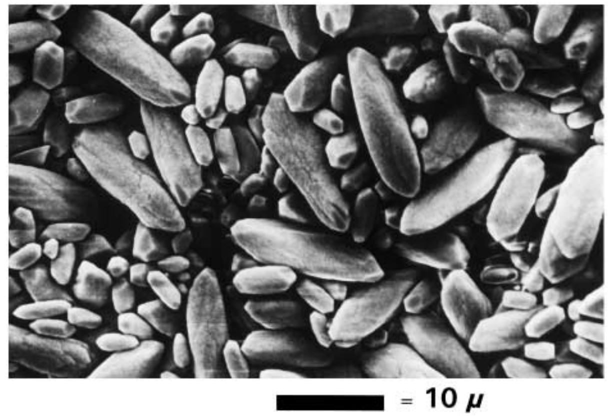

Fig. 1. Otoconia on the saccule of hamsters showing normal vestibular functioning.

Hamster I showed severe swimming disturbances, as could be seen when circling under water. This hamster often had to be saved from drowning (no mean swimming speed available).

Furthermore, when tested on the tubes, this hamster was not able to stand on the tube or walk on the tube. Histological examination of the otoconial layer showed that both saccules were covered with spherulites (Fig. 2) and no otoconia were present. The otoconial layer of the utricles had normal otoconia.

Hamster II showed no difference from the control hamsters in swimming and balancing during the first 10 weeks of testing. After this period, this hamster had a decreased swimming ability which was shown by difficulty in keeping its head above water level and swimming with a horizontal body position. Furthermore, the swimming speed (mean speed 0.14 m/sec, range 0.07–0.25 m/sec) was lower for this hamster than for the rest of the group. The ability to keep its balance on the tube also decreased: in 46% of the trials this hamster was unable to stand on the tube. The otoconia of both the saccules and the utricles were malformed (Fig. 3).

Hamster III had a decreased swimming ability that was shown by difficulty in keeping its head above water level and swimming with a horizontal body position during the whole period of testing. Furthermore, this hamster swam more slowly than the other hamsters (mean speed 0.08 m/sec, range 0.07–0.2 m/sec). These differences were observed during the whole period of testing. Balancing on the tubes was within range of the rest of the group. The otoconia of the saccules appeared to be malformed (multifaceted, Fig. 4), while the otoconia from the utricle looked normal.

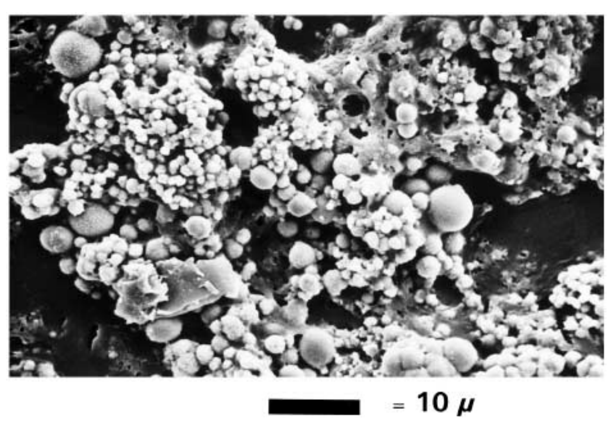

Fig. 2. Apatite spherulites on the saccular otoconial patch of hamster I.

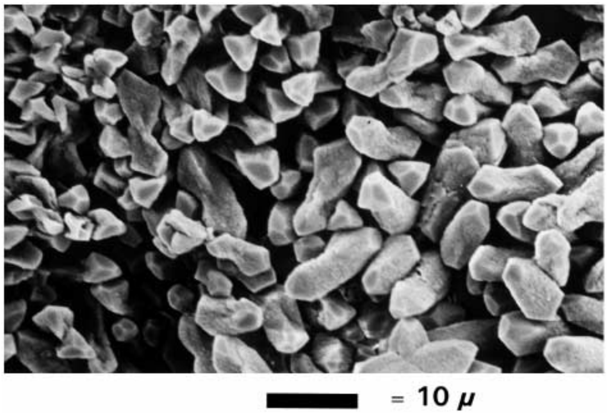

Fig. 3. Malformed otoconia on the saccular otoconial patch of hamster II.

Hamster IV showed a decreased swimming ability during week 1–12, which was observable in circling during swimming, and a decreased swimming speed (mean speed 0.12 m/sec, range 0.09–0.14 m/sec) compared with the control group. Sometimes the hamster had to be saved from drowning. After week 13, the swimming ability of this hamster improved and was not different from the other hamsters during the rest of the experiment. No difficulties were found in balancing on the tubes. The otoconia of the saccules and the utricles were malformed (Fig. 5).

DISCUSSION

Alterations in otoconia morphology seem to lead to disturbances in vestibular behaviour, such as ataxia, head-tilting and disorientation during locomotion, swimming and air-righting (1, 2). Swimming disturbances were found in animals with otoconial malformations or with a total absence of otoconia. These otoconia alterations were caused either by ototoxic agents or by genetic mutations (1, 2, 6). Until now it

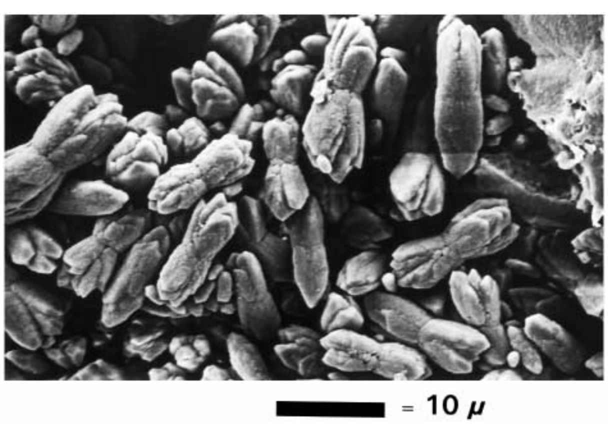

Fig. 4. Multifaceted otoconia on the saccular otoconial patch of hamster III.

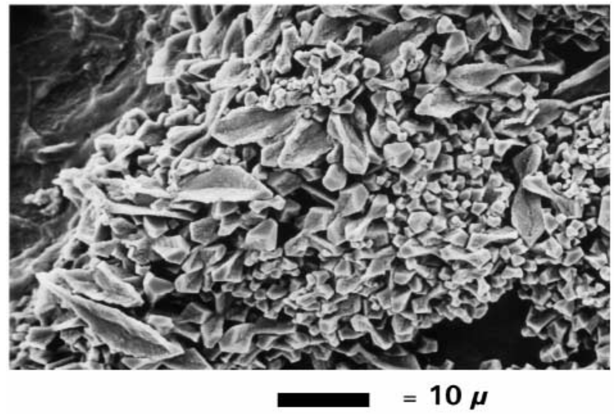

Fig. 5. Malformed otoconia on the utricular otoconial patch of hamster IV.

has been suggested that vestibular function only decreases when the malformations in the otoconial layer are severe enough. For example, Gray et al. (7) found that destruction of more than two of the four otolith organs of mice led to disturbances in swimming behaviour. The data from hamster I suggest that behavioural disturbances may occur even when only two otolith organs are affected by alterations. The spherulites observed on both saccular maculae of this hamster (Fig. 2) resembled those observed in the otolith organs of a patient described by Johnsson et al. (8). This patient had a hereditary congenital deafness and complained about moments of dizziness. Post-mortem study of the otolith organs revealed that the utricles and saccules were covered with apatite spherulites. The same kind of spherulites was also found in some labyrinths of deaf Dalmatian dogs (9). However, in the last study no systematic research into abnormal vestibular behaviour was included. Thus, spherulites are found both in humans and in other species and are most probably the cause of the observed behavioural disturbances. Rouse et al. (9) hypothesized that a disturbed endolymphatic homeostasis causes these otoconial abnormalities. Through phosphatization, the calcite structure of the otoconia is transformed into an apatite structure resulting in spherulites.

Animals with either otoconial malformations or multifaceted otoconia in the otolith organs had less severe problems with performance on the vestibular tasks (hamsters II, III and IV).

Furthermore, our data indicate that these disturbances in behaviour occurred at an earlier stage of otoconia malformation in the hamster than in mice, rats or guinea-pigs (1–3). The reason for this higher sensitivity to otoconial alterations in the hamsters might be that the hamster depends more on vestibular information than the other species do, because of its poorly developed visual system. However, we do not know whether the underlying layers of the otolith organs were malformed, as examination of these layers was not included in our experiment.

The multifaceted appearance of the otoconia of hamster III (Fig. 4) was previously described in fetal rodents and chickens (10–12). However, the multifaceted form of the otoconia is normally replaced by smooth otoconia during maturation (11). We suggest that either the multifaceted otoconia in hamster III never matured or they replaced the normal matured otoconia after an inner ear dysfunction.

The otoconial malformations found in hamsters II and IV (Figs. 3 and 5) resemble the collapsed otoconia after decalcification, described by Lim (13). Again, a disturbed endolymphatic homeostasis could be one cause of these malformations, as is the case with the development of spherulites. The data from hamster II, which showed behavioural disturbances after week 10, suggest that such otoconia malformations can develop after birth. The behavioural data from hamster IV (behavioural disturbances during the first 12 weeks) seem to indicate that a young animal with these otoconial malformations can overcome the vestibular deficit.

Our data confirm the conclusion that normal otoconia are necessary for normal vestibular function. However, disturbances in behaviour that occur because of otoconial malformations are sometimes only found in specific vestibular information-sensitive tasks. The spherulites seem to be a sign of severe vestibular damage in both humans and other animals, resulting in behavioural disturbances in both groups, while the otoconia malformations observed in the other animals seem to present a milder form of vestibular damage.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Netherlands Organization for Scientific Research (NWO) for funding this project. This research was supported by a grant from the Foundation for Behavioural and Educational Sciences of this organization (575-62-049).

REFERENCES

1. Lim DJ, Erway LC. Influence of manganese on genetically defective otolith: A behavioral and morphological study. Ann Otol 1984; 83: 565–81.
2. Douglas RJ, Clark GM, Erway LC, Hubbard DG, Wright CG. Effects of genetic vestibular defects on behavior related to spatial orientation and emotionality. J Comp Phys Psych 1979; 93: 467–80.
3. Johnsson L-J, Wright CG, Preston RE, Henry PJ. Defects of the otoconial membranes in normal guinea pigs. Acta Otolaryngol (Stockh) 1980; 89: 93–104.
4. Sondag HNPM, de Jong HAA, van Marle J, Oosterveld WJ. Effects of sustained acceleration on the morphological properties of otoconia in hamsters. Acta Otolaryngol (Stockh) 1995; 115: 227–30.
5. Sondag HNPM, de Jong HAA, Oosterveld WJ. Behavioural disturbances in hamsters subjected to hypergravity; adaptation and re-adaptation to increased gravity forces. Acta Otolaryngol (Stockh) 1996; 116: 192–7.
6. Huygen PLM, Fischer AJEM, Kuijpers W. The vestibular function in the manganese-deficient rat. Acta Otolaryngol (Stockh) 1986; 101: 19–26.
7. Gray LE, Rogers ME, Ostby JS, Kavlock RJ, Ferrell JM. Prenatal dinocap exposure alters swimming behavior in mice due to complete otolith agenesis in the inner ear. Toxicol Appl Pharmacol 1988; 92: 266–73.
8. Johnsson L-G, Rouse RC, Wright, CG, Henry PJ, Hawkins JE. Pathology of neuroepithelial suprastructures of the human inner ear. Am J Otolaryngol 1982; 3: 77–90.
9. Rouse RC, Johnsson L-G, Wright CG, Hawkins JE. Abnormal otoconia and calcification in the labyrinths of deaf Dalmatian dogs. Acta Otolaryngol (Stockh) 1984; 98: 61–71.
10. Ballarino J, Howland HC, Skinner CW, Brothers EB, Bassett W. Studies of otoconia in the developing chick by polarized light microscopy. Am J Anat 1985; 174: 131–44.
11. Mann S, Parker SB, Ross MD, Skanulis AJ, Williams RPJ. The ultrastructure of the calcium carbonate balance organs of the inner ear: An ultrahigh resolution electron microscopy study. Proc R Soc Lond (Biol) 1983; 218: 415–24.
12. Salamat MS, Ross MD, Peacor DR. Otoconial formation in the fetal rat. Ann Otol 1980; 80: 229–38.
13. Lim DJ. Formation and fate of the otoconia: Scanning and transmission electron microscopy. Ann Otol 1973: 82; 23–35.

Submitted November 29, 1996; accepted April 2, 1997

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