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Dynamic characteristics of otolith ocular response during counter rotation about dual yaw axes in mice

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Abstract

The central vestibular system plays an important role in higher neural functions such as self-motion perception and spatial orientation. Its ability to store head angular velocity is called velocity storage mechanism (VSM), which has been thoroughly investigated across a wide range of species. However, little is known about the mouse VSM, because the mouse lacks typical ocular responses such as optokinetic after nystagmus or a dominant time constant of vestibulo-ocular reflex for which the VSM is critical. Experiments were conducted to examine the otolith-driven eye movements related to the VSM and verify its characteristics in mice. We used a novel approach to generate a similar rotating vector as a traditional off-vertical axis rotation (OVAR) but with a larger resultant gravito-inertial force ($>1\text{ g}$) by using counter rotation centrifugation. Similar to results previously described in other animals during OVAR, two components of eye movements were induced, i.e. a sinusoidal modulatory eye movement (modulation component) on which a unidirectional nystagmus (bias component) was superimposed. Each response is considered to derive from different mechanisms; modulations arise predominantly through linear vestibulo-ocular reflex, whereas for the bias, the VSM is responsible. Data indicate that the mouse also has a well-developed vestibular system through otoliths inputs, showing its highly conserved nature across mammalian species. On the other hand, to reach a plateau state of bias, a higher frequency rotation or a larger gravito-inertial force was considered to be necessary than other larger animals. Compared with modulation, the bias had a more variable profile, suggesting an inherent complexity of higher-order neural processes in the brain. Our data provides the basis for

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further study of the central vestibular system in mice, however, the underlying individual variability should be taken into consideration.

Keywords

eye movement; vestibular; otolith; off-vertical axis rotation (OVAR); velocity storage mechanism

1 Introduction

The vestibular sensory structures consist of semicircular canals and otolith organs. These two sets of vestibular sensors are highly conserved in the vertebrate inner ear and specialized to detect different sensory stimuli, i.e. angular acceleration and linear acceleration or head tilt, respectively. Together with visual and proprioceptive inputs, peripheral information converges towards a multisensory process in the central vestibular system which is known as “velocity storage mechanism” (VSM; Raphan et al., 1979). Its precise function under natural behavior still remains controversial, however, it is generally accepted that the VSM plays an essential role in higher level functions such as self-motion perception and spatial orientation (Cohen et al., 2003; Dai et al., 2011; Laurens and Angelaki, 2011). One of the apparent manifestations of the VSM is the prolongation of the time constant of the angular vestibulo-ocular reflex (VOR) compared with that of cupular in the semicircular canal, as observed in a variety of species including: monkey (16 s; Waespe and Henn, 1977), cat (15 s; Robinson, 1976) and rat (11 s; Tempia et al., 1991). By extending peripheral canal inputs, rotation velocity is estimated through VSM for more accurate gaze and posture control (Raphan and Cohen, 1985, 2002). In parallel, optokinetic after-nystagmus (OKAN), which is another major representation of the VSM through visual system activation (Cohen et al., 1987), has also been thoroughly investigated. Previous extensive studies based on these ocular responses have demonstrated its inherent complexity and strong multimodal aspects (see reviews by Raphan and Cohen, 2002; Laurens and Angelaki, 2011). In contrast, the mouse lacks a dominant time constant of the angular VOR (2–4 s; Killian and Baker 2002; Stahl et al., 2006) or an OKAN under traditional structured stimulation (van Alphen et al., 2001). Thus, the characteristics of mouse VSM has yet to be defined. The purpose of this study was to obtain an accessible procedure for adequate ocular performance linked to the VSM and other central estimate of tilt and to verify their response properties in mice.

Vestibular-only neurons in the vestibular nuclei, that are considered to be responsible for the VSM, have activity that is not only related to the dominant time constant of angular VOR and to OKAN through canal and visual inputs, but also to the otolith induced eye movements observed as sustained horizontal nystagmus or bias components during off-vertical axis rotation (OVAR) (Reisine and Raphan, 1992; Yokota et al., 1992). In this paradigm, the semicircular canal output decays during the on-going constant rotation, while the otolith organs are activated by the rotating vector around the subject. To date, ocular performances to OVAR have been widely investigated across various species from lateral-eyed to frontal-eyed animals. It produces qualitatively similar responses in different species, including rat (Hess and Dieringer, 1990), rabbit (Maruta et al., 2001), cat (Darlot and

Denise, 1988) and primates (Kushiro et al., 2002), showing a highly conserved vestibular system throughout mammals. The induced horizontal slow phase eye movements consist of two components; a bias component and modulation components as the head changes orientation relative to gravity (Guedry 1965; Benson and Bodin 1966). It is generally accepted that each response was generated by two different mechanisms. For the bias component, the VSM is responsible (Hain 1986; Schnabolk and Raphan, 1992; Angelaki et al., 1992), whereas cyclic modulations arise predominantly through linear VOR orientation mechanisms (Darlot et al., 1988; Angelaki and Hess, 1996; Wood et al., 2007). In human subjects, these eye movements during OVAR are likely associated with motion sickness showing a clear correlation between bias components and its sensitivity (Ventre-Dominey et al., 2008; Dai et al., 2011). Although there are several studies of mouse OVAR, so far only data from the limited range of stimulus are available (Killian and Baker, 2002; Harrod and Baker, 2003; Beraneck et al., 2012; Romand et al., 2013). In this study, we used a counter rotation centrifuge to generate a dynamic rotational force (pseudo-OVAR) greater than that which can be generated by conventional OVAR stimulation at any tilt angle or velocity (Kaufman et al., 2002, 2005). Here, we show the dynamic characteristics of mouse otolith ocular responses to a wide range of rotational force. The results indicate that the mouse also has well-organized orientation mechanisms and that central neural process related to VSM is highly conserved in mice.

2 Methods

2.1 Animals

Total of 19 C57BL/6J strain mice obtained from The Jackson Laboratory (Bar Harbor, ME) were tested for this study. All mice were housed on a 12-hour light, 12-hour dark cycle and most experiments took place during the afternoon or evening periods of the light-dark cycle. The animals were 3–6 months old at the time of recordings. All of the experiments and procedures were approved by the Institutional Animal Care and Use Committee at University of Texas Medical Branch and with the guidelines laid down by the NIH in the US regarding the care and use of animals for experimental procedures (Protocol number: 511072, May 31, 2013).

2.2 Eye movement recording

Most of the techniques for eye movement recording were similar to those described in a previous study in our laboratory (Makishima et al., 2011). Before each trial session, mice were briefly anesthetized with Isoflurane and treated with Pilocarpine (2%) eye drops to limit pupil dilation in darkness. The body was wrapped in a close fitting plastic bag and then was mounted on a custom made platform. The head was stabilized by clamping the maxilla firmly to a bite bar which was also attached to the platform. The head position during recordings was maintained in a position where the lateral semicircular canals were tilted up ~30 deg from the earth-horizontal position (Calabrese and Hullar, 2006). Ten minutes were allowed to elapse before the start of data acquisition so that the pupil size could become constant and to allow the mouse to become alert as confirmed by observation of spontaneous limb movements (Katoh et al., 2005; van Alphen et al., 2010; Romand et al., 2013). Eye movement recordings were performed in complete darkness using a video-oculography at

60Hz sampling rate. We studied movements of one eye in two-dimensions because of equipment limitations. The frontal view of the right eye was monitored by an infrared camera and processed by a custom pupil tracking system (Sung et al., 1995). Horizontal and vertical positions of the pupil center were stored on a computer, along with centrifuge parameters, including velocity and position profiles of motion control axes and used for data analysis.

2. 3 Stimulus apparatus and Protocols

A schematic overview of the counter rotation for pseudo-OVAR stimulus was provided in Fig. 1. To produce pseudo-OVAR eye movements, the animals were rotated about two independent earth-vertical axes. A horizontal arm mounted in the center to the main servocontrolled rotating motor supported an eccentric turntable on one end and control and power equipment for counterbalance on the other end (Kaufman et al., 2002, 2005). The eccentric axis crossed the midpoint of the interaural axis of the animal. The radius from the center of the eccentric turntable to the main axis was 45 cm. Before presentation, each axis was set in the zero position where animals were facing out from the center of the main axis (Fig. 1 and 2). The mice were rotated about each axis at each constant velocity in the different directions. This counter rotation could generate a larger magnitude rotational vector (i.e. resultant of gravity and centrifugal force) depending on the main axis rotation (>1 g) than can be achieved using conventional OVAR (Fig. 1). Each rotation axis provides a different alignment of the linear force vectors for activation of otolith organs. Velocity of the eccentric turn table was varied to examine the effect of eccentric axis velocity or head frequency and velocity of the main axis rotation was varied to examine the effect of resultant gravito-inertial force. We designed two patterns of different counter rotation paradigms to examine otolith-ocular performance either (1) at different eccentric velocity or rotational frequencies at a fixed resultant tilt angle without stopping in-between different stimulus frequencies (continuous rotation) or (2) at different frequencies and resultant tilt angles with a complete stop in-between each stimulus presentation (consecutive rotation). In addition to exploring different magnitudes of centrifugal accelerations in this second paradigm, we were also able to examine differences in responses following longer periods of continuous rotation with briefer periods of rotation.

Continuous Rotation—First, the main axis was accelerated at 60 deg/s^2 to a constant angular velocity of 245 deg/s in the counter-clockwise direction, so that a centrifugal acceleration of 0.84 g was projected in the horizontal plane of animals upon the eccentric turntable. The resultant force of the centrifugal acceleration relative to the gravity vector was tilted 40 deg relative to earth vertical. This induced the initial eye movements through canal and otolith co-activation at this phase of the paradigm that declined to zero as this off-axis rotation continued. The eccentric turntable rotation then started in the clockwise direction and progressively increased from 18 to 144 deg/s (0.05 – 0.4 Hz) for 5 – 35 cycles of rotation while data were collected. The eccentric rotational velocities remained constant at 18 (0.05 Hz), 36 (0.1 Hz), 72 (0.2 Hz), 108 (0.3 Hz) and 144 deg/s (0.4 Hz) for 90 – 120 s in this experimental paradigm and animals were exposed to continuous 10 min rotation. The recording was conducted once a day, 2 – 3 times per mouse. The data under same stimulus condition were averaged.

Consecutive Rotation—The main axis was rotated at an angular acceleration of 60 deg/s² to reach a constant velocity of 112, 161, 203 and 245 deg/s in the counter-clockwise direction. These main axis rotations generated each centrifugal force of 0.17, 0.35, 0.55 and 0.84 g respectively, along in the horizontal head plane. The resultant gravito-inertial force of the centrifugal acceleration and the gravity was tilted at a vector sum from earth vertical of 10, 20, 30 and 40 deg, respectively. After subsidence of transient eye movements in a similar fashion as observed in continuous rotation, the eccentric turntable was then rotated in the clockwise direction, while the main axis was still rotating at a constant velocity. The eccentric velocity was kept constant at 18, 36, 72, 108 or 144 deg/s for periods of 60–90 s. After data for 5–20 cycles of rotation were collected, both axis rotations were stopped in every stimulus condition before next trial began (inter-trial interval of 1 min). Recording for the same stimulus condition was conducted on each separate day and repeated 2–6 times per mouse.

2. 4 Data analysis

Eye position signals were calibrated off-line using a linear scale factor that accounted for the average ocular globe diameter relative to the number of pixels from our video images. These eye calibration scale factors had been previously verified by rotating a camera concentrically about the mouse eye at a fixed-radius (Makishima et al., 2011). After applying the eye calibration, pupil position was differentiated and desaccaded using velocity thresholds and then verified using a custom interactive script (MATLAB, The MathWork Inc., Natic, MA). To avoid any influence of canal stimulation during the recordings, 50 s were allowed to elapse after every change in eccentric rotational velocity during the continuous rotation paradigm and 30 s during the consecutive rotation. Eventually, a subset of traces in which eye movement amplitude was constant for the cycles of eccentric axis rotation, typically 3–10 cycles, was used for the data analyses. Each rotation cycle was located using synchronized trigger pulses that indicated the start of a new rotation cycle, or zero position (Fig. 1 and 2E). The amplitude of the eye movement modulation and the steady state bias velocity were derived from least-squares sinusoidal fits of horizontal and vertical eye position and velocity. An F-test was used for statistical analysis.

3 Results

All studied mice exhibited basic features of the OVAR observed in the previous reports of other species. Horizontal and vertical eye movements showed cyclic modulation of the eye position onto which horizontal nystagmus was superimposed as long as dual axis rotation continued (Fig. 2, A and B). The modulation of the beating field was correlated with the orientation of the head with respect to gravito-inertial force and driven in the same direction to that of yaw eye deviations generated by OVAR in rats (Hess and Dieringer, 1990) and rabbits (Maruta et al., 2001). This is opposite to that of monkey (Kushiro et al., 2002), in which different ocular kinematic mechanisms might account for differences between lateral eyed and frontal eyed animals (Robinson, 1982). Mostly, non-sinusoidal modulations of the beating fields were observed, presenting a quick shift in nasal-temporal direction, whereas gradually drifting in temporal-nasal direction during a cycle of the eccentric rotation (Fig. 2). These asymmetrical response curves were horizontally inverted during opposite counter

rotation (Fig. 3). This non-sinusoidal response property has been already noted in rodents (Hess and Dieringer, 1990; Killian and Baker, 2002). These data suggest that different functional mechanisms may be at work in each direction in these animals. For the analysis described in the text, we mainly used a full-cycle sinusoidal curve fit following the previous studies of OVAR. However, for evaluation of fairly large asymmetric responses, we applied two sinusoidal fits separately to temporal-nasal and nasal-temporal response for close measurements in addition to simple full-cycle analysis, but the extracted data currently studied were not significantly different in each sinusoidal analysis. The induced counter-clockwise slow phases of nystagmus were compensatory as the eccentric axis rotated in clockwise direction, resulting in the offset of horizontal mean eye velocity in the negative direction (Fig. 2C, dashed horizontal line). This slow phase mean velocity, or bias velocity, was dependent on the eccentric axis rotation at lower velocities. For higher velocities, it was independent of eccentric turn rotation (Fig. 4C). Bias velocity increased with increase in equivalent tilt angle or gravito-inertial force. However, there were large variations in horizontal bias components across the animals used (Fig. 7C). Vertical eye position also changed periodically with maximal elevation and depression when the centrifugal force generated by the main axis rotation was aligned with interaural axis (Fig. 2, B and D). The vertical component associated with the continuous nystagmus was negligible (Fig. 2B), thus vertical mean eye velocity modulated approximately around zero (Fig. 2D, solid horizontal line).

3. 1 Effects of eccentric velocity (frequency)

A continuous counter rotation was first conducted using 14 mice. The eccentric turn table was rotated at 18–144 deg/s while the main axis angular velocity was constant at 245 deg/s, which produced a centrifugal force of 0.84 g in the horizontal plane (Fig. 1). The evoked eye movements to the different eccentric axis rotations were plotted in Fig. 4. The ocular performance of modulation components showed similar properties across the animals (thin lines in Fig. 4, A and B). At all eccentric rotation velocities, amplitudes of vertical modulations in eye positions and velocities were greater than those horizontal modulations. The amplitudes of the modulation of horizontal beating field decreased as eccentric velocity increased, while the vertical modulation decreased slightly or rather remained stable (Fig. 4A). The amplitude of horizontal and vertical components in slow phase eye velocity increased with increases in the velocity of eccentric axis rotation (Fig. 4B). The average magnitude of bias velocity increased with eccentric axis rotation and reached a maximum value at 21.2 ± 9.3 deg/s (mean $\pm$ SD) at rotation velocity of 72 deg/s, then remained stable (Fig. 4C). Averaged amplitudes of the horizontal slow phase eye velocity and the bias velocity showed similar values at each eccentric axis rotation velocity, however, there were significantly larger inter-animal variances in bias velocity at 72–144 deg/s (note thin lines for each mouse in Fig. 4, one-tailed F-test, $**P < 0.01$). In 10 mice, the bias velocity reached a maximum value at 72 or 108 deg/s, whereas 4 mice showed an increase in bias at up to 144 deg/s.

To examine the characteristics of bias components more closely, we selected 5 mice based on the results of the continuous rotation test. Among these mice, three reached the highest bias velocity at rotational velocity of 72 or 108 deg/s (including mouse M, dashed line), and

two did not show a plateau during continuous rotation (including mouse N, gray line). Animals were rotated at the main axis velocity of 112–245 deg/s and the eccentric axis velocity of 18–144 deg/s. As observed during the continuous rotation, essentially similar properties of modulation components were obtained across the animals, thus data of modulation were averaged. Fig. 5 indicates the average data of oscillatory eye movements obtained from five mice as a function of the eccentric rotation velocity during consecutive rotation. The amplitudes of the horizontal modulations in eye position decreased, while the changes of vertical eye position remained stable as eccentric velocity increased (Fig. 5, A and B). The amplitude of horizontal and vertical velocity increased with velocity of eccentric axis rotation (Fig. 5, C and D). Results at the equivalent vector tilt angle of 20 and 40 deg are shown. Amplitudes of eye position changes and eye velocities increased with increase in equivalent tilt angles. On the other hand, the characteristics of bias components showed large inter-animal variations. Fig. 6 shows the properties of bias velocity obtained from mouse M (Fig. 6, A and B) and mouse N (Fig. 6, C and D) as a function of the eccentric rotation velocity. It is clear that increase in bias approached plateau values at 72 or 108 deg/s at each equivalent tilt angle in mouse M (Fig. 6A), whereas the bias velocity steadily increased with increasing eccentric rotation velocity in mouse N (Fig. 6C). The following analysis revealed that bias velocity at the equivalent tilt of 40 deg reached the highest values at 72 or 108 deg/s in two mice. In three mice, no plateau was observed within the range of tested eccentric rotation velocities.

3. 2 Effects of resultant gravito-inertial force

The effects of gravito-inertial force were studied by employing a consecutive rotation stimulus. Data of the bias velocity from mouse M and mouse N were plotted in Fig. 6, B and D as a function of equivalent tilt angle of 10–40 deg. There were monotonic increases in the bias velocity relative to increases in gravito-inertial force at lower eccentric velocities (< 36 deg/s). However, at higher rotation velocities (> 72 deg/s), dynamics of ocular performance in each mouse showed different response properties. In mouse M (Fig. 6B), the bias velocity reached a saturation value of 26.3 ± 3.7 deg/s (mean $\pm$ SD) at 30 deg for eccentric velocities of 72 deg/s, which was essentially close to those attained under rotation of 108 and 144 deg/s (22.9 and 24.9 deg/s). In contrast, no plateau value was attained at the equivalent tilt of 10–40 deg in mouse N (Fig. 6D). The bias velocity, increased with the increase in equivalent tilt angle, and the higher the eccentric rotation velocity, the more rapidly the bias velocity increased.

To further characterize the effects of gravito-inertial force on the bias velocity, 14 mice were rotated at 10–40 deg of equivalent tilt while eccentric velocity was remained at a constant eccentric velocity of 72 deg/s. Fig. 7 indicates an average of horizontal and vertical modulations in eye position and eye velocity and bias velocity as a function of the equivalent tilt. The amplitudes of modulations in eye positions and eye velocities monotonically increased with increases in the equivalent tilts (Fig. 7, A and B). At all equivalent tilt angles, amplitudes of vertical modulations in eye positions and velocities were greater than those horizontal modulations. The average magnitude of bias also increased with gravito-inertial force from 4.6 ± 3.8 deg/s (mean $\pm$ SD) at the equivalent tilt angle of 10 deg, to 21.0 ± 5.6 deg/s (mean $\pm$ SD) at 40 deg (Fig. 7C). In most mice, the bias

velocity increased with the equivalent tilt angles, however, two mice approached saturation at 30 deg of equivalent tilt (including mouse M, dashed line). The variances of horizontal slow phase eye velocity and bias velocity were statistically significant at all equivalent tilt angles (F-test, $*P < 0.05$, $**P < 0.01$).

4 Discussion

To date, given the ample studies of OVAR in various species, little is known about its characteristics in mice. There are several experiments about mouse OVAR, but mostly, they focused on the estimation of peripheral otolith function, one aspect of this paradigm, at a single tilt angle (Harrod and Baker, 2003; Beraneck et al., 2012; Romand et al., 2013). On the other hand, Killian and Baker (2002) specifically investigated a higher vestibular system by observing bias components at 90 deg of tilt. The impaired bias after medial cerebellar lesion including the nodulus might be considered as the evidence of central neural process closely related to the mouse VSM. For further evaluation in this small species, we used a dual yaw axes counter rotation presenting a similar rotating vector as OVAR but with a larger gravito-inertial force (pseudo-OVAR; Kauffmann et al., 2002, 2005). Both pseudo-OVAR and OVAR present sinusoidal oscillation of the gravitational force along the interaural and naso-occipital axis and induce dynamic eye movements from otolith activation. To produce pseudo-OVAR, first, the animals are stimulated with a directionally fixed centrifugal force in the horizontal plane by a constant velocity main axis rotation. The following eccentric turn table rotation can generate dynamic rotating vector that acts on the vestibular organs (Fig. 1). Because the effect on the semicircular canals decays over time during constant velocity rotation, once the initial canal-related eye movement has decayed, neural activity responsible for the steady-state continuous response to this counter rotation is considered to arise primarily from the excitation of otolith organs. The rotating centrifugal force activating sensory cells in the macula of the utricle is identical to the horizontal projection of the gravity force during OVAR. However, there are several differences between two stimulus conditions. For instance, OVAR produces eye movements by actual tilt of the body vertical in space, but the dual vertical axes rotation in pseudo-OVAR induces responses without changing the spatial orientation of semicircular canals and otolith organs relative to gravity similar to linear motion on a sled or a parallel swing stimulus. Second, because the animals are exposed to the earth-vertical rotation without tilting the rotational axis during pseudo-OVAR, the dorso-ventral shear force remains constant at 1 g, while in OVAR, the dorso-ventral force varies depending on the angle of tilt of the rotation axis (<1 g). In other words, the resultant gravito-inertial force of rotating vector (i.e. gravity and centrifugal force) in pseudo-OVAR becomes increasingly stronger depending on the main axis rotation (>1 g), while it is fixed to 1 g during OVAR.

Under static stimulus condition, the additional inputs to otolith organs along dorso-ventral axis were reported to alter ocular performance. In human subjects, a direct comparison of static otolith-ocular response to the eccentric rotation centrifuge and roll-tilt apparatus with an equivalent interaural shear force has been conducted (MacDougall et al., 1999). Significant difference in torsion, which was greater on the centrifuge than on the static tilted-chair, suggested that the dorso-ventral shear force was important for generating a larger ocular torsion, otherwise, it could be due to the larger gravity vector acting on the

otolith organs and body tilt receptors (Miller and Graybiel, 1971; Yates et al., 2000). In the past, a study of the mouse OVAR at a single tilt angle of 17 deg reported that the vertical eye position modulated ± 8.5 deg for the rotation velocity of 50 deg/s (Beraneck et al., 2012). Compared with this value, our data showed a slightly larger value (± 10.9 deg) at 72 deg/s about equivalent tilt of 20 deg. However, a close comparison may be difficult for the differences in the experimental paradigms and conditions.

During OVAR, modulatory responses are associated with the orienting eye movements through linear VOR (Hess and Dieringer, 1990; Angelaki and Hess, 1996; Maruta et al., 2001; Kushiro et al., 2002; Wood et al., 2007). The evoked oscillations of mouse eye during pseudo-OVAR had similar properties to those of other animals in OVAR. The horizontal and vertical eye positions shifted in the same directions as the orienting responses to static tilt (Oommen and Stahl, 2008), which modulated as a function of head position with regard to the centrifugal shear force. The magnitudes of modulation decreased with increased rotational velocities (Fig. 4A), reflecting the low-pass characteristics of the otolith system, which may be suitable to complement the poor performance of canal reflexes at low frequencies (Fernandez and Goldberg, 1971; Baarsma and Collewin, 1975; Maruta et al., 2001). At all eccentric rotation velocities, vertical change in eye positions were larger than that of horizontal change, consistent with the previous studies of other species. On the other hand, changes in velocity modulation showed different features from frontal eyed animals. In cats and monkeys, the amplitude of both horizontal and vertical velocity modulation equally increased with increase in the rotational velocities (Darlot and Denise, 1988; Kushiro et al., 2002), whereas, in mice, an increase in horizontal velocity modulation were smaller than that of vertical velocity (Fig. 4B), similar to the observation in rat (Hess and Dieringer, 1990). The different response properties of modulations across lateral and frontal eyed animals may be caused largely by the distinct differences in ocular kinematic mechanisms between these species. As shown in Fig. 5 and 7, we revealed that the modulation components were dependent on the equivalent tilt angle from earth gravity. This property of mouse eye movements is in agreement with that of observed under static stimulation (Oommen and Stahl, 2008). So far, the effects of axis tilt, or gravito-inertial force on modulation components during OVAR have not been well investigated in lateral eyed animals, however a similar dependence was observed in frontal eyed animals such as cats and primates (Darlot and Denise, 1988; Kushiro et al., 2002). These similarities indicate that the mouse also has well-organized orientation mechanisms like other species, which are more consistent with lateral eyed animals.

The otolith induced eye movements during OVAR are overlapped with a number of neural interactions. We have presented here the ocular performance that can be explained by the velocity feedback loop. Horizontal slow phase mean eye velocity, or bias velocity during pseudo-OVAR showed similar response properties to OVAR of other species. In 10 mice, the bias velocity reached a plateau value at 72–108 deg/s of eccentric velocity at equivalent tilt of 40 deg (Fig. 4C). Such a plateau state has been reported previously. In cats, the bias velocity increased with the head rotation and remained stable at 60 deg/s and 15 deg tilt (Darlot and Denise, 1988). In primates, the magnitude of bias velocity was dependent on both rotation velocity and tilt angle, reaching a maximum plateau value at 40–70 deg/s rotation velocity at 90 deg tilt (Raphan et al., 1981; Kushiro et al., 2002). It is proposed that

bias velocity produced by OVAR is closely related to the storage capabilities in the vestibular system, determined from the time constant of VOR through canal activation (Kushiro et al., 2002). Thus, the plateau state of bias is indicated that saturation of VSM has been reached in this experimental procedure (Raphan et al., 1985; Darlot and Denise, 1988). In contrast, no such plateau state was attained during OVAR in mice over a range of head velocity at 16–200 deg/s at 90 deg tilt (Killian and Baker, 2002), indicating that saturation of VSM was not attained under this experimental condition. Given that magnitude of bias increases with the equivalent tilt angle (Fig. 7C), at steeper tilt, mice might need to be rotated more rapidly to produce saturation of VSM. Otherwise, otolithic activation with stronger shear forces, as we applied here, might be better suited to observe the saturation of mouse VSM. Additionally, a plateau of bias was also not observed in rats with rotational velocities up to 70 deg/s at 30 deg tilt (Hess and Dieringer, 1990). Functional significance of this shift toward higher head velocity for a plateau of bias in rodents than larger animals is unclear. However, one reason for the shift to higher velocity may result from different aspects of their locomotor behaviors. The locomotion of smaller species is characterized by higher frequency limb motions than that of larger species and potentially, more rapid head motions (Spoor, 2003). Although the precise function of VSM still remains controversial, it is generally proposed to provide compensation for head velocity to stabilize retinal image at low-, or more recently, mid-frequency rotation (Raphan et al., 1981; Raphan and Schnabolk, 1988; see Raphan and Cohen, 1985; Laurens and Angelaki, 2011 for review). The extended linear range of the mouse response may be related to the relative increase in head velocities observed in normal mouse locomotion, along with head stabilization mechanisms to minimize the associated head movements (Takemura and King, 2005).

While the otolith organs are sensitive to tilt or linear acceleration of the head, the semicircular canals, another component of the peripheral vestibular system, are specialized to detect angular acceleration. Why VSM through canal activation is less prominent in mice is still an open question. Together with the indistinct OKAN, the time constant of the mouse VOR is remarkably short around 2 s or less than 1 s reflecting mainly the cupular time constant (van Alphen et al., 2001; Stahl et al., 2006). Indeed, decay of the horizontal nystagmus was immediate after the initial main axis rotation under the current experimental condition. Histologically, it is reported that the mouse has equivalent size of crista surface area to other rodents, given the body weight of animals (Desai et al., 2005). On the other hand, physiologically, it is postulated that animals with higher head movements typically have less sensitive canals (Clarke, 2005). Vestibular afferent sensitivity to sinusoidal head rotations showed a close relationship to canal size across a variety of mammalian species, and there was a significant difference between the dynamic responses of mouse afferents and those of other animals showing the considerably low sensitivity of mouse fibers (Yang et al., 2007). Thus, in the mouse, the limited signals for neural processing from canal inputs may be associated with the fact that the traditional rotation stimulation is insufficient to provide enough angular velocity from canal activation to affect the dominant time constant of VOR. Eventually, an ability to store head angular velocity from canal or visual inputs may be of little biological importance in mice, showing that the long time constant is not a universal attribute of VSM in the mammalian vestibular system. Nevertheless, in such a small species, it may be expected that they are more susceptible to gravitational instability

(e.g. falling or being lifted and carried) than other larger mammals requiring well-developed compensatory and orienting eye movements through vestibular system. To adapt to these vulnerable circumstances, the central VSM processing from otolith related inputs may be regulated to stabilize retinal image, regardless of the prolongation of the time constant.

Recent studies have utilized the evaluation of bias components during OVAR to examine the mechanisms of motion sickness comparing inter-individual sensitivity (Ventre-Dominey et al., 2008; Dai et al., 2011). One important aspect of the current study was that individual variability was also confirmed in mice of the same strain. The C57BL/6J mouse tested here is the same background strain commonly used as knockout and transgenic mouse models in neurobehavior study, including vestibular research (Sakatani et al., 2007; Shimizu et al., 2010, Tabata et al., 2010; see Stahl, 2008 for review). Compared with the simultaneously evoked modulatory eye movements, there was a significantly larger variance in bias components (Fig. 4 and 7). These results indicate that it is necessary to take into account the individual nature of mouse eye movements when focusing on the higher neural processes in the vestibular system (Fig. 6). Otherwise, obtained data could be misinterpreted as plausible different outcomes, if a specific procedure was applied to a small number of selected subjects. In addition, it is known that higher-order central processes play an essential role in multimodal vestibular function as motion perception and special orientation. The distinct response properties of bias may imply the inherent complexity of the central neural circuits in the vestibular system, while a smaller inter-animal variability in response to head oscillation may represent its more reflexive nature. Further studies are needed to test this hypothesis. In summary, we revealed that mouse eye movements induced by dynamic stimulation of the otoliths organs with a wide range of gravito-inertial force. The otoliths activating ocular performance which was highly conserved among other mammals was also prominent in mice. The data presented here indicate the possibility of applying further genetically manipulated mice to obtain additional insight into the molecular and cellular bases for understanding of higher-order neural processes in the vestibular system.

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Abbreviations

GIF	gravito-inertial force
OKAN	optokinetic after nystagmus
OVAR	off-vertical axis rotation
VOR	vestibulo-ocular response
VSM	velocity storage mechanism

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Highlights

- We report the mouse ocular performance evoked by dynamic otolith stimulation.
- Counter rotation enabled to evaluate with a wide range of gravito-inertial force.
- Well conserved response properties in other species were also prominent in mice.
- For study of mouse VSM, individual diversity should be taken into account.

pseudo-OVAR **OVAR**

C; centrifugal force

G; gravity force

R; resultant gravito-inertial force

H; horizontal projection of gravity

V; vertical projection of gravity

 θ ; equivalent tilt**Fig. 1.**

Scheme of counter rotation centrifuge for pseudo off-vertical axis rotation (OVAR) stimulus. In this position, animal is facing out from the center of the main axis, which represents the nose-out position. θ indicates an angle of resultant gravito-inertial force (GIF) from the earth vertical, which is equivalent to the tilt angle of rotation axis during OVAR. See also figure in the box. Both the magnitude and tilt of the resultant GIF during pseudo-OVAR is a function of the radius (fixed at 45 cm) and velocity of the main axis. While the magnitude of the resultant GIF during pseudo-OVAR is greater than 1g as it is in OVAR, the modulation of linear acceleration about a specific head axis in both paradigms is achieved by rotating the animal at an axis that is tilted with respect to the resultant GIF. Therefore, during pseudo-OVAR the main axis velocity determines the magnitude of the resultant GIF while the velocity of the eccentric rotation determines the frequency of the linear acceleration modulation. In the *continuous rotation* paradigm, the main axis were kept rotating at 245 deg/s for 10 min and velocity of eccentric rotation increased in ascending order. In the *consecutive rotation*, each counter rotation was stopped once before next trial began.

Fig. 2.

Eye movements from one mouse at 72 deg/s of eccentric rotation velocity when the resultant force of centrifugal force and gravity was tilted 40 deg relative to earth vertical. (A, B) Horizontal and vertical eye positions. Eye movements in the nasal-to-temporal and upward directions are positive. (C, D) Horizontal and vertical eye velocities. The dashed line in C indicates the mean slow phase velocity (bias velocity). The solid line in D indicates zero velocity. (E) Eccentric table position signal that reset every 360 degree. The positions of the mouse at various points are shown in the inset (nose-out, left side out, nose-in and right side out). (F) Egocentric frame of reference. Arrows indicate the direction of centrifugal shear force generated by main axis rotation at each point. The horizontal bar indicates the time scale.

Fig. 3. Horizontally inverted non-sinusoidal response curve of eye position trace during opposite counter rotation (i.e. main axis rotation in the clockwise direction and eccentric axis in the counter-clockwise direction).

Fig. 4.

Results of 14 mice as a function of eccentric axis rotation velocity with 40 deg of the equivalent tilt during continuous rotation. Data of 2–3 trials are shown for each mouse (thin lines) and average (thick line). (A, B) Amplitudes of modulations of eye position and eye velocity. Filled diamond and square symbols indicate horizontal and vertical responses, respectively. (C) Properties of the horizontal bias velocity. Note the inter-animal variation. Asterisk indicates that variance of bias velocity was significantly different from that of horizontal slow phase eye velocity at each corresponding eccentric rotation (1-tailed F-test,

****** $P < 0.01$). The results of mouse M and mouse N are shown as a dashed and a gray line, respectively. Error bars indicate 1 SD.

Eye position change

Slow phase velocity

Fig. 5.

Average responses of modulation components obtained from 5 mice during consecutive rotation. Amplitude of horizontal and vertical eye position change (A, B) and eye velocity (C, D) as a function of the velocity of eccentric axis rotation are shown. Results of the equivalent tilt angles of 20 (gray line) and 40 deg (black line) are shown. Each data point represents the average for 2–6 trials per mouse. Error bars indicate 1 SD.

Fig. 6.

Variation of velocity profiles of bias components as a function of the eccentric rotation velocity (A, C) and the angle of equivalent tilt (B, D) from two mice. Each data point represents the averages of 2–6 trials under each condition in mouse M (A, B) and mouse N (C, D). In A and C, gray dashed, gray solid, black dashed and black solid lines correspond to the equivalent tilt angles of 10, 20, 30 and 40 deg, respectively. In B and D, gray dashed, black thin, black solid, black dashed and gray solid lines indicate the eccentric rotation velocities of 18, 36, 72, 108 and 144 deg/s, respectively.

Fig. 7.

Average results of 14 mice as a function of gravito-inertial force at a constant eccentric rotation velocity of 72 deg/s. The main axis was rotated at 112–245 deg/s to generate gravito-inertial forces tilted from the earth vertical at 10–40 deg. Amplitudes of modulations of eye position (A) and eye velocity (B). Open diamond symbols indicate horizontal response and open squares indicate vertical response. (C) Properties of the horizontal bias velocity. Data are shown for each mouse (thin lines) and average (thick line). The results of mouse M and mouse N are superimposed as a dashed and a gray line, respectively. Asterisk

shows that variance of bias velocity and slow phase velocity at each corresponding equivalent tilt was statistically significant (1-tailed F-test, $**P < 0.01$, $*P < 0.05$). Each point represents the average of 2–6 trials in each condition. Error bars indicate 1 SD.