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EFFECTS OF MODERATE NOISE EXPOSURE ON HEARING FUNCTION IN C57BL/6J MICE

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Abstract

Objective To study characteristics of hearing loss after exposure to moderate noise exposure in C57BL/6J mice. **Methods** Male C57BL/6J mice with normal hearing at age of 5-6 weeks were chosen for this study. The mice were randomly selected to be studied immediately after exposure (Group P0), or 1 day (Group P1), 3 days (Group P3), 7 days (Group P7) or 14 days (P14) after exposure. Their before exposure condition served as the normal control. All mice were exposed to a broad-band white noise at 100 dB SPL for 2 hours, ABR thresholds were used to estimate hearing status at each time point. **Results** ABR threshold elevation was seen at every tested frequency at P0 ($P<0.01$). Elevation at high-frequencies (16 kHz and 32 kHz) was greater than at lower frequencies (4 kHz and 8 kHz, $P<0.05$). From P1 to P14, ABR thresholds continuously improved, and there was no significant difference between P14 and before exposure ($P>0.05$). **Conclusion** There is a frequency specific response to 100 dB SPL broad-band white noise in C57BL/6J mice, with the high-frequency being more susceptible. Hearing loss induced by moderate noise exposure appears reversible in C57BL/6J mice.

Key Words: C57BL/6J mice; Noise exposure; Hearing loss; Temporary noise-induced hearing loss; ABR threshold shift

Nowadays, noise pollution has become a public health problem[1-3]. Overexposure to noisy environment can cause various kinds of dysfunction, such as annoyance[4], sleep disturbance[5], cognition impairment[6], hypertension and cardiovascular diseases[7,8], besides hearing impairment [9] which is the primary noise-related dysfunction.

For the auditory system, overexposure to intense sound can cause temporary or permanent noise-induced hearing loss (NIHL) [10,11]. Permanent noise-induced hearing loss is a hot research focus to many scholars. However, compared to long time, high intensity narrow-band noise, exposure to moderate intensity broad-band environmental noise of variable time course seems more practically relevant due to the high variable noise sources in modern society. In addition, noises from high-speed railway, automobiles, aircrafts, music and radio players are usually characterized by moderate intensities and broad frequency spectrum.

Researchers have found that temporary NIHL can be associated with changes in microscopic physiological structures in the cochlea. For example, Kujawa and Liberman successfully established a temporary NIHL model using CBA mice which were exposed to an oc-

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tave-band noise of 100 dB sound pressure level (SPL) for 2 hours [12]. The study showed that the threshold elevation at each frequency fully recovered to its pre-exposure level over 2 weeks after overexposure, and that acoustic overexposures could cause acute loss of afferent nerve terminals and delayed degeneration of the cochlear nerve, although cochlear sensory cells were intact [12]. Lin et al subsequently established a temporary NIHL model using guinea pigs and obtained similar results [13]. However, no ideal temporary NIHL model using C57BL/6J mice has been reported until now. This may be due to relatively few studies on C57 mice which tend to show presbycusis early and are seemingly not suitable for long-term studies as a simple NIHL model. While recent studies have showed that hearing at 32 kHz and higher frequencies in C57 mice begins to gradually decline at 3 months after birth, there is no hearing loss at lower frequencies at six months or later [14-16], indicating the feasibility for studies on temporary NIHL in these mice in the first three months. Davis et al once carried out hearing sensitivity studies with the C57 and CBA mice using narrow-band noise of various intensities, and demonstrated appropriate noise conditions which could induce permanent hearing loss (HL) for C57 mice. But when C57 mice were exposed to noises at relatively low intensities, no consistent temporary or permanent threshold shifts could be established [17]. We have successfully constructed a hearing loss animal model of aminoglycoside induced hearing impairment using C57BL/6J mice[18, 19]. Therefore, establishing a NIHL model in C57 mice may benefit future studies on the complex hearing dysfunction in presbycusis and aminoglycoside induced hearing loss. In this study, we chose broad-band white noise as the exposure to study its effect on hearing in C57BL/6J mice.

Materials and methods

Animals

Male C57BL/6J mice (5-6 weeks of age) with normal auditory brainstem response (ABR) thresholds and no middle or inner ear disease were provided by Animal Center of Chinese Military Medical Sciences Academy in this study. The mice were randomly divided into five groups (5-7 mice in each group) to be studied immediately after exposure (P0) or 1 day (P1), 3 days (P3), 7 days (P7) or 14 days (P14) after noise exposure. Their condition before exposure were used as the normal control. All procedures were approved by the Animal Care and Use Committee of PLA General Hospital.

Acoustic Overexposure

All experimental mice were exposed to a broad-band white noise at 100 dB SPL for 2 hours. During the exposure, the animal was kept in a 6cm×6cm×10cm wire cage unrestrained (1 animal/cage). The cage was suspended directly below the horn of the sound-delivery loudspeaker in a small, reverberant chamber. Noise calibration to target SPL was performed immediately before each exposure session with a standard sound level meter. During calibration, the probe of sound level meter was placed inside the cage, and the variation of the sound level was less than 1 dB SPL in various parts of the cage.

ABR Testing

The ABR threshold was acquired pre-exposure and at different post-exposure time points with the TDT hardware and BioSig software (USA). Mice were anesthetized with 10% chloral hydrate (0.0045-0.005 ml/g, i.p.) and kept warm on a heating pad in a soundproof electrically shielded room. Subdermal needle electrodes were inserted at the vertex and ventrolaterally to both ears after anesthesia. The distance between testing earphone and the external ear canal was approximately 0.5 cm. Acoustic stimuli used in our study were clicks and tone bursts (rise/fall time: 1 ms, duration: 4 ms) presented in 5 dB steps descending from 90 dB SPL. Scanning time was 10 ms, and 1024 sweeps were averaged with 300-3000 Hz filtering band-width. At each frequency, threshold was determined as the lowest level at which a repeatable wave III could be obtained,

Preparation of the Organ of Corti

After ABR testing, mice were sacrificed by cervical dislocation. The temporal bone was removed after decapitation, and the cochlea was quickly separated.

Under dissecting microscope, a hole was opened at the apex of the cochlea and the round and oval windows were opened with a needle. The cochlea was carefully perfused with 4% paraformaldehyde (PFA) solution via the apex, then fixed with 4% PFA solution at 4°C overnight. Next, the specimen was decalcified in 10% ethylenediaminetetraacetic acid (EDTA) solution for 3.5 hours. The cochlea shell was removed from apex to base under a dissecting microscope in 0.01 mmol/L PBS solution. The basilar membrane was separated, and the vestibular membrane and tectorial membrane were removed.