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Contribution of vestibular efferent system alpha-9 nicotinic receptors to vestibulo-oculomotor interaction and short-term vestibular compensation after unilateral labyrinthectomy in mice

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HIGHLIGHTS

- Saccades occur at higher frequencies of yaw head rotations in $\alpha9^{-/-}$ mice vs. WT mice.
- After UL, no ipsilesional saccades occurred in WT mice in contrast to $\alpha9^{-/-}$ mice.
- After UL, $\alpha9^{-/-}$ mice and WT mice have different time course of SN suppression.
- Pre-UL, $\alpha9^{-/-}$ mice have a longer vestibulo-ocular reflex time constant vs. WT mice.
- After UL, better recovery of time constant occurred in $\alpha9^{-/-}$ mice than in WT mice.

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ABSTRACT

Sudden unilateral loss of vestibular afferent input causes nystagmus, ocular misalignment, postural instability and vertigo, all of which improve significantly over the first few days after injury through a process called vestibular compensation (VC). Efferent neuronal signals to the labyrinth are thought to be required for VC. To better understand efferent contributions to VC, we compared the time course of VC in wild-type (WT) mice and α9 knockout ($\alpha9^{-/-}$) mice, the latter lacking the α9 subunit of nicotinic acetylcholine receptors (nAChRs), which is thought to represent one signaling arm activated by the efferent vestibular system (EVS). Specifically, we investigated the time course of changes in the fast/direct and slow/indirect components of the angular vestibulo-ocular reflex (VOR) before and after unilateral labyrinthectomy (UL). Eye movements were recorded using infrared video oculography in darkness with the animal stationary and during sinusoidal (50 and 100°/s, 0.5–5 Hz) and velocity step (150°/s for 7–10 s, peak acceleration 3000°/s^2) passive whole-body rotations about an Earth-vertical axis. Eye movements were measured before and 0.5, 2, 4, 6 and 9 days after UL. Before UL, we found frequency- and velocity-dependent differences between WT and $\alpha9^{-/-}$ mice in generation of VOR quick phases. The VOR slow phase time constant (TC) during velocity steps, which quantifies contributions of the indirect component of the VOR, was longer in $\alpha9^{-/-}$ mutants relative to WT mice. After UL, spontaneous nystagmus (SN) was suppressed significantly earlier in WT mice than in $\alpha9^{-/-}$ mice, but mutants achieved greater recovery of TC symmetry and VOR quick phases. These data suggest (1) there are significant differences in vestibular and oculomotor functions between these two types of mice, and (2) efferent signals mediated by α9 nicotinic AChRs play a role during VC after UL.

1. Introduction

Unilateral afferent vestibular loss causes oculomotor, postural and motion symptoms and signs including vertigo, spontaneous nystagmus, ocular counter-roll and skew deviation, roll and yaw head tilt, contralateral limb extension and ipsilateral limb flexion, barrel rolling and circular walking [1]. Most symptoms and signs of a large difference in vestibular input from the two ears following unilateral labyrinthectomy (UL) decrease markedly over the first few days to weeks after injury, through a process of central nervous system adaptation commonly called vestibular compensation (VC). Immediately after UL, asymmetry in activity of neurons in the vestibular nuclei (VN) elicits oculomotor and postural symptoms because VNs neurons provide the major premotor drive

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to motoneurons mediating the vestibulo-ocular and vestibulospinal reflexes [2]. VC mechanisms rebalance activity between ipsi- and contralesional VN via inhibitory commissural GABAergic and glycinergic connections [3,4]. Suppression of oculomotor and postural symptoms over the first 48 h after UL corresponds to a marked increase of GABA release in the ipsilesional VN and a subsequent return to near-normal levels by 96 h after UL [3]. Following these rapid compensatory changes, ipsilesional VN gliosis, neurogenesis and plasticity mediated in part by changes in extracellular matrix also contribute to long-term VC [4].

The importance of cerebellar contributions to VC is demonstrated by the fact that compensatory recovery of VOR symmetry and suppression of spontaneous nystagmus (SN) after UL is severely delayed or abolished by prior ipsilesional flocculectomy [5] or genetic cerebellar deficiency [6].

The efferent vestibular system (EVS) is also thought to play a role in VC [7]. Vestibular efferent neurons synapse upon both hair cells and primary vestibular afferents [8,9]. In reptiles, birds, and mammals the EVS has strong synaptic inputs to calyx afferents, type II hair cells, and bouton afferents innervating type II hair cells [10]. Vestibular efferent neurons use acetylcholine receptors (AChRs) for synaptic transmission, in particular, an efferent-mediated inhibition is provided by activation of α9/α10 nicotinic AChRs on only the type II hair cells, while the α4β2* nicotinic AChRs and muscarinic AChRs contribute to fast and slow efferent-mediated excitation in type II hair cells and calyx afferents [11].

The role of the commissural inhibitory system in short-term VC has been extensively investigated [3,4]; however, few published data are available to describe the contributions of the EVS, particularly efferent-mediated inhibition, to vestibulo-oculomotor function during VC after partial vestibular loss. Release of ACh due to EVS stimulation does act via α9/α10 nAChRs on type II hair cells, leading to Ca^{2+} influx, activation of Ca^{2+}-dependent potassium channels, and a rise of potassium efflux, which in turn causes hyperpolarization of type II hair cells. This hyperpolarization subsequently reduces Ca^{2+} influx, mainly through action on voltage-dependent calcium channels, thereby reducing depolarization of hair cells [10,12].

To investigate contributions of the efferent-mediated inhibitory system, we examined VOR responses during VC in alpha-9 knockout ($\alpha9^{-/-}$) mice lacking the α9 subunit of the nAChR. Specifically, we sought to quantify differences between WT mice and $\alpha9^{-/-}$ mice with regard to the action of two conceptually distinct (but likely overlapping and interrelated) pathways of the VOR: the direct/fast pathway, thought to be predominantly mediated by second order vestibular eye movement related neurons [2], and the indirect/slow pathway, which is thought to be mediated mainly by pure vestibular related neurons in VN [13]. These two different pathways of the commissural system, which have been localized to the caudal and rostra-medial parts, respectively, of medial vestibular nuclei, have been studied extensively in primates [14]. However, compensation of the fast and slow components of the VOR after UL has not yet been fully described in mice undergoing VC. We therefore investigated the early time course of VC by quantifying changes in fast and slow components of VOR over the first 9 days after UL.

2. Methods

2.1. Subjects and surgery

Breeding of CBACaJ;129S-*Chrna9*^{tm1Bedv}/J and C57BL/6 (The Jackson Laboratory) was performed to obtain F2 homozygous $\alpha9^{-/-}$ knockout mice and homozygous $\alpha9^{+/+}$ wild-type (WT) mice. Six WT mice and three $\alpha9^{-/-}$ mice of same mixed genetic background were used. We performed all experiments on adult male mice at ≥3 months of age with fully maturated vestibular system [15]. All protocol procedures were approved by the Johns Hopkins Animal Care and Use Committee consistent with European Community Directive 86/609/EEC.

Isoflurane anesthesia (3–5% in O_2) was used for all surgical procedures. A head holder was implanted on the skull using steel screws and dental cement. Two WT and three $\alpha9^{-/-}$ mice underwent UL on the right side without violation of bone over the paraflocculus. Each animal fully recovered from anesthesia prior to eye movement measurements. Apart from VOR testing sessions, animals resided under normal diurnal lighting and feeding conditions. Second left-side labyrinthectomy was performed 10 days post-UL.

2.2. Eye movement recording

Horizontal and vertical eye angular positions were recorded in darkness using a video-oculography technique based on a system previously described in detail [16] but adapted to perform pupil tracking under infrared illumination without application of corneal markers that might otherwise interfere with vision in between VOR measurements (Fig. 1). To constrict the pupil during video eye tracking, each eye was topically treated with pilocarpine 1%.

To confirm calibration of video system, one deeply anesthetized mouse with 0.3 mm marker affixed to the cornea (red square, Fig. 1**Bd**) was positioned in a gimbal so that the mouse could be rotated about an Earth-vertical axis through the eye with respect to an Earth-fixed camera. After visual inspection confirmed absence of VOR responses, the animal was reoriented at different angles over a ±40° range in 5° increments (Fig. 1**Ad** and **C**), while the recording system identified the pixel centroid of the constricted pupil (Fig. 1**Bc**). Horizontal angle of eye rotation was calculated:

$$H_{deg} = \arcsin\left(\frac{H_{pix} \times 0.3\text{mm}}{1.69\text{mm} \times M_{pix}}\right) \times \frac{180}{\pi} \quad (1)$$

where H_{deg}, H_{pix} = horizontal position deviation from starting center position, measured in degrees and in pixels, respectively; M_{pix} = size of side of square marker in pixels; 1.69 mm is the mean radius of an adult mouse eye [17]. Although the mouse eye is not spherical and refractive effects of the cornea result in small differences between true angular vs. measured angle of the eye positions, errors due to this approximation were very small and therefore neglected. There is perfect correlation of recorded and actual eye angles using this recording system (Fig. 1**C**). A similar procedure was performed with a marker placed briefly on the eye at the end of each experiment, to measure a marker's size in pixels (Fig. 1**Bd**). To minimize blinks during imaging of marker, the cornea was anesthetized with proparacaine (0.5% topical), which was not used for eye movement recording.

The center of oculomotor range was determined in each experiment to account for apparent changes in VOR gain as a function of ocular deviation from this center. To estimate the center of the oculomotor range, pupil position was measured during horizontal sinusoidal rotations in darkness at 1 Hz, and the average position calculated before, after and during these rotations was taken as the starting eye position (x_o, y_o), where x is horizontal and y is vertical eye angular position in the eye frame of reference. Thereafter, horizontal eye position was estimated as:

$$H_{deg} = \arcsin((H_{pix} - x_o) \times 0.3/(1.69 \times M_{pix} \times \cos[\arcsin(V_{pix} - y_o) \times 0.3/1.69 \times M_{pix}])) \times \frac{180}{\pi}$$

where x_o and y_o are the pixel coordinates of the center pupil position; V_{pix} is the eye vertical angular position measured in pixels; H_{deg}, H_{pix} are the horizontal position measured in degrees