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Research paper

Large-scale phenotyping of noise-induced hearing loss in 100 strains of mice

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ABSTRACT

A cornerstone technique in the study of hearing is the Auditory Brainstem Response (ABR), an electrophysiologic technique that can be used as a quantitative measure of hearing function. Previous studies have published databases of baseline ABR thresholds for mouse strains, providing a valuable resource for the study of baseline hearing function and genetic mapping of hearing traits in mice. In this study, we further expand upon the existing literature by characterizing the baseline ABR characteristics of 100 inbred mouse strains, 47 of which are newly characterized for hearing function. We identify several distinct patterns of baseline hearing deficits and provide potential avenues for further investigation. Additionally, we characterize the sensitivity of the same 100 strains to noise exposure using permanent thresholds shifts, identifying several distinct patterns of noise-sensitivity. The resulting data provides a new resource for studying hearing loss and noise-sensitivity in mice.

1. Introduction

Hearing loss is the most common sensory impairment in the world and is estimated to affect more than 278 million individuals of all ages, causing significant reduction in quality of life and socioeconomic impairment (Shearer and Smith, 2012).

Over the past several decades, human studies of sensorineural hearing loss (SNHL) have made abundantly clear that many forms of hearing loss possess a strong genetic contribution. There are approximately 67 genes that have been found to result in non-syndromic hearing loss (NSHL) that affect a broad range of components within the Organ of Corti (Shearer and Smith, 2012). Likewise, twin studies of noise-induced hearing loss (NIHL) indicate that approximately 36% of the disorder is heritable and candidate gene studies have identified a small number of potential NIHL susceptibility genes (Van Eyken et al., 2007; Fortunato et al., 2004; Van Laer et al., 2006; Konings et al., 2007, 2009a). Age-related hearing impairment (ARHI) shows a clear familial aggregation: the National Academy of Science–National Research Council (NAS–NRC) aging twin panel study has estimated the heritability of ARHI to be approximately 61% (Reed et al., 2000).

Despite the remarkable progress in our understanding of clinical hearing loss, human studies are met with several obstacles such as limited statistical power, difficulties in reproducibility, difficulties in controlling environmental factors such as noise exposure and ototoxic medications, and the considerable task of organizing large observational studies. Mice provide a useful complementary platform to the study of hearing loss. Given the existence of deafness in mice, similarity between mouse and human inner ears, genetic homology between mice and humans, and the molecular tools afforded by a model organism, mice have proven invaluable in the study of the heredity and molecular pathogenesis of hearing loss.

An important technique in hearing research, the auditory brainstem response (ABR) is a widely used electrophysiological technique that utilizes pure-tone bursts of varying frequency to stimulate the auditory pathway and detects the resulting activity in characteristic waveforms that serve as a quantitative measure of

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hearing function. A particularly useful ABR metric is hearing threshold, which is determined by subjecting an individual to increasing intensities of noise stimuli until the characteristic ABR waveform is detected. Several large scale studies have characterized ABR thresholds across different strains of mice, providing a valuable resource for interstrain comparisons of hearing function and genetic mapping of hearing traits. A study by Zheng and colleagues (Zheng et al., 1999) reported the ABR thresholds of 80 classic inbred mouse strains, 35 of which displayed varying degrees and onsets of hearing loss. Another study by Willott and colleagues reported the ABR thresholds and spiral ganglia morphologies for 25 recombinant inbred (RI) BXD strains (Willott and Erway, 1998). Lastly, a study by Johnson and colleagues utilized the ABR phenotypes of another set of BXD strains to identify the *ahl8* locus, elucidating its role in hearing loss and characterizing its epistasis with another key hearing loss gene *Cdh23* (Johnson et al., 2008).

While the database for baseline hearing traits has grown impressively, there are still many strains yet to be characterized that could provide useful models for hearing loss. In this study, we performed a superficial screening study of baseline hearing function in 100 inbred strains of mice, 47 of which have never been studied for hearing traits. We characterized the baseline hearing function of these 100 strains using ABR and identified several distinct patterns of baseline hearing impairment. Additionally, we characterized the sensitivity of the same 100 strains to noise-exposure through the use of permanent threshold shifts (PTS) and identified several distinct forms of noise sensitivity, providing new phenotypic data and potential models for future investigation of baseline hearing impairment and NIHL.

2. Materials and methods

2.1. Animal research ethics and handling

This study was carried out in strict accordance with the recommendations of the American Association for Laboratory Animal Sciences (AALAS) and the EU Directive 2010/63/EU for animal experiments. The protocol and all studies performed on the mice were approved by the University of Southern California Institutional Animal Care and Use Committee (Permit Number: 12033) and the Department of Animal Resources.

Animals were housed with ambient noise not exceeding that of normal air conditioning. All techniques were performed on mice under intraperitoneal anesthesia (ketamine 80 mg/kg body weight and xylazine 16 mg/kg body weight) and all efforts were made to minimize suffering.

2.2. Noise exposure

6 week old mice were exposed for 2 h to octave band noise (OBN) with a center frequency of 10 kHz using a method adapted from Kujawa and Liberman (Kujawa and Liberman, 2009). Mice were placed in a circular ¼ inch wire-mesh exposure cage with four shaped compartments and were able to move about within the compartment. The cage was placed in a MAC-1 sound-proof chamber designed by Industrial Acoustics (IAC, Bronx, NY) and the sound chamber was lined with sound-proofing acoustical foam to minimize reflections. Noise recordings were played with a Fostex FT17H Tweeter Speaker built into the top of the sound chamber. The damaging noise was measured across the sound chamber with a B&K sound level meter and adjusted to an intensity of 108 dB SPL with a variation of 1.5 dB across the cage.

2.3. Audiometric equipment and assessment of ABR thresholds

For inclusion in the study, data from at least three members of each strain was required (with the exception of strain AXB10/PgnJ). The number of mice evaluated per strain is listed in Supplemental Table 1. Mice 5–8 weeks of age were chosen as the optimal age for evaluation to avoid confounding of data from ARHI. Only female mice were evaluated as significant gender differences in hearing loss are known to exist (Henry, 2004).

All ABRs were performed inside a MAC-1 sound-proof chamber designed by Industrial Acoustics (IAC, Bronx, NY) to eliminate both environmental and electrical noise. Auditory stimuli were generated with a data acquisition board from National Instruments (National Instruments Corporation, Austin, Texas) and were delivered using an Intelligent Hearing Systems speaker (Intelligent Hearing Systems, Miami, Florida) attached to an 8–in. long tube that was inserted into the ear canal with sound pressure measured by a condenser microphone. Stainless-steel electrodes were placed subcutaneously at the vertex of the head and the right mastoid with a ground electrode at the base of the tail. Body temperature was maintained throughout the procedure on a heating pad kept at body temperature and an artificial tear ointment was applied to the eyes.

Auditory signals were presented to the right ear only as tone pips with a rise and a fall time of 0.5 msec and a total duration of 5 ms at the frequencies 4, 8, 12, 16, 24, and 32 kHz. Tone pips were delivered below threshold and then increased in 5 dB increments up to 100 dB SPL. Signals were presented at a rate of 30/second. They were sent to an amplifier and then to a sound transducer from Intelligent Hearing Systems. Physiologic responses were recorded with a 20,000 analog-to-digital rate and sent to an 8 channel 150-gain AC/DC headbox and then onto a secondary Synamps signal amplifier of 2500 gain before analysis. Responses were filtered with a 0.3–3 kHz pass-band. 512 waveforms were averaged for each stimulus intensity. Hearing thresholds were determined by visual inspection of ABR waveforms and defined as the minimum intensity at which a wave 1 complex could be distinguished. Post-noise exposure thresholds were evaluated by the same method 2 weeks post exposure. ABR Peak Analysis Software Version 0.9.0.2 ©Copyright 2007 Speech and Hearing Bioscience and Technology was used to analyze ABR waveforms and determine thresholds.

2.4. Determination of baseline hearing patterns

Mean ABR thresholds of each strain were graded for severity relative to the corresponding mean thresholds of CBA/J mice at the same test frequencies. Similar to the strategy employed by Zheng et al. (Zheng et al., 1999), strains with mean baseline thresholds more than 3 standard deviations greater than the corresponding CBA/J baseline mean at a given frequency were categorized as hearing-impaired at that frequency, and any strain with hearing impairment at any frequency was considered to be an overall hearing-impaired strain. Cutoffs were determined as follows: 78 dB (for 4 kHz), 62 dB (for 8 kHz), 43 dB (for 12 k Hz), 42 dB (for 16 kHz), 36 dB (for 24 kHz), and 44 dB (for 32 kHz). Hearing impaired strains were further graded at each frequency as mildly, moderately, or severely impaired if the strain mean was <20 dB, 20–40 dB, or >40 dB above the cutoff at that frequency, respectively. To exclude the possibility of middle ear pathology, absolute wave latencies were reviewed as wave latencies become prolonged in conductive hearing loss (McGee and Clemis, 1982).

2.5. Determination of PTS and noise-sensitivity patterns

PTS was derived from the difference between the mean post-