

Experimentally Induced Motion Sickness in Fish: Possible Role of the Otolith Organs

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Objective—Although the occurrence of motion sickness is largely attributed to sensory conflict, the reasons for the highly variable inter-individual susceptibility to motion sickness remain unclear. One cause may be asymmetry between otoconial masses in the right and left inner ear. This hypothesis has been supported by experiments in fish under conditions of prolonged weightlessness and parabolic flight. In swordtail fish (*Xiphophorus helleri*) we examined the extent to which a Coriolis force environment, which has a strong motion sickness-triggering effect, disturbs swimming behaviour or produces motion sickness.

Material and Methods—In a sealed aquarium, fish ($n = 22$) were subjected to Coriolis stimulation by constant vertical axis rotation, combined with pendular oscillation around a horizontal axis.

Results—Initially, at low stimulus intensity, all fish showed active compensatory swimming behaviour. However, while the majority ($n = 19$) maintained active compensation movements at higher stimulus levels, the others ($n = 3$) entered a passive uncoordinated state, from which they recovered again after reduction of stimulus intensity. On examination of the otoconial mass, we found asymmetries between the right and left utricles in all three cases and between the saccules in one case.

Conclusion—We conclude that a lateral preponderance of the otoconial masses in the utricle or saccule promotes motion sickness under experimental conditions. The utricle appears to play a more important role in this than the saccule. *Key words: motion sickness, otolith asymmetry, saccule, swordtail fish, utricle.*

INTRODUCTION

The sensory mismatch theory is generally accepted as an explanation for the development of motion sickness (1). It is understood that in most cases contradictory visual and vestibular information leads to the development of the classical symptoms of motion sickness. Recently, Bles et al. (2) compared this classic concept to a more specific conflict in the processing of the subjective vertical, i.e. between the perceived vertical and that known from previous experience. This would favour the role of the otolith organs in the aetiology of motion sickness. Earlier experiments provided initial evidence of otoconial involvement in motion sickness. Von Baumgarten et al. (3) and Wetzig (4) observed disturbed swimming behaviour with screw and looping movement patterns under microgravity, which they attributed to an otoconial mass asymmetry (OMA) of the two labyrinths. Under normal conditions, this OMA is presumably compensated for by appropriate neuronal weighting in the central vestibular system. This compensation process may be disrupted by changes in gravitational conditions or by unfamiliar movement patterns. It has long been known that Coriolis forces (5), as well as pseudo-Coriolis effects (6), are capable of triggering motion sickness. In addition, a genetic component of motion sickness has been indicated by Ijiri (7), who bred two different strains of fish (*Oryzias latipes*) and then observed differences in swimming

patterns during parabolic flight. One breed showed the typical screw and looping movements described by von Baumgarten et al. (3) and Wetzig (4), while the other had completely normal swimming behaviour. In humans, the OMA theory is supported by ocular torsional disconjugacy observed in phases of microgravity during parabolic flight (8).

Given these findings, OMA may well be a contributing factor to the large inter-individual variation in susceptibility towards motion sickness. Earlier studies in fish confirmed that the otoconial mass is regulated very precisely (9, 10) and that a pronounced lateral preponderance only exists in individual cases (11). This is true for both the saccule and utricle. Statistical analyses of otoconial mass (11) demonstrate that separate regulatory processes for otoconial formation exist in the saccule and utricle. The development of the otoliths under reduced (12) or increased gravitation (13) argues in favour of gravity-dependent regulation of otoconial formation.

In an earlier publication, McKenzie (14) observed that cod kept in fish tanks over a period of months exhibited vomiting behaviour during subsequent transport by sea. This vegetative symptom provides further evidence that fish are subject to motion sickness. In a previous study, it could be shown that disturbed swimming behaviour, or motion sickness, is triggered in fish by Coriolis forces (10). However, the question of whether a correlation exists between motion sickness and OMA has remained unresolved.

MATERIAL AND METHODS

We examined 22 adult fish (*Xiphophorus helleri*) from the same stock, following the principles of laboratory animal care (authorization was given for the animal experiment).

Aquarium design and Coriolis stimulation

Fish were examined in a specially constructed aquarium with a volume of ≈ 5 l (Fig. 1). The aquarium was completely filled to eliminate any air pockets and prevent large wave-like circulation of the contents. The aquarium was illuminated exclusively from the top and movement of the fish was recorded using a video camera mounted on the front. During testing the laboratory was darkened to eliminate any optokinetic stimulation. The Coriolis force environment was generated by means of a motor-driven, dual-axis rotator (Neuro Kinetics Inc., Pittsburgh, PA).

Before beginning stimulation, the fish were given a few minutes to become accustomed to their environment. The aquarium was rotated eccentrically (radius 50 cm) around the vertical axis (yaw) on a turntable at velocities of 60–240°/s. An additional pendular oscillation of 0.2–0.33 Hz about the horizontal axis (pitch) was started after ≈ 1 min at a constant angular velocity about the vertical axis. The fish were tested in male/female pairs and could be distinguished by the gender difference in tail form. Swimming movements were observed throughout on a video monitor and recorded for later analysis.

Otoconial mass determination

The fish were decapitated and the labyrinths exposed under a microscope (M650; Wild) in a fresh, unfixed state. To extirpate the otoliths, the skull cap was removed and the brain lifted out of the skull with a small suction tube. This revealed the vestibular or-



Fig. 1. The experimental set-up showing the aquarium bolted to the turntable. The video camera at the front, the light source at the top and the closed tube used for filling the aquarium are clearly visible.

gans with the utricle and semicircular canal ampullae in the lateral and the saccule in the basal skull region. As the otoliths consist of a relatively soft mineralized type of calcium carbonate (aragonite), special care had to be taken to protect the fine crystalline structure. After opening the membranous labyrinth, the otoliths were removed using micro-forceps. The otoliths were rinsed with distilled water, individually placed in marked micro test tubes (1.5 ml; Eppendorf) and dried in a drying kiln at 50°C for several hours. The mass of the otoliths was determined using a high-precision electronic microscale (model 2405; Sartorius) with a measurement range of 10^{-2} – 10^{-7} g. The mean error was 0.1% in the 10^{-6} g range and 1% in the 10^{-7} g range. The scale was placed on a vibration-free table in a chamber at constant relative humidity (60%) and temperature (25°C). To avoid any further contact with the otoliths, each specimen was placed on the scale together with a lid of a test tube.

Statistical analysis

Owing to the small sample size, non-parametric statistics were employed for analysis. The Spearman rank correlation coefficient (r_s) was calculated for the following: right and left otoconial masses in the saccules and utricles; the relationship of the saccular to the utricular otoconial mass in the ipsilateral labyrinth; and the relative asymmetry between the saccule and utricle (expressed as a percentage). The mean OMA between the right and left sides, expressed as a percentage difference, was calculated as follows:

$$\frac{|\text{right} - \text{left}| \times 2}{|\text{right} + \text{left}|} \times 100$$

RESULTS

Swimming behaviour during Coriolis stimulation

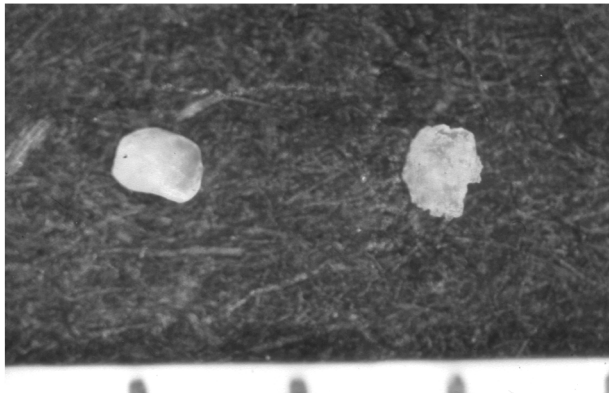
Prior to rotation, the fish were observed to swim randomly around the aquarium. At the start of rotation, the fish aligned themselves with the longitudinal axis of the aquarium, counter to the direction of rotation. All fish showed this alignment, and spontaneous free swimming around the aquarium was no longer observed. This alignment became even more consistent when the yaw velocity was increased to 240°/s. Additional pitch oscillation at 0.2 Hz was then introduced. This Coriolis stimulation caused active compensatory swimming behaviour with upward and downward attitude changes. When the pitch frequency was increased to 0.33 Hz fish Nos. 9, 10 and 16 ceased to actively compensate and appeared to enter a passive uncoordinated state, sinking

to the bottom of the aquarium. However, dorsal alignment with the light source remained constant throughout the trial. This behavioural state was defined as motion sickness. Fish Nos. 9, 10 and 16 were again only capable of active orientation and spatial alignment after a marked reduction in their vertical motion and showed no abnormality after a few minutes of rest. This method enabled the classification of fish into two groups: those susceptible and non-susceptible to motion sickness.

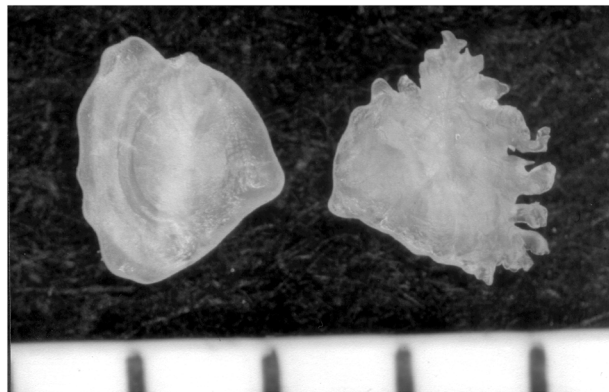
Otoconial masses

Fig. 2 gives an impression of the size and mass differences of the otoliths in the saccule and utricle. The mean otoconial mass (± 1 SD) was 1.044 mg (± 0.344 mg) in the saccule and 0.064 mg (± 0.024 mg) in the utricle. All values are presented in Fig. 3, and otoliths with a deviant form are specially marked. On average the saccular otoliths were ≈ 16 times heavier than those of the utricle.

Statistical analysis revealed a very high correlation between the right and left otoconial masses for both the saccule ($r_s = 0.892$; $p < 0.0001$) and utricle ($r_s =$

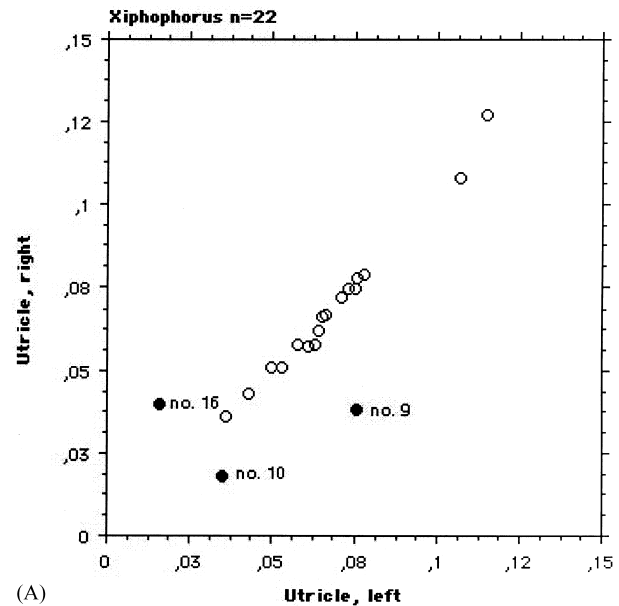


(A)

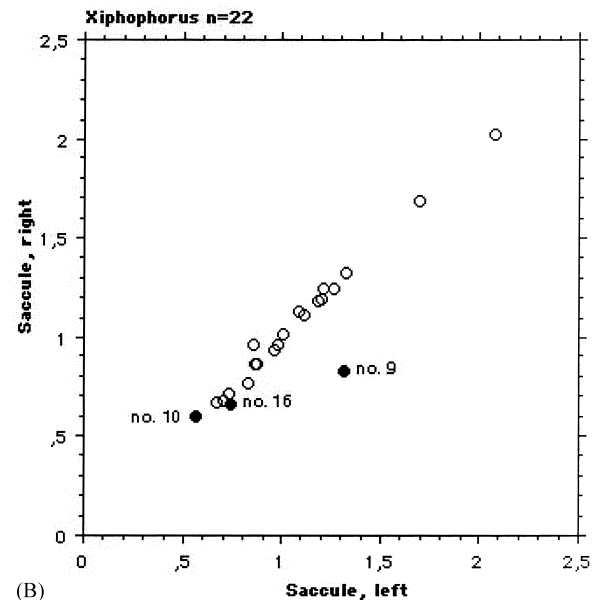


(B)

Fig. 2. (A) The left and right utricular otoliths of fish No. 9, showing the distinct differences in mass, and to a lesser degree, form (1 scale division corresponds to 1 mm). (B) The left and right saccular otoliths of fish No. 9, showing the pronounced OMA and difference in morphology.



(A)



(B)

Fig. 3. (A) The right and left otoconial masses (mg) of the utricle are plotted, clearly illustrating the close symmetry between the right and left sides. The numbered points designate those fish (Nos. 9, 10 and 16) that were found to deviate in terms of otolith mass, form and swimming behaviour. (B) Distribution of the saccular otoconial masses (mg).

0.842; $p < 0.0002$). Analysis of the otoconial mass of the saccule and utricle in each ear yielded a correlation coefficient of $r_s = 0.615$ ($p = 0.0001$) over all examined ears.

The mean OMA between the right and left sides, expressed as a percentage difference, was found to be 4.8% (minimum 0%, maximum 45.1%) for the saccules and 13.1% (minimum 0%, maximum 85.7%) for the utricles.

Finally, the correlation coefficient for saccular asymmetry against utricular asymmetry was $r_s = 0.105$ ($p = 0.65$). Thus the OMA for the saccules appears to be independent of that for the utricles.

Correlation of OMA and swimming behaviour

Measurements in the utricle showed a pronounced OMA in all 3 fish (Nos. 9, 10 and 16) with abnormal swimming behaviour (Fig. 2A). Additionally, in fish No. 9 the saccule showed marked OMA (Fig. 2B). Saccular OMA was not found in any other fish, regardless of swimming behaviour.

DISCUSSION

Yegorov and Samarin (15) were the first to consider OMA as a factor in the causation of motion sickness. Some time later von Baumgarten and Thümler (16) presented a corresponding model. Von Baumgarten et al. (3) found evidence for this hypothesis in their observations of looping and spinning swimming patterns in fish under altered gravitational conditions. Such typical looping and spinning movements were confirmed in later experiments by Ijiri (7), who examined various genetic strains of medaka fish (*Oryzias latipes*) during parabolic and space flight. His results indicated that the process of otolith formation could be genetically manipulated. While this would indirectly support the otolith asymmetry hypothesis, no OMA measurement data are available for medaka fish. To date there is little confirmed knowledge regarding the exact regulating mechanisms for otoconial formation in mammals (17). Furthermore, it is likely that these mechanisms differ between fish and higher vertebrates (18). More recently, Anken et al. (13) also reported looping and spinning movement patterns in cichlid fish (*Oreochromis mossambicus*). They maintained that such behaviour was present in 60% of a fish sample that had been reared under 3g centrifuge-generated hypergravity, and then transferred to the normal 1g environment. They related this disturbed swimming behaviour to their morphometric data, calculated as the right–left asymmetry of maximum otolith radius. However, their use of the otolith radius, as is common in the fishing industry to determine age, must be questioned in the present context. Given that the otolith organ operates as an inertial mass accelerometer, its mass or volume, rather than the one-dimensional radius, must be measured in order to obtain a relevant measure of otoconial asymmetry. For example, as illustrated in Fig. 2B, the radii of the left and right saccular otoliths of fish No. 9 appear to be approximately equal, but their masses were found to differ considerably. It is also puzzling that the data of Anken et al. demon-

strate that the 1g control group manifested a significantly higher radius asymmetry than the 3g-reared group.

The present results demonstrate a high degree of symmetry of otoconial mass in the majority of the fish examined. This implies that such symmetry is of considerable advantage for sensorimotor coordination, and ultimately for the survival of the individual fish. However, it remains unclear why there is such a consistently large difference between saccular and utricular otoconial mass. In swordtail fish, for example, the saccular otoliths are 16 times heavier than the utricular otoliths, and similar large differences have been observed in other species of fish (11). In the case of fish with clear OMA, it can be assumed that neural compensation, either in the peripheral or central vestibular system, provides normal sensorimotor coordination in the familiar 1g environment. Thus, according to the OMA hypothesis the abnormal spinning and looping movement occurs in the unaccustomed microgravity environment in which compensatory weighting is no longer adequate. By the same token, this neural compensation would also become inadequate in a Coriolis force environment. Firstly, it was remarkable how an upright orientation was consistently maintained in the majority of fish during aquarium rotation. This clearly demonstrates the dominant role of the dorsal light reflex. Further, as the rotatory speed increased, spontaneous swimming clearly resolved into a pattern compensatory to the predominant centrifugal and/or Coriolis forces. Both of these strategies reduce the effects of the Coriolis forces on the otolith organs, which could lead to disorientation if spontaneous movement patterns were maintained in the rotating aquarium.

In this study the Coriolis force environment was enhanced by oscillation of the aquarium about the pitch axis during yaw rotation. Here too, the fish appeared to correct their attitude to compensate for the existing forces. Only at the higher Coriolis force levels did this vestibularly driven compensation appear to break down in fish Nos. 9, 10 and 16. It is noteworthy that all fish nevertheless maintained their upright orientation via the dorsal light reflex (19). The higher weighting of optical over vestibular information could be existentially important to orientation in turbulent waters. Stimulus of the dorsal light reflex was deliberately maintained in this experiment by using a light source mounted in the roof of the aquarium. The influence of the dorsal light reflex may explain the different response observed in this study, i.e. adoption of a passive, uncoordinated state, compared to the spinning and looping patterns observed in other experiments. In the experiments reported by von Baumgarten et al. (3), the goldfish were blinded,

in order to completely eliminate the dorsal light reflex (3). Wetzig (4), in addition to blinding, also removed the otoliths on one side prior to observing the swimming behaviour of the fish in a microgravity environment (4). Both of these surgical measures are relatively traumatic insults to the vestibular system. For this reason, it is difficult to find a common denominator for the decisive differences in the changes in swimming behaviour.

The question must be raised as to what constitutes motion sick behaviour in fish. Strictly speaking the looping and spinning behaviour cannot be equated with motion sickness, but rather with a physiological response to the force environment. However, the passive or lethargic behaviour observed in a small number of fish corresponds more to the recognized signs of motion sickness.

REFERENCES

- Reason JT. Motion sickness adaptation: a neural mismatch model. *J R Soc Med* 1978; 71: 819–29.
- Bles W, Bos JE, de Graaf B, Groen E, Wertheim AH. Motion sickness: only one provocative conflict? *Brain Res Bull* 1998; 47: 481–7.
- von Baumgarten R, Baldrigi G, Schillinger GL. Vestibular behavior of fish during diminished G-force and weightlessness. *Aerospace Med* 1972; 43: 626–32.
- Wetzig J. Untersuchungen über das Schwimmverhalten einseitig entstateter Fische unter kurzzeitiger Einwirkung von Schereelosigkeit [PhD dissertation]. Mainz, Germany: University of Mainz, 1983.
- Sjöberg AA. Experimentelle Studien über den Auslösemechanismus der Seekrankheit. *Acta Otolaryngol Suppl* (Stockh) 1931; 14: 1–136.
- Bles W, Bos JE, Kruit H. Motion sickness. *Curr Opin Neurol* 2000; 13: 19–25.
- Ijiri K. How the four fish astronauts were selected. In: Ijiri K, ed. *The first vertebrate mating in space—a fish story*. Tokyo: Ricut, 1995: 39–50.
- Diamond SG, Markham CH. Validating the hypothesis of otolith asymmetry as a cause of space motion sickness. *Ann N Y Acad Sci* 1992; 656: 725–31.
- Watanabe S, Takabayashi A, Tanaka M, Yanagihara D. Neurovestibular physiology in fish. *Adv Space Biol Med* 1991; 1: 99–128.
- Scherer H, Helling K, Hausmann S, Clarke AH. On the origin of interindividual susceptibility to motion sickness. *Acta Otolaryngol* (Stockh) 1997; 117: 149–53.
- Helling K, Hausmann S, Flöttmann T, Scherer H. Untersuchungen zur interindividuell unterschiedlichen Kinetoseempfindlichkeit. *HNO* 1997; 45: 210–5.
- Lychakov DV, Lavrova EA. Structure of the vestibular apparatus and ionic composition of the body of *Xenopus laevis* larvae as affected by weightlessness. *Kosm Biol Aviakosm* 1985; 19: 48–52.
- Anken RH, Kappel T, Rahmann H. Morphometry of fish inner ear otoliths after development at 3g hypergravity. *Acta Otolaryngol* (Stockh) 1998; 118: 534–9.
- McKenzie RA. Codfish in captivity. *Progress reports of the Atlantic biological station, Vol. 16, Note No. 47*, Halifax, Canada, 1935: 7–10.
- Yegorov BB, Samarin GI. Possible change in the paired operation of the vestibular apparatus during weightlessness. *Kosm Biol Aviakosm* 1970; 4: 85–6.
- von Baumgarten RJ, Thümler RA. A model for vestibular function in altered gravitational states. *Life Sci Space Res* 1978; 17: 161–70.
- Salamat MS, Ross MD, Peacor DR. Otoconial formation in the fetal rat. *Ann Otol Rhinol Laryngol* 1980; 89: 229–38.
- Mann S, Parker SB, Ross MD, Skarnulis AJ, Williams RJP. The ultrastructure of the calcium carbonate balance organs of the inner ear: an ultra-high resolution electron microscopy study. *Proc R Soc Lond* 1983; 218: 415–24.
- von Holst E. Die Arbeitsweise des Statolithenapparates bei Fischen. *Z Vergl Physiol* 1950; 32: 60–120.

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