Otolith deprivation induces optokinetic compensation

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Abstract

According to the multisensory integration theory vestibular, optokinetic and proprioceptive inputs act in concert to maintain a stable retinal image of the visual world. Yet, it remains elusive to what extent the otolith organs contribute to this process and whether a specific loss of otolith input is compensated for. Here we investigated the compensatory eye movements in tilted mice, which lack otoconia due to a mutation in otopetrin 1. Tilted mice showed very small displacements of the eyes in the orbit during static roll paradigms, suggesting the absence of functional otolith organs. Independent of head position with respect to gravity, the gain and phase lead of angular vestibulo-ocular reflex of tilted mice were decreased and increased, respectively (frequencies 0.2 to 1 Hz and peak accelerations 8 to 197 deg/sec^2, respectively). Furthermore, lack of otolith input increases the dependency of the vestibular system on stimulus frequency. In contrast, the gain of optokinetic reflex in tilted mice was significantly higher in the low frequency range than in control mice, regardless of the position of the mice in space or the plane of the eye movements. To explain these results, a simple model was used in which a multisensory integration unit was embedded. With this model, we were able to simulate all the behaviours observed. Thus our data and the model support the presence of the multisensory integration system and revealed a compensatory enhanced optokinetic reflex in tilted mice, indicating an adaptive synergism in the processing of otolith and visually driven signals.

Keywords: Otolith organs, tilted mice, multisensory integration theory, otopetrin 1 gene
Introduction

To maintain a stable retinal image one needs access to information provided by sensory systems controlling the vestibulo-ocular reflex (VOR), optokinetic reflex (OKR) and cervico-ocular reflex. Moreover, the control systems of these individual reflexes need to be sufficiently integrated in the central nervous system in order to weigh the impact and need of the different components under a wide variety of physical circumstances (Raphan et al. 1979; Merfeld and Zupan 2002; Mergner et al. 2003; Angelaki et al. 2004). Otolith organs provide a specific contribution to this multisensory system. Electrical stimulation of otolith organs (Fluur and Mellstrom 1971) or otolith nerves (Suzuki et al. 1969) demonstrated that otoliths control eye movements. The otolith signals are conveyed via primary otolith afferents (Fernandez and Goldberg 1976a, b) to the vestibular nuclei (Bush et al. 1993). These signals are able to generate an angular maculo-ocular reflex, which operates as a low-pass filtered response (rabbits: Barmack 1981; Van der Steen and Collewijn 1984; rats: Brettler et al. 2000; mice: Harrod and Baker 2003; cats: Rude and Baker 1988; Tomko et al. 1988; monkeys: Paige and Tomko 1991; Angelaki and Hess 1996). In addition, information provided by the otolith organs influences the orientation of the eyes with regard to gravity and linear acceleration (Baarsma and Collewijn 1975; Cohen et al. 2001). This information must be combined with input from semicircular canals to obtain proper compensatory eye movements (Angelaki et al. 2004). Moreover, converged otolith-canal neural activity significantly changes its modulation in different behavioral contexts such as active and passive head movements (McCrea and Luan 2003). In some of these situations the otolith information may be used to transform primary semicircular canal signals into space-reference angular motion.
(Angelaki and Hess 1995). Thus, several lines of evidence suggest that the otolith organs control eye movements via vestibular and oculomotor nuclei that are also used by the semicircular canal system.

According to the multisensory integration theory, one expects not only that deficits in the otoliths can cause a variety of problems in the canal-driven system, but also that compensation must take place. Yet, at present it is not clear whether dysfunctional otoliths can be compensated for, and if so, how and under which circumstances such compensations can occur. Eye movement recordings of primates under microgravity in space have not been conclusive due to small sample sizes, limited experimental time and the fact that the otoliths can still sense accelerations in this situation (Dizio and Lackner 1992; Clement et al. 1993; Correia 1998; Moore et al. 2003). Moreover investigations on this topic have been hampered by the inability to mechanically lesion the otolith organs or nerves without affecting the input from the semicircular canals or without the loss of afferent fibers that will induce a reactive synaptogenesis (Goto et al., 2002).

To investigate potential vestibular compensatory processes, unilateral stimulation experiments on patients with unilateral vestibular nerve dissections (Clarke and Engelhorn 1998) and gravity-aligned/misaligned rotation experiments on patients with vestibular neuritis were performed (Schmid-Priscoveanu et al. 2004). However, these pathological circumstances were not specific enough to elucidate the compensatory mechanism induced by dysfunctional otoliths.

In the present study, we investigated potential mechanisms for compensation using tilted mice, which lack otoconia due to a spontaneous recessive mutation in otopetin 1 gene (Otop 1) located on chromosome 5 (Hurle et al. 2003). Although their vestibular ganglion does develop relatively slowly (Smith et al. 2003), the
projections from the otolith organs to the vestibular nuclei seems to be at least grossly normal in these mutant mice (Crapon de Caprona et al. 2004). Furthermore, *tilted* mice do not show any permanent abnormal phenotype in organ systems other than the otoliths (Ornitz et al. 1998). The linear vestibular evoked potential (linear VsEPs) is absent in *tilted* mice (Jones et al. 2004), as a consequence of the absence of otoconia. Apart from confirming their deficiency in gravito-inertial information by determining their eye position following static roll paradigms, we investigated their angular vestibulo-ocular reflex in the dark (aVOR) and the light (angular visually enhanced VOR or aVVOR) as well as their optokinetic reflex (OKR) over a wide range of stimulus parameters. The lack of otoconia decreases the gains and increases the phase errors not only during “otolith-mediated” aVOR but also during “canal-mediated” aVOR and increases the vestibular system dependency on frequency, especially at stimulus frequencies smaller than 1 Hz. We demonstrate that a frequency dependent enhancement of the optokinetic system can be used as a compensatory mechanism for a lack of functional otoliths. Furthermore, a simple model structure was explored in order to interpret the experimental data, to formulate a possible physiological template of how the canal, otolith and visual signals share centrally processing and to obtain insight in the consequences of otolithic dysfunction.
Materials and Methods

In this study, we used eighteen homozygous tilted mice (Otop 1<sup>tl</sup>) and twenty-two heterozygous control littermates (age: 12 - 20 weeks; The Jackson Laboratory). The recessive tlt mutation arose spontaneously in 1983 on the STOCK p<sup>6H</sup>/p<sup>d</sup> and was backcrossed onto the C57BL/6J background. The mutant mice were not deaf or blind (Ornitz et al. 1998). They were housed on a 12 hour light/dark cycle with food and water available ad libitum. All animal procedures described were in accordance with the guidelines of the ethical committee of Erasmus MC, Rotterdam.

Phenotype assessment

The homozygous tilted mice were easily identified by their inability to swim when they were dropped from at least 20 cm height into a deep tank of water. Tilted mice cannot find the surface of the water and need rescuing to prevent drowning. Heterozygous control littermates mice can find the surface of the water and swim easily (Ornitz et al. 1998).

Surgical procedures

An acrylic pedestal was formed on the animal’s skull under general anesthesia of a mixture of isofluoran (Isofloran 1-1.5%; Rhodia Organique Fine Ltd), nitrous oxide and oxygen. The pedestal construction was made as follows: a midline incision was made to expose the dorsal cranial surface and four stainless steel screws (1x1.5mm) were implanted in the calvarium and then embedded in dental acrylic. A prefabricated piece equipped with two nuts was attached to the pedestal in order to fixate the mouse in the restrainer device.
Video eye movement recording apparatus

After a recovery period (3 days), each mouse was handled daily for 2 days. During the experiment it was placed in an acrylic tube, with their head secured. The tube was inserted into the setup via a carrier that allowed orientation of the mouse (from mouse upright to mouse with its nose up or down; ±90 degree). The carrier on which the mouse was fixed also permitted translation of the mouse in the left-right direction and near-far direction from the camera. The purpose of these translations was to position the mouse’s eyeball on the rotation axis of the video camera, which ran through the center of the table (Stahl et al. 2000).

A cylindrical screen (diameter 63 cm) with a random-dotted pattern (each element 2°) surrounded the turntable (diameter 60 cm). Both the surrounding screen and the turntable were driven independently by an AC servo-motor (Harmonic Drive AG, The Netherlands). The table and drum position signal were measured by potentiometers, filtered (cut-off frequency 20 Hz), digitized (CED Limited, Cambridge, UK) and stored on a computer.

Three infrared emitters (maximum output 600 mW, dispersion angle 7°, peak wavelength 880 nm) illuminated the eye during the recording. The camera and two infrared emitters were fixed to the turntable. The third infrared emitter was connected to the camera and aligned horizontally with the camera’s optical axis. This third emitter produced the tracked corneal reflection (CR).

The eye movements were recorded using the eye-tracking device of Chronos Vision. The images of the eye were captured using an infrared sensitive CMOS camera (frame rate 50 Hz) and were relayed to a personal computer equipped with acquisition software from Chronos Vision (IRIS).
Behavioral testing

A head-fixed coordinate frame was defined as follows: the yaw (z) axis was the ventro-dorsal axis, the roll (x) axis was naso-occipital and the pitch (y) axis was interaural. Four different approaches were used to test the eye movement performance. First, the eye movement counterroll performances were measured during different static horizontal roll stimuli. Mice in upright stance (naso-occipital axis along an earth-horizontal plane) were positioned at different roll angles between ± 20°. The mice were rotated very slowly (5°/s) around their naso-occipital axis from one to another position. All tilt positions of the mouse were held at least for 20 sec or until the eye position was stable. Second, the optokinetic eye movements (OKR), the angular vestibulo-ocular eye movements (aVOR) and the angular visually enhanced vestibulo-ocular eye movements (aVVOR) were measured during different paradigms. The amplitude was kept at 5° while the frequency of the sinusoidal stimulus ranged from 0.2 to 1 Hz (generating a peak velocity between 6 deg/sec and 31 deg/sec, and a peak acceleration between 8 deg/sec² and 197 deg/sec²) during the following paradigms:

1. Dynamic horizontal yaw (Yh): mice upright (naso-occipital axis along the earth-horizontal plane), rotation around ventro-dorsal axis,
2. Dynamic vertical roll (Rv): mice nose up (naso-occipital axis along the earth-vertical plane), rotation around naso-occipital axis,
3. Dynamic horizontal roll (Rh): mice upright (naso-occipital axis along the earth-horizontal plane), rotation around naso-occipital axis,

Third, aVOR was tested at constant peak velocities (8 deg/sec and 30 deg/sec) while the frequency varied between 0.1 and 1.6 Hz. Fourth, aVOR was tested at constant peak acceleration (18 deg/sec²) while the frequency varied between 0.1 and 1.6 Hz.
The constant peak velocity and acceleration were tested using the dynamic horizontal yaw paradigm (Yh).

Each paradigm was presented to the mice at least for three days, but not all the paradigms were delivered on the same day. Each animal was recorded no more than once a day. Before aVOR recordings, pilocarpine 4% (Laboratories Chauvin, France) was used to limit the pupil dilatation in darkness.

Data analysis
A calibration was made before any of the recordings were started. The camera was rotated several times by ± 10° around the earth-vertical axis passing through the center of the table. The positions of the pupil (P) and corneal reflection (CR) recorded at the extreme positions of the camera rotation were used to calculate Rp, the radius of rotation of the pupil (Stahl et al. 2000).

The gain and the phase of the eye movements were calculated by using a custom-made Matlab programme (Mathworks Inc., Natick, USA). The eye position (E) was calculated using the CR and P positions from the recorded file and the Rp value was computed from the calibration (Stahl et al. 2000).

\[ E = \arcsin \left( \frac{\text{CR}-P}{R_p} \right) \]

To obtain slow-phase eye velocity the eye-position data (E) was differentiated. The quick phases were identified using a velocity-threshold filter. The trace parts containing the quick phase (0.02 sec before the saccades and 0.08 sec after the saccades) were removed from the eye velocity data. To obtain stimulus velocity, the stimulus (table or drum) trace was also differentiated. Both eye and stimulus velocity
signals were filtered by a Butterworth low pass filter with a cut off frequency of 40 Hz, before they were fitted by a sine wave function using a least-squares method. Gain was computed as the ratio of eye velocity to stimulus velocity whereas phase was expressed as the difference (in degrees) between the eye velocity and stimulus velocity traces. In the static roll paradigm, gain was computed as a ratio of eye position (degrees) to head position (degrees), whereas the sensitivity was computed as a ratio of eye position (degrees) to the sine of the head roll angle, which is equal with the linear acceleration along the interaural axis (head y axis) in unit of $g = 981 \text{ cm/s}^2$ (Maruta et al. 2001).

Model description and simulation

The model is a feedback model (Fig. 6A) for roll aVORs and OKRs and represents an extension of that proposed by Green and Galiana (1998). The boxes in the model represent dynamic element and circles represent summing junctions. The first order approximations of semicircular canals $C(s)=1/(T_c s+1)$, otolith organs $O(s)=1/(T_o s+1)$, neural feedback filter $F(s)=1/(T_f s+1)$, eye plant $P(s)=1/(T_p s+1)$ and retinal slip integrator $R(s)=1/s$ were implemented with $T_c= 3 \text{ sec}$ (Jones and spells, 1963; Curthoys, 1982: rat), $T_o= 0.016 \text{ sec}$, and $T_f=T_p=0.24 \text{ sec}$ (Fernández and Goldberg, 1976c; Robinson, 1981; Galiana and Outerbridge, 1984; Green and Galiana, 1998). The retinal slip velocity signal was saturated before it entered the model (saturation threshold 2 degr./s), because of the limited sensitivity of the retinal ganglion cells (Oyster et al., 1972; Collewijn, 1972). Model parameters associated with the gains of different pathways (see Fig 6A: a, b, c and d) were chosen to satisfy the following criteria: 1) first, weight $a$ (vestibular nuclei projection) was chosen to reproduce experimentally observed horizontal roll aVOR gains of tilted mice ($a=0.28$); 2) then
projection weight $b$ (otoliths afferent projection) was chosen to fit the static otolith sensitivity of 26 °/g of control mice ($b=0.121$); 3) the remaining projection weights $c$ (retina projection) and $d$ (cerebellar projection) were chosen to reproduce the OKR in control mice ($c=0.10$ and $d=6.5$).

The model was implemented using Matlab simulation toolbox Simulink (Mathworks, Natick, MA). Model simulations were performed using a fixed-step Runge-Kutta integration routine (ode45) with time steps of 0.01 s. Model predictions were compared to experimental data.

**Statistics**

To compute the session average, gain and phase values were combined per trial. Session averages from at least three days were used to calculate the final gain and phase value per mouse. Data were presented as mean ± SD. For statistical comparisons we used the two-way ANOVA for repeated measures and the standard $t$-test. Statistical analysis was performed using the commercial software package SPSS 11.0 (SPSS Inc.).
Results

a. Otolithic function during static roll

*Tilted* mice lack otoconia in both otolith organs (Ornitz et al. 1998). To test the function of the otolith organs, mice were subjected to static horizontal roll. Mice were rotated very slowly (5°/s) towards the end-point roll angle where they were held at least for 20 sec or until the eye position was stable. Head roll angles around an earth horizontal axis varied between ± 20° generating a projection of the gravity vector along the interaural axis ranging from -0.34 g to +0.34 g. Sensitivity and gain of the eye counterroll in *tilted* mice were 3 ± 3°/g and 0.06 ± 0.005 (n = 7), respectively (Fig. 1A-B). Both values were significantly lower than those in control mice (n = 7; sensitivity 26 ± 4°/g, p < 0.001, t-test; gain 0.45 ± 0.07, p < 0.001, t-test; Fig. 1A-B). In control mice, but not in *tilted* mice, there was a linear relationship ($r^2 = 0.79$) between eye position and linear acceleration along the interaural axis. *Tilted* mice did not show any relationship between the eye position and the head roll angle ($r^2 = 0.002$). Together, these data indicate that the static contribution of the otoliths to compensatory eye movements is negligible in otoconia-deficient mice.

b. Contribution of otoliths to the VOR

If the static contribution of otoliths to the eye position is affected in the mutants, one expects that the dynamic contribution of otoliths to the VOR is also affected. We therefore subjected control and *tilted* mice to horizontal roll (Rh), which activates conjunctively the vertical semicircular canals and otolith organs (Fig. 2A). The
vertical roll (Rv; Fig. 2B) and horizontal yaw (Yh; Fig. 2C) paradigms were used to
dynamically stimulate the vertical semicircular canals or the horizontal semicircular
canals, respectively. An eye movement recording from each stimulus paradigm is
shown in Fig. 2.

In control mice (n = 8) the gains of the horizontal roll (Rh) aVOR varied from
0.38 ± 0.11 at 0.2 Hz to 0.59 ± 0.1 at 1.0 Hz, while their phase leads were relatively
fixed around 10 degrees at all frequencies (Fig. 3A). Tilted mice (n = 7) had
significantly lower gains (varying from 0.14 ± 0.08 at 0.2 Hz to 0.44 ± 0.12 at 1 Hz; p
< 0.005, ANOVA) and significantly higher phase leads (varying from 93.00 ± 19.8° at
0.2 Hz to 33.6 ± 4.9° at 1 Hz; p < 0.001, ANOVA) at all frequencies. Both the gain
and phase differences between mutants and control mice decreased as the frequency
increased. The finding that the eye movement performance during horizontal roll is
severely affected in tilted mice does not necessarily mean that the eye movement
performance during vertical roll and horizontal yaw stimuli are also impaired, because
these are more selectively driven by the vertical and horizontal semicircular canals,
respectively. On the other hand, these types of reflexes may also be impaired in tilted
mice, if otolith information is needed for the central integration process preceding
these compensatory eye movements. We therefore investigated the aVOR during
vertical roll and horizontal yaw. Although the differences between mutants (n = 9)
and control mice (n = 7) were less prominent than during horizontal roll, vertical roll
paradigm showed lower gains and higher phase leads in tilted mice (Fig. 3B; for gain
and phase values p = 0.08 and p < 0.01 (ANOVA), respectively).

The otolith input contribution to the aVOR can be determined by subtracting
the vertical roll from horizontal roll eye responses in control mice or by subtracting
the horizontal roll responses in tilted mice from the horizontal roll responses in
control mice. Figure 3C shows that these two subtractions do not lead to the same outcome in terms of gain or phase. It appears unlikely that this difference is due to some interaction between the horizontal and vertical canals, because subtraction of the eye movement performance during vertical roll in tilted mice from that during horizontal roll in tilted mice renders an outcome of approximately zero (Fig. 3C). Thus, it is likely that a static-otolith driven component combined with a dynamic-otolith-canals driven component lead to higher gains and lower phases in control mice during horizontal roll aVOR as compare to tilted mice during vertical roll aVOR and horizontal roll aVOR.

As the aVOR over the studied frequency range depend on peak acceleration (van Alphen et al. 2001), we tested the horizontal yaw aVOR not only during constant amplitude (5 degrees) but also during constant peak velocity and constant peak acceleration paradigms. In control mice (n = 8) the gains of the horizontal yaw (Yh) aVOR at constant amplitude stimulation varied from 0.43 ± 0.11 at 0.2 Hz to 0.77 ± 0.11 at 1 Hz, whereas their phase leads decreased from 27.9 ± 10.2° at 0.2 Hz to 6.1 ± 2.6° at 1 Hz (Fig. 4A). Tilted mice (n = 7) had significantly lower gains (varying from 0.15 ± 0.04 at 0.2 Hz to 0.44 ± 0.07 at 1 Hz; p < 0.005, ANOVA) and significantly higher phase leads (varying from 103.6 ± 23.2° at 0.2 Hz to 23.2 ± 4.7° at 1 Hz; p < 0.001, ANOVA). When the performance of the horizontal yaw (Yh) aVOR was tested during constant peak velocity (8 deg/sec and 30 deg/sec; Fig. 4B) and constant peak acceleration (18 deg/sec²; Fig. 4C), tilted mice (n=5) showed again significantly lower gains and significantly higher phase leads than control mice (for all gain and phase values p < 0.001 (ANOVA)). The aVOR gains and phases in control mice were dependent not only on frequency but also on acceleration of the stimulus (Fig. 4C), increasing and decreasing, respectively, as the acceleration increased. If in tilted mice
the aVOR gains and phases had been also dependent on amplitude and/or acceleration of the stimulus, then separate curves should have emerged in figure 4B. In tilted mice aVOR gains do not depend on amplitude and/or acceleration at low frequencies (0.1 and 0.2 Hz), but depend only on the frequency of the stimulus, whereas aVOR phases depend only on stimulus frequency over the studied frequency range.

These data indicate that inputs from the otolith organs improve the eye movement performance during aVOR especially at the lower frequencies. In the absence of the otolith input, at low frequencies, the vestibular system of the mouse converts from a system dependent on frequency, peak velocity and peak acceleration of the stimulus to a system primarily dependent on the frequency of the stimulus.

c. OKR compensation

The data described above showed that the otolith organs can improve both via static and dynamic mechanisms the eye movement performance during aVOR around different axes in space. This contribution is most prominent at lower frequencies. These findings raise the question whether the deficits that occur in otoconia-deficient mice during aVOR can be compensated by a secondary enhanced OKR, which can particularly dominate oculomotor performance at the lower frequency range (Collewijn and Grootendorst 1979). We therefore tested the OKR under the same set of body orientations and frequencies that were used for the experiments described above. For the vertical eye movement OKR, i.e. that of the horizontal roll and vertical roll, the gain values of the OKR of tilted mice (n = 11) were significantly higher than those of control mice (n = 10) at the two lowest frequencies of 0.2 Hz and 0.4 Hz which correspond to velocities 6 deg/sec and 8 deg/sec but not at the higher
frequencies (Fig. 5A-B). In control mice (n = 8) the gains of the horizontal yaw OKR varied from $0.69 \pm 0.08$ at 0.2 Hz to $0.15 \pm 0.04$ at 1 Hz whereas *tilted* mice (n = 7) had significantly higher gains (varying from $0.80 \pm 0.05$ at 0.2 Hz to $0.26 \pm 0.08$ at 1 Hz; data not shown). The significance levels varied from $p < 0.05$ in vertical roll position to $p < 0.001$ in yaw position (ANOVA). In contrast, no significant differences were observed in the phase values of the OKR among the mutants and controls (p-levels varied from 0.54 in horizontal roll to 0.98 in vertical roll; ANOVA). The subtraction of the OKR gain values of control mice in horizontal roll position from those of *tilted* mice in the same position did not differ from the same subtraction in vertical roll position (Fig. 5D). Thus, the position of the mouse does not influence the gains of the vertical eye movement OKR. Although the optokinetic compensation was significant and robust in all body positions in *tilted* mice, it was not sufficient to obtain a normal gain of the VVOR (Table I). For example, the VVOR gains during yaw movements were significantly higher in control mice (n = 9; $0.88 \pm 0.06$) than in *tilted* mice (n = 8; $0.80 \pm 0.06$); ($p < 0.01$, ANOVA).

d. Model simulations

The dynamic behaviours of the horizontal and vertical roll aVOR and OKR of control and tilted mice were simulated by the model shown in figure 6A. To simulate the horizontal roll aVOR of control mice, vestibular nuclei projection weight $a$ was set to 0.28, otoliths projection weight $b$ was set to 0.121, retina projection weight $c$ and cerebellar projection weight $d$ were set to zero. In order to mimic the vertical roll aVOR of control mice, the otolithic projection weight $b$ was reduced. In tilted mice this projection weight $b$ was set to zero.
Figure 6B shows the experimental and predicted data of the roll aVOR of control and tilted mice. The predicted frequency response of the horizontal roll aVOR of control and tilted mice are consistent with our experimental data. The vertical roll aVOR of control mice was simulated by reducing the projection weight $b$ by 35%.

Simulations of the model for the optokinetic response of control and tilted mice are illustrated in Fig. 6C. To simulate roll OKR of control mice, vestibular nucleus projection weight $a$ was set to 0.28, otolith projection weight $b$ was set to zero, retina projection weight $c$ was set to 0.1 and cerebellar projection weight $d$ was set to 6.5. Increment of the cerebellar projection weight $d$ from 6.5 to 8.7 mimicked the optokinetic compensation observed in tilted mice. Both the experimental data as well as the predicted data show that this compensatory mechanism is frequency dependent.
Discussion

Our data show 1) that absence of otoconia leads to dysfunctional otolith organs impairing both the static contribution to the vestibulo-ocular counterroll and the static and dynamic contribution to the angular vestibulo-ocular reflex; 2) that the deficits occur most prominently at the lower frequencies of the aVOR; 3) that the absence of functional otolith organs results in greater frequency dependence of the aVOR; 4) that in light these deficits are to a large extent compensated by an enhanced optokinetic response; and 5) that a simple model in which the vestibular nucleus was embedded as a multisensory integration unit, could simulate all eye movement behaviours observed. In conjunction, they provide supportive evidence for an adaptive multisensory integration system for stabilizing the retinal image.

VOR deficits

The lack of otoconia in tilted mice resulted in dysfunctional otolith organs that were virtually unable to evoke correct eye movement responses following static or dynamic displacement of the head. With regard to the static stimuli, we found that the gain and sensitivity of their eye-counterrolls were approximately 10% of those in control mice littermates. The residual counterroll eye movements in tilted mice might be driven by inputs from extracervical somato-sensory receptors (Krejcova 1971; Yates et al. 2000) or by inputs from giant otoconia that are sometimes present in tilted mice (Ornitz et al. 1998). The sensitivity in control mice (26°/g) was in between that of rabbits (17°/g) (Maruta et al. 2001) and fish (30°/g) (Benjamins 1918; Cohen et al. 2001). With regard to the dynamic stimuli in tilted mice, we found the most prominent aberrations
during horizontal roll, indicating that this paradigm evokes a relatively high activity in the otolith organs. Interestingly, comparison between control mice and tilted mice also revealed deficits in eye movement performance following horizontal yaw and vertical roll stimulation even though these paradigms are thought to evoke relatively little dynamic activity in the otolith organs (see also Harrod and Baker 2003). Moreover, we showed that subtraction of the eye movement performance during horizontal roll in the mutants from that during horizontal roll in control mice is not equal to the difference between eye movement performance during vertical roll in control mice and that during horizontal roll in control mice (Fig. 3C). Altogether, these results suggest the presence of an otolith component during both horizontal yaw and vertical roll stimulations and show that by placing control mice in the vertical position, the contribution of the otolith organs to aVOR is not completely removed. This possibility is confirmed by the model suggested (Fig 6A), which indicates that in vertical position the otolith organs of control mice still give a functional contribution (Fig 6B). The most obvious explanation of these unexpected otolith contributions in this situation is that the otolith organs are statically stimulated and that this static otolith signal is partially able to correct the “vertical semicircular canals aVOR” in these control mice (Fig. 3B and 6B). The presence of a static otolith signal during rotation of mice in the plane of the horizontal canals will also explain the eye movement aberration found in tilted mice during horizontal yaw stimulations. An alternative explanation is that otolith organs were not precisely placed in the centre of rotation during stimulation. Consequently, a dynamic stimulation of the otolith organs was induced that would be large enough to contribute substantially to the aVOR. This possibility is very unlikely, because the tangential and centripetal acceleration are under these circumstances too low (tangential acceleration = 0.002g; centripetal
acceleration = 0.0002g) to elicit a response (Clarke and Engelhorn 1998). The tilt angle of the rotation axis with respect to gravity elicits the otolith responses, therefore the facts that the macular surface of the otolith organs is curved, not planar (Flock 1964) and that otolith organs do not lie in the plane of the semicircular canals (Curthoys et al. 1999) are also no plausible explanations for our results. Nevertheless, it remains unclear whether the mechanism suggested, is entirely responsible for this large otolith-dependent aVOR component. The possibility that otolithic deprivation in tilted mice altered the neuronal activity of utricular afferents still needs to be elucidated. In order to unravel this mechanism, electrophysiological measurements of either utricular afferents or vestibular nuclei neurons are necessary.

The similarities in eye movement responses evoked by horizontal linear acceleration and off-vertical axis rotation in rat led to the conclusion that utricular driven eye movement in rodents complements the semicircular canal activations in order to achieve gaze stability during horizontal roll stimulation (Hess and Dieringer 1990, 1991). The horizontal roll aVOR can be explained by the fact that signals derived from otolith organs and semicircular canals converge at the level of vestibular neurons, which send their eye movement commands to the oculomotor nuclei (Sato et al. 2000; Zhang et al. 2001; Dickman and Angelaki 2002; Zhang et al. 2002). The missing static otolith correction in the tilted mice and the convergence of the otolith and canal driven signals might also explain our finding that the aberrations in aVOR of tilted mice were frequency-dependent not only during horizontal roll, but also during vertical roll and horizontal yaw. This is the first study that shows that in the absence of otolith input, the dependency of the vestibular system on the frequency of the stimulus is increased. Taken together our aVOR data support the multisensory integration theory in that the brain must combine information from semicircular
canals and otolith organs in order to make proper compensatory eye movements (Harrod and Baker 2003; Angelaki et al. 2004).

**OKR compensation**

We found that OKR gain values of tilted mice were significantly increased. Several findings support the argument that this increase reflects a mechanism that will compensate for deficits in the aVOR. First, the increases in OKR gain occurred in every position at which deficits in the aVOR were detected, i.e. that of the horizontal roll, horizontal yaw and vertical roll. Second, the increases in OKR gain occurred predominantly at the lower frequencies, which corresponds to the frequency range at which the aVOR gain values were most prominently affected. Furthermore, the VVOR gain values were not increased in tilted mice, suggesting that the OKR increase was not a primary effect but a secondary effect in an attempt to correct the VVOR gain values that were partly reduced. 

The mechanism that underlies OKR compensation in tilted mice probably resembles that underlying OKR and VOR adaptation following visuo-vestibular or visual training paradigms (Collewijn and Grootendorst 1979; Nagao 1983; Iwashita et al. 2001). During these adaptations OKR or VOR gain values change in response to enhanced retinal slip. While a change in VOR gain depends on the direction of retinal slip in relation to the direction of the eye movement, the OKR gain always increases when there is enhanced retinal slip, independent from the direction of the slip (Collewijn and Grootendorst 1979; De Zeeuw et al. 1998). In tilted mice the aVOR gains are reduced due to dysfunctional otoliths, which in turn increase the retinal slip triggering a compensatory change in the OKR. Similarly, the low-frequency aVOR
can be enhanced as a mechanism to compensate for a decrease in OKR gain; this reversed process occurs in lurcher mice, which suffer from reduced OKR gain values due to a lack of floccular Purkinje cells (Van Alphen et al. 2002). Even so, it should be noted that a total blockage of the VOR such as occurs in shaker mutants, Usher Syndrome Type 1B patients or subjects after bilateral labyrinthectomy does not necessarily result in increased OKR gains (Cohen et al. 1973; Barmack et al. 1980; Sun et al. 2001). In these cases the vestibular deficits fall too much in the high frequency range and/or the increased retinal slip levels fall outside the optimal range that can drive optokinetic signals mediating adaptation in the flocculus of the cerebellum (Simpson et al. 1996). Thus, the optokinetic system may be particularly suited to compensate for the lack of otolith-driven information necessary for a proper aVOR as both systems have similar low pass filter characteristics, while it may not be well designed to compensate for deficits in the vestibular-canal system, which dominates the higher frequencies. These observations correspond with the behaviour of our model. Alterations in the weight of cerebellar projection affect the responses in a similar low pass filter characteristic way as described above (Fig 6C), suggesting that the cerebellar cortex is a suitable site for this OKR compensatory mechanism.

In conclusion, by analysing mutants with specific deficits in their otoliths we provide evidence that the otolithic input shows central cross-talk with the input of the semicircular canals and that the otolith organs provide indispensable information for the angular vestibulo-ocular reflex. The lack of otolith input increases the dependency of the vestibular system on the stimulus frequency. The optokinetic reflex can compensate for the lack of gravito-inertial perception in the low frequency range. By using a simple model, in which the vestibular nucleus was embedded as a
multisensory integration unit, we were able to simulate all behaviours observed in control and in *tilted* mice. All these phenomena support the presence of an **adaptive** multisensory integration system that combines information from otolith organs, semicircular canals, and retina in order to make proper compensatory eye movements.
Acknowledgments

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References


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Figure legends

Figure 1. Static contribution of otolith organs to compensatory eye movement in normal and tilted mice. In A), one example is given for how the sensitivity was calculated: the eye position was plotted against the sine of the roll angle (the linear acceleration along the interaural axis) for a control mouse (filled squares) and a tilted mouse (open squares). B) Eye positions recorded during different static roll angles showed smaller sensitivity and gain values in tilted mice. Data present the mean + SD.

Figure 2. Example of compensatory eye movements during sinusoidal rotation in dark at 0.6 Hz and 5 degree. Vertical eye movements measured during horizontal roll aVOR (Rh; A) and vertical roll aVOR (Rv; B), and horizontal eye movements during yaw aVOR (Yh; C) are shown. Inset: 5 degree amplitude (vertical) and 0.4 sec (horizontal).

Figure 3. Tilted mice show deficits during roll aVOR. A) During horizontal roll at which both the semicircular canals and otoliths are stimulated both gain and phase values of tilted mice (open circles) are severely affected. B) During vertical roll at which only the vertical canals are directly stimulated both gain and phase values of tilted mice (open triangles) are moderately affected. C) Subtractions of the vertical roll from horizontal roll eye responses in control mice (diamonds) and tilted mice (triangles), as well as subtractions of the horizontal roll eye responses in tilted mice from horizontal roll eye responses in control mice (circles) are shown. Dynamic stimulation of otolith organs increases the gain values and decreases the phase leads of the aVOR in a frequency dependent manner (diamonds) less than the combined
dynamic-static stimulation of the otolith organs (circles). In tilted mice there is no difference between canals only and canals-otolith mediated aVOR (triangles). Rh and Rv indicate horizontal roll and vertical roll, respectively. Data present the mean + or - SD.

**Figure 4.** Tilted mice show deficits during yaw aVOR. During horizontal yaw in which only the horizontal canal is directly stimulated both gain and phase values of tilted mice are moderately affected in all tested conditions: A) constant amplitude (5 degree), B) constant velocities (8 deg/sec and 30 deg/sec) and C) constant acceleration (18 deg/sec/sec). Data present the mean + or - SD.

**Figure 5.** Low-frequency OKR compensation due to otolith dysfunction. A-C) Tilted mice show higher gains during horizontal roll OKR (A) and vertical roll OKR (B) at the lower frequencies, while their phase values are normal at all frequencies (C). D) The position of the mouse does not influence the compensatory OKR gains (circles and triangles). Rh and Rv indicate horizontal roll and vertical roll, respectively. Data present the mean + or - SD.

**Figure 6.** The proposed compensatory eye movement model with multisensory integration of otolith, canal and retina signals. A) Schematic representation of the model for roll aVOR and OKR in which semicircular canals C(s), otolith O(s) and retina R(s) signals are incorporated (Green and Galiana, 1998). Inputs to the model are head acceleration (\(\mathbf{a}\)), gravity induced interaural acceleration (\(\mathbf{a}_g\)) and retinal slip velocity (\(\mathbf{s}\)). Output of the model is eye position (E). Boxes are dynamic elements that represent a sensor [C(s), O(s) and R(s)], a motor plant [P(s)], or a neural
filter \([F(s)]\). Circles are summing junctions used to represent particular cell populations including neurons of the vestibular nucleus (VN), neurons of the oculomotor nucleus (OMN) and neurons of the prepositus hypoglossi (PrH). The model parameters \(a, b, c\) and \(d\) associate with the projection weight of the different pathways. Detailed description of the model parameters can be found in the material and methods section. B) Comparison of model predictions and experimental horizontal roll aVOR of control (filled circles; \(b = 0.121\)) and tilted mice (open circles; \(b = 0\)). The vertical roll aVOR of control mice was simulated by changing the projection weight \(b\) into 0.079. C) Comparison of model predictions and experimental optokinetic responses of control (filled circles; \(d = 6.5\)) and tilted mice (open circles; \(d = 8.7\)).
Table legends

Table I. Data are presented as mean ± SD. Gain values (eye velocity / stimulus velocity) and phase values (eye velocity - stimulus velocity in degrees) of the angular visually enhanced vestibulo-ocular reflex (aVVOR) at stimulus frequencies ranging from 0.2 to 1 Hz during horizontal roll (Rh), vertical roll (Rv) and horizontal yaw (Yh).
Table I A.) Angular visually enhanced vestibulo-ocular reflexes induced by three different vestibular stimuli in wild type and *tilted* mice (gains).

<table>
<thead>
<tr>
<th>Paradigm</th>
<th>Mice</th>
<th>0.2 Hz</th>
<th>0.4 Hz</th>
<th>0.6 Hz</th>
<th>0.8 Hz</th>
<th>1 Hz</th>
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</thead>
<tbody>
<tr>
<td>Rh</td>
<td>Wt (n = 8)</td>
<td>0.78 ± 0.11</td>
<td>0.82 ± 0.11</td>
<td>0.82 ± 0.10</td>
<td>0.83 ± 0.13</td>
<td>0.82 ± 0.13</td>
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<tr>
<td></td>
<td>Tlt (n = 10)</td>
<td>0.59 ± 0.06</td>
<td>0.57 ± 0.04</td>
<td>0.58 ± 0.05</td>
<td>0.63 ± 0.07</td>
<td>0.67 ± 0.06</td>
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<tr>
<td>Rv</td>
<td>Wt (n = 12)</td>
<td>0.70 ± 0.13</td>
<td>0.68 ± 0.14</td>
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<td>0.73 ± 0.15</td>
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<td>Tlt (n = 7)</td>
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<td>0.62 ± 0.08</td>
<td>0.63 ± 0.08</td>
<td>0.67 ± 0.11</td>
<td>0.71 ± 0.09</td>
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<tr>
<td>Yh</td>
<td>Wt (n = 9)</td>
<td>0.85 ± 0.06</td>
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<tr>
<td></td>
<td>Tlt (n = 8)</td>
<td>0.84 ± 0.05</td>
<td>0.76 ± 0.09</td>
<td>0.78 ± 0.04</td>
<td>0.81 ± 0.06</td>
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Table I B Angular visually enhanced vestibulo-ocular reflexes induced by three different vestibular stimuli in wild type and *tilted* mice (phases).

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<thead>
<tr>
<th>Paradigm</th>
<th>Mice</th>
<th>0.2 Hz</th>
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<th>0.6 Hz</th>
<th>0.8 Hz</th>
<th>1 Hz</th>
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<tr>
<td>Rh</td>
<td>Wt (n = 8)</td>
<td>0.4 ± 2.8</td>
<td>-0.8 ± 2.4</td>
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<td>-0.5 ± 1.5</td>
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<td>Tlt (n = 10)</td>
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<td>8.6 ± 5.4</td>
<td>5.2 ± 4.0</td>
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<tr>
<td>Rv</td>
<td>Wt (n = 12)</td>
<td>3.9 ± 2.4</td>
<td>1.5 ± 2.3</td>
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<td>2.7 ± 5.3</td>
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<tr>
<td></td>
<td>Tlt (n = 7)</td>
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<td>1.0 ± 4.9</td>
<td>3.8 ± 7.2</td>
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<tr>
<td>Yh</td>
<td>Wt (n = 9)</td>
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<td>0.1 ± 1.3</td>
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<td>Tlt (n = 8)</td>
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<td>0.5 ± 3.7</td>
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<td>1.6 ± 5.1</td>
<td>3.5 ± 4.1</td>
</tr>
</tbody>
</table>
Figure 1

A

Eye pos. (deg)

Linear acceleration (g)

B

Sensitivity (deg/g)

Gain

Cntrl  Tilt

Cntrl  Tilt
Figure 2

A  Horizontal roll (Rh)  B  Vertical roll (Rv)  C  Horizontal yaw (Yh)

Eye Ctrl  
Eye tilt  
Stimulus  

(Stimulus lines 0.4 s, 5 degrees)
Figure 3
Figure 5

A

Horizontal OKR

B

Vertical OKR

C

Horizontal and vertical OKR

D

Subtractions

Gain

Phase (deg.)

Frequency (Hz)
Figure 6

A

B

C

Roll aVOR

Roll OKR

Gain

Phase (deg.)

Frequency (Hz)

Frequency (Hz)