Mice misunderstand tilt during translational motion: evolution of the otolith-ocular reflex and a new index for assessing the otolith-ocular reflex in mice

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Abstract

In the interaural direction, translational linear acceleration is loaded during lateral translational movement and gravitational acceleration is loaded during lateral tilting movement. These two types of acceleration induce eye movements via two kinds of otolith-ocular reflexes to compensate for movement and maintain clear vision: horizontal eye movement during translational movement, and ocular counter roll during tilting movement. Although the two types of acceleration cannot be discriminated, the two otolith-ocular reflexes can distinguish them effectively. In the current study, we tested whether lateral-eyed mice exhibit both of these otolith-ocular reflexes. In addition, we propose a new index for assessing the otolith-ocular reflex in mice. During lateral translational movement, mice did not show appropriate horizontal eye movement, but exhibited unnecessary vertical ocular counter roll-like eye movement that compensated for the angle between the body axis and gravito-inertial acceleration (GIA; i.e., the sum of gravity and inertial force due to movement) by misunderstanding GIA as gravity. Using the new index (amplitude of vertical component of eye movement) / (angle between body axis and GIA), the mouse otolith-ocular reflex can be assessed without determining whether the otolith-ocular reflex is induced during translational movement or during tilting movement.

Main

The otolith is a sensory organ that responds to linear acceleration. In the interaural direction, linear acceleration is loaded during lateral translational motion, and gravity acceleration is loaded during lateral tilting movement. According to Einstein's equivalence principle, the two acceleration types cannot be discriminated. However, humans are typically able to discriminate the two acceleration types perceptually, and act in space accordingly. Thus, previous studies have reported that humans use two types of otolith-ocular reflexes^{1,2} that control the gaze to ensure stable perception of the environment during movement³. One type of otolith-ocular reflex induces horizontal eye movement during lateral translational motion¹ and the other type induces ocular counter roll (OCR) during lateral tilting movement². Horizontal eye movement and OCR are exhibited even in darkness and are induced by the otolith-ocular reflex as well as by optic flow^{1,4}. The appropriate use of two different otolith-ocular reflexes requires higher-order neural pathways combining signals from multiple sensors, including the semi-circular canals and the visual system⁵⁻⁷. In the current study, we sought to examine whether lateral-eyed mice possess both types of otolith-ocular reflexes exhibited by species with progressive frontalization of the eyes, including humans and monkeys. Using binocular three-dimensional analysis of the otolith-ocular reflex in mice⁸, the current study revealed that mice only exhibited OCR-like movement, both during translational linear motion and during tilting movement.

Balance-related falls have been reported to cause more than half of accidental deaths among older people (NIH 1991 Annual Report, National Deafness and Other Communication Disorders Advisory Board). The inner ear is mainly responsible for balance, and the function of the otolith system in the inner ear has been examined in evolutionary studies, clinical studies, and studies of physical principles⁹. In

addition, loss of otolith function has been found to lead to patient reports of disorientation and postural unsteadiness¹⁰. Despite the significant morbidity and mortality associated with loss of otolith function, the pathophysiology of otolith-related diseases remains to be elucidated. A major cause of this lack of research is the absence of an effective functional test of otolith function in mice. The majority of studies of normal and abnormal development of the otolith system have focused on mutant mice¹¹. Therefore, the secondary purpose of the current study was to develop an index for evaluating otolith-ocular reflexes in mice, and to identify the normal value of the index, providing a new otolith function test in mice.

Results

Ten male C57BL/6J mice at 9–10 weeks of age weighing 20–26 g were used in this study. To induce the otolith-ocular reflex, the otolith was stimulated by linear acceleration using a simple method involving lateral translational linear motion. Ten mice were reciprocated left and right for a one-way length of 1800 mm in five round trips in light conditions. The profile of the mouse's position, velocity and acceleration is shown in Fig. 1A, with a maximum velocity of 3.25 m/s and maximum acceleration of 0.9G. During the trips, the movement of both eyes was recorded using 240-Hz high-speed infrared cameras. The data were collected during the third and fourth trips. Figure 1B shows movement data from both eyes of the mouse during the lateral translational motion shown in Fig. 1A. Although humans show conjugate horizontal eye movement during reciprocating left and right in light by discriminating between gravity acceleration and translational linear acceleration¹² (Fig. 1C), mice exhibited disconjugate eye movement, in which the main component was vertical (Fig. 1B, D). In addition, the waveform of the vertical component of the eye position data (second column in Fig. 1B) differed from the waveform of the mouse's position data (first column in Fig. 1A). This eye movement not only failed to stabilize gaze in space during motion but also increased error in the stabilization of gaze. Therefore, this eye movement did not appear to be induced by optic flow. Even in darkness, similar eye movement was observed (attached movie), and the amplitude of the vertical component was proportional to the magnitude of maximum linear acceleration in the interaural direction (Fig. 1F). These results indicate that eye movement was induced by linear acceleration in an interaural direction (i.e., otolith-ocular reflex). However, this eye movement is clearly different from that induced by the otolith-ocular reflex in humans (Fig. 1C vs 1D). Therefore, we suspected that this eye movement involved the other type of otolith-ocular reflex (i.e., OCR), which occurs during lateral tilting movement in humans (Fig. 2A). During lateral translational motion, the mouse was not tilted against gravity. The otolith-ocular reflex corresponding to the OCR in humans may have been induced in mice during translational motion because the mouse misunderstood gravito-inertial acceleration (GIA; i.e., the sum of gravity and inertial force due to translational motion; Fig. 1D) as gravity. This misunderstanding would be expected to cause a misunderstanding of the sense of tilting (Fig. 1E). We calculated the misunderstanding of tilt angle (i.e., the tilt angle of GIA) (Fig. 1D, E and G). The waveforms of vertical eye movement in the left eye were a mirror image of the waveform of GIA tilt angle (Fig. 1F vs G), which indicates that vertical eye movement compensated for the GIA tilt angle. This result suggests that the mouse attempted to set a straight line connecting two centres of pupils of both eyes to be parallel to the imaginary ground (i.e., perpendicular to the direction of GIA) (Fig. 1E). The rotation of the

line (red arrow in Fig. 1E) appears to compensate for the mouse's misunderstanding of the tilt angle. Therefore, we consider that the lateral-eyed mouse's disconjugate vertical eye movement corresponds to OCR in frontal-eyed humans, which suggests that this disconjugate vertical eye movement would be seen during lateral tilting movement.

To verify this hypothesis, we analysed the eye movement of 10 mice during lateral tilting movement in darkness (Fig. 2B). As shown in Fig. 2A, the frontally positioned eyes of humans rotate around the X-axis when tilting laterally. Therefore, the OCR that compensates for tilting movement (black curved arrows in Fig. 2A) is the torsional eye movement that rotates around the X-axis (red curved arrows in Fig. 2A). However, as shown in Fig. 2B, the laterally positioned eyes of mice rotate around the Y-axis when tilting laterally. Thus, the mouse's eye movement compensates for the tilting movement with eye movement that rotates around the Y-axis, not around the X-axis. Therefore, the mouse's eye movement should be vertical, in contrast to the torsional eye movement observed in humans. To prevent contamination of the semi-circular canal-ocular reflex, the mouse was tilted as slowly as possible, and remained still for approximately 5 seconds at each 10° interval (Fig. 2C). As shown in Fig. 2D, as expected, mice showed disconjugate vertical eye movement (attached movie) and the straight line connecting the centres of the pupils of both eyes rotated in the opposite direction to the mouse's tilting movement (red curved arrow vs. black curved arrow in Fig. 2E). The results revealed that the line was kept in space (** in Fig. 2E). These findings indicate that the otolith-ocular reflex corresponding to OCR in humans (hereafter referred to as OCR-like eye movement) functioned correctly during lateral tilting movement (Fig. 2E) and functioned incorrectly during translational motion (Fig. 1D).

To verify whether the same OCR-like eye movement was exhibited both during translational motion and during tilting movement, we analysed eye movements of 10 mice during translational motion and tilting movement in dark conditions, as shown in Fig. 2F. The average maximum shift angle of vertical component of both eyes of the 10 mice during lateral translational motion (Fig. 1D, F) were set on the ordinate axis and the maximum tilting angle of gravito-inertial acceleration from gravity (Fig. 1D, E, $\tan^{-1}$ [value of maximum interaural translational linear acceleration / value of gravity acceleration]) was set on the abscissa axis. In the graph, the average shift angle of the vertical component of both eyes of the 10 mice during lateral tilting movement (Fig. 2E) was set on the ordinate axis and the tilting angles (Fig. 2E) were set on the abscissa axis. These data were plotted on the same straight line. The results indicate that similar OCR-like eye movements were exhibited in mice during both translational motion and tilting movement, and that mice did not exhibit an otolith ocular reflex to compensate for translational motion by discriminating between gravity acceleration and translational linear acceleration.

To test this possibility further, we investigated the eye movement of 10 mice when loaded with linear acceleration in a direction other than the interaural direction (i.e., the naso-occipital direction) during back and forth translational linear motion and during forward and backward tilting movement. As expected, during motion in light conditions, disconjugate torsional, conjugate vertical and disconjugate horizontal eye movement compensated for the tilt of GIA (Fig. 3A–D) and did not compensate for translational motion to stabilize gaze in space; if a mouse's eye movement compensated for motion, the eye

movement would be disconjugate pure horizontal eye movement, both when looking forward and when looking sideways, as shown in Fig. 3E. In dark conditions, the same eye movement was observed (Fig. 3F). During forward and backward tilting movement in dark conditions, the same disconjugate torsional, conjugate vertical and disconjugate horizontal eye movement corresponding to OCR-like eye movement was observed (Fig. 4A–C). As shown in Fig. 4D, the line that was constructed using the average vertical shift angle data of the left eye (Fig. 3C, F) of 10 mice during back and forth translational motion in dark (red line) was the same as the line constructed using the data (Fig. 4B, C) during forward and backward tilting movement in dark conditions (blue line). These results indicate that the mouse exhibited only one type of otolith-ocular reflex, OCR-like eye movement, when linear acceleration was loaded in the naso-occipital direction and did not exhibit any otolith-ocular reflex to compensate for translational motion (Fig. 3C vs 3E).

These findings revealed that mice exhibited inappropriate eye movement during translational motion, using only OCR-like eye movement, which suggests that mice have not evolved two types of otolith-ocular reflexes.

Discussion

Using binocular three-dimensional eye movement analysis, the current study indicated that mice have not evolved two types of otolith-ocular reflexes. Rather, the findings suggested that lateral-eyed animals, such as mice, possess only a primitive otolith-ocular reflex that compensates for tilting movement: OCR-like eye movement (Fig. 2E). The ancestor of modern primates, frontal-eyed shoshonius, evolved from oldest primates, lateral-eyed purgatories¹³. The progressive frontalization of the eyes resulted in overlap of the left and right visual fields, which led to stereopsis that enabled the perception of depth in the overlapped narrow visual field¹⁴. In addition, because species with frontalization of the eyes acquired fovea, to which optimal focus is confined, there was a need to direct the optic axis to targets¹⁵. Because clear vision during translational motion in humans and monkeys requires stabilization of gaze in space via eye movement to compensate for translational motion, the primitive otolith-ocular reflex evolved into an additional type of otolith-ocular reflex that compensates for translational motion (Fig. 1C) by combining signals from multiple sensory pathways, such as semi-circular canals and the visual system^{5–7}. As a result, the primitive otolith-ocular reflex (OCR-like eye movement) degenerated and became vestigial in humans and monkeys. Evidence from previous studies suggests that the OCR in humans and monkeys cannot compensate for tilting movement, because the ratio of torsional angle of the eye (the angle indicated by the red curved arrow in Fig. 2A, E) against the head tilting angle (the angle indicated by the black curved arrow in Fig. 2A, E) (OCR gain) is smaller than that in lateral-eyed mice, rats and rabbits (Fig. 4E)^{2,16–18}. Without the evolved otolith-ocular reflex, lateral-eyed animals can ensure stable perception of the environment during translational motion because the lateralization of both eyes acquires a whole panoramic field of view. The current results may contribute to a better understanding of the evolution of the otolith-ocular reflex.

The secondary purpose of the current study was to develop an appropriate index for evaluation of the otolith-ocular reflex in mice, and to determine the normal value of the index. To the best of our knowledge, the current study is the first to analyse the eye-movements of both eyes in mice three-dimensionally (i.e., with six parameters). As shown in Figs. 1B, 2D, 3B and 4B, regardless of whether eye movement was conjugate or disconjugate, the amplitude of both eyes was the same for each of three components; thus, analysis of either the left or right eye was sufficient. In OCR-like eye movement during lateral translational motion and lateral tilting movement, the vertical component was largest among the torsional, vertical, and horizontal components, and the other components were extremely small, as shown in Figs. 1B and 2D; thus, the vertical component was the optimal parameter under these conditions. In OCR-like eye movement during back and forth translational motion and forward and backward tilting movement, the torsional and vertical components were larger than the horizontal component and the amplitudes of the two components were almost the same as those shown in Figs. 3B and 4B; thus, either the torsional or vertical component is the optimal parameter under these conditions. When analysing eye movement from video-recordings, analysis of the vertical component is easier than analysis of the torsional component because the vertical component can be analysed only by measuring the two-dimensional coordinates of the centre of the pupil in the video. However, to analyse the torsional component, it was necessary to measure the two-dimensional coordinates of both the centre of pupil and an iris freckle in the video (appendix figure). Therefore, the vertical component was the best parameter under these conditions.

Regarding whether light or dark conditions are better for recording the otolith-ocular reflex in mice, the current results indicated that either condition was acceptable, because the otolith-ocular reflex in dark conditions was similar to that in light conditions (Fig. 1B vs Fig. 1F, Fig. 3B vs Fig. 3F). As above, we propose that the vertical component is the best parameter for evaluating the otolith-ocular reflex. If optic flow induces eye movement in light conditions, when eye movement is purely horizontal, as shown in Figs. 1C and 3E, the vertical component is not influenced by optic flow. Therefore, when the vertical component is used as an index of the otolith-ocular reflex in mice, either dark or light conditions are acceptable. However, dark conditions may be safer than light conditions because of the possible influence of “crosstalk” on the vertical component. “Crosstalk” refers to diagonal slow-phase eye movement that is evoked during strictly horizontal or vertical optic flow stimuli¹⁹. Overall, for the analysis of the otolith-ocular reflex in mice, measurement of the vertical component in dark conditions represents the optimal parameter, and the analysis of a single eye is sufficient. In the current study, we adopted left eye analysis (Figs. 2F and 4D).

When assessing the otolith-ocular reflex, each of the two types of otolith-ocular reflex (i.e., horizontal eye movement during lateral translational motion [Fig. 1C] and OCR [Fig. 2A]) have different indexes. When assessing horizontal eye movement during lateral translational motion, the sensitivity of eye movement was assessed using several indexes, such as (amplitude of eye movement) / (amplitude of translational motion) (in units of deg/cm)²⁰, (slow phase eye angular velocity) / (loaded acceleration) (in units of deg/s/g), (amplitude of eye movement) / (loaded acceleration) (in units of deg/g)²¹, and (amplitude of eye movement) / (amplitude of translational motion) / MA (MA refers to meter-angles, the index of

vergence) (in units of deg/cm/MA)²². When assessing OCR, the index is the absolute number, the ratio of eye torsional angle (red curved arrow in Fig. 2A) against the head tilting angle (black curved arrow in Fig. 2A). When assessing the otolith-ocular reflex in frontal-eyed animals such as humans and monkeys, it is necessary to use the different indexes appropriately. However, because lateral-eyed animals such as mice have only one-type of otolith-ocular reflex (OCR-like eye movement) one index is sufficient for assessing the otolith-ocular reflex both during tilting movement and during translational motion. During tilting movement, the index is the same as that for frontal-eyed animals (i.e., absolute number, the ratio of amplitude of vertical component of eye movement against head tilting angle (Figs. 2E and 4C). During translational movement, the index is also the absolute number, the ratio of amplitude of vertical component of eye movement against the tilting angle of GIA (Figs. 1DE and 3CD). These two absolute numbers can be combined into one (i.e., the ratio of amplitude of vertical component of eye movement against the angle between the body axis and GIA). During tilting movement, because GIA refers to gravity itself, the angle between the body axis and GIA is the head tilting angle in space (Figs. 2E and 4C). During translational motion, the angle between the body axis and GIA is the tilt angle of GIA (Figs. 1CD and 3CD). Based on the above findings, we propose a new index for assessing the otolith-ocular reflex in mice, the absolute number, (amplitude of vertical component of eye movement) / (angle between body axis and GIA). Using this index, it is not necessary to determine whether the otolith-ocular reflex is induced by tilting movement or by translational motion, and, as shown in Figs. 2F and 4D, both otolith-ocular reflexes induced by tilting movement and translational motion can be assessed equivalently.

The utricle and saccule are the two organs of the otolith. The left and right utricular maculae are in approximately the same horizontal plane, and, because of this position, are more useful for providing information about the position of the head's side-to-side tilt when in an upright position²³. Therefore, the otolith-ocular reflex (i.e., OCR-like eye movement when mice tilt laterally, as shown in Fig. 2B, E) is induced mainly by the utricle. During lateral translational motion (Fig. 1A, D), mice showed OCR-like eye movement that responded to the tilt of GIA (Fig. 1B, E), the same as that during lateral tilting movement; thus, the otolith-ocular reflex during lateral translational motion is induced mainly by the utricle. By calculating our new index, (amplitude of vertical component of eye movement) / (angle between body axis and GIA), during lateral tilting movement (Fig. 2E) or lateral translational motion (Fig. 1E), the function of the utricle can be evaluated. The index reflects the inclination of the straight red and blue lines shown in Fig. 2F. The normal value of the index for assessing the function of the utricle is 0.297 (the average of the two inclinations). In the same way, the saccular maculae are in parallel vertical planes and are likely to respond more to forward and backward tilting of the head²³; thus, the otolith-ocular reflex during back and forth translational motion is induced mainly by the saccule. Therefore, by calculating our new index, during forward and backward tilting movement (Fig. 4C) or back and forth translational motion (Fig. 3D), the function of the saccule can be evaluated. The index reflects the inclination of the straight red and blue straight lines shown in Fig. 4D. The normal value of the index for assessing the function of the saccule is 0.173 (the average of the two inclinations).

In conclusion, the current findings demonstrated that mice have not evolved two types of otolith-ocular reflexes, and exhibit only one type (OCR-like eye movement). We propose a new index for assessing the otolith-ocular reflex in mice, absolute value, (amplitude of vertical component of eye movement) / (angle between body axis and GIA). The normal value of the index reflecting the function of the utricle is 0.297, and the normal value of the index reflecting the function of the saccule is 0.173.

Declarations

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Author contributions

S.H. and T.I. designed, performed and analysed the experiments. T.I made the figures and wrote the manuscript. Y.T. prepared the experimental equipment and acquired preliminary data. N.T., T.K., Y.O., T.S., T.K., and M.K. substantially contributed to interpretation of data. S.S. and H.I. supervised the work.

Competing interests

The authors declare no competing interests.

Data availability statement

We have attached the raw data used to construct Figs. 2F and 4D. In addition, we have attached a movie showing that the mouse exhibited disconjugate vertical eye movement during reciprocating lateral motion at a maximum acceleration of 0.9 G (maximum velocity of 3.25 m/s) in darkness. Data from this movie were used to construct Fig. 2F. We have also attached a movie showing that the mouse exhibited disconjugate vertical eye movement while tilting leftward in darkness. The video is shown at 3× actual speed. Data from this movie were used to construct Fig. 2F.

Methods

Animals

Ten male C57BL/6J mice at 9–10 weeks of age weighing 20–26 g were used in this study. The animals were purchased from Japan SLC Inc. (Hamamatsu, Japan). This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The Osaka University School of Medicine Animal Care and Use Committee approved the protocol of the study (Permit Numbers: 21- 086-0, 27-043-000). All surgery was performed under anaesthesia, and every effort was made to minimize animal suffering and reduce the number of

animals used. After the experiments, we euthanized the animals by intraperitoneal injection of sodium pentobarbital (Nembutal; 200 mg/kg body weight).

Surgical procedure

Mice were anaesthetized with an intraperitoneal injection of a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) in conjunction with local anaesthesia (1% lidocaine). We made a small incision in the mouse's head skin and fixed a small metal plate with a screw hole to the centre of the skull using dental cement (Sun Medical, Shiga, Japan). After surgery, mice were isolated and closely observed for 48 h.

Stimulation of linear acceleration during translational motion

The experiment was carried out using 10 mice. The mice were subjected to movement in light and dark conditions. A mouse was placed on a linear sled constructed from a plastic cylindrical container and a computer-controlled motor. The mouse was fixed to the sled with a screw by a metal plate attached to the head, and the mouse's head remained firmly fixed during movement of the sled. The head position was fixed at a position where the bregma-lambda axis was parallel to the ground. The sled moved on a linear stainless steel rail that was parallel to the ground (Figs.1D, 3D, 5A). The sled and rail were constructed by Bio-Medica Co., Ltd (Osaka, Japan). From the rightmost edge, the sled was accelerated, then decelerated, and travelled 1800 mm to the leftmost edge. The sled was accelerated, then decelerated, and returned to the rightmost edge. The sled made five round trips. At both the rightmost and leftmost edges, the sled was stationary for approximately 0.3 s. Five settings were used, with maximum acceleration and velocity as follows: 1.3 G (3.25 m/s), 0.9 G (3.25 m/s), 0.7 G (3.06 m/s), 0.3 G (2.24 m/s) and 0.2 G (1.69 m/s). The mouse was placed in the sled in transverse and longitudinal orientations. When transverse, the mouse was moved rightward and leftward, and linear acceleration was loaded in the interaural direction (dark grey arrow in Fig. 1D). When longitudinal, the mouse was moved forward and backward, and linear acceleration was loaded in the naso-occipital direction (dark grey arrow in Fig. 3D). The settings were performed in random order. When calculating the maximum shift angle of vertical component of both eyes for making Fig. 2f, the following formula was used:

$$((\text{maximum shift angle of vertical component of the left eye}) - (\text{maximum shift angle of vertical component of the right eye})) / 2$$

The above value was calculated both during leftward acceleration and during rightward acceleration.

When calculating the maximum shift angle of vertical component of both eyes for making Fig. 4D, the following formula was used:

$$((\text{maximum shift angle of vertical component of the left eye}) + (\text{maximum shift angle of vertical component of the right eye})) / 2$$

The above value was calculated both during forward acceleration and during backward acceleration.

Static tilt

The experiment was carried out using 10 mice. The experiment was performed in dark conditions. The mouse was placed in a plastic cylinder container and fixed to the device with a screw by a metal plate attached to the head. The container was fixed to a board. The board had a gear with 36 teeth positioned at 10° intervals and meshed at 10° (Fig. 5B). The mouse was fixed to the board in two body positions. In one body position, the mouse was rotated laterally about the roll axis (Figs. 2B, 2E, and 5B). In the other body position, the mouse was rotated back and forth about the pitch axis (Fig. 4C). The board was rotated manually and was held at the 0°, 10°, 20°, 30°, 40°, 50°, 60° and 70° rotated positions for approximately 5 s. The rotated position was changed slowly (3–5°/s) over 2–3 s (Figs. 2C, 4A). The direction of rotation was selected randomly.

When calculating the shift angle of vertical component of both eyes for making Fig. 2F, the following formula was used:

$$\frac{((\text{shift angle of vertical component of the left eye}) - (\text{shift angle of vertical component of the right eye}))}{2}$$

When calculating the shift angle of vertical component of both eyes for making Fig. 4D, the following formula was used:

$$\frac{((\text{shift angle of vertical component of the left eye}) + (\text{shift angle of vertical component of the right eye}))}{2}$$

Eye movement recording

To record the movement of both eyes during stimulation by translational linear acceleration, a high-speed infrared camera (sampling rate 240 Hz) (STC-CL338A Sentech Co., Ltd, Kanagawa, Japan) was used. The acquisition of images of both eyes was synchronized using a software program (Stream-Pix; NorPix, Montreal, Canada). In light conditions, as a preliminary experiment, we set the angles of the right and left camera axis at 60° to the interaural axis to minimize obstruction of the field of view (Fig. 5C). During the experiment, we set the angles of the right and left camera axis at 30° to the interaural axis to obtain clear images of the eyes (Fig. 5D). By comparing the data of the actual experiment and preliminary experiment, we confirmed that the data were similar in both experiments and confirmed that the camera position did not affect eye movement during the movement. In dark conditions, the cameras were set directly beside the eyes (Fig. 5A). Movements of the eyes during the third and fourth trips were analysed.

The infrared camera (sampling rate 60 Hz) (GR200HD2-IR, Shodensha Co., Ltd, Osaka, Japan) was used to record the movement of both eyes during tilting movement in dark conditions. The acquisition of images of both eyes was synchronized using a colour quad processor (SG-202II; Daiwa, Japan). The cameras were set directly beside the eyes (Fig. 5B).

When recording eye movement in darkness, the pupils were contracted with an ophthalmic solution (1% pilocarpine hydrochloride; Nippon Tenganyaku Kenkyusho, Nagoya, Japan).

Three-dimensional analysis of eye movements

The eye movement images were analysed using an algorithm developed in our laboratory^{8,24} (see Appendix 1). The eye position is represented by a vector around the axis, of which the length is proportional to the angle of rotation. The reference position was defined as the eye position when remaining stationary. The head coordinates for analysing left and right eye movements, as measured by referencing the centre of the pupil and an iris freckle, were reconstructed in three dimensions and defined as shown in Fig. 2B. The X-, Y-, and Z-components mainly reflect the torsional, vertical, and horizontal components, respectively. For the X-component, “right torsional” and “left torsional” indicate that the superior pole of the eyeball rotated to the right and left, respectively. The rotation vector $\mathbf{r}$ describing a rotation of θ around the axis $\mathbf{n}$ was given by the formula $\mathbf{r} = \mathbf{n} \tan(\theta/2)$, with $\mathbf{n}$ being the unit vector, the direction of which represents its axis. The value of the axis angle refers to the Euler angle, and not to $\tan(\theta/2)$. Accordingly, we used the Euler angle parameter, given as $2 \tan^{-1}$ (magnitude of rotation vector), to represent eye position as an axis-angle representation²⁵. Because the camera axis was set at 30° to the interaural axis during translational motion in light conditions (Fig. 5D), $\mathbf{r}$ of the left eye was calculated using the following formula.

$$\mathbf{r} = \begin{pmatrix} \cos 30^\circ & -\sin 30^\circ & 1 \\ \sin 30^\circ & \cos 30^\circ & 0 \\ 0 & 0 & 0 \end{pmatrix} (\text{analysed } \mathbf{r})$$

$\mathbf{r}$ of the right eye was calculated using the following formula.

$$\mathbf{r} = \begin{pmatrix} \cos(-30^\circ) & -\sin(-30^\circ) & 1 \\ \sin(-30^\circ) & \cos(-30^\circ) & 0 \\ 0 & 0 & 0 \end{pmatrix} (\text{analysed } \mathbf{r})$$

Recording and analysis of mouse movement

To record mouse movement during stimulation by translational linear acceleration, the marker was set on the sled and the movement of the marker was recorded using a high-speed infrared camera (STC-CL338A). The acquisition of images of the marker was synchronized with eye images using software (Stream-Pix). The coordinates of the centre of the marker were extracted by binarizing the image of the marker (Fig. 5E). The position of the mouse was calculated from the coordinate.

To record mouse movement during tilting movement in dark conditions, two markers were set on the board and the movement of the markers was recorded using the infrared camera (GR200HD2-IR). The acquisition of images of the markers was synchronized with images of the eyes using a colour quad processor (SG-202II). The coordinates for the centre of gravity of the two markers were extracted (Fig.

5F). The tilt angle of the mouse was calculated from the tilting angle of the line connecting the two centres.

References

1. Busetтини, C., Miles, F., Schwarz, U. & Carl, J. Human ocular responses to translation of the observer and of the scene: dependence on viewing distance. *Exp Brain Res.* **100** (3), 484–494 <https://doi.org/10.1007/BF02738407> (1994).
2. Ooka, T. *et al.* Clinical application of 3D-VOG analysis for quantitative evaluation of Otolith-Ocular reflex in the roll and pitch planes. *Acta Otolaryngol.* **140** (11), 925–929 <https://doi.org/10.1080/00016489.2020.1816657> (2020).
3. Green, A. & Angelaki, D. Resolution of sensory ambiguities for gaze stabilization requires a second neural integrator. *J Neurosci.* **23** (28), 9265–9275 <https://doi.org/10.1523/JNEUROSCI.23-28-09265.2003> (2003).
4. Jáuregui-Renaud, K., Faldon, M., Gresty, M. & Bronstein, A. Horizontal ocular vergence and the three-dimensional response to whole-body roll motion. *Exp Brain Res.* **136** (1), 79–92 <https://doi.org/10.1007/s002210000559> (2001).
5. Liu, S. & Angelaki, D. Vestibular signals in macaque extrastriate visual cortex are functionally appropriate for heading perception. *J Neurosci.* **29** (28), 8936–8945 <https://doi.org/10.1523/JNEUROSCI.1607-09.2009> (2009).
6. Imai, T., Takimoto, Y., Takeda, N., Okumura, T. & Inohara, H. Three-dimensional analysis of linear vestibulo-ocular reflex in humans during eccentric rotation while facing downwards. *Exp Brain Res.* **235** (8), 2575–2590 <https://doi.org/10.1007/s00221-017-4990-8> (2017).
7. Merfeld, D., Zupan, L. & Peterka, R. Humans use internal models to estimate gravity and linear acceleration. *Nature.* **398** (6728), 615–618 <https://doi.org/10.1038/19303> (1999).
8. Imai, T. *et al.* High-Speed Video-Oculography for Measuring Three-Dimensional Rotation Vectors of Eye Movements in Mice. *PLoS One.* **11** (3), e0152307 <https://doi.org/10.1371/journal.pone.0152307> (2016).
9. de Ramos, A., Zarowski, A., Sluydts, M., Ramos Macias, A. & Wuyts, F. L. The Superiority of the Otolith System. *Audiol Neurotol.* **25**, 35–41 <https://doi.org/10.1159/000504595> (2020).
10. Zhou, Y., Wu, Y. & Wang, J. Otolithic organ function in patients with profound sensorineural hearing loss. *J Otol.* **11**, 73–77 <https://doi.org/10.1016/j.joto.2016.05.002> (2016).
11. Hughes, I., Thalmann, I., Thalmann, R. & Ornitz, D. M. Mixing model systems: using zebrafish and mouse inner ear mutants and other organ systems to unravel the mystery of otoconial development. *Brain Res.* **1091**, 58–74 <https://doi.org/10.1016/j.brainres.2006.01.074> (2006).
12. Bronstein, A. M. & Gresty, M. A. Short latency compensatory eye movement responses to transient linear head acceleration: a specific function of the otolith-ocular reflex. *Exp Brain Res.* **71**, 406–410 <https://doi.org/10.1007/bf00247500> (1988).

13. Masters, J. P. & Evolution Biological Science Fundamentals And Systematics Edited by Giancarlo Contrafatto, Alessandro Minelli, Encyclopedia of Life Support Systems (EOLSS), Pris, France, 226–252(2009).
14. Gonzalez, F. & Perez, R. Neural mechanisms underlying stereoscopic vision. *Prog Neurobiol.* **55** (3), 191–224 [https://doi.org/10.1016/s0301-0082\(98\)00012-4](https://doi.org/10.1016/s0301-0082(98)00012-4) (1998).
15. Martin, R. *Primate Origins and Evolution: A Phylogenetic Reconstruction* (1990).
16. Walker, M. *et al.* Lesions of the cerebellar nodulus and uvula in monkeys: effect on otolith-ocular reflexes. *Prog Brain Res.* **171**, 167–172 [https://doi.org/10.1016/S0079-6123\(08\)00622-5](https://doi.org/10.1016/S0079-6123(08)00622-5) (2008).
17. Maruta, J., Simpson, J., Raphan, T. & Cohen, B. Orienting otolith-ocular reflexes in the rabbit during static and dynamic tilts and off-vertical axis rotation. *Vision Res.* **41** (25–26), 3255–3270 [https://doi.org/10.1016/s0042-6989\(01\)00091-8](https://doi.org/10.1016/s0042-6989(01)00091-8) (2001).
18. Hamann, K., Reber, A., Hess, B. & Dieringer, N. Long-term deficits in otolith, canal and optokinetic ocular reflexes of pigmented rats after unilateral vestibular nerve section. *Exp Brain Res.* **118** (3), 331–340 <https://doi.org/10.1007/s002210050287> (1998).
19. Hainline, L., Lemerise, E., Abramov, I. & Turkel, J. Orientational asymmetries in small-field optokinetic nystagmus in human infants. *Behav Brain Res.* **13**, 217–230 [https://doi.org/10.1016/0166-4328\(84\)90164-5](https://doi.org/10.1016/0166-4328(84)90164-5) (1984).
20. Paige, G. D. The influence of target distance on eye movement responses during vertical linear motion. *Exp Brain Res.* **77**, 585–593 <https://doi.org/10.1007/bf00249611> (1989).
21. Angelaki, D. E. & Hess, B. J. Three-dimensional organization of otolith-ocular reflexes in rhesus monkeys. I. Linear acceleration responses during off-vertical axis rotation. *J Neurophysiol.* **75**, 2405–2424 <https://doi.org/10.1152/jn.1996.75.6.2405> (1996).
22. Telford, L., Seidman, S. H. & Paige, G. D. Dynamics of squirrel monkey linear vestibuloocular reflex and interactions with fixation distance. *J Neurophysiol.* **78**, 1775–1790 <https://doi.org/10.1152/jn.1997.78.4.1775> (1997).
23. Highstein, S. How does the vestibular part of the inner ear work? Disorders of the vestibular system. edited by Balow RW and Halmagyi GM, Oxford(1996).
24. Imai, T. *et al.* Rotation Vector Analysis of Eye Movement in Three Dimensions with an Infrared CCD Camera. *Acta Otolaryngol (Stockh).* **119** (1), 24–28 (1999).
25. Schnabolk, C. & Raphan, T. Modeling three-dimensional velocity-to-position transformation in oculomotor control. *J Neurophysiol.* **71** (2), 623–638 (1994).
26. Abadi, R. & Bjerre, A. Motor and sensory characteristics of infantile nystagmus. *Br J Ophthalmol.* **86** (10), 1152–1160 (2002).

Figures

Figure 1

Mice did not exhibit an otolith ocular reflex to compensate for lateral translational motion in light conditions (A) The change in position of the mouse during lateral translational motion First column: Mouse position data; Second column: Mouse velocity data; Third column: Mouse acceleration data in the interaural direction. The mouse was reciprocated left and right for a one-way length of 1800 mm in five round trips. The mouse remained at the leftmost and rightmost edges for 0.3 s. The data were collected

during the third and fourth trips. The mouse moved at a maximum velocity of 3.25 m/s, and a maximum acceleration of 0.9 G. (B) Three-dimensional data from both eyes of a mouse that showed inappropriate disconjugate vertical component during lateral translational motion in light conditions. We recorded movement of both eyes of the mouse using high-speed cameras and analysed the recorded images using an offline computer image analysis system. These data represent the three-dimensional movement of both eyes during the motion shown in Fig. 1A. In the present study, eye movements can be three-dimensionally described by axis angle, which characterizes the eye positions around a single rotation. The three-dimensional coordinates of the eye were defined as follows (Fig. 2B): the X-axis parallel to the interaural axis (positive left in left eye, positive right in right eye), the Y-axis parallel to the naso-occipital axis (positive backward in left eye, positive forward in right eye), and the Z-axis normal to the X–Y plane (positive upwards). The X-, Y-, and Z-components mainly reflect the torsional, vertical, and horizontal components, respectively. The direction of rotation was described from the mouse's point of view. For the X-component, "right torsional" and "left torsional" indicate that the superior pole of the eyeball rotated to the right and left, respectively. The main component was the vertical component and the waveform (second column) was similar to the waveform of mouse acceleration (third column in Fig. 1A), not to the waveform of mouse position (first column in Fig. 1A). The vertical eye movements were disconjugate (i.e., during rightward acceleration, the left eye moved upward and right eye moved downward, and vice versa). The conjugate horizontal eye movement compensating for lateral translational motion observed in humans (Fig. 1C) was not exhibited by the mouse. All 10 mice showed disconjugate vertical eye movement in light conditions. (C) Otolith ocular reflex during lateral translational motion in humans compensates for translational motion. During lateral translational motion in humans, the individual is loaded with inertial force in the interaural direction and the inertial force stimulates the otolith, which responds to linear acceleration. The otolith induces conjugate horizontal eye movement and is one of the otolith-ocular reflexes that serves to stabilize gaze in space by moving the eyes in the opposite direction to the movement. (D) Schema showing a mouse exhibiting inappropriate disconjugate vertical eye movement when accelerating rightward. Although the mouse moved laterally on the stainless steel rail, the eyes moved vertically. This eye movement was not able to control gaze appropriately and disturbed the stabilization of gaze in space during the motion. The mouse was loaded with leftward inertial force in the interaural direction. As a result, the mouse was loaded with gravito-inertial acceleration (GIA). (E) Schema illustrating the mouse's disconjugate vertical eye movement corresponding to ocular counter roll in human induced by misunderstanding of GIA as gravity by the mouse. If the mouse misunderstands GIA as gravity, the sense of tilting would also be misunderstood. In this situation of misunderstanding, the imaginary ground is perpendicular to the direction of GIA. During this movement, if the lateral-eyed mouse attempted to set the line passing through the centres of both eyes parallel to the imaginary ground, as in ocular counter roll in frontal-eyed humans (Fig. 2A), disconjugate vertical eye movement would be induced. (F) During lateral translational motion under dark conditions at five different maximum accelerations, the same vertical eye movement in the mouse's left eye could be seen in light conditions. The mouse was reciprocated left and right with a one-way length of 1800 mm in five round trips at five different maximum accelerations under dark conditions (Fig. 1A). In all 10 mice, the same characteristic eye movement was observed in light conditions (Fig. 1B). This graph shows the vertical component of the

left eye position data in a mouse during the third trip at each of five different accelerations. The maximum shift angle was proportional to the maximum acceleration. This result indicates that these eye movements were induced by the otolith-ocular reflex, not by optic flow during motion. (G) GIA tilt angle, of which the waveform was a mirror image relative to the vertical position data of the left eye. The GIA tilt angle was calculated using acceleration data at the third trip of five trips when the maximum acceleration was 0.9 G (the first half of the third column in Fig. 1A). The tilt angle of GIA suggests misunderstanding of the tilt angle of the mouse (Fig. 1E). The shapes of the waveform are similar among the five different maximum accelerations, although the maximum values of the GIA tilt angle are different. The shape of the waveform was a mirror image relative to the vertical position data of the left eye shown in Fig. 1F. This indicates that left eye vertical movement compensated for the misunderstanding of tilt angle of the mouse (Fig. 1E).

Figure 2

Mice exhibited an appropriate otolith ocular reflex to compensate for lateral tilting movement in dark conditions (A) Three-dimensional eye coordinates and ocular counter rolling that compensates for lateral tilting movement in humans In frontal-eyed humans, three-dimensional coordinates of the eye can be defined as follows: the X-axis parallel to the naso-occipital axis, the Y-axis parallel to the interaural axis, and the Z-axis normal to the X–Y plane. The coordinates of both eyes are similar. When tilting laterally,

the individual rotates around the X-axis. Therefore, the ocular counter roll that compensates the tilting movement (black curved arrows) is the torsional eye movement that rotates around the X-axis (red curved arrows). When tilting, the individual is loaded with inertial force in the interaural direction. This inertial force stimulates the otolith, which induces ocular counter roll, the other type of otolith-ocular reflex, rather than conjugate horizontal eye movement during lateral translational motion, as shown in Fig. 1C. (B) Three-dimensional coordinates of both eyes of the mouse that differed from those in humans In the lateral-eyed mouse, each eye has three-dimensional coordinates. The three-dimensional coordinates of the eyes were defined as follows: the X-axis parallel to the interaural axis (positive left in left eye, positive right in right eye), the Y-axis parallel to the naso-occipital axis (positive backward in left eye, positive forward in right eye), and the Z-axis normal to the X–Y plane (positive upwards). In the present study, eye movements can be three-dimensionally described by axis angle, characterizing the eye positions around a single rotation. Therefore, the X-, Y-, and Z-components mainly reflect the torsional, vertical, and horizontal components, respectively. The direction of rotation was described from the mouse's point of view. When tilting laterally, the mouse rotates around the Y-axis, whereas the human rotates around the X-axis (Fig. 2A). Thus, the mouse's eye movement compensates for the tilting movement with eye movement that rotates around the Y-axis, not around the X-axis. Therefore, the eye movement in the mouse is vertical eye movement, not torsional eye movement as seen in humans (Fig. 2A). (C) The tilt angle of the mouse The mouse was first tilted leftward (Fig. 2B) then tilted rightward. To prevent contamination of the semi-circular canal-ocular reflex, the mouse was tilted as slowly as possible, and remained still for approximately 5 s at each 10° interval. (D) Three-dimensional position data for both eyes of the mouse during lateral tilting movements in dark conditions that compensated for the tilting movements These data show the three-dimensional positions of the movement of both eyes when tilting in dark conditions, as shown in Fig. 2C. As explained in Fig. 2B, both eyes showed no torsional component (first column) but did show a vertical component. As expected from the eye movement results during translational motion (Fig. 1A, B), disconjugate vertical eye movement was observed (second column). When tilting leftward in dark conditions (Fig. 2B), both eyes rotated around the Y-axes, the left pupil moved upward, and the right pupil moved downward (second column). Both eyes rotated rightward slightly (third column). Therefore, the line passing through the centres of both pupils (the line indicated by ** in Fig. 2E) tilts rightward. When tilting rightward, the line tilted leftward because the left pupil moved downward and the right pupil moved upward (second column). Both eyes rotated leftward slightly (third column). All 10 mice showed the same disconjugate vertical eye movements. (E) Schema illustrating the mouse's disconjugate vertical eye movement corresponding to ocular counter roll in humans, induced when tilting leftward The line passing through the centres of both pupils (the line shown by **) is tilted rightward. As a result, the line is kept stable in space and almost parallel to the ground by compensating the tilt angle of the mouse. The rotation of the line appears to be similar to the ocular counter roll (OCR) in humans. Therefore, the mouse's disconjugate vertical eye movement appears to correspond to OCR in humans (red curved arrow in Fig. 2A vs. red curved arrow in Fig. 2B). This disconjugate vertical eye movement is referred to as OCR-like eye movement hereafter. (F) The line created using the maximum vertical shift angle data during translational motion in dark conditions was same as the line created using vertical shift angle data during lateral tilting in dark conditions in 10 mice The average maximum

shift angle of vertical component of both eyes of 10 mice during the lateral translational motion (Fig. 1D, F) was set on the ordinate axis, and the maximum tilting angle of gravito-inertial acceleration (GIA) from gravity (Fig. 1d, e, $\tan^{-1}[\text{the value of maximum interaural translational linear acceleration} / \text{the value of gravity acceleration}]$) was set on the abscissa axis. The maximum shift angle of vertical component of both eyes was calculated using the formula: $((\text{maximum shift angle of vertical component of the left eye}) - (\text{maximum shift angle of vertical component of the right eye})) / 2$. The maximum shift angle during rightward acceleration (for example, the shift angle shown by the red bidirectional arrow in Fig. 1F) was plotted on the negative side of the abscissa axis. The maximum shift angle during leftward acceleration (for example, the shift angle shown by the green bidirectional arrow in Fig. 1F) was plotted on the positive side of the abscissa axis. Red squares were plotted and a red approximate straight line was generated. In the graph, the average shift angle of the vertical component of both eyes of 10 mice during the lateral tilting movement (Fig. 2E) was set on the ordinate axis, and the tilting angles (Fig. 2E26) were set on the abscissa axis. The shift angle of the vertical component of both eyes was calculated using the formula: $((\text{shift angle of vertical component of the left eye}) - (\text{maximum shift angle of vertical component of the right eye})) / 2$. The shift angle during leftward tilt was plotted on the negative side of the abscissa axis. The maximum shift angle during rightward tilt was plotted on the positive side of the abscissa axis. Blue rhomboids were plotted and a blue approximate straight line was generated. The error bar shows the 95% confidence interval. The red line and blue line were almost the same. This result indicates that during both lateral translational motion and lateral tilting movement, the same otolith-ocular reflex (OCR-like eye movement) was exhibited.

Figure 3

Mice did not exhibit an otolith ocular reflex to compensate for back and forth translational motion in light conditions (A) The change of mouse position during back and forth translational motion First column: Mouse position data, Second column: Mouse velocity data, Third column: Mouse acceleration data in the naso-occipital direction. The mouse was reciprocated back and forth for a one-way length of 1800 mm in five round trips. The mouse remained at the leftmost and rightmost edges for 0.3 s. The data were

recorded during the third and forth trips. The mouse moved with a maximum velocity of 3.25 m/s, and a maximum acceleration of 0.9 G. The waveforms of position, velocity and acceleration were similar to the waveform shown in Fig. 1A. (B) Three-dimensional data from both eyes of a mouse that could not compensate for back and forth translational motion in light conditions. These data are three-dimensional position data for the movement of both eyes during the motion shown in Fig. 3A. The eye movements were three-dimensionally described in the same way shown in Figs. 1B and 2D. Disconjugate torsional, conjugate vertical and disconjugate horizontal eye movement were observed. These waveforms have a similar shape to the waveform of the mouse's acceleration (third column in Fig. 3A), but not similar to the waveform of the mouse's position (first column in Fig. 3A). This result indicates that eye movement responded to the linear acceleration in the naso-occipital direction and did not compensate the motion of the mouse to stabilize gaze in space. (C) Schema of the observed left eye movement in mice that cannot compensate for translational motion in the naso-occipital direction when accelerating forward. When accelerating forward, the left eye showed left torsional and downward movement. This indicates that the mouse did not exhibit an otolith-ocular reflex compensating for translational motion. The mouse was loaded with backward inertia force in the naso-occipital direction. As a result, the mouse was loaded with gravito-inertial acceleration (GIA). (D) Schema illustrating the mouse's left eye movement that compensates for GIA tilt angle by misunderstanding GIA as gravity. If the mouse misunderstands GIA as gravity, the sense of tilting would also be misunderstood. If the mouse attempted to keep the pupil of their left eye still in space during this time, right torsional movement (red curved arrow) would be required to compensate for the tilting movement (black curved arrow), and downward (blue arrow) and leftward (green arrow) movement need would be required to compensate for upward head movement against the imaginary ground. Therefore, the left eye movement shown in the boxed part of Fig. 3B was the eye movement that compensated for the tilting movement of GIA. (E) Schema of ideal eye movement when gaze is stabilized in space during forward translational motion. When looking forward during forward translational motion, close-set eye movement should be induced, and when seeing sideways, open-set eye movement should be induced, to stabilize the gaze in space. Eye movements should be disconjugate pure horizontal movements and should not have torsional or vertical components. In reality, eye movements exhibit torsional and vertical components and minor horizontal components (Fig. 3B). Therefore, actual eye-movements were not ideal for stabilizing gaze in space. (F) During back and forth translational motion under dark conditions at five different maximum accelerations, the same vertical eye movement in the mouse's left eye could be seen in light conditions. The mouse was reciprocated back and forth for a one-way length of 1800 mm in five round trips at five different maximum accelerations in dark conditions (Fig. 3A). In all 10 mice, the same characteristic eye movement was observed in light conditions (Fig. 3B). This graph shows the vertical component of left eye position data of a mouse during the third trip at each of five different accelerations. The maximum shift angle was proportional to the maximum acceleration. This result indicates that these eye movements were induced by the otolith-ocular reflex, not by optic flow during motion.

Figure 4

Mice exhibited an appropriate otolith ocular reflex to compensate for forward and backward tilting movement in dark conditions and ocular counter roll gain of frontal-eyed animals was lower than that in lateral-eyed animals (A) The tilt angle of the mouse The mouse was first tilted forward then tilted backward. To prevent contamination of the semi-circular canal-ocular reflex, the mouse was tilted as slowly as possible, and remained still for approximately 5 seconds at each 10° position. (B) Three-

dimensional position data of both eyes of the mouse during forward and backward tilting movement in dark conditions that compensated for the tilting movement. These data are three-dimensional movement data of both eyes when tilting in dark conditions, as shown in Fig. 4A. As expected from the eye movement results during back and forth translational motion (Fig. 3B, D), disconjugate torsional, conjugate vertical and disconjugate horizontal eye movements could be seen. When tilting forward in dark conditions, the left eye showed left torsional movement and the right eye showed right torsional movement to compensate for the tilting movement. The left eye showed upward and rightward movement and the right eye showed upward and leftward movement to compensate the downward head movement. When tilting backward in dark conditions, the left eye showed right torsional movement and the right eye showed left torsional movement to compensate for the tilting movement. The left eye showed downward and leftward movement and the right eye showed downward and rightward movement to compensate for the upward head movement. All 10 mice showed the same disconjugate torsional, conjugate vertical and disconjugate horizontal eye movements. (C) Schema illustrating the mouse's observed left eye movement when tilting backward. Backward tilting movement induces upward head movement. Therefore, to stabilize gaze in space (***) during tilting backward, not only torsional eye movement but also oblique eye movement are needed. The left eye compensated for the tilting movement (black curved arrow) by right torsional movement (red curved arrow) and compensated for the upward head movement by downward (blue arrow) and leftward (green arrow) eye movement. (D) The line made by using the maximum vertical shift angle data during back and forth translational motion in dark conditions was the same as the line made by using the vertical shift angle data during forward and backward tilting in dark conditions in 10 mice. The average maximum shift angle of vertical component of the left eye of the 10 mice during back and forth translational motion (Fig. 3D, F) was set on the ordinate axis, and the maximum tilting angle of gravito-inertial acceleration (GIA) from gravity (Fig. 3D, $\tan^{-1}[\text{value of maximum naso-occipital translational linear acceleration} / \text{value of gravity acceleration}]$) was set on the abscissa axis. The maximum shift angle of vertical component of both eyes was calculated using the formula: $((\text{maximum shift angle of vertical component of the left eye}) + (\text{maximum shift angle of vertical component of the right eye})) / 2$. The maximum shift angle during forward acceleration (for example, the shift angle shown by red bidirectional arrow in Fig. 3F) was plotted on the negative side of the abscissa axis. The maximum shift angle during backward acceleration (for example, the shift angle shown by green bidirectional arrow in Fig. 3F) was plotted on the positive side of the abscissa axis. Red squares were plotted and a red approximate straight line was generated. On the graph, the average shift angle of the vertical component of the left eye of the 10 mice during the forward and backward tilting movement (Fig. 4C) was set on the ordinate axis, and the tilting angles were set on the abscissa axis. The shift angle of the vertical component of both eyes was calculated using the formula: $((\text{shift angle of vertical component of the left eye}) + (\text{maximum shift angle of vertical component of the right eye})) / 2$. The shift angle during forward tilt was plotted on the negative side of the abscissa axis. The maximum shift angle during backward tilt was plotted on the positive side of the abscissa axis. Blue rhomboids were plotted and a blue approximate straight line was generated. The error bar shows the 95% confidence interval. The red line and blue lines were almost identical. This result indicates that during both back and forth translational motion and forward and backward tilting movement, the same otolith-

ocular reflex that compensated for the tilting movement was exhibited. (E) Ocular counter roll gains of frontal-eyed animals were lower than those of lateral-eyed animals. In this graph, OCR gains of mouse, rat, rabbit, monkey and human are shown. OCR gain refers to the ratio of torsional angle of the eye (the angle indicated by the red curved arrow in Fig. 2A) against the head tilting angle (the angle indicated by the black curved arrow in Fig. 2A). Large OCR gain indicates that the primitive otolith-ocular reflex (OCR or OCR-like eye movement) functions well, and small OCR gain indicates that the primitive otolith-ocular reflex does not function. The primitive otolith-ocular reflex degenerated in frontal-eyed humans and monkeys, with OCR gains of humans and monkeys being smaller than OCR gains of lateral-eyed rabbits, rats and mice. The OCR gains of rats were calculated using data from Hamann KF et al 1998¹⁸. The OCR gains of rabbits were calculated using data from Maruta J et al 2001¹⁷. OCR gains of monkeys were calculated using data from Walker MF et al 2008¹⁶. OCR gains of humans were calculated using data from Ooka T et al 2020². Regarding OCR gains of mice and rats, the ratio of the amplitude of OCR-like eye movement (the angle indicated by red curved arrow in Fig. 2E) against the mouse tilting angle (the angle indicated by black curved arrow in Fig. 2E) was calculated. Because the OCR gains of mice were calculated using the data shown in Fig. 2F, the OCR gains of mice reflect the inclination of the blue line shown in Fig. 2F.

Figure 5

Methods figure (A) The stainless steel rail and sled for the stimulation of linear translation This setting is for the recording of eye movement during translational motion in dark conditions. Infrared high speed cameras were set just beside the mouse. (B) The gear and container for the stimulation of static tilt in dark conditions Infrared cameras were set just beside the mouse. (C) High speed camera setting in the preliminary experiment during translational motion in light conditions We set the angles of the right and

left camera axis at 60° to the interaural axis to minimize obstruction of the field of view. During lateral translational motion, the random dot pattern was set in front of the mouse, and, during back and forth translational motion, the random dot patterns were set at both sides of the mouse. (D) High speed camera setting in the actual experiment during translational motion in light conditions We set the angles of the right and left camera axes at 30° to the interaural axis. During lateral translational motion, the random dot pattern was set in front of the mouse, and during back and forth translational motion, the random dot patterns were set at both sides of the mouse. By comparing the data from the actual experiment and preliminary experiment (Fig. 5C), we confirmed that the results were similar in both experiments, and confirmed that the camera position did not affect eye movement during movement. (E) The method for analysing mouse position data during translational motion The upper column shows the original image during translational motion. The coordinates of the centre of the marker that was set on the sled were extracted by binarizing the image of the marker (lower column). (F) The method for analysing mouse tilt angle during tilting movement The left picture shows the original image during tilting movement. The coordinates of the centre of the two markers that were set on the container were extracted by binarizing the image of the marker, and we calculated the angle between the horizontal line and the line connecting the two centres (right picture).

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