The mammalian efferent vestibular system plays a crucial role in the high-frequency response and short-term adaptation of the vestibuloocular reflex

The mammalian efferent vestibular system plays a crucial role in the high-frequency response and short-term adaptation of the vestibuloocular reflex. J Neurophysiol 114: 3154–3165, 2015. First published September 30, 2015; doi:10.1152/jn.00307.2015.—Although anatomically well described, the functional role of the mammalian efferent vestibular system (EVS) remains unclear. Unlike in fish and reptiles, the mammalian EVS does not seem to play a role in modulation of primary afferent activity in anticipation of active head movements. However, it could play a role in modulating long-term mechanisms requiring plasticity such as vestibular adaptation. We measured the efficacy of vestibuloocular reflex (VOR) adaptation in α9-knockout mice. These mice carry a missense mutation of the gene encoding the α9 nicotinic acetylcholine receptor (nAChR) subunit. The α9 nAChR subunit is expressed in the vestibular and auditory periphery, and its loss of function could compromise peripheral input from the predominantly cholinergic EVS. We measured the VOR gain (eye velocity/head velocity) in 26 α9-knockout mice and 27 cba129 control mice. Mice were randomly assigned to one of three groups: gain-increase adaptation (1.5×), gain-decrease adaptation (0.5×), or no adaptation (baseline, 1×). After adaptation training (horizontal rotations at 0.5 Hz with peak velocity 20°/s), we measured the sinusoidal (0.2–10 Hz, 20–100°/s) and transient (1,500–6,000°/s²) VOR in complete darkness. α9-Knockout mice had significantly lower baseline gains compared with control mice. This difference increased with stimulus frequency (∼5% < 1 Hz to ∼25% > 1 Hz). Moreover, vestibular adaptation (difference in VOR gain of gain-increase and gain-decrease adaptation groups as % of gain increase) was significantly reduced in α9-knockout mice (17%) compared with control mice (53%), a reduction of ∼70%. Our results show that the loss of α9 nAChRs moderately affects the VOR but severely affects VOR adaptation, suggesting that the EVS plays a crucial role in vestibular plasticity.

Despite considerable effort, the functional role of the mammalian efferent vestibular system (EVS) is poorly understood. Anatomically, the EVS is a well-documented and extensive efferent pathway from the brain stem to the inner ear that can modify afferent output of the peripheral vestibular organs (Gacek and Lyon 1974; Goldberg and Fernandez 1980; Highstein 1991; Marco et al. 1993; Purcell and Perachio 1997). In some nonmammalian species, i.e., fish and reptiles, the EVS prepares the vestibular organs in anticipation of active head movements with gaze shift, presumably in an effort to suppress the vestibuloocular reflex (VOR) response (Brichta and Goldberg 2000; Highstein 1991; Highstein and Baker 1985). However, this does not seem to be the role of the mammalian EVS (Cullen and Minor 2002; Sadeghi et al. 2007). A study by Cullen and Minor (2002) showed that the resting discharge rate and rotational sensitivity of semicircular canal afferents during different conditions of head and eye movement did not change, suggesting that the mammalian EVS did not play a role in modifying primary vestibular afferent signals over the short time course of their experiment.

Two findings support the hypothesis that the mammalian EVS modulates afferent activity during longer processes requiring plasticity, such as vestibular adaptation and compensation. First, contralateral and ipsilateral vestibular efferent neurons have extensive collateral projections to cerebellar flocculus and ventral paraflocculus, two regions known to be crucial for vestibular plasticity (Shinder et al. 2001). Second, changes have been observed in the proportion of tonic vs. phasic central vestibular neurons after unilateral labyrinthectomy, consistent with an increase in the proportion of irregular-to regular-discharging afferents (Beraneck et al. 2003, 2004; Beraneck and Idoux 2012; Cullen et al. 2009; Pfanzelt et al. 2008; Sadeghi et al. 2007; Straka et al. 2005). A prime candidate driving these peripheral changes could be the EVS (Sadeghi et al. 2007).

Minor et al. (1999) suggested that vestibular signal processing predominantly occurs along two pathways: a velocity-sensitive pathway with tonic dynamics and an acceleration-sensitive pathway with phasic dynamics (Clendaniel et al. 2001, 2002; Lasker et al. 1999, 2000; Migliaccio et al. 2003, 2004, 2008; Minor et al. 1999). This model is based on primate data and has been shown to account for the normal VOR response (Migliaccio et al. 2003; Minor et al. 1999), VOR compensation (Lasker et al. 1999, 2000), VOR adaptation (Clendaniel et al. 2001, 2002), and VOR viewing-context modification (Migliaccio et al. 2004, 2008). These studies demonstrated that VOR changes were predominantly mediated by the highly modifiable phasic pathway. The dynamic responses (sensitivity and latency) of these tonic and phasic pathways resemble those of regular- and irregular-discharging vestibular primary afferents (Hullar et al. 2005; Hullar and Minor 1999) as well as vestibular nuclei neurons (Dickman and Angelaki 2004). If the mammalian EVS modulates the relative contribution of the phasic pathway by controlling the proportion of regular/irregular activity, then it could directly control vestibular plasticity. The mammalian EVS was shown to exert its largest effects on irregular-discharging (phasic) afferents,
which is consistent with this notion (Goldberg and Fernandez 1980; Marlinski et al. 2004).

We hypothesize that a compromised mammalian EVS results in reduced vestibular plasticity, so we tested VOR adaptation in the α9-knockout mouse. This knockout strain carries a missense mutation of the gene encoding the α9 nicotinic acetylcholine (ACh) receptor (nAChR) subunit found in efferent synapses, which alters peripheral input from the EVS. Apart from a compromised EVS, these mice have no other obvious phenotype and show no difficulties with balance, movement, or vision (Terreros et al. 2015; Vetter et al. 1999). Previous studies have shown that 1) α9 nAChR subunits are specifically expressed in the vestibular and auditory periphery, 2) ACh is the predominant neurotransmitter of vestibular efferents and functions by activating nicotinic receptors (nAChRs) located in the vestibular periphery, and 3) ACh can induce inhibition and/or excitation of vestibular afferents (Anderson et al. 1997; Elgoyhen et al. 1994; Hiel et al. 1996; Holt et al. 2001; Jagger et al. 2000; Katz et al. 2000; Vetter et al. 1999; Zhou et al. 2013). For a review about efferent synaptic mechanisms see Jordan et al. (2013). The cholinergic component of EVS activation likely functions by inhibition of type II hair cells (i.e., strictly a reduction of resting discharge rate and attenuation of sensitivity/gain) via α9 nAChRs coupled to calcium-activated potassium (SK) channels (Holt et al. 2006; Poppi et al. 2014) and excitation of afferents (Boyle and Highstein 1990; Goldberg and Fernandez 1980), through nAChRs that contain α4, α6, and β2 subunits (Holt et al. 2015). In α9-knockout mice this inhibition/excitation dual effect would be partially compromised. Nonfunctional α9 nAChRs would prevent EVS inhibition of type II hair cells. However, because of the presence of alternative types of nAChRs (i.e., α4, α6, and β2 subunits) on calyx-bearing afferents, the excitatory afferent effect could still operate. Our results suggest that the loss of the α9 nAChR subunit moderately affects the VOR but severely affects VOR adaptation, suggesting that the EVS plays a crucial role in vestibular plasticity.

MATERIALS AND METHODS

Animal groups and surgical preparation. We obtained data from 26 α9-knockout mice and 27 control mice (both sexes, aged 11–14 wk). The mouse strain carrying the α9-knockout mutation has been maintained on a CBA/CaJ × 129/SvEv background line by The Jackson Laboratories (stock no. 005696). We set up an independent colony of these mice at the facilities of the University of New South Wales. We obtained data from 26 α9-knockout mice and 27 control mice (both sexes, aged 11–14 wk). The mouse strain carrying the α9-knockout mutation has been maintained on a CBA/CaJ × 129/SvEv background line by The Jackson Laboratories (stock no. 005696). We set up an independent colony of these mice at the facilities of the University of New South Wales. We then drilled three guide holes into the skull (2 lateral of bregma and 1 lateral of lambda) and inserted three stainless steel anchoring screws (no. 0 × 1/8, Micro Fasteners, Thomastown, VIC, Australia). A lightweight countersunk metal screw was placed with the flat head down between the three anchoring screws, and all were embedded in a thick layer of dental composite (Protemp IV, 3M). Also, while mice were still under anesthesia, we shortened eyelashes and vibrissae to minimize irritation and to facilitate placement of the marker arrays onto the eyes immediately prior to VOR testing and after VOR adaptation training. After surgery animals were allowed to recover for 2 h in a separate cage before they were restrained and placed on the rotator platform.

All surgical and experimental procedures were approved by the Animal Care and Ethics Committee of the University of New South Wales.

Adaptation and eye movement recording. Upon full recovery, mice were restrained in a close-fitting plastic capsule and both capsule and animal were mounted on a rotary platform driven by a high-torque servomotor (GOLDLINE DDR D083, Danaher). The head pedestal was pitched ~30° “nose down” so that the rotation of the servomotor maximally stimulated the horizontal semicircular canals (Calabrese and Hullar 2006). To evoke adaptation of the VOR we used a custom-built planetarium projector system, which projected a random pattern of light spots onto a dome surrounding the animal. The projector unit was driven by a small high-resolution servomotor, which was synchronized with the rotary platform with an electronic gearing system (latency < 0.1 ms). This system has been successfully used in previous adaptation studies in our laboratory (see Hübner et al. 2014).

For VOR adaptation we chose a sinusoidal vestibular stimulus at 0.5 Hz with peak velocity of 20°/s. These parameters were chosen as optimal based on our experience and reports from other laboratories (De Zeeuw et al. 1998; Kimpo et al. 2005). The visual projector was set to rotate in the opposite direction of the vestibular stimulus with amplitude of 1.5× (gain increase) and 0.5× (gain decrease) of the vestibular stimulus velocity. We kept adaptation training to one 40-min session.

After VOR adaptation was completed, we measured VOR gain in complete darkness with a binocular three-dimensional video-oculography system (Hübner et al. 2013, 2014; Migliaccio et al. 2005, 2010a). To facilitate recording we placed marker arrays onto both eyes, which allowed us to accurately measure VOR eye movement components in all three dimensions: horizontal, vertical, and torsional. Because the marker arrays are affixed to the eye with cyanoacrylate, removal causes temporary corneal swelling that likely affects vision. To ensure ideal vision during adaptation training we recorded predadaptation VOR gains in a separate group of mice, rather than comparing pre- and postadaptation gains within the same mouse.

We tested the VOR in response to horizontal whole body: sinusoidal oscillation 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 and transient acceleration stimuli at 1,500, 3,000, and 6,000°/s² reaching a velocity plateau of 100, 150, and 300°/s, respectively. We refer to transient stimulus conditions with the abbreviations 1.5k100, 3k150, and 6k300, respectively. Data analysis. To analyze the three-dimensional VOR data, we converted eye movements acquired in eye coordinates into rotation vectors in head coordinates. Eye velocities traces with quick phases were removed because they are not part of the VOR. A radical oscillation 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 and transient acceleration stimuli at 1,500, 3,000, and 6,000°/s² reaching a velocity plateau of 100, 150, and 300°/s, respectively. We refer to transient stimulus conditions with the abbreviations 1.5k100, 3k150, and 6k300, respectively. Data analysis. To analyze the three-dimensional VOR data, we converted eye movements acquired in eye coordinates into rotation vectors in head coordinates. Eye velocities traces with quick phases were removed because they are not part of the VOR. A
sine waves with fixed frequency and variable amplitude and phase. VOR gain was then calculated as the average ratio of eye/head velocity peak amplitude of the least-square fits. To analyze the effects caused by changes in stimulus acceleration we calculated the first derivative of the eye and head velocity signal for each stimulus frequency-velocity combination. Peak accelerations that occurred at all three test stimulus peak velocities (20, 50, and 100°/s, at respective frequencies) were compared.

For transient steps of acceleration, we fit least-square linear regressions to the constant-acceleration and constant-velocity part of eye and head velocity traces. Using these fits, we calculated three parameters: acceleration gain ($G_a$), constant-velocity gain ($G_v$), and latency.

![Graphs A1 and A2](image1.png)

![Graphs B](image2.png)

![Graphs C1 and C2](image3.png)
 ROLE OF MAMMALIAN EVS IN VOR ADAPTATION

G, was calculated as the average ratio of eye/head acceleration (using the slopes of the constant-acceleration fit). G, was calculated as the average ratio of eye/head velocity (using the point-by-point offset of the constant-velocity fit) during the 200 ms to 400 ms interval after stimulus onset.

Quick-phase eye movements are closely related to saccadic eye movements, and so their duration and peak velocity can be related to their amplitude to generate a “main sequence” (Stahl et al. 2006). We analyzed quick phases for sinusoidal stimuli at 0.2, 0.4, 0.5, 0.8, and 1 Hz at 20, 50, and 100°/s. Quick phases were either removed or extracted with a semiautomatic desaccading technique that we optimized based on several published algorithms (Fauconneau et al. 2007; Migliaccio et al. 2006). However, instead of manually adjusting the acceleration threshold for each individual trace, we automated the process by fixing the acceleration threshold at 5,000°/s². Amplitude was defined as the difference between mouse type baseline gains or phases.

RESULTS

Baseline VOR response to sinusoidal rotation. Figure 1, A1 and A2, show superimposed VOR responses to sinusoidal rotations (1 Hz at 100°/s) for one cba129 (control) mouse and one α9-knockout mouse prior to adaptation training. We measured the baseline VOR response (no prior adaptation) in α9-knockout and cba129 mice. Figure 1B shows a direct comparison of cba129 and α9-knockout baseline VOR gain (top) and phase (bottom) across frequencies 0.2–10 Hz and velocities of 20, 50, and 100°/s. For both mouse types the VOR gain significantly increased with stimulus peak velocity for quick-phase amplitude, peak velocity, and duration. All variables were included in the model initially, and those found insignificant were subsequently removed. Post hoc tests were performed with t-tests with multiple-comparison correction in case of significant ANOVA results. Probability values ≥ 0.001 are reported with numeric value; the rest are reported as < 0.001. Unless otherwise stated all results are reported as means ± SD.

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Fig. 1. A: the vestibuloocular reflex (VOR) response to sinusoidal rotations (1 Hz at 100°/s) for 1 cba129 (control) mouse (A1) and 1 α9-knockout mouse (A2) prior to adaptation training. Eye velocity (dark gray) is inverted to allow easier comparison with head velocity. For each stimulus frequency 10–100 individual cycles were superimposed and quick-phase eye movements (light gray) were removed. Least-square pure sine waves were fit to the head velocity stimuli and eye velocity responses to calculate VOR gain and phase. B: baseline VOR gain (top) and phase (bottom) in cba129 control mice and α9-knockout mice. The VOR was measured for frequencies from 0.2 to 10 Hz at peak-velocities of 20, 50, and 100°/s. Error bars indicate mean ± SE. Unlike cba129, α9-knockout mice did not show a characteristic increase in VOR gain for frequencies < 1 Hz at peak velocity of 20 and 50°/s. In addition, α9-knockout mice had significantly lower VOR gains at frequencies > 1 Hz, at all 3 test peak velocities. VOR phase in α9-knockout mice significantly differed from cba129 at low and high frequency extremes. At frequencies < 0.8 Hz at peak velocity 20°/s VOR phase was less than in control mice, while at 10 Hz at all 3 test peak velocities VOR phase was more than in control mice. C: comparison of baseline VOR gain across sinusoidal test stimulus peak velocities (C1) and peak accelerations (C2). Peak acceleration was calculated using the 1st derivative of the velocity stimulus profile. Unlike cba129 mice, the VOR gain decreased as stimulus peak acceleration increased in α9-knockout mice, whereas the VOR gain increased as peak velocity increased in both mouse types but more so in α9-knockout mice. *Significant difference between mouse type baseline gains or phases.
gain of α9-knockout mice stayed at 0.74 ± 0.09 and 0.8 ± 0.13 at 0.2 and 1.6 Hz, respectively.

In addition to the difference in VOR gains between mouse types over the 0.2–1 Hz frequency range, α9-knockout mice had significantly lower VOR gains than cba129 mice at frequencies >1 Hz. At 20°/s the VOR gain in α9-knockout mice remained low over the whole range of test frequencies (0.2–10 Hz). In comparison, at 50 and 100°/s there was a frequence threshold of 1.6–2 Hz above which a pronounced VOR gain reduction in α9-knockout mice became apparent (see Fig. 1B, top). The average difference between cba129 and α9-knockout mice at frequencies >2 Hz was 0.23 ± 0.02 (25% difference) \( [t_{194.56} = 10, P < 0.001] \).

A difference between cba129 and α9-knockout mice was also observed when baseline VOR gains were compared across stimulus peak velocities (Fig. 1C1) and peak accelerations (Fig. 1C2). VOR gains of both mouse types increased as peak velocity increased. This effect was more pronounced in α9-knockout compared with cba129 mice. Peak acceleration did not affect VOR gains in cba129 mice but had an effect on the VOR gain of α9-knockout mice. The latter showed significantly decreased VOR gain as peak stimulus acceleration increased.

While the general phase response was similar between cba129 and α9-knockout mice, there were differences in VOR phase at both low and high frequency extremes (see Fig. 1B, bottom). For stimulus frequencies <0.8 Hz with stimulus peak velocity 20°/s there was a clear decrease in the phase lead of α9-knockout mice. This decrease was most pronounced at 0.2 Hz, the lowest frequency tested, with a phase lead of 29.44 ± 6.93° in cba129 mice vs. 21.70 ± 4.88° in α9-knockout mice \( [t_{32.08} = 3.86, P < 0.001] \). A similar effect was observed at 10 Hz at all three test stimulus peak velocities. cba129 mice showed a steadily increasing phase lag with zero-phase crossover at ~3 Hz and a maximum phase lag of ~11.01 ± 3.25° at 10 Hz. α9-knockout mice also demonstrated increasing phase lag with frequency with zero-phase crossover at ~2 Hz, but the maximum phase lag was only ~5.68 ± 5.06° at 10 Hz. This difference of ~5.33 ± 0.81° was statistically significant \( [t_{96.56} = -6.58, P < 0.001] \).

**Baseline VOR response to transient steps of acceleration.** Figure 2A shows horizontal VOR response to transients high-acceleration whole body rotations (acceleration 1.5k and velocity 100°/s) for one cba129 mouse and one α9-knockout mouse prior to adaptation training. We measured the baseline acceleration gain \( (G_{\text{Pre}}) \) during the initial “constant-acceleration part” of transient step stimului. cba129 mice had an average \( G_{\text{Pre}} \) of 1.14 ± 0.19. In contrast, the average \( G_{\text{Pre}} \) of α9-knockout mice was significantly lower at 1.02 ± 0.21 \( [t_{1,24} = 4.445, P = 0.037] \) (see Fig. 2B, left). This difference in \( G_{\text{Pre}} \) between mouse strains was similar for all three stimulus accelerations [interaction “Strain × Acceleration”: \( F_{(2,48)} = 0.222, P = 0.802 \)].

We also analyzed the prepredation VOR gain response during the “constant velocity plateau” \( (G_{\text{Vpre}}) \) of transient step stimulus. There was no difference in \( G_{\text{Vpre}} \) between α9-knockout and cba129 mice [factor “Species”: \( F_{(1,24)} = 2.275, P = 0.144 \)]. Also, the interaction between mouse strain and velocity was not significant [interaction “Strain × Velocity”: \( F_{(1,50)} = 0.628, P = 0.432 \)]. The average \( G_{\text{Vpre}} \) of cba129 and α9-knockout mice was 0.82 ± 0.2 and 0.79 ± 0.27, respectively.

\( G_{\text{Vpre}} \) was similar for stimulus velocities of 100 and 150°/s, with an average gain of 0.87 ± 0.2 (pooled α9-knockout and cba129 mice), but decreased to 0.65 ± 0.26 when tested at 300°/s.

**VOR quick-phase main sequence.** Quick-phase eye movements were typically observed during sinusoidal frequencies ≤1 Hz. Figure 1A shows superimposed quick-phase responses to sinusoidal rotations (1 Hz at 100°/s) for one cba129 (control) mouse and one α9-knockout mouse prior to adaptation training.

There was no difference in the number of quick phases per stimulus cycle between α9-knockout and cba129 mice \( [F_{(1,29)} = 3.83, P = 0.063] \). The mean quick-phase amplitude for cba129 and α9-knockout mice was ~8°, showing no significant difference \( [t_{(1798)} = 1.676, P = 0.094] \). The mean quick-phase peak velocity for cba129 and α9-knockout mice across stimulus conditions was ~450°/s with median ~400°/s, showing no significant difference \( [t_{(1798)} = 0.0502, P = 0.960] \). The slope of the linear fit between quick-phase peak velocity and amplitude for cba129 and α9-knockout mice was not significant \( [t_{(1798)} = -1.11, P = 0.268] \).

In cba129 mice the mean quick-phase duration was 45.36 ± 22.83 ms, whereas for α9-knockout mice it was ~10% faster at 41.12 ± 20.93 ms \( [t_{(1798)} = 4.073, P < 0.001] \). The \( K \) and \( \tau \) terms in the nonlinear fit between quick-phase duration and amplitude were similar between cba129 and α9-knockout mice \( [K_{(1,1798)} = 0.43, P = 0.671; \tau_{(1,1798)} = -0.34, P = 0.734] \).
α9-Knockout mice, compared with cba129 mice (controls), showed significantly reduced adaptation across all test frequencies and velocities. At 0.5 Hz and 20°/s, the same stimulus used during adaptation training, we measured an overall effect of adaptation training of 0.45 ± 0.05 in cba129 mice but only 0.12 ± 0.07 in α9-knockout mice, a reduction of 0.33 (73%) \([t(12.93) = −1.76, P = 0.10]\). The average reduction at 20°/s when calculated across all tested frequencies was 0.26 (65%).

Similar to cba129, α9-knockout mice demonstrated velocity-selective adaptation behavior. The largest overall adaptation effect was measured at 20°/s, which was the velocity used during adaptation training \(F_{(1,13)} = 3.90, P = 0.07\). Frequency did not affect adaptation \(F_{(1,112)} = 0.33, P = 0.57\). Overall adaptation was governed purely by gain-increase adaptation. No gain-decrease adaptation was observed at either velocity. At 50°/s overall adaptation was minimal at 0.11 ±

Fig. 2. Baseline VOR in response to transient steps of acceleration. A: example of overlaid slow-phase eye velocities (inverted; gray) in response to sudden steps of acceleration (1,500°/s²) followed by a constant velocity plateau (100°/s) in 1 cba129 (control) mouse (A1) and 1 α9-knockout mouse (A2). Individual VOR gains were measured during the constant velocity part and constant acceleration part of the stimulus. Latency of the VOR response was determined with the zero velocity intercept of head acceleration and eye acceleration linear fits. B: acceleration gain (GA; left) and velocity gain (GV; right) in response to 3 acceleration and velocity stimuli. α9-Knockout mice demonstrated significantly lower GA compared with cba129 mice at 6,000°/s². No difference between mouse types was observed for GV. *Significant difference between mouse type baseline gains.
0.03 \( [F_{(1,13)} = 1.03, P = 0.33] \), a reduction of 0.11 (50%) compared with cba129 mice. At 100°/s no adaptation occurred \( [F_{(1,13)} = 0.09, P = 0.76] \). The generalization index for gain-increase adaptation across test velocities was 0.49 ± 0.34, which is considerably more generalized compared with results in cba129 mice.

Unlike the VOR gain response, there was no significant difference in VOR phase between cba129 and α9-knockout
mice \( F(1,27) = 0.01, P = 0.93 \). We did not observe an independent effect of adaptation training on VOR phase \( F(1,27) = 1.56, P = 0.22 \). However, for both mouse types there was a phase-crossover between their gain-increase and gain-decrease response curves at 0.5 Hz and 20\( \times \)s, the exact stimulus parameters used during adaptation training (Fig. 3D) \( F(1,534) = 2.81, P = 0.094 \), which was not seen at stimulus peak velocities of 50 and 100\( \times \)s.

VOR response to transient steps of acceleration following adaptation training. In cba129 mice we observed a pronounced adaptation effect for the acceleration gain \( G_A \) for all three acceleration stimuli (see Fig. 4, top left). The average difference of \( G_A \) between gain-increase vs. gain-decrease adaptation was 0.12 ± 0.03 \( t(145.93) = -4.12, P < 0.001 \). Post hoc pairwise comparison revealed that this difference was mostly due to gain-decrease adaptation resulting in \( G_A \) significantly lower than baseline for all three acceleration stimuli (1.5k: \( P < 0.001 \), 3k: \( P < 0.001 \), 6k: \( P < 0.001 \)). In contrast, \( \alpha \)-9-knockout mice demonstrated adaptation of \( G_A \) compared with baseline only at the 6k acceleration stimulus (gain decrease: \( P < 0.01 \), gain increase: \( P < 0.01 \); Fig. 4, bottom left), with \( G_A \) decreasing after both gain-decrease and gain-increase adaptation training. Analysis using ANOVA indicated that the interaction effect between “Mouse Strain” and “Adaptation Training” was significant \( F(4.98) = 2.57, P = 0.043 \).

In cba129 mice we observed a pronounced adaptation effect for the velocity gain \( G_V \) for all three velocity stimuli (Fig. 4, top right). Post hoc pairwise comparison revealed that this difference was mostly due to gain-decrease adaptation resulting in \( G_V \) significantly lower than baseline for all three velocity stimuli (100: \( P < 0.05 \), 150: \( P < 0.05 \), 300: \( P < 0.05 \)). In contrast, \( \alpha \)-9-knockout mice did not demonstrate adaptation of \( G_V \) at any test velocity stimulus.

**DISCUSSION**

We sought to determine whether the EVS affects vestibular adaptation. Our findings suggest that the EVS has minimal to no effect on the oculomotor system, moderately affects the VOR gain, but severely affects VOR adaptation. The main sequence was similar between \( \alpha \)-9-knockout and control mice (cba129), suggesting that the quick-phase/saccadic oculomotor gain-decrease adaptation the angular velocity of the projected light spots was set to 1.5

| **Fig. 3. VOR response following visual-vestibular mismatch adaptation. A:** a custom-built planetarium projector unit mounted beneath the head of the mouse was used to project a pattern of random light spots onto a surrounding dome surface. After adaptation this dome was removed and 2 high-speed cameras were used to record binocular 3D eye movements. B: the projected light spots moved synchronously with the head but in the opposite direction. During gain-increase and gain-decrease adaptation the angular velocity of the projected light spots was set to 1.5 \( \times \) and 0.5 \( \times \) the head velocity stimulus. C: both cba129 (top) and \( \alpha \)-9-knockout mice (bottom) demonstrated maximal adaptation at 20\( \times \), the velocity used during adaptation training. However, in \( \alpha \)-9-knockout mice the overall adaptation effect (difference between gain-increase vs. gain-decrease VOR gains) was ~70% smaller compared with cba129. This was true across all test stimulus frequencies. D: VOR phase in both mouse types showed a crossover between gain-increase and gain-decrease responses at 0.5 Hz and 20\( \times \), the same stimulus parameters used during adaptation training (black arrow). E: comparison of gain-increase (○) and gain-decrease (●) VOR gains across all test velocities (frequency pooled). The baseline VOR response is illustrated as a dashed line (mean) surrounded by gray shading (SD). cba129 mice showed strong velocity-selective VOR adaptation. At 20\( \times \) the effect of adaptation (difference between gain-increase vs. gain-decrease VOR gains) was maximal. As velocity increased the effect of adaptation continuously decreased. Similar velocity selective effects were also observed in \( \alpha \)-9-knockout mice. However, in \( \alpha \)-9-knockout mouse the adaptation effect was minimal at 20\( \times \) and disappeared at higher velocities. *Significant difference between post gain-decrease and post gain-increase adaptation training gain. **Significant difference between baseline gain and post gain-increase adaptation training gain. *Significant difference between baseline gain and post gain-decrease adaptation training gain. |
both type I and type II hair cells (Baird et al. 1988). As shown in turtles (Holt et al. 2006), and more recently in mammals (C57BL/6 and cba129 mice: Poppi et al. 2014), EVS activation, specifically the cholinergic component, is thought to have a dual effect. One effect of EVS activation is the inhibition of type II hair cells (i.e., strictly a reduction of resting discharge rate and attenuation of sensitivity/gain) via $\alpha_9$ nAChRs coupled to SK channels (Holt et al. 2006; Poppi et al. 2014). The other effect is the excitation of afferents (Boyle and Highstein 1990; Goldberg and Fernandez 1980), through nAChRs that contain $\alpha_4$, $\alpha_6$, and $\beta_2$ subunits (Holt et al. 2015). In $\alpha_9$-knockout mice this inhibition/excitation dual effect would be partially compromised. Since $\alpha_9$ nAChRs are nonfunctional in these knockout mice, this would prevent EVS inhibition of type II hair cells seen in control mice. This lack of inhibition would allow the normally suppressed type II hair cells to “contribute” to the overall afferent activity (particularly dimorphs) during EVS activation in $\alpha_9$-knockout mice. Simultaneously, because of the presence of alternative types of nAChRs (i.e., $\alpha_4$, $\alpha_6$, and $\beta_2$ subunits) on calyx-bearing afferents, the excitatory EVS effect would still be operating in $\alpha_9$-knockout mice. In short, the predicted overall effect of EVS activation on dimorphic afferents in $\alpha_9$-knockout mice is increased afferent discharge but with an additional input from normally EVS-suppressed type II hair cells (i.e., increased type II hair cell gain and resting discharge rate compared with controls) leading to an increase in afferent regularity and corresponding shift in afferent dynamic response. This hypothesis is supported by a preliminary single-unit vestibular afferent study in the $\alpha_9$-knockout mouse (Han et al. 2007) that compared the single-unit vestibular afferent response during 2-Hz whole body rotations between $\alpha_9$-knockout and C57BL/6 mice (note that C57BL/6 is not the appropriate control background strain for the $\alpha_9$ knockout). That study reported three major differences between these two mouse types. First, regularly (tonic) discharging afferents (defined as having a CV* < 0.1; CV* is the normalized coefficient of variation in the interspike interval of afferent background discharge) were more sensitive to head rotations, i.e., for the same head velocity the firing

Fig. 4. Acceleration gain ($G_A$; left) and constant-velocity gain ($G_V$; right) after sinusoidal visual-vestibular mismatch adaptation training. cba129 mice (top) demonstrated pronounced changes in $G_A$ after adaptation training with a significant effect of adaptation, measured as the difference between gain-increase and gain-decrease conditions. This difference was mostly due to gain-decrease adaptation resulting in significantly lower than baseline gains at all stimulus conditions. In contrast, $\alpha_9$-knockout mice (bottom) showed adaptation of $G_A$ only at 6k acceleration but none for $G_V$ at any stimulus condition. Significant difference: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. 

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Can changes in EVS activity affect central adaptation mechanisms? In addition to the vestibular sensory neuropetithelium, EVS neurons project collaterals to the flocculus and ventral parafloucculus bilaterally (Shinder et al. 2001). These collateral projections suggest that the EVS may directly influence the part of the cerebellum involved with regulation and adaptation of the VOR, specifically, the Purkinje cells of the flocculus. Purkinje cells in the floccular lobe of the cerebellum receive information about head and eye movement through parallel fiber synapses and information about image motion through climbing fibers from the inferior olive and therefore are well positioned to sense and reduce VOR error resulting in retinal slip (Ito 1982), i.e., via the modifiable pathways involving the floccular target neurons (FTNs) (Lisberger and Pavelko 1986). A well-tested theory is that cerebellar learning depends on long-term depression (LTD) of synapses from parallel fibers onto Purkinje cells (for review see De Zeeuw and Yeo 2005; Gittis and du Lac 2006). Targeted genetic disruption of LTD in Purkinje cells has little effect on baseline oculomotor function but impairs short-term learning in the VOR that is induced by visual-vestibular mismatch training (de Zeeuw et al. 1998), such as ours, so a possibility is that the EVS affects LTD in Purkinje cells. It has also been proposed that the EVS could vary the proportion of irregular to regular afferent signal input going to the FTNs (Lisberger 1994), i.e., similar to the effect the EVS might have on the firing of vestibular primary afferents. Varying the signal going to the FTNs in this way could be important for encoding and processing signals across a wide range of stimuli, i.e., avoiding signal cutoff or saturation (Goldberg 2000; Shinder et al. 2001). In addition, the proportion of irregular to regular afferent signal might need to be modified under different behavioral circumstances (Chen-Huang and McCrea 1998) or to compensate for differences in the dynamic loads of the various reflexes (Boyle et al. 2001). Therefore, the difference in VOR adaptation between \( \alpha \)-knockout and control mice may be due to not only differing primary (peripheral) vestibular afferent signals but also changes in central adaptation mechanisms.

Can decrease in proportion of irregular- to regular-firing vestibular primary afferents explain decrease in baseline high-frequency VOR response and decrease in adaptation? The observed differences in the baseline VOR response between \( \alpha \)-knockout and cba129 mice may be explained by differences in EVS activity, i.e., reduced EVS activity in the \( \alpha \)-knockout mouse leading to vestibular dimorphic afferents with discharge rate that is more regular (tonic). The nonlinear (phasic) and linear (tonic) components of the behavioral VOR can be considered as two pathways: one irregular, consisting of predominantly irregularly discharging afferents and mostly irregularly discharging (type B) medial vestibular neurons, the other regular, consisting of predominantly regularly discharging afferents and mostly regularly discharging (type A) medial vestibular neurons (e.g., Beraneck et al. 2004). This idea is supported by the observation that the transfer function of regularly discharging canal afferents fits well with the tonic component of the behavioral VOR that is predominantly dependent on stimulus (head) velocity (Minor et al. 1999). Similarly, the transfer function of irregularly discharging canal afferents fits well with the phasic component of the behavioral VOR that has a dependence on both stimulus velocity and frequency corresponding closer to head acceleration (Minor et al. 1999). It is this acceleration signal that causes the VOR gain to increase when the stimulus frequency, and consequently acceleration, increases (Minor and Lasker 2009). Thus the phasic pathway significantly “augments” the VOR response during high-acceleration stimuli, such as those with frequencies \( > 2 \) Hz. The phasic pathway may also contribute, albeit to a lesser extent, to the low-frequency VOR response; however, it has been shown that during low-frequency/amplitude sinusoids the contribution of irregularly discharging afferents is minimal (Minor and Goldberg 1991). If a compromised EVS (which we hypothesize is similar to an inhibited EVS) results in a decrease in the ratio and sensitivity of irregular-firing afferents leading to a decrease in the phasic pathway, then one would predict that the high-frequency VOR would be affected most, which is what we observed in the \( \alpha \)-knockout mouse. However, a decrease in the phasic pathway would minimally affect velocity selectivity, because velocity selectivity is likely to be mediated mostly by tonic (velocity sensitive) pathways (Hübner et al. 2014). This may explain why velocity selectivity in \( \alpha \)-knockout mice was similar to that in cba129 mice. Cullen and Minor (2002) showed in primates that the resting discharge rate and rotational sensitivity of semicircular canal afferents do not change for different conditions of head and eye movement, suggesting that tonic EVS activity does not change in mammals such as mice, at least over the short time course of an experiment such as ours. Therefore, VOR adaptation as observed in our control mice is unlikely to be due to EVS-induced short-term changes in type II hair cell gain or afferent background discharge rate as shown to occur in fish and reptiles. Rather, the adaptation differences are likely due to preexisting differences in EVS activity before training (which affects the regularity of afferents) and due to differences in the way irregular and regular pathway signals are processed centrally. Rather than inducing short-term changes in the behavioral VOR, we hypothesize that the normal EVS shifts the regularity of dimorphic afferents over a long time course as part of the process of maintenance and calibration (Sadeghi et al. 2007), i.e., not during short-term adaptation or other tasks requiring immediate changes in VOR response (e.g., Cullen and Minor 2002).

Taken together, our data suggest that unlike the EVS in fish and reptiles that modulates the primary afferent firing rate during large or active head movements, the mammalian EVS changes the proportion of irregular- to regular-discharging afferents. We hypothesize that the EVS changes occur over a long time course as part of the process of maintenance and calibration. When the EVS activity is significantly reduced, the contribution from the highly plastic phasic pathway is also reduced, and this leads to a reduction in behavioral vestibular plasticity as shown in this study. Our results not only provide a pivotal contribution toward understanding the role of the mammalian EVS but also identify for the first time a crucial part of the vestibular system that upon stimulation, e.g., elec-
trically or via EVS agonists, could potentially boost vestibular plasticity.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: P.P.H. and S.I.K. analyzed data; P.P.H. and A.A.M. interpreted results of experiments; P.P.H. prepared figures; P.P.H. and A.A.M. drafted manuscript; P.P.H., S.I.K., and A.A.M. edited and revised manuscript; P.P.H., S.I.K., and A.A.M. conceived and designed research.

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