Role of the mammalian EVS in VOR adaptation

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

Patrick P. Hübner\textsuperscript{1,2}, Serajul I. Khan\textsuperscript{1,2} and Americo A. Migliaccio\textsuperscript{1,2,3}

\textsuperscript{1}Balance and Vision Laboratory, Neuroscience Research Australia, Sydney, NSW, 2031, Australia
\textsuperscript{2}University of New South Wales, Sydney, NSW, 2033, Australia
\textsuperscript{3}Department of Otolaryngology - Head and Neck Surgery, Johns Hopkins University, Baltimore, MD, 21205, USA

running Head: Role of the mammalian EVS in VOR adaptation

\#words in abstract = 249
\#main text pages = 22
\#figures = 4
\#tables = 0

Address for correspondence
Associate Professor Americo Migliaccio
Balance and Vision Laboratory
Neuroscience Research Australia
Cnr Barker Street & Easy Street
Randwick, 2031, NSW, Australia
Tel: +61 2 9399 1030
Email: a.migliaccio@neura.edu.au
Role of the mammalian EVS in VOR adaptation

Abstract

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 vestibulo-ocular reflex (VOR) adaptation in α9-knockout mice. These mice carry a missense mutation of the gene encoding the α9 nicotinic acetylcholine receptors (nAChRs) 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 controls. Mice were randomly assigned to one of three groups: gain-increase adaptation (x1.5), gain-decrease adaptation (x0.5) or no adaptation (baseline, x1). Following adaptation training, (horizontal rotations at 0.5Hz with peak-velocity 20°/s) we measured the sinusoidal (0.2-10Hz, 20-100°/s) and transient (1500-6000°/s²) VOR in complete darkness. α9-knockout mice had significantly lower baseline gains compared to control mice. This difference increased with stimulus frequency (~5%<1Hz to ~25%>1Hz). Moreover, vestibular adaptation (difference in VOR gain of gain-increase and gain-decrease adaptation groups as a percentage of gain-increase) was significantly reduced in α9-knockout mice (17%) compared to controls (53%), a reduction of ~70%. Our results show that the loss of alpha9-nAChRs moderately affects the VOR, but severely affects VOR adaptation, suggesting the EVS plays a crucial role in vestibular plasticity.
Role of the mammalian EVS in VOR adaptation

Keywords: efferent vestibular system, vestibular adaptation, vestibular plasticity, vestibulo-ocular reflex, α9-knockout mice
Role of the mammalian EVS in VOR adaptation

**Introduction**

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 brainstem to the inner ear that can modify afferent output of the peripheral vestibular organs (Gacek and Lyon 1974; Goldberg and Fernandez 1980; Marco et al. 1993; Highstein 1991; Purcell and Perachio 1997). In some non-mammalian 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 vestibulo-ocular 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. 2006). The 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 versus phasic central vestibular neurons following unilateral labyrinthectomy, consistent with an increase in the proportion of irregular to regular discharging afferents (Beraneck et al. 2003 Beraneck et al. 2004; Beraneck and Idoux 2012; Cullen et al. 2009; Pfanzelt et al.2008; Sadeghi
Role of the mammalian EVS in VOR adaptation

et al. 2007; Sadeghi et al. 2006; Straka et al. 2005). A prime candidate driving these peripheral changes could be the EVS (Sadeghi et al., 2006).

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 and Minor 1999; Hullar et al. 2005), 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 hypothesise 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 receptor (nAChR) subunit found in efferent synapses, which alters peripheral input from the EVS. Apart from a compromised EVS
Role of the mammalian EVS in VOR adaptation

these mice have no other obvious phenotype and show no difficulties with balance, movement or vision (Vetter et al. 1999; Terreros et al., 2015). Previous studies have shown that: 1) α9 nAChR subunits are specifically expressed in the vestibular and auditory periphery; 2) acetylcholine (ACh) is the predominant neurotransmitter of vestibular efferents and functions by activating nicotinic receptors (nAChRs) located in the vestibular periphery, and 3) acetylcholine 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. 200; 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. Non-functional α9 nAChRs would prevent EVS inhibition of type II hair cells. However, due to 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 alpha9 nAChR subunit moderately affects the VOR, but severely affects VOR adaptation, suggesting that the EVS plays a crucial role in vestibular plasticity.
Role of the mammalian EVS in VOR adaptation

Materials and Methods

Animal groups and surgical preparation

We obtained data from 26 α9-knockout mice and 27 controls (both sexes, aged 11-14 weeks). The mouse strain carrying the α9-knockout mutation has been maintained on a CBA/CaJ x 129/SvEv background line by The Jackson Laboratories (Stock Number: 005696). We set up an independent colony of hybrid CBA/CaJ x 129/SvEv (from here on referred to as cba129) that we used as the controls. When the homozygous α9-knockout breeders became old, new breeders were selected from different heterozygous (cba129 x α9-knockout) breeding pairs. Both, α9-knockout and cba129 mice were randomly assigned to one of three groups: 1) no prior adaptation (i.e. normal gain ≈ 1); 2) gain-increase adaptation (gain = 1.5); or 3) gain-decrease adaptation (gain = 0.5). The baseline VOR response (no prior adaptation) was measured in 22 mice: 11 α9-knockout and 11 cba129 mice. Post adaptation data was acquired from a total of 31 mice, which contributed to one of four groups: cba129 - gain-increase (n = 8), cba129 - gain-decrease (n = 8), α9 - gain-increase (n = 7) and α9 - gain-decrease (n = 8).

To facilitate head immobilization during VOR recording, we implanted a pedestal onto the skull of each animal on the day of the experiment. The exact implantation technique has been described previously (Migliaccio et al. 2005, 2010a; Hübner et al. 2013). In short, we anaesthetised mice using general inhalation anaesthesia (Isoflurane 2 - 4 %). While under anaesthesia we made a midline incision to expose the skull from Bregma to Lambda. We stripped the periosteum and dried the bony surface using sterile cotton buds. We then drilled 3 guide holes into the skull (2 lateral of Bregma and 1 lateral of Lambda) and inserted 3 stainless steel anchoring screws (#0 x 1/8, Micro Fasteners Pty. Ltd., Thomastown, VIC, Australia).
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 still under anaesthesia, we shortened eyelashes and vibrissae to minimise 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 hours in a separate cage before they were restrained and placed onto 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 onto a rotary platform driven by a high torque servomotor (GOLDLINE DDR D083, Danaher). The head pedestal was pitched ~30 degrees ‘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 synchronised with the rotary platform using an electronic gearing system (latency <0.1ms). 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 0.5Hz with peak-velocity 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
Role of the mammalian EVS in VOR adaptation

The opposite direction of the vestibular stimulus with amplitude of 1.5x (gain-increase) and 0.5x (gain-decrease) of the vestibular stimulus velocity. We kept adaptation training to one, 40 minute session.

After VOR adaptation was completed, we measured VOR gain in complete darkness using a binocular 3D video-oculography system (Hübner et al. 2013, 2014; Migliaccio et al. 2005, 2010). 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 using cyanoacrylate, removal causes temporary corneal swelling that likely affects vision. To ensure ideal vision during adaptation training we recorded pre-adaptation VOR gains in a separate group of mice, rather than comparing pre and post adaptation 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 1500, 3000 and 6000 °/s² reaching a velocity plateau of 100, 150 and 300 °/s, respectively. From here on, we refer to transient stimulus conditions using the following abbreviations: 1.5k100, 3k150 and 6k300.

Data analysis

To analyse the three-dimensional VOR data, we converted eye movements acquired in eye coordinates into rotation vectors in head coordinates. Eye velocity traces with quick-phases removed, were inverted so that an ideal VOR would yield a gain (eye velocity / head velocity) of +1 and phase of 0 degrees. Positive phase lead denotes eye velocity leading head velocity. The
Role of the mammalian EVS in VOR adaptation

methods of analysis are similar to those that have been described previously (Hübner et al. 2013, 2014; Migliaccio et al. 2010, 2010a). Unless otherwise stated all results are reported as mean ±1SD.

For sinusoidal rotations, we fit individual cycles of head and eye velocity (10 to 100, depending on frequency) using least-square pure 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 analyse 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. $G_A$ was calculated as the average ratio of eye/head acceleration (using the slopes of the constant-acceleration fit). $G_V$ 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 analysed 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 using a semi-automatic de-saccading technique that we optimised based on several published algorithms (Migliaccio et al., 2006;
Role of the mammalian EVS in VOR adaptation

Faucheux et al., 2007). However, instead of manually adjusting the acceleration threshold for each individual trace, we automated the process by fixing the acceleration threshold parameter to 5,000 °/s². Amplitude was defined as the difference in horizontal eye position between the quick-phase start and end. Peak velocity was defined as the maxima of the horizontal eye velocity trace during the quick-phase. Because quick-phase peak velocity and amplitude histograms were Weibull distributions, we report the median in addition to the mean, quick-phase peak velocity and amplitude. The relationship between peak velocity and amplitude was determined by a least-square linear fit. The relationship between amplitude and duration was determined by a non-linear regression fit of the form:

\[ D = K(1 - e^{-A/\tau}) \]

where D stands for quick-phase duration and A for quick-phase amplitude. K and \( \tau \) are fit constants for the exponential equation and represent the saturated duration and non-linear component of the fit, respectively (Sakatani and Isa 2007).

**Analysis of Adaptation Selectivity**

Similar to previous studies (Hübner et al. 2014) we compared VOR adaptation selectivity between gain-increase versus gain-decrease adaptation training by calculating a generalisation index across frequencies and velocities. The generalisation index was defined as the fraction of adaptive gain change when the testing stimulus frequency and velocity matched the adaptation training stimulus, compared to the average adaptation at all other testing stimulus frequencies and velocities.
Role of the mammalian EVS in VOR adaptation

\[
\text{Generalisation index} = \frac{1}{\Delta \text{gain}_{\text{training}}} \left( \sum_{i} \Delta \text{gain}_{i} \right), \text{where } i \neq \text{training}
\]

\(\Delta \text{gain}_{\text{training}}\) represents the amplitude of VOR gain adaptation at the same frequency/velocity used during adaptation training. \(\Delta \text{gain}_{i}\) represents the amplitude of VOR gain adaptation at one of \(j\) stimulus frequencies/velocities other than the adaptation training frequency/velocity (e.g., for adaptation training peak velocity 20 °/s, \(i = \{50, 100\} \times 10 \circ/s, j = 2\)). A generalisation index close or equal to 1 (\(\Delta \text{gain}_{i} \approx \Delta \text{gain}_{\text{training}}\)) indicates broad generalisation of VOR gain adaptation, while a generalisation index close or equal to 0 indicates stimulus specific VOR adaptation.

### Statistical Analysis

Because each mouse only contributed to one group (gain-increase, or gain-decrease, or gain-baseline) it was not possible to perform a pairwise analysis of adaptation within mice. Instead we used a multivariate ANOVA with mouse strain (\(\alpha 9\)-knockout and cba129) and adaptation type (gain-increase, gain-decrease and gain-baseline) as main factors. For sinusoidal rotations additional independent factors were: frequency, peak-velocity and peak-acceleration, and dependent factors were: gain and phase. For transient steps of acceleration an additional independent factor was: stimulus (1.5k100, 3k150, 6k300), and dependent factors were: \(G_A\) and \(G_V\). For quick-phase analysis, an additional independent factor was: stimulus (0.2 Hz, 0.4 Hz, 0.5 0.8 Hz, 1 Hz), and dependent factors were 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 using \(t\) tests with multiple–comparison correction in case of significant ANOVA results. Probability values \(\geq 0.001\) were reported with
numeric value, the rest were reported as < 0.001. Unless otherwise stated all results are reported as mean ± standard deviation.
Results

Baseline VOR Response to Sinusoidal Rotation

Figure 1 A1-A2 shows 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 1 B shows a direct comparison of cba129 and α9-knockout baseline VOR gain (top) and phase (bottom) across frequencies 0.2 to 10 Hz and velocities of 20, 50 and 100 °/s. For both mouse types the VOR gain significantly increased with stimulus peak-velocity ($F_{(1,335)} = 547.95$, $P < 0.001$). In cba129 mice the average VOR gain at 20 °/s pooled across frequencies was 0.73 ± 0.21 while at 100 °/s the average VOR gain was 0.98 ± 0.13. α9-knockout mice demonstrated a similar increase in VOR gain with increasing stimulus peak-velocity, with average 0.59 ± 0.17 and 0.87 ± 0.16 at 20 and 100 °/s, respectively.

In cba129 mice the VOR gain in response to vestibular stimulation at 20 °/s (top left panel of Figure 1 B) showed a steady increase with frequency for frequencies <1 Hz. At 0.2 and 1 Hz the VOR gains were 0.46 ± 0.12 and 0.81 ± 0.2, respectively ($t_{(25.56)} = -6.08$, $p < 0.001$). In contrast, α9-knockout mice showed almost no change in VOR gain with gains of 0.54 ± 0.14 and 0.59 ± 0.16 at 0.2 and 1 Hz, respectively ($t_{(25.94)} = -0.91$, $p = 0.37$). This absence of gain increase with stimulus frequency was also observed when the vestibular stimulus peak-velocity was 50 °/s. While the VOR gain of cba129 mice climbed from 0.77 ± 0.15 at 0.2 Hz to a maximum of 0.97 ±
0.16 at 1.6 Hz, the VOR 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 to 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 to 10 Hz). In comparison, at 50 and 100 °/s there was a frequency threshold of 1.6 to 2 Hz above which a pronounced VOR gain reduction in α9-knockout mice became apparent (see top panel of Fig. 1B). The average difference between cba129 and α9-knockout mice at frequencies >2 Hz was 0.23 ± 0.02 (a 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 (Figure 1 CI) and peak-accelerations (Figure 1 C2). VOR gains of both mouse types increased as peak-velocity increased. This effect was more pronounced in α9-knockout compared to 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 bottom panel of Figure 1 B). 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 degrees in cba129 mice versus 21.70 ± 4.88 degrees 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
Role of the mammalian EVS in VOR adaptation

increasing phase lag with zero-phase crossover at ~3 Hz and a maximum phase lag of -11.01 ± 3.25 degrees 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 degrees at 10 Hz. This difference of -5.33 ± 0.81 degrees was statistically significant ($t_{(96.56)} = -6.58, p < 0.001$).

Baseline VOR Response to Transient Steps of Acceleration

Figure 2 A shows horizontal VOR response to transient 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 ($GAP_{\text{pre}}$) during the initial "constant-acceleration phase" of transient step stimuli. cba129 mice had an average $GAP_{\text{pre}}$ of 1.14 ± 0.19. In contrast, the average $GAP_{\text{pre}}$ of α9-knockout mice was significantly lower at 1.02 ± 0.21 ($F_{(1,24)} = 4.445, p = 0.037$) (see left panel of Figure 2 B). This difference in $GAP_{\text{pre}}$ between mouse strains was similar for all three stimulus accelerations (Interaction "Strain x Acceleration": $F_{(2,48)} = 0.222, p = 0.802$).

Figure 2

We also analysed the pre-adaptation VOR gain response during the "constant velocity plateau" ($GV_{\text{pre}}$) of transient step stimuli. There was no difference in $GV_{\text{pre}}$ 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 x Velocity": $F_{(1,50)} = 0.628, p = 0.432$). The average $GV_{\text{pre}}$ of cba129 and α9-knockout mice was 0.82 ± 0.2 and 0.79 ± 0.27, respectively. $GV_{\text{pre}}$ 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 τ terms in the non-linear fit between quick-phase duration and amplitude were similar between cba129 and α9-knockout mice (K: $t_{(1798)} = 0.43, p = 0.671$; τ: $t_{(1798)} = -0.34, p = 0.734$).

**Sinusoidal VOR Response following Adaptation Training**

We measured the VOR gain response after 40 minutes of horizontal sinusoidal gain-increase (x1.5) and gain-decrease (x0.5) visual-vestibular adaptation training with a vestibular stimulus of
Role of the mammalian EVS in VOR adaptation

0.5 Hz and 20 °/s (see Figure 3 A and 3 B). Figure 3 C displays the post adaptation VOR gains for cba129 (top row) and α9-knockout mice (bottom row) across frequencies from 0.2 to 10 Hz with peak-velocities of 20, 50 and 100 °/s. cba129 mice exhibited robust adaptation across all stimulus conditions \(F(1,419) = 8.82, p = 0.003\). The adaptation effect for each test peak-velocity, calculated as the difference between gain-increase versus gain-decrease adaptation pooled across frequencies, was maximal at 0.4 ± 0.02 when the test peak-velocity matched the adaptation training peak-velocity of 20 °/s. In contrast, for test peak-velocities of 50 and 100 °/s the adaptation training only averaged 0.22 ± 0.02 and 0.15 ± 0.02, respectively. This velocity-selective training effect was evident in our statistical model as a highly significant interaction between "training" and "velocity" factors \(F(1,454) = 69.87, p < 0.001\).

Figure 3

Velocity- and frequency-selective effects were analysed separately for gain-increase and gain-decrease adaptation. Velocity-selectivity was most evident for gain-increase adaptation. The generalisation-index across velocities, a measure of the degree of velocity-selectivity, was 0.03 ±0.26 (highly selective) for gain-increase adaptation and 0.77 ± 0.4 (generalised across velocities) for gain-decrease adaptation \(t_{(11.85)}= -4.22, p = 0.001\). Velocity-selectivity during gain-increase adaptation is best seen in Figure 3 E. At 20 °/s, the VOR gain difference (adaptation effect) between the baseline and gain-increase VOR compared to the difference between the baseline and gain-decrease VOR, were nearly equal in magnitude. No gain-increase adaptation training was observed at 50 and 100 °/s \(F(1,17) = 0.21, p = 0.65\).

Figure 4
α9-knockout mice, when compared to cba129 (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 behaviour. 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 ± 0.03 ($F_{(1,13)} = 1.03, p = 0.33$), a reduction of 0.11 (50%) compared to cba129 mice. At 100 °/s no adaptation occurred ($F_{(1,13)} = 0.09, p = 0.76$). The generalisation index for gain-increase adaptation across test velocities was 0.49 ± 0.34, which is considerably more generalised compared to 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 °/s, the exact stimulus parameters used during adaptation training (Figure 3 $D$) ($F_{(1,534)} = 2.81, p = 0.094$), which was not seen at stimulus peak-velocities of 50 and 100 °/s.
Role of the mammalian EVS in VOR adaptation

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 Figure 4, top left). The average difference of $G_A$ between gain-increase versus gain-decrease adaptation was $0.12 \pm 0.03$ ($t_{(145.93)} = -4.12, p < 0.001$). 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, α9-knockout mice demonstrated adaptation of $G_A$ compared to baseline at only the 6k acceleration stimulus (gain-decrease: $p < 0.01$; gain-increase: $p < 0.01$; Figure 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 (Figure 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, α9-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 α9-knockout and control mice (cba129) suggesting that the quick-phase / saccadic oculomotor system is not affected by loss of α9 nAChR function. The α9-knockout mice had significantly lower baseline VOR gains (x1) compared to cba129 mice. This difference in gain widened with increasing stimulus frequency. At stimulus frequencies <1 Hz the difference was ~5 % compared to ~25 % at frequencies >1 Hz. These findings are consistent with a recent study showing that calcitonin-gene related peptide (CGRP) knockout mice (CGRP is expressed in vestibular efferents and important for EVS function) also had a reduction (~50%) in baseline VOR gain (Luebke et al. 2014). In contrast, the baseline VOR phase between α9-knockout and control mice was similar and did not change as a result of VOR adaptation training. The baseline VOR gain in the cba129 mouse was almost identical to that observed in the wild-type C57BL6 mouse tested under similar conditions (Hübner et al. 2014). The capacity for vestibular adaptation, measured as the difference in mean VOR gains between gain-increase (x1.5) and gain-decrease (x0.5) adaptation training groups, was significantly smaller in α9-knockout mice compared to cba129. The VOR gains of cba129 mice ranged from ~0.41 after gain-decrease adaptation to ~0.88 after gain-increase adaptation, a difference of (0.47) ~53 %. In contrast, the VOR gains of α9-knockout mice ranged from ~0.60 to ~0.73, a difference of only (0.13) ~17 %. This represents a 70 % reduction of vestibular adaptation in the α9-knockout mouse. Could this reduction be due to poor vision and / or poor generation of a retinal image slip signal to drive adaptation? We did not measure optokinetic function in these mice, but there are several lines of evidence suggesting
that these mice have adequate vision and slip signal for adaptation. First, qualitatively these mice react to light and visual movement in the same way as other mice we have tested. The fact that the α9-knockout was able to perform a baseline visual discrimination task described by Terreros et al. (2015) just as well as the wild-type mice also suggests that they have normal vision. Second, the α9-knockout mice we tested did show adaptation, especially during gain-increase training when the test stimulus peak-velocity was 20 °/s, suggesting they have an image slip signal large enough to drive adaptation. Third, we recently demonstrated in C57BL6 mice that VOR gain changes as a result of adaptation training are maximal when the stimulus testing velocity matches the adaptation training velocity (Hübner et al. 2014). In the present study we observed similar velocity-selective adaptation in α9-knockout and cba129 mice. We also observed in both mouse types stronger velocity-selective adaptation for gain-increase compared to gain-decrease adaptation, which also matches our previous observations in the C57BL6 mouse (Hübner et al. 2014).

*Can changes in EVS activity affect the proportion of irregular to regular firing vestibular primary afferents?*

It is important to note that dimorphic afferents account for ~75-80 % of all mammalian vestibular afferents, and receive input from 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 (C57BL6 and cba129 mice: Poppi et al. 2014, 2015), 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 α9 nAChRs coupled to calcium-activated potassium (SK) channels (Holt et al. 2006; Poppi et al. 2014, 2015).
Role of the mammalian EVS in VOR adaptation

The other effect is the 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. Since α9 nAChRs are non-functional 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 α9-knockout mice. Simultaneously, due to the presence of alternative types of nAChRs (i.e. α4, α6, and β2 subunits) on calyx-bearing afferents, the excitatory EVS effect would still be operating in α9-knockout mice. In short, the predicted overall effect of EVS activation on dimorphic afferents in α9-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 to 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 α9-knockout mouse (unpublished: poster abstract Han et al. 2007) that compared the single unit vestibular afferent response during 2 Hz whole-body rotations between α9-knockout and C57BL6 mice (Note that the C57BL6 is not the appropriate control background strain for the α9 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 normalised coefficient of variation in the inter-spike interval of afferent background discharge) were more sensitive to head rotations, i.e., for the same head velocity, the firing rates of regular discharging afferents were higher in α9-knockout compared to C57BL6 mice. Second, irregularly (phasic) discharging afferents (defined as having a CV* >
Role of the mammalian EVS in VOR adaptation

0.1) were less sensitive to head rotations in α9-knockout compared to C57BL6 mice. Third, the ratio of regularly versus irregularly afferents was higher in the α9-knockout mouse.

Can changes in EVS activity affect central adaptation mechanisms?

In addition to the vestibular sensory neuroepithelium, EVS neurons project collaterals to the flocculus and ventral paraflocculus 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 fibre synapses and information about image motion through climbing fibres 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 fibres 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 cut-off or saturation (Goldberg, 2000; Shinder et al., 2001). In addition, the proportion of irregular to
Role of the mammalian EVS in VOR adaptation

regular afferent signal might need to be modified under different behavioural circumstances (Chen-Huang and McCrea, 1998) or to compensate for differences in the dynamic loads of the various reflexes (Boyle et al., 1992). Therefore, the difference in VOR adaptation between α9-knockout and control mice may not only be due to differing primary (peripheral) vestibular afferent signals, but also changes in central adaptation mechanisms.

Can a decrease in the proportion of irregular to regular firing vestibular primary afferents explain the decrease in the baseline high-frequency VOR response and decrease in adaptation?

The observed differences in the baseline VOR response between α9-knockout and cba129 mice may be explained by differences in EVS activity, i.e., reduced EVS activity in the α9-knockout mouse leading to vestibular dimorphic afferents with discharge rate that is more regular (tonic). The nonlinear (phasic) and linear (tonic) components of the behavioural 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 behavioural 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 behavioural VOR that has a dependency 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
Role of the mammalian EVS in VOR adaptation

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 hypothesise 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 α9-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 α9-knockout mice was similar to cba129 mice.

Cullen and Minor (2002) showed in primates that the resting discharge rate and rotational sensitivity of semicircular canal afferents does 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 pre-existing 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 behavioural VOR, we hypothesise 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
Role of the mammalian EVS in VOR adaptation

et al. 2006), 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 modulate the primary afferent firing rate during large or active head movements, the mammalian EVS changes the proportion of irregular to regular discharging afferents. We hypothesise 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 behavioural vestibular plasticity as shown in this study. Our results not only provide a pivotal contribution towards understanding the role of the mammalian EVS, but also identifies for the first time a crucial part of the vestibular system that upon stimulation, e.g., electrically or via EVS agonists, could potentially boost vestibular plasticity.
Role of the mammalian EVS in VOR adaptation

Acknowledgements

A. A. Migliaccio and this work were supported by a National Health and Medical Research Council of Australia (NHMRC) Biomedical Career Development Award CDA-568736 and NHMRC Project Grant APP1010896. P. P. Hübner was supported by a University of New South Wales (UNSW) International Research Scholarship and a Neuroscience Research Australia (NeuRA) supplementary scholarship. We would like to thank Alan M. Brichta for his help in revising the manuscript.
Role of the mammalian EVS in VOR adaptation

References


Role of the mammalian EVS in VOR adaptation


Role of the mammalian EVS in VOR adaptation


Role of the mammalian EVS in VOR adaptation


Role of the mammalian EVS in VOR adaptation


Role of the mammalian EVS in VOR adaptation


Cholinergic efferent synaptic transmission regulates the maturation of auditory hair cell

Jordan PM, Parks XX, Contini D, Holt JC. A review of synaptic mechanisms of vestibular
efferent signaling in turtles: Extrapolation to efferent actions in mammals. *J Vestibul Res*,
**23**:161-175, 2013.

Katz E, Verbitsky M, Rothlin CV, Vetter DE, Heinemann SF, Elgoyhen AB. High calcium
permeability and calcium block of the alpha9 nicotinic receptors. *Hear Res* **141**:117-128,
stimulus generalization of increases and decreases in VOR gain. *J Neurophysiol* **94**:3092–
3100, 2005.

Kimpo RR, Boyden ES, Katoh A, Ke MC, Raymond JL. Distinct patterns of stimulus
generalization of increases and decreases in VOR gain. *J Neurophysiol* **94**:3092–3100,
2005.

Lasker DM, Backous DD, Lysakowski A, Davis GL, Minor LB. Horizontal vestibuloocular
reflex evoked by high-acceleration rotations in the squirrel monkey. ii. responses after canal

Lasker DM, Hullar TE, Minor LB. Horizontal vestibuloocular reflex evoked by high-acceleration
rotations in the squirrel monkey. III. Responses after labyrinthection. *J
Role of the mammalian EVS in VOR adaptation


Role of the mammalian EVS in VOR adaptation


Role of the mammalian EVS in VOR adaptation

Minor LB, Goldberg JM. Vestibular-nerve inputs to the vestibulo-ocular reflex: a functional- 

Murthy V, Taranda J, Elgoyhen AB, Vetter DE. Activity of nAChRs containing alpha9 subunits 
modulates synapse stabilization via bidirectional signaling programs. *Dev Neurobiol* 

Pfanzelt S, Rössert C, Rohregger M, Glasauer S, Moore LE, Straka H. Differential dynamic 
processing of afferent signals in frog tonic and phasic second-order vestibular neurons. *J 

Poppi LA, Tabatabaee H, Callister RJ, Lim R, Brichta AM. Cholinergic activity of the peripheral 

Purcell I, Perachio A. Three-dimensional analysis of vestibular efferent neurons innervating 

Sadeghi SG, Minor LB, Cullen KE. Response of Vestibular-Nerve Afferents to Active and 
Passive Rotations Under Normal Conditions and After Unilateral Labyrinthectomy. *J 

Sadeghi SG, Minor LB, Cullen KE. Response of vestibular-nerve afferents to active and passive 
rotations under normal conditions and after unilateral labyrinthectomy. *J Neurophysiol 

Sakatani T, Isa T. Quantitative analysis of spontaneous saccade-like rapid eye movements in 
Role of the mammalian EVS in VOR adaptation


Terreros G, Jorratt P, Elgoyhen AB, Delano P. Selective attention to visual stimuli in α9-Nicotinic Acetylcholine receptor knock-out mice. ARO Midwinter Meeting, Baltimore, **38**:32, 2015.


Figure Captions

Figure 1. (A1-A2) The VOR response to sinusoidal rotations (1 Hz at 100 °/s) for one cba129 (control) mouse and one α9-knockout mouse prior to adaptation training. Eye velocity (dark-grey) 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-grey) 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 controls (filled circles) and α9-knockout (open circles) The VOR was measured for frequencies from 0.2 to 10 Hz at peak-velocities of 20, 50 and 100 °/s (columns). Error bars indicate mean ± standard deviation. Unlike cba129, α9-knockout mice did not show a characteristic increase in VOR gain for frequencies < 1 Hz at peak-velocity 20 and 50 °/s. In addition, α9-knockout mice had significantly lower VOR gains at frequencies > 1 Hz, at all three 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 controls, while at 10 Hz at all three test peak-velocities VOR phase was more than controls. (C) Comparison of baseline VOR gain across sinusoidal test stimulus peak-velocities (1) and peak-accelerations (2). Peak-acceleration was calculated using the first 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. (*) denotes a significant difference between mouse type baseline gains or phases.
Figure 2. Baseline VOR in response to transient steps of acceleration. (A1-A2) An example of overlayed slow-phase eye velocities (inverted; grey) in response to sudden steps of acceleration (1500 °/s²) followed by a constant velocity plateau (100 °/s) in one cba129 (control) mouse and one α9-knockout mouse. Individual VOR gains were measured during the constant velocity part and constant acceleration part of the stimulus. Latency of the VOR response was determined using the zero velocity intercept of head acceleration and eye acceleration linear fits. B Acceleration gain ($G_A$; left panel) and velocity gain ($G_V$; right panel) in response to three acceleration and velocity stimuli. α9-knockout mice demonstrated significantly lower $G_A$ compared to cba129 mice at 6000 °/s². No difference between mouse types was observed for $G_V$. (*) denotes a significant difference between mouse type baseline gains.

Figure 3. The 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. Following adaptation this dome was removed and two high speed cameras were used to record binocular 3D eye movements. (B) The projected light spots moved synchronously with the head but in opposite direction. During gain-increase and gain-decrease adaptation the angular velocity of the projected light spots was set to 1.5x and 0.5x the head velocity stimulus. (C) Both cba129 (top row) and α9-knockout mice (bottom row) demonstrated maximal adaptation at 20 °/s, the velocity used during adaptation training. However, in α9-knockout mice the overall adaptation effect (difference between gain-increase versus gain-decrease VOR gains) was ~70 % smaller compared to cba129. This was true across all test stimulus frequencies and velocities. (D) VOR phase in both mouse types showed a cross-over between gain-increase and gain-decrease responses at 0.5 Hz and 20 °/s, the same stimulus parameters used during adaptation training (black arrow). (E) Comparison of gain-
Role of the mammalian EVS in VOR adaptation

increase (open circles) and gain-decrease (closed circles) VOR gains across all test velocities (frequency pooled). The baseline VOR response is illustrated as a dashed line (mean) surrounded by grey shadow (standard deviation). Cba129 mice showed strong velocity selective VOR adaptation. At 20 °/s the effect of adaptation (difference between gain-increase versus gain-decrease VOR gains) was maximal. As velocity increased the effect of adaptation continuously decreased. Similar velocity selective effects were also observed in α9-knockout mice. However, in α9-knockout mice the adaptation effect was minimal at 20 °/s and disappeared at higher velocities. (*) denotes a significant difference between post gain-decrease and post gain-increase adaptation training gain. (▲) denotes a significant difference between baseline gain and post gain-increase adaptation training gain. (▼) denotes a significant difference between baseline gain and post gain-decrease adaptation training gain.

Figure 4. Acceleration gain (\(G_A\); left panel) and constant-velocity gain (\(G_V\); right panel) following sinusoidal visual-vestibular mismatch adaptation training. cba129 mice (top row) demonstrated pronounced changes in \(G_A\) following adaptation training with a significant effect of adaptation, measured as the difference between gain-increase (light grey) and gain decrease (dark grey) conditions. This difference was mostly due to gain-decrease adaptation resulting in significantly lower than baseline gains at all stimulus conditions. In contrast, α9-knockout mice (bottom row) showed adaptation of \(G_A\) only at 6k acceleration, but none for \(G_V\) at any stimulus condition. (***\() denotes significant difference \(p < 0.001\), (**) \(p < 0.01\), and (*) \(p < 0.05\).
**A1 (Cba129)**

**A2 (Alpha9)**

**Gain (GA)**

- **Time (ms)**
  - 0 500 1000 1500

**Gain (GV)**

- **Velocity (°/s)**
  - 0 50 100 150 200

**B**

**Gain (GA)**

- **Acceleration (°/s²)**
  - 1500 3000 6000

**Gain (GV)**

- **Velocity (°/s)**
  - 100 150 300