Angular vestibulo-ocular reflex responses in Otop1 mice. II. Otolith sensor input improves compensation after unilateral labyrinthectomy

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Abstract

The role of the otoliths in mammals on the normal angular vestibulo-ocular reflex (VOR) was characterised in an accompanying study based on the Otopetrin1 (Otop1) mouse, which lacks functioning otoliths due to failure to develop otoconia but seems to have otherwise normal peripheral anatomy and neural circuitry. That study showed that otoliths do not contribute to the normal horizontal (rotation about Earth-vertical axis parallel to dorso-ventral axis) and vertical angular VOR (rotation about Earth-vertical axis parallel to inter-aural axis), but do affect gravity context-specific VOR adaptation. By using these animals, we sought to determine whether the otoliths play a role in the angular VOR after unilateral labyrinthectomy when the total canal signal is reduced. In 5 Otop1 and 5 control littermates we measured horizontal and vertical left-ear-down (LED) and (RED) sinusoidal VOR (0.2-10Hz, 20-100°/s) during the early (3-5 days) and plateau (28-32 days) phases of compensation following unilateral labyrinthectomy and compared these measurements with baseline preoperative responses from the accompanying study. From similar baselines, acute gain loss was ~25% less in control mice, and chronic gain recovery was ~40% more in control mice. The acute data suggest that the otoliths contribute to the angular VOR when there is a loss of canal function. The chronic data suggest that a unilateral otolith signal can significantly improve angular VOR compensation. These data have implications for vestibular rehabilitation of patients with both canal and otolith loss and the development of vestibular implants, which currently only mimic the canals on one side.

Keywords: angular vestibulo-ocular reflex (VOR), VOR compensation, Otolith contribution to angular VOR, Otop1 mouse model, otoconia deficient mice.

New & Noteworthy

This is the first study examining the role of the otoliths (defined here as the utricle and saccule) on the acute and chronic angular vestibulo-ocular reflex (VOR) after unilateral labyrinthectomy in an animal model where the otoliths are reliably inactivated and the semicircular canals preserved. This study shows that the otolith signal is used to augment the acute angular VOR and help boost VOR compensation after peripheral injury.

Introduction

Most angular head movements stimulate the three semicircular canals and two otolith organs, the utricle and saccule. It has been suggested that the signals from each are organised in a synergistic way to optimize the vestibulo-ocular reflex (VOR), which is our main gaze-stabilising mechanism during rapid head motion (e.g., Angelaki et al., 1999; Merfeld et al., 1999). In an accompanying study, we examined the role of the otoliths on the normal angular VOR and its adaptation using the Otop1 mouse model (Khan et al., 2018). Otop1 mice lack functioning otoliths due to failure to develop otoconia crystals, but they have normal functioning semicircular canals and have a distinct vestibular phenotype, but seem to have a normal phenotype in organ systems other than the otoliths (Ornitz et al., 1998). Our study showed that ocular counter-roll responses were minimal in the Otop1 mouse, but that the horizontal (rotation about Earth-vertical axis parallel to dorso-ventral axis) and vertical (rotation about Earth-vertical axis parallel to inter-aural axis) angular VOR was normal. Horizontal and vertical VOR measures were analogous in that study because the angular vertical VOR was measured with the animal on its side while being rotated about an Earth-vertical axis, i.e. only the translation component of the otolith signal, not the gravity component, contributes to the VOR. In addition, the Otop1 mouse had normal angular VOR adaptation for training that sought to increase or decrease the VOR gain (eye velocity / head velocity). The main finding from that study was that gravity context-specific adaptation was absent in the Otop1 mouse, suggesting that the otolith signal provides the main sensory cue for gravity context-specific adaptation. Taken together the results from that study suggest that when both otolith and canal angular head rotation signals are available, the redundant translation otolith signal is not used to drive the angular VOR. In addition, our findings suggest that the Otop1 angular VOR and associated adaptive neural

circuitry is unaffected by the absence of otoliths. We sought to determine whether the otoliths played a role in the angular VOR after unilateral labyrinthectomy - a situation where the total canal signal is reduced by half. Given our prior findings, it is unlikely that central nervous system circuits that mediate VOR compensation are affected in this mouse model and so any differences in compensation time course will likely be due to the lack of otolith signal input. Another reason why examination of responses to unilateral labyrinthectomy in the Otop1 mouse model is particularly informative is because this experimental paradigm physiologically mimics the current generation of vestibular implants, which so far are unilaterally implanted and only stimulate the ampullary nerves of the three semicircular canals. The normal mammalian physiological VOR response when only the canals on one side drive the total vestibular response is unknown, because there is no surgical technique that can reliably and selectively ablate the otoliths without damaging the semicircular canals. It is well established that the adaptive capacity of the vestibulo-cerebellar circuits is sufficient to ensure that individuals with a single, normal vestibular organ, e.g. after unilateral injury or infection, typically compensate well enough to suffer little disability in activities of daily life (Curthoys and Halmagyi, 1995; Black et al., 1998). However, it is not yet clear whether the same will be true when a bilaterally vestibular-deficient individual is treated with a vestibular implant that can only partially restore unilateral function of the three ipsilateral semicircular canals, without providing prosthetic signals intended for the two otolith organs. Another advantage of the Otop1 model is that because the sensitivity of mouse vestibular afferents are 2-4 times lower than those in primates (Lasker et al., 2008), a unilateral vestibular organ should be sufficient to encode head rotations in both inhibitory and excitatory directions, as does the vestibular prosthesis. We hypothesised that signals from three semicircular canals on one side only, like a vestibular prosthesis, would result

in reduced angular VOR compensation compared to when the otoliths on that side are also present. In order to determine whether the otoliths contribute to the angular VOR during acute and chronic compensation, we measured the horizontal and vertical sinusoidal VOR (0.2 - 10Hz, 20 - 100°/s) in Otop1 and control mice 3-5 days and 28-32 days after unilateral labyrinthectomy and compared these with the baseline responses measured in the accompanying study (Khan et al., 2018).

Materials and Methods

Animal groups and surgical preparation

All surgical and experimental procedures were approved by the Animal Care and Ethics Committee of the University of New South Wales, Australia. Data were obtained from five Otop1 mice and five control littermates (males, 11-14 weeks) from a colony bred and maintained at the Australian BioResources animal facility in Moss Vale, New South Wales, Australia. Male mice only were used to negate the possible effects of estrous cycle on mouse behaviour (Sanchez-Alavez et al., 2011; See explanation in Khan et al., 2018). We imported ten heterozygous B6.Cg-Otop1^{tilt}/J (stock number: 001104) mice that carry the Otop1 knockout mutation from Jackson Laboratories, USA. We set up a colony of B6.Cg-Otop1^{tilt}/J x B6.Cg-Otop1^{tilt}/J (het x het pairs) to produce homozygous unaffected (controls), homozygous affected (Otop1), and heterozygous (breeding pairs) mice for our study. The horizontal and vertical VOR responses were measured in each animal on days 3-5 (acute) and days 28-32 (chronic) after unilateral labyrinthectomy. For each of the two time points, i.e. acute and chronic, the horizontal and vertical responses were measured in pseudo-randomised order over separate days. These data were compared with the horizontal and vertical baseline VOR responses of control and Otop1 mice described in the accompanying study by Khan et al. (2018).

To facilitate head immobilization during eye movement recording, we implanted a light-weight (0.5 grams), low-profile head-post adapter plate onto the skull under general anaesthesia at least seven days before unilateral labyrinthectomy. The exact implantation technique has been described previously (Hübner et al., 2014, 2017; Khan et al., 2017).

All surgical and experimental procedures were approved by the Animal Care and Ethics Committee of the University of New South Wales and were in strict compliance with the Australian Code of Practice for the Care and USE of Animals for Scientific Purpose.

Unilateral Labyrinthectomy

Surgical preparation and postoperative care after unilateral labyrinthectomy has been described in detail by Hübner et al. (2015). Briefly, anaesthesia was introduced using a mix of isoflurane (1.5-3%) and oxygen (2-3 l/min). Once anaesthetized, a subcutaneous injection of 0.003 ml of carprofen (5 mg/ml) was given as an analgesic, and antibiotic treatment and 0.5 ml of sterile saline was injected intraperitoneally to prevent dehydration during surgery. The oval window and stapes footplate, the entire round window niche, and the stapedial branch of the internal carotid artery were exposed via a partial transcanal approach. The stapedial artery was cauterized and sectioned to gain access to the vestibular organ. After removal of the stapes, gentle suction was used to aspirate endolymph and perilymph fluid exiting the vestibule. The edges of the oval window were out-fractured, and the sensory epithelia of the utricle, as well as those of the lateral and anterior semicircular canals, were excised with the use of fine biological forceps. The saccule was destroyed mechanically by aspiration of the medial aspect of the vestibule. Suction applied to the opening of the lateral and anterior canals destroyed the posterior crista. After complete destruction of the vestibular end organ, the vestibule and the cavity were packed with Gelfoam soaked in 1 µl of gentamicin-buffered solution (20 mg/ml). Analgesic treatment using subcutaneous injection of buprenorphine (twice a day) was continued for up to 3 days post-surgery or until the animal recovered. Mice regained consciousness 10–15 min after inhalation anesthesia was discontinued.

Once awake both control and Otop1 mice displayed clearly deviated posture and strong tendency to body roll about their main axis towards the lesioned ear. These behaviours were not evident prior to labyrinthectomy. In addition, both mouse types displayed clearly visible spontaneous nystagmus with quick-phase eye movements beating away from the lesioned side. For control mice, body roll, circling, and spontaneous nystagmus usually ceased two days after labyrinthectomy; whereas in Otop1 mice, body roll when picked up by the tail and circling persisted.

Data Acquisition

The method of recording three dimensional binocular eye movements with the use of high-speed video-oculography and the techniques used for the offline analysis of VOR responses have been described previously (Migliaccio et al., 2005, 2010, 2011; Hübner et al., 2013, 2014, 2015, 2017, 2018; Khan et al., 2017). In brief, the VOG system tracks a marker array that is placed onto each eye, which allows accurate measurement of eye movement components in all three dimensions: horizontal, vertical and torsional (about the line-of-site). All VOR data were recorded using two high-speed infrared sensitive cameras (DX-COL-CS, Point-Grey, Canada), each one operating at 200 frames per second to capture binocular 3D eye movements. Calibration consisted of aligning the camera's optical axis with the centre of corneal curvature and calculating the pixel size in mm by using the known distance (200 μm) between squares on the marker array (Migliaccio et al., 2005). VOR responses to horizontal (rotation about Earth-vertical axis parallel to dorso-ventral axis) and vertical (rotation about Earth-vertical axis parallel to inter-aural axis, left-ear down [LED] and right-ear-down [RED]) whole-body sinusoidal oscillations at 0.2, 0.4, 0.5, 0.8, 1, 1.6, 2, 5 and 10 Hz with peak-velocities of 20, 50, and 100°/s were measured acutely and chronically after unilateral labyrinthectomy.

175 ***Data analysis***

176 Eye and head rotation data were converted to rotation vectors in head coordinates and analysed
177 off-line. The methods of analysis are the same as those described previously (Hübner et al.,
178 2017). In brief, quick-phases were detected using the method outlined in Hübner et al. (2013,
179 2015). To avoid bias in slow-phase analyses, the values of data points between quick-phase starts
180 and ends were not interpolated or included in the mean response analysis for stimulus
181 frequencies ≤ 1 Hz. For frequencies > 1 Hz quick-phases were rare and often distorted the whole
182 cycle, so the whole cycle was removed. VOR responses to sinusoidal rotations were analyzed
183 separately for rotations towards each side (left/right for horizontal, up/down for vertical). Only
184 the eye velocity component in the same plane as the head rotation (i.e. the largest component)
185 was analysed, e.g., horizontal eye velocity during horizontal head rotation. The other two
186 components were < 10% of the largest component. The eye and head velocity traces were split at
187 the point where the full-cycle sinusoidal head velocity fit intersected the zero velocity axis,
188 which corresponds to a direction change in position (Hübner et al., 2017). Head and eye velocity
189 traces (10 to 100 cycles, depending on frequency) had least-square pure sine waves with fixed
190 frequency and variable amplitude fit to the half-cycles of head and eye velocity to compute VOR
191 gain to each side. VOR gain was calculated as the average ratio of eye/head velocity peak-
192 amplitude of the least square fits. An ideal VOR would yield a gain of +1 and phase of 0^{o}, and
193 positive phase lead denotes eye velocity leading head velocity.

194 ***Statistical Analysis***

195 Statistical analysis was performed using SPSS version 24. We used repeated measures ANOVA
196 to analyse the VOR data. The independent factors were mouse type (control, Otop1), time-point
197 (baseline, acute, chronic), stimulus peak-velocity (20, 50 and 100^{o}/s), and stimulus frequency

198 (0.2, 0.4, 0.5, 0.8, 1, 1.6, 2, 5 and 10 Hz). Dependent variables gain and phase were analysed for
199 the horizontal and vertical VOR separately using four ANOVAs. Whenever the ANOVA showed
200 a significant main effect or interaction, post hoc *t-tests* were used to identify differences within
201 and between factors. Data are reported as mean ± SD. The significance level was $P < 0.05$.
202

Results

Horizontal VOR compensation time course

There was no difference in acute horizontal VOR gain responses between ipsilesional and contralesional rotations for both mouse types (ANOVA: control, $F_{(1,4)} = 2.44$, $P = 0.19$; Otop1, $F_{(1,4)} = 5.25$, $P = 0.08$). Figure 1A shows the pooled (ipsilesional and contralesional rotation sides) acute horizontal VOR gain during sinusoidal rotations (0.2 to 10 Hz) with peak velocity 20°/s (left column), 50°/s (middle column) and 100°/s (right column) for control (solid circles) and Otop1 (open circles) mice. For both mouse types, acute horizontal VOR gains were significantly lower than baseline gains (ANOVA: control, $F_{(1,4)} = 66.25$, $P < 0.001$; Otop1, $F_{(1,4)} = 2700.04$, $P < 0.0001$). There was also a significant difference in acute horizontal VOR gains between mouse types (ANOVA: $F_{(1,8)} = 19.19$, $P < 0.005$). The only other significant factor to affect gain was stimulus frequency (ANOVA: $F_{(1,8)} = 464.83$, $P < 0.0001$), i.e. the gain increased with frequency. There was no difference between baseline and acute horizontal VOR phases (data not shown), except for an increasing phase lag at the higher frequencies (ANOVA: 2, 5 and 10 Hz; $F_{(2,16)} = 178.68$, $P < 0.0001$). There was no difference in VOR phase between mouse types (ANOVA: $F_{(1,8)} = 2.29$, $P = 0.17$).

Similar to the acute data, there was no difference in chronic horizontal VOR gain responses between ipsilesional and contralesional rotations for both mouse types (ANOVA: control, $F_{(1,4)} = 2.62$, $P = 0.18$; Otop1, $F_{(1,4)} = 1.16$, $P = 0.34$). Figure 1B shows the pooled (rotations sides) chronic horizontal VOR gains for control and Otop1 mice. For control mice, chronic horizontal gains were significantly lower than the baseline values across velocities (ANOVA: $F_{(2,8)} = 8.76$, $P < 0.02$) and frequencies (ANOVA: $F_{(8,32)} = 45.77$, $P < 0.001$). There was a significant difference between acute and chronic gains (ANOVA: $F_{(1,4)} = 14.77$, $P < 0.02$) with the

difference larger at lower frequencies (ANOVA, $F_{(8,32)} = 112.94$, $P < 0.001$) and lower velocities (ANOVA, $F_{(2,8)} = 6.61$, $P < 0.02$). For Otop1 mice, there was no significant difference between acute and chronic gains (ANOVA: $F_{(1,4)} = 0.63$, $P = 0.47$). There was no difference between baseline and chronic horizontal VOR phases (data not shown) (ANOVA: control, $F_{(1,4)} = 4.57$, $P = 0.10$; Otop1, $F_{(1,4)} = 3.53$, $P = 0.13$). Figure 1C shows the horizontal VOR gains pooled across rotation sides, velocities and frequencies, showing the baseline, acute and chronic time-point VOR gains for control and Otop1 mice.

Vertical VOR compensation time course

There was no difference in acute vertical VOR gain responses between upward and downward rotations for both mouse types (ANOVA: control, $F_{(1,4)} = 0.06$, $P = 0.82$; Otop1, $F_{(1,4)} = 0.31$, $P = 0.61$). Figure 2A shows the pooled (rotation sides) acute vertical VOR gain during sinusoidal rotations (0.2 to 10 Hz) with peak velocity 20°/s (left column), 50°/s (middle column) and 100°/s (right column) for control (solid circles) and Otop1 (open circles) mice. For both mouse types, acute vertical VOR gains were significantly lower than baseline gains (ANOVA: control, $F_{(1,4)} = 68.10$, $P < 0.001$; Otop1, $F_{(1,4)} = 900.50$, $P < 0.001$). There was also a significant difference in acute vertical VOR gains between mouse types (ANOVA: $F_{(1,8)} = 7.85$, $P < 0.05$). The other significant factors that affected gain were stimulus frequency (ANOVA: $F_{(1,8)} = 43.39$, $P < 0.001$) and velocity (ANOVA: $F_{(2,8)} = 65.28$, $P < 0.001$), with the gain increasing with frequency and velocity. There was no difference between baseline and acute vertical VOR phases (data not shown), except for an increasing phase lag at the higher frequencies (ANOVA: 2, 5 and 10 Hz; $F_{(2,16)} = 58.95$, $P < 0.0001$). There was no difference in VOR phase between mouse types (ANOVA: $F_{(1,8)} = 1.41$, $P = 0.27$).

Similar to the acute data, there was no difference in chronic vertical VOR gain responses between upward and downward rotations for both mouse types (ANOVA: control, $F_{(1,4)} = 0.25$, $P = 0.75$; Otop1, $F_{(1,4)} = 0.14$, $P = 0.73$). Figure 2B shows the pooled (rotations sides) chronic vertical VOR gains for control and Otop1 mice. For both mouse types, chronic vertical gains were significantly lower than the baseline values (ANOVA: control, $F_{(1,4)} = 25.98$, $P < 0.01$; Otop1, $F_{(1,4)} = 64.99$, $P < 0.001$) across velocities (ANOVA: control, $F_{(2,8)} = 45.41$, $P < 0.001$; Otop1, $F_{(2,8)} = 120.10$, $P < 0.001$) and frequencies (ANOVA: control, $F_{(8,32)} = 57.40$, $P < 0.001$; Otop1, $F_{(8,32)} = 23.21$, $P < 0.001$). For both mouse types, there was a significant difference between acute and chronic gains (ANOVA: control, $F_{(1,4)} = 16.58$, $P < 0.02$; Otop1, $F_{(1,4)} = 12.50$, $P < 0.02$) with the difference larger at lower frequencies (ANOVA: control, $F_{(8,32)} = 35.62$, $P < 0.001$; Otop1, $F_{(8,32)} = 40.61$, $P < 0.001$) and lower velocities (ANOVA: control, $F_{(2,8)} = 48.15$, $P < 0.001$; Otop1, $F_{(2,8)} = 78.31$, $P < 0.001$). There was a significant difference in chronic vertical VOR gains between mouse types (ANOVA: $F_{(1,8)} = 5.42$, $P < 0.05$)There was no difference between baseline and chronic vertical VOR phases (data not shown) (ANOVA: control, $F_{(1,4)} = 2.10$, $P = 0.22$; Otop1, $F_{(1,4)} = 1.00$, $P = 0.41$). Figure 2C shows the vertical VOR gains pooled across rotation sides, velocities and frequencies, showing the baseline, acute and chronic time-point VOR gains for control and Otop1 mice.

Discussion

From similar pre-surgery baseline gains, the horizontal VOR acute gain loss in control mice was 21% (significantly) smaller than in Otop1 mice, suggesting that the otoliths were contributing to the acute horizontal angular VOR in control mice. Similarly, the vertical VOR acute gain loss in control mice was 33% (significantly) smaller than in Otop1 mice. This increased difference (21% vs 33%) between mouse types for the acute horizontal and vertical VOR, suggests that the otoliths were contributing more to the acute vertical VOR than the acute horizontal VOR in control mice.

The gimbal and rotator axes passed through the stereotaxic centre of the mouse skull so that the otoliths were positioned ~3.5 mm away from the axis of rotation during both horizontal and vertical whole-body rotations (Calbrese and Hullar, 2006; Watson et al., 2012). Because both horizontal and vertical head rotations were about earth-vertical axes (for the vertical VOR animals were positioned on their sides) the utricle and saccule could predominantly sense translational, and minimally gravity, forces during sinusoidal angular rotation. For the stimuli used in this study the maximum tangential acceleration at 3.5 mm was 0.384 m/s^2 at the highest frequency and velocity, which corresponds to 0.04 g-force. Given the similarity of otolith stimulation between horizontal and vertical rotations in our study, the significantly higher difference in acute VOR gain between mouse types during the vertical VOR is likely attributable to central differences on how the horizontal and vertical VOR pathways weight otolith input. One could assume that otolith input during typical vertical angular head motion, i.e. when the head rotating about an Earth-horizontal axis when the animal starts in its normal upright position, can potentially contribute more to the angular VOR because the otoliths sweep through the gravity vector, generating a signal that can be used to encode vertical head rotation. In contrast,

during typical horizontal head motion, the otoliths can only sense translational forces and therefore provide a weaker signal encoding horizontal head rotation. If the otolith signal were centrally weighted more during vertical compared to horizontal head rotations, then this could explain how control mice acute gains decreased least for the vertical VOR.

There are relatively few mouse studies that have measured the VOR gain after unilateral labyrinthectomy using video-oculography, which is the gold standard technique (Stahl et al., 2000). The main difference between our study and two other studies that examined the VOR in mice after unilateral labyrinthectomy using video-oculography (C57Bl6: Beraneck et al., 2008; CBA129: Hübner et al., 2017) is that we did not detect a significant difference between ipsilesional and contralesional rotation horizontal VOR gains. However, albeit significant, the difference between ipsilesional and contralesional rotations was smaller in Hübner et al. (2017) compared to Beraneck et al. (2008), and smaller when compared to other mammalian species (e.g., monkey: Lasker et al., 2000). This lack of directional asymmetry, which in other species increases with frequency, was especially unexpected for the acute horizontal data where compensation mechanisms are yet to significantly complete/stabilise. One possible explanation is that compared to other mammals, mouse vestibular primary afferents have 2-4 times lower rotational sensitivity, likely due to their smaller canal size (e.g., Hullar, 2006; Yang and Hullar, 2007), suggesting that mouse canal afferents can encode a larger range of head velocities and frequencies compared to other species (Lasker et al., 2008). For example, at 4 Hz C57Bl6 regular afferents have a sensitivity of 0.13 spikes/s per ^{o}/s with baseline firing rate of 55 spikes/s (measured when the animal is stationary) and irregular afferents have a sensitivity of 0.37 spikes/s per ^{o}/s with baseline rate 37 spikes/s (See Table 3 in Lasker et al., 2008). Based on these numbers contralesional irregular afferents would be driven into inhibitory cut-off during

ipsilesional rotations of 100°/s and regular afferents at rotations of 423°/s, so one would not expect to see large directional asymmetries for head rotations below 100 °/s, i.e., VOR gain directional asymmetries are harder to elicit in mice with unilateral lesions. The present C57Bl6 control data are consistent with the CBA129 control data we collected 5 and 30 days after unilateral labyrinthectomy in a prior study (Hübner et al., 2017, see figure 2A). In that study, side asymmetries were most evident during steps of acceleration, which have higher velocity and frequency content than the sinusoidal stimuli used in the present study. One should note that for the present study the difference between ipsilesional and contralesional acute horizontal VOR gains were close to 5% significant in Otop1 mice (P = 0.08; for control mice, P = 0.19), and that a larger sample size might have detected a difference as it did in our prior study (Hübner et al., 2017). The VOR gains measured at the different frequencies and velocities in the C57Bl6 control mice in the present study followed the same pattern as observed in the CBA129 control in our prior study in that the sinusoidal VOR gain increased with frequency (up to 10 Hz) and velocity (up to 100 °/s) (Hübner et al., 2017). However, this same pattern was less pronounced in the Beraneck et al. (2008) study on day 5 post labyrinthectomy (frequencies ranging 0.2 to 4 Hz, at peak-velocity 40 /s). We have previously reported baseline horizontal and vertical VOR gains in the C57Bl6 mouse (Migliaccio et al., 2011, see figure 4). In that study, with increasing stimulus velocity the horizontal VOR changed more than the vertical VOR, also the high-frequency vertical VOR gain was greater than the horizontal gain, both characteristics of the present study. However, the overall gains were lower and the mid-frequency gain trajectories were flatter in the prior study compared to the present. This difference in part could be due to refinement of our technique, e.g., in that study the marker array placed on the eye (covering the pupil) was exposed to a visible ultraviolet light that was fixed with respect to the animal, potentially driving VOR

suppression, whereas in the present study all testing was performed under infrared lighting only, ensuring the absence of any potential suppression stimulus. A possible behavioural explanation for the flatter mid-frequency vertical VOR is that for a lateral eyed animal, such as a mouse where the eyes at rest point along an axis ~30 degrees with respect to the inter-aural axis, a pitch head movement results in a mostly torsional eye rotation. Torsional retinal image slip might be more tolerated by the mouse VOR, as it is in the human VOR (Schubert et al., 2012), because unlike horizontal and vertical image slip, the image remains on the central retinal area where photoreceptor density is highest. Consequently, there is less need for a highly-compensatory vertical VOR gain during mid-frequency head rotations, whereas at higher frequencies torsional retinal slip might be less tolerated. Another difference between the present study with those from other species and the mouse Beraneck et al. (2008) study is that we did not observe an increasing VOR gain loss with increasing frequency. This also might be explained by the mouse afferent data. The larger decrease in VOR gain at higher frequencies in other species is thought to occur because irregular afferents (thought to mediate the phasic pathway) contribute more to the VOR at higher frequencies, and so in a normal animal, results in a VOR gain boost at high frequencies (e.g., Minor et al., 1999). In a lesioned animal this boost is missing because contralesional irregular afferents quickly go into inhibitory cut-off during ipsilesional rotations, so a larger decrease in gain is seen at higher frequencies. However, because the sensitivity of mouse irregular afferents are 2-4 times lower than in other species, one would not expect to observe the same large decrease in VOR gain at higher frequencies, which is consistent with the present data.

For control mice, horizontal chronic VOR gains were 27% significantly larger than their acute gains, bringing their chronic VOR gain to 76% of baseline. In contrast, Otop1 mice did not show any significant improvement in chronic gains. The situation was different for the vertical

chronic VOR because both mouse types showed significant improvement. In magnitude, the improvement from acute to chronic gain was ~25% for both mouse types, but when the improvements were described with respect to baseline values, i.e. to control for mouse type, chronic recovery was higher in control mice compared to Otop1 mice, so that the chronic VOR gain was 86% of baseline for the control mouse, but only 66% of baseline for the Otop1 mouse. It is important to note that the magnitude of vertical VOR compensation was similar to that of the horizontal VOR, but because the vertical VOR baseline gains were lower than the horizontal gains, vertical compensation was greater when expressed as a percentage increase from baseline. Taken together these data suggest that not only does the translational otolith signal contribute to the angular VOR when the total canal signal is reduced, as occurs during unilateral labyrinthectomy, but also that the addition of an otolith signal improves VOR compensation, at least over the period between acute and chronic measures.

There could be several explanations for the poor compensation of Otop1 mice compared to controls. For example, proprioceptive input could be reduced in the Otop1 mouse as a result of the Otopetrin1 gene, which is expressed in the dorsal root ganglia (Tu et al., 2018). However, it has been shown that non-lesioned Otop1 mice have a normal capacity for balance training during low-frequency head moving tasks with a tendency to use their tails more for support and presumably proprioceptive input (de Caprona et al., 2004; for discussion on the possible effects of Otopetrin1 mutation on proprioception see Khan et al., 2018). Another possibility is that the Otopetrin1 mutation has affected the efferent vestibular system (EVS; Balaban et al., 2002) shown to be important for VOR adaptation and compensation (Hübner et al., 2015 and 2017). However, our accompanying study has shown that the magnitude of VOR adaptation in the Otop1 mouse is the same as controls suggesting the EVS is unaffected (Khan et al., 2018).

Vestibular velocity storage has been shown to depend on gravity (e.g. Laurens and Angelaki et al., 2018). We did not measure the velocity storage time constant in Otop1 mice. The typical time constant for mice is smaller at 2-4 s (e.g. Eron et al., 2015) compared to 15-25 s in primates (e.g. Raphan et al., 1979). However, it is possible that a further reduction in velocity storage could hinder compensation especially at low-frequencies, which is consistent with our data. Another possibility is that the Otop1 mouse has learnt to rely more on proprioception than control mice, which would work best for low-frequency VOR enhancement due to its long-latency. So when the Otop1 mouse is introduced to a non-familiar vestibular stimulus as in our study, the low-frequency VOR loss is exposed.

It is tempting to compare our findings from otolith deficient mice with those obtained during space flight where there is a lack of gravity. However, comparison of the present mouse data with that obtained during space flight, mainly from humans, is problematic. Those data have shown that the baseline VOR gain changes during space flight (e.g., Tomilovskaya et al., 2013) and that there are adaptive changes to velocity storage due to microgravity (e.g., Oman and Balkwill, 1993). However, during space flight the otoliths are functioning, but only stimulated when the head accelerates during linear or rotational motion and so that situation is more analogous to angular VOR testing in the control mouse, rather than the Otop1, because for both horizontal and vertical VOR measures the mouse was positioned so that its functioning otoliths did not sweep the gravity vector. Another reason why our data cannot be compared to that during space flight is that our mice were always exposed to gravity, whereas the space flight data comes after exposure to a period of non-gravity, so other adaptive processes may be occurring (e.g., Oman and Balkwill, 1993).

Data from the present study have implications for rehabilitation of patients with vestibular peripheral hypofunction. First, they suggest that recovery of the angular VOR, which is often the focus of vestibular rehabilitation, will be greater in patients that have functioning otoliths, and therefore that assessment of utricle and saccule function may have prognostic value in predicting outcomes and tailoring rehabilitation exercise regimens after activation of a unilateral vestibular implant intended to provide semicircular canal stimulation. Rehabilitation paradigms that exercise and thereby strengthen residual otolith-driven signal pathways in these patients are likely to help further improve angular VOR recovery. Our findings also have implications for the development of future vestibular prostheses, because these data show that unilateral otolith input not only augments the angular VOR response, but also provides input that facilitates VOR compensation. Addition of even a crude stimulus targeting the otolith end organs may therefore facilitate angular VOR responses to prosthetic input targeting the semicircular canals. Otolith contribution could also explain why a recent vestibular prosthesis study in monkeys showed better restoration of angular VOR compared to humans (Monkey: Sun et al., 2015; Human: Schoo et al., 2018). That study used intra-tympanic gentamicin to damage the peripheral vestibular organ resulting in significantly reduced angular VOR responses, but normal utricular and saccular responses (Sun et al., 2015). In contrast, in the human study, the otoliths were not functioning (Schoo et al., 2018).

In summary, our findings suggest that the translational otolith signal does not normally contribute to the angular VOR due to the redundancy of the otolith signal encoding head rotation; however, after unilateral labyrinthectomy the remaining labyrinth's otolith signal is used to augment the angular VOR and to help boost VOR compensation.

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Figure Legends

Figure 1. A) Pooled (across rotation sides) acute horizontal VOR gain during sinusoidal rotations (0.2 to 10 Hz) with peak velocity 20°/s (left column), 50°/s (middle column) and 100°/s (right column) for control (solid circles) and Otop1 (open circles) mice. Error bars indicates the mean ± standard deviation. **B**) Same as above except this data shows the chronic horizontal VOR. **C**) Horizontal VOR gains pooled across rotation sides, velocities and frequencies, showing the baseline, acute and chronic time-point VOR gains for control and Otop1 mice.

Figure 2. A) Pooled (across rotation sides) acute vertical VOR gain during sinusoidal rotations (0.2 to 10 Hz) with peak velocity 20°/s (left column), 50°/s (middle column) and 100°/s (right column) for control (solid circles) and Otop1 (open circles) mice. Error bars indicates the mean ± standard deviation. **B**) Same as above except this data shows the chronic vertical VOR. **C**) Vertical VOR gains pooled across rotation sides, velocities and frequencies, showing the baseline, acute and chronic time-point VOR gains for control and Otop1 mice.