Hearing Research 335 (2016) 118–127

Contents lists available at ScienceDirect

Hearing Research

journal homepage: www.elsevier.com/locate/heares

Research paper

Effects of high intensity noise on the vestibular system in rats

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ARTICLE INFO

Article history:
Received 8 December 2015
Received in revised form
22 February 2016
Accepted 7 March 2016
Available online 10 March 2016

Keywords:
Noise
Vestibular
Hair cell
Vestibular afferent
Vestibulo-ocular reflex (VOR)
Rat

ABSTRACT

Some individuals with noise-induced hearing loss (NIHL) also report balance problems. These accompanying vestibular complaints are not well understood. The present study used a rat model to examine the effects of noise exposure on the vestibular system. Rats were exposed to continuous broadband white noise (0–24 kHz) at an intensity of 116 dB sound pressure level (SPL) via insert ear phones in one ear for three hours under isoflurane anesthesia. Seven days after the exposure, a significant increase in ABR threshold (43.3 ± 1.9 dB) was observed in the noise-exposed ears, indicating hearing loss. Effects of noise exposure on vestibular function were assessed by three approaches. First, fluorescein-conjugated phalloidin staining was used to assess vestibular stereocilia following noise exposure. This analysis revealed substantial sensory stereocilia bundle loss in the saccular and utricular maculae as well as in the anterior and horizontal semicircular canal cristae, but not in the posterior semicircular canal cristae. Second, single unit recording of vestibular afferent activity was performed under pentobarbital anesthesia. A total of 548 afferents were recorded from 10 noise-treated rats and 12 control rats. Noise exposure produced a moderate reduction in baseline firing rates of regular otolith afferents and anterior semicircular canal afferents. Also a moderate change was noted in the gain and phase of the horizontal and anterior semicircular canal afferent's response to sinusoidal head rotation (1 and 2 Hz, 45°/s peak velocity). Third, noise exposure did not result in significant changes in gain or phase of the horizontal rotational and translational vestibulo-ocular reflex (VOR). These results suggest that noise exposure not only causes hearing loss, but also causes substantial damage in the peripheral vestibular system in the absence of immediate clinically measurable vestibular signs. These peripheral deficits, however, may lead to vestibular disorders over time.

1. Introduction

Hearing loss as a result of high intensity noise exposure is an unavoidable aspect of many occupations, particularly those associated with industry and the military services. It has been reported that some individuals with noise-induced hearing loss (NIHL) also suffer from balance disorders (Oosterveld et al., 1982; Juntunen et al., 1987; Golz et al., 2001). Reduced vestibular caloric response (Manabe et al., 1995; Golz et al., 2001), reduced vestibular-evoked myogenic potentials (VEMP) (Wang et al., 2006; Wang and Young, 2007; Kumar et al., 2010; Akin et al., 2012; Zuniga et al., 2012), nystagmus (Man et al., 1980; Shupak et al., 1994; Oosterveld et al., 1982; Golz et al., 2001), and increased body sway (Ylikoski, 1988; Kilburn et al., 1992) have been reported. Despite our understanding of the effect of noise on auditory function, the mechanisms underlying noise-induced vestibular deficiency remain to be elucidated.

The vestibular system is exquisitely sensitive to head rotation, translation, and changes in orientation with respect to gravity (for

Abbreviations: ABR, Auditory brainstem response; AC, anterior canal afferents; CR, corneal reflection; CV*, normalized coefficient of variation of interspike intervals; dB, decibel; FFT, fast Fourier transform; HC, horizontal canal; LE, Long-Evans; NIHL, noise-induced hearing loss; PC, posterior semicircular canal; SO, superior branch otolith organ afferents; PFA, paraformaldehyde; PBS, phosphate-buffered saline; pSPL, peak Sound Pressure Level; SD, Sprague–Dawley; VOR, vestibulo-ocular reflex

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http://dx.doi.org/10.1016/j.heares.2016.03.002
0378-5955/

review, Goldberg et al., 2012). However, because vestibular end organs share the same fluid environment with the auditory end organ, they are also impacted by intense acoustic waves. Acoustic activation of the vestibular system occurs not only in pathological conditions where the bony canal is compromised by fenestration or by canal dehiscence (Tullio, 1929; Minor et al., 1998), but also occurs in healthy human subjects (Parker et al., 1978) and in animal models with intact labyrinths (Young et al., 1977, Xu et al., 2009, monkeys; Wit et al., 1984, pigeons; McCue and Guinan, 1994a, 1994b, 1995, 1997, cats; Murofushi et al., 1995, Murofushi and Curthoys, 1997, Curthoys et al., 2006, 2012; Curthoys and Vulovic, 2011, guinea pig; Carey et al., 2004, chinchilla; Zhu et al., 2011, 2014, rats). Electrophysiological and anatomical studies indicate that both the otolith organs and the semicircular canals are activated by loud sound (80 dB above ABR threshold), although the strongest excitations are from otolith organ organs (Zhu et al., 2011, 2014). Nevertheless, it is unclear whether high-intensity noise exposure produces damage in the five vestibular end organs. The current study employed three approaches to examine effects of exposure to high intensity broadband noise on the vestibular system of rodents, i.e., analysis of vestibular hair cell morphology, singe unit recording of vestibular afferents and testing of the rotational and translational vestibulo-ocular reflex (VOR). Our results show that a single high-intensity noise exposure results in substantial damage to the peripheral vestibular end organs, in the absence of immediate signs of vestibular dysfunction.

2. Methods

2.1. Animals

Adult male Sprague–Dawley (SD) rats (Harlan Sprague–Dawley, Indianapolis, IN) weighing 250–350 g were used in the morphological and neurophysiological studies. Pigmented female Long-Evans rats (Harlan Labs, Indianapolis, IN) weighing 175–225 g were used for VOR testing. All procedures were carried out in accordance with NIH guidelines and approved by the Institutional Animal Care and Use Committee at the University of Mississippi Medical Center.

2.2. High intensity noise exposure

Rats were held under isoflurane anesthesia and exposed to broadband white noise [0–24 kHz, 116 dB sound pressure level (SPL)] that was delivered continuously to the left external ear canal for three hours. The contralateral external ear canal was blocked with an ear plug. During the noise exposure, body temperature was maintained at 36–37 °C with a heating pad (Frederick Haer, Bowdoinham, ME, USA). Noise was generated through a MA3 stereo microphone amplifier (Tucker–Davis Technologies, Alachua, FL) and delivered to the external ear canal via sound-conducting tubing, connected to an insert ear phone (ER-3A, Etymotic Research, Inc., Elk Grove Village, IL) with a 3.5 mm infant tip adapter. Control rats received a sham noise exposure under isoflurane anesthesia for three hours while connected to an inset ear phone without noise exposure. Before the ear phone was inserted, the ear canal was checked with an otoscope to ensure patency. Noise intensity was calibrated by a sound level meter (Brüel and Kjoer, Copenhagen, Denmark).

2.3. Auditory brainstem response

Auditory brainstem response (ABR) was measured under isoflurane anesthesia before and seven days after noise exposure. Stainless steel subdermal electrodes were placed at the vertex (active), behind the stimulated ear (reference), and in the hind leg (ground) (Simpson et al., 1985). The sound stimulus was a 0.1 ms broadband click of alternating polarity. Clicks were generated by a MA3 stereo microphone amplifier and delivered via an insert ear phone (ER-3A) at a rate of 25.1clicks/s. EEG signals were amplified (×100,000), filtered (100 Hz–3 kHz), averaged over 2000 trials and digitized at 20 kHz over a 15-ms epoch (ICS Chartr EP 200 evoked potential assessment device; GN Otometrics, Taastrup, Denmark). Click intensity began at 110 dB peak sound pressure level (pSPL) and was lowered by 5 dB until ABR threshold was obtained. ABR threshold was determined as the lowest intensity at which clicks generate well-defined and reproducible wave I. Animals with elevated ABR thresholds (>70 dB pSPL before noise or sham exposure) were excluded due to possible hearing loss.

2.4. Vestibular afferent recording

Seven days after noise or sham exposure, vestibular afferent activity was recorded under pentobarbital sedation (50 mg/kg, i.p.) as described previously (Zhu et al., 2011, 2014). Sedation was maintained by injection of a 5 mg/kg dose of pentobarbital as needed. The rat body temperature was monitored and maintained at 36–37 °C with a heating pad. Surgical procedures were conducted aseptically. Each rat's head was secured on a stereotaxic frame (David Kopf Instruments, Tujunga, CA) via a surgically implanted head holder. The left side occipital bone was opened and the cerebellum was exposed. With the assistance of a surgical microscope, the left cerebellar hemisphere, flocculus, and paraflocculus were removed by aspiration (Model 130 Schuco-Vac, Allied Healthcare Products Inc., St Louis, MO) to access the 8th nerve (Zhu et al., 2011, 2014).

Single unit recording of vestibular afferent activity was performed on the side ipsilateral to the noise or sham-exposed ear. Rats were secured on a gimbaled structure that allowed roll and pitch tilts. The structure was mounted on the platform of a rotator for small animals, which was servo-controlled to deliver angular and linear accelerations (Neuro Kinetic, Inc., Pittsburgh, PA, USA). A quartz microelectrode (Sutter Instruments, Novato, CA, USA), filled with 3 M sodium chloride (10–20 MΩ) was positioned over the superior branch of the vestibular nerve with the assistance of a surgical microscope. Every spontaneously active nerve fiber encountered was tested. First, at least 30 s of background discharge activity was recorded for calculating the regularity and baseline firing rate of the afferent. Next, each semicircular canal was brought into the plane of earth-horizontal rotation and whether the isolated afferent's firing rate was modulated by sinusoidal earth-horizontal rotations or by sinusoidal pitch rotations (dynamic vertical head tilt) was tested. Extracellular recording was obtained using a MNAP system (Plexon Inc., Dallas, TX, USA). The rotational stimuli were delivered at 0.5, 1, and 2 Hz, at a peak velocity of 45°/s. Single-unit data along with horizontal and vertical head-position signals were recorded for 30 cycles.

Extracellular voltage signals were sampled by a CED Power 1401 system (Cambridge Electronics Devices, Cambridge, UK) at 20 kHz with 16-bit resolution and a temporal resolution of 0.01 ms. Head position signals were sampled at 1 kHz. Offline data analysis was performed on PC workstations using Spike 2 (Cambridge Electronics Devices, Cambridge, UK), MATLAB (MathWorks, Inc., MA, USA) and SigmaPlot (Systat Software Inc., CA, USA) software. Regularity of vestibular afferents was determined by calculating their normalized coefficient of variation of interspike intervals, i.e., CV*s, using the methods described in Lasker et al. (2008). Vestibular afferents were classified as regular (CV*<0.1) or irregular (CV*>0.2) units based on their CV* (Goldberg et al., 1984; Young et al., 1977). Permutation analyses were carried out, as described by Liu and