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Title: 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.

Keywords: Hearing loss; noise; mouse; inbred strain

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[1].

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 [1]. 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 [2–6]. 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% [7].

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 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 [8] 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 [9]. 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* [10].

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 80mg/kg body weight and xylazine 16mg/kg body weight) and all efforts were made to minimize suffering.

2.2 Noise Exposure

6 week old mice were exposed for 2 hours to octave band noise (OBN) with a center frequency of 10 kHz using a method adapted from Kujawa and Liberman [11]. 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 [12].

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 msec 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 to 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 [8], 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 12k 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, 20-40dB, 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 [13].

2.6 Determination of PTS and Noise-Sensitivity Patterns

PTS was derived from the difference between the mean post-exposure threshold and mean baseline threshold for each strain. Strains with PTS<20 at all frequencies were considered noise-resistant, whereas strains with PTS ≥20 at any frequency were considered noise-sensitive. A cutoff of 20dB was determined based on usage by prior studies [14,15]. Noise-sensitivities at each frequency were further categorized as mild (20≤PTS<30dB), moderate (30≤PTS<40dB), or severe (≥40dB).

3 Results

3.1 Establishing Baseline Hearing Thresholds

To assess the 100 inbred strains for baseline hearing function, ABR thresholds for each strain were determined prior to noise exposure (Supplemental Table 1). Strains were categorized at each test frequency as normal hearing, mildly impaired, moderately impaired or severely impaired using the inbred strain CBA/J as an internal reference for normal hearing as described in the methods [16–18].

Several distinct patterns of hearing loss were apparent: high-frequency hearing loss, high-and low-frequency hearing loss, flat hearing loss, and notch-type hearing loss (Figure 1). The vast majority of strains (49 strains) fell into the high-frequency hearing loss group in which hearing loss was most pronounced in the 24-32 kHz range. This group was further broken down into mild, moderate, and severe high-frequency impairment. Normal hearing strains were the second largest group, comprising 36 strains. Four strains exhibited combined high and low-frequency impairment with deficits at 4 kHz and 32 kHz. Flat loss strains (7) had deficits of similar magnitude across all frequencies. Notch-type strains (4) had steeply sloping peak deficits in intermediate frequencies of 16 kHz and/or 24 kHz. No strains were identified with isolated low-frequency hearing impairment. For clarity of interpretation, the baseline hearing data is replotted in alphabetical order in Supplemental Figure 1, with fewer strains per graph and standard error included.

3.2 Sensitivity to Noise Exposure

In addition to baseline hearing function, we also characterized the sensitivity of the same 100 strains to acoustic insult (Supplemental Table 1). Strains were exposed to damaging levels of noise then reevaluated two weeks later by ABR for post-exposure thresholds. PTS values were then calculated from the difference between pre-noise-exposure (baseline hearing threshold) and post-noise-exposure mean thresholds. Strains were categorized at each test frequency as either noise-resistant (PTS<20) or noise-sensitive (PTS≥20), and noise-sensitive thresholds were further categorized as mildly, moderately, or severely sensitive as described in the methods.

Several discernable patterns of noise-sensitivity were apparent: noise-resistant, high-frequency sensitivity, broad-frequency sensitivity, multimodal sensitivity, middle-frequency sensitivity, notch-type

sensitivity, and progressively sloping sensitivity (Figure 2 and Supplemental Figure 2). The 9 broadly sensitive strains exhibited PTS across multiple consecutive frequencies, such as BALB/cByJ which had moderate-to-severe PTS across all frequencies. The 4 strains with high-frequency sensitivity demonstrated peak PTS at 24 kHz and 32 kHz. There were 30 strains with middle-frequency sensitivity, comprising the largest group and demonstrating peak PTS at consecutive frequencies of 12 and 16 kHz. This group was further broken down into mild, moderate, and severe middle-frequency sensitivity. The 7 strains categorized as notch-type sensitive each exhibited peak PTS at a single isolated frequency; for example BXD42/TyJ was severely sensitive at 12 kHz but resistant at all other frequencies. Multimodal sensitivity strains exhibited peak PTS of similar magnitude at two or more non-consecutive frequencies, such as FVB/nJ which had peak PTS at 12 and 24 kHz. Progressive-sloping sensitivity strains demonstrated progressively greater noise-sensitivity with higher frequencies; for example, BXA16/PgnJ had mild PTS in the 12 and 16 kHz range but moderate PTS in the 24 and 32 kHz range. 14 noise-resistant strains showed minimal PTS at all frequencies tested. No strains with isolated low-frequency noise-sensitivity were identified.

Notably, the majority of strains demonstrated threshold shifts within the dynamic range of testing (0-100 dB SPL). However, several strains had, at specific frequencies, such severe baseline hearing deficits that categorization of subsequent PTS as noise resistant or sensitive according to our strategy was not reliable. For example, NOD/ShiLtJ had baseline mean thresholds of 85.8, 93.3, and 92.5 dB SPL and PTS values of 10.8, 3.3, and 5.0 dB at test frequencies of 16, 24, and 32 kHz, respectively. These PTS values met our technical criteria for resistance but were a product of significant baseline deficits rather than "true" noise resistance. As noted by Lin et al., a possible explanation for this phenomenon is a "ceiling effect", in which there are a limited number of damage-susceptible elements in the inner ear, and the more elements that are already damaged from prior causes, the fewer elements remain to be damaged by further noise exposure [19]. In total, sixteen strains were excluded from noise-sensitivity-pattern categorization. However, the data for these strains is still provided (Supplemental Table 1 and Supplemental Figure 2).

3.3 Baseline hearing impairment and noise sensitivity

Prior studies have demonstrated that preexisting SNHL reduces subsequent threshold shifts from noise exposure [19–21], a trend which we also observed during our phenotyping of noise-sensitivity. As noted above, this is likely partly due to the ceiling effect, which becomes progressively more relevant as thresholds near the upper limit of testing.

Given the above observation, we felt it important to include a plot of PTS as a function of baseline ABR threshold to aid the interpretation of noise-sensitivity with consideration for severity of baseline hearing deficit. Strains were plotted as a function of baseline ABR threshold and post-noise-exposure PTS at each of the test frequencies from 4-32 kHz (Figure 3 and Supplemental Figures 3-7). The 16 kHz plot was selected for Figure 3 because the behavior at this frequency was most representative of behavior at other test frequencies; however, plots at other test frequencies are included in the Supplemental Material.

4 Discussion

Our focus was to expand upon the existing hearing phenotype literature by characterizing baseline hearing in 47 strains not present in the literature. Additionally, no group has published large scale phenotypic data of noise-sensitivity in mice. Thus, the noise-sensitivity data presented in this study provides a new resource for the study of NIHL.

4.1 Choice of Strains

Our lab studies the genetics of common forms of hearing loss in mice, including age-related and noise-induced hearing loss. The 100 mouse strains used in this study were selected from the Hybrid Mouse Diversity Panel (HMDP), which is a library of inbred mouse strains designed for use with Genome-Wide-Association Studies (GWAS) [22]. The HMDP is a powerful resource for dissecting the

genetic variation underlying common traits and is powered to detect genetic variation responsible for as little as 5% of the phenotypic variance [23]. The 30 common inbred strains and 70 recombinant strains that comprise the HMDP provide high statistical power and mapping resolution [24]. In particular, the recombinant inbred strains, which include AXB, BXA, BXD, BXH, and CXB, are derived from pairwise crosses of classical inbred strains; their inclusion in the HMDP significantly increases the statistical power to detect single-nucleotide polymorphisms (SNPs) associated with complex traits [25]. We recently published a genome-wide association study utilizing the HMDP to identify NADPH oxidase3 (*Nox3*) as a NIHL susceptibility gene [26]. In this manuscript, we present the complete 100 strain panel of baseline ABR threshold phenotypes and noise sensitivity phenotypes with the hope that this data will facilitate future investigations in hearing research.

Many inbred mouse strains possess distinct biological traits that make them useful models for human diseases; such traits are also a convenient means of studying relationships between hearing loss and other disease processes. For example, NZB/BinJ and NZW/LacJ, which were identified as noise-resistant in our study, are both models of autoimmune disorders [27] and may provide a useful platform for studying the role of autoimmunity in the development of or resistance against NIHL. C3H/HeJ mice have reduced reactive-oxygen-species (ROS) generation and cellular immunity, making them highly susceptible to gram-negative bacterial challenge [28]. Interestingly, this deleterious trait may prove advantageous in regard to hearing loss as we identified this strain as noise-resistant. This finding supports the notion that oxidative stress plays a role in mediating hair cell damage during hearing loss [29]. BTBR and I/LnJ both lack a corpus callosum, which contains nerve projections from the primary and secondary auditory cortices; these strains interestingly show divergent noise-sensitivity phenotypes with the former being severely noise sensitive and the latter being noise resistant.

In addition to unique biological traits, the genetic diversity provided by the HMDP allows for the study of genetic background effects on allelic penetrance and expressivity. Several of the HMDP strains possess the *Cdh23*^{ahl} allele, which leads to progressive hearing loss of variable timing and severity depending on the genetic background. The recombinant inbred strains in particular provide a useful model for dissecting such effects because of the heterogeneity of their genetic makeup, which is derived from various crosses of classical inbred strains. Inspection of different recombinant strains with the *Cdh23*^{ahl} allele sharing common progenitors may reveal divergent phenotypes that arise from subtle differences in genetic background. For example, the hearing loss phenotypes of the BXD strains, which are derived from C57BL/6J and DBA/2J crosses, have been shown to vary substantially in onset, progression, and severity; this variation is determined in part by the number of AHL genes inherited from each progenitor strain and by genetic background effects [9]. Thus, the strains and phenotypic data included in our panel provide a useful platform for further identification of modifying genes in hearing loss. Moreover, as previously noted by Zheng et al.[8], genetic background can confound analysis of hearing experiments or behavioral tests which rely on hearing for experimental output, so the characteristic baseline hearing ability and noise sensitivity for a given strain are important considerations in any experimental design relying on hearing for accurate interpretation of results.

4.2 Patterns of baseline hearing impairment and noise-sensitivity

Audiometric patterns of hearing loss have a long history in both clinical studies and animal models, and different patterns have been shown to reflect distinct underlying pathophysiological mechanisms [30,31]. A classic example is the audiometric pattern of ARHI. Characterized by flat hearing loss of similar magnitude across low frequencies and progressively more severe loss at higher frequencies, ARHI arises from a gradual loss of the endocochlear potential (EP) over time and the differential response of the basal and apical portions of the cochlea to this loss of EP [30,32–37]. Other classic examples include the audiometric profiles of NIHL and toxin-induced hearing loss, which are both characterized by a notch of well-defined hearing loss at high frequencies and arise from damage to the cochlear amplifier [38–40]. Thus, the distinct audiometric patterns of hearing loss and noise-sensitivity described in this study may provide further insight into the mechanisms underlying hearing loss.

Indeed, much progress has already been made in regard to the complex pathophysiology of hearing loss, particularly the role of genetics. The role of heredity in hearing loss is supported by the strain-specificity of hearing impairment patterns in inbred mouse strains [29,41,42]. For example, in their phenotypic profiling of common inbred strains, Zheng et al. observed frequency-specific impairment patterns unique to certain strains, noting that A/J mice have a specific hearing impairment at 16 kHz whereas C57BR/cdJ and C57L/J mice are least impaired at that frequency [8]. Targeted gene deletion studies have further delineated the genetic and cellular components important for normal development and function of the auditory system. Li et al. demonstrated that deletion of the gene *Aquaporin4* (AQP4) on a CD1 background causes broad-frequency hearing impairment, which they attribute to the inability of epithelial cells of the organ of Corti to adapt to large potassium fluxes during mechano-electric signal transduction [43]. Young mice with defects in *Barhl1*, a mouse homolog for the Drosophila BarH homeobox genes, develop a distinct low-frequency hearing loss at 4 kHz that progresses to higher frequency hearing loss with age; hearing loss correlates with progressive OHC degeneration that begins at the cochlear apex and spreads to the base [44]. Developmental abnormalities of the inner ear and central auditory pathways may also account for distinct patterns of hearing impairment. *Kcnq4* [45]and *Bdnf* [46] each have distinct developmental gradients in cochlear hair cells along the longitudinal axis of organ of Corti. Dysfunction in these genes and others with location-specific developmental roles could give rise to distinct patterns of hearing loss.

Similarly, noise-sensitivity can demonstrate distinct patterns. We recently demonstrated that mice possessing a *Nox3* mutant allele have relatively normal baseline hearing but demonstrate a selective vulnerability to noise-induced hearing loss at 8 kHz based on data from ABR studies and distortion product otoacoustic emissions; this audiometric phenotype was reflected by a decrease in synaptic ribbons at the corresponding tonotopic location in the cochlea [26]. Thus, further study of the HMDP strains and noise sensitivities provided in this study may reveal other genes accounting for the distinct patterns of noise sensitivity observed here.

4.3 Relationship between baseline hearing function and PTS

As previously mentioned, past studies have demonstrated that preexisting SNHL reduces subsequent threshold shifts from noise exposure [19–21], a trend which we also observed during our phenotyping of noise-sensitivity. As Lin et al. note, a possible explanation for this phenomenon is a "ceiling effect." According to this explanation, there are a limited number of damage-susceptible elements in the inner ear; the more elements that are already damaged from prior exposures or from inherited defects as in this study, the fewer elements remain to be damaged by further noise exposure [19]. A prime substrate for hearing loss is the cochlear amplifier and its major components: the outer hair cells (OHC) and the stria vascularis. The cochlear amplifier is an anatomically and physiologically complex organ critical for the sensitivity and frequency-specificity of hearing [47], and dysfunction of the cochlear amplifier will significantly impact these functions [38,48–50]. In the case of the inbred strains used in our study, inherited differences in cochlear amplifier function may account for baseline defects in hearing that will limit further threshold shifts in response to noise-exposure, although other factors not addressed in our experimental design may be involved as well.

An alternative theory is that there may be an active physiological process that, in response to preexisting SNHL, may function to reduce subsequent acoustic trauma. Prior studies have demonstrated the effects of acoustic "toughening", in which pre-conditioning with moderate-level acoustic stimulus can reduce damage from later exposure to the same stimulus at high intensity [19,21,51,52], although the protective effects of such pre-exposure are transient. Notably, these studies are focused on noise as a means of pre-conditioning, whereas in this study the "source" of preconditioning would be preexisting deficits due to genetic differences, a topic for which there is little study to date.

4.4 Limitations

Noise vulnerability changes as a function of age, such that young humans and animals are particularly sensitive to acoustic insult. In mice, this sensitivity period (alternatively known as the

"critical period" or "early window") peaks around 6-8 weeks then gradually diminishes to permanent adult levels around 4 months [53]. Our study utilized 5-8 week old mice to avoid confounding by ARHI, but it must be noted that younger and older age groups should be viewed as mechanistically distinct models and that our 'early window' noise-sensitivity results are most appropriately used with this consideration in mind.

Moreover, the noise exposure conditions used in this study were subject to variation in several parameters that are important to consider. For example, it has been shown that hypothermia (30ºC) is protective for NIHL while hyperthermia (40ºC) exacerbates NIHL [54]. To reduce possible confounding artifacts from fluctuating body temperature, the mice were left awake during the exposure. Additionally, as the presence of solid materials within the exposure cage can block transmission of sound waves, mice were housed in a pie-shaped wire-mesh exposure cage with four compartments using a circular design to ensure equivalent SPLs between mice. The mice were separated to minimize huddling that might reduce sound transmission. ¼ inch wire mesh was used for the cage body to allow mouse waste to drop away from the animals. We selected the Fostex FT17H Tweeter Speaker due to the low variation (± 3 dB) in its frequency response curve, but there were still unavoidable variations inherent to the equipment that are worthy of mention.

Lastly, it should be noted that this work implicitly references a noise level-versus-PTS function that may change if we altered the set age, noise level, or frequency of noise exposure. We do not know the shape of this function or if/how the shape varies with strain or age, which is an important limitation to bear in mind. However, this caveat is present in any noise exposure study and due to the limitation of resources and time, we chose to expand upon the research by including more strains.

5 Conclusions

In this study, we report the results of a superficial screening study of baseline hearing ability and noise sensitivity in 100 inbred mouse strains, 47 of which have never been characterized for hearing traits. We report the baseline ABR thresholds for these 100 strains and identify several distinct patterns of baseline hearing impairment. Secondly, we report the noise vulnerability of these same 100 strains as measured by PTS and identify several distinct patterns of noise-sensitivity. Lastly, we make the complete phenotypic dataset available for general use. This data establishes a new resource for the study of NIHL in mice and adds 47 newly characterized strains to the existing baseline hearing literature.

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Figure Legends

Figure 1. Inbred strains of mice show distinct patterns of hearing impairment.
Baseline hearing for each strain is shown in audiogram format, with mean ABR threshold (in dB SPL) plotted as a function of auditory stimulus frequency (in kHz). The following patterns of baseline hearing function were observed: **(A)** normal hearing strains [AXB1/PgnJ, AXB10/PgnJ, AXB24/PgnJ, AXB6/PgnJ, AXB8/PgnJ, BALB/CJ, BTBR_T_tf/J, BXA12/PgnJ, BXA14/PgnJ, BXD13/TyJ, BXD28/TyJ, BXD31/TyJ, BXD74/RwwJ, BXH14/TyJ, BXH22/KccJ, BXH4/TyJ, BXH7/TyJ, BXH9/TyJ, C3H/HeJ, C57BL/6J, CBA/J, CXB1/ByJ, CXB11/HiAJ, CXB12/HiAJ, CXB2/TyJ, FVB/nJ, KK/HIJ, MRL/MpL, NON/ShiLtJ, NZB/BinJ, NZW/LacJ, PL/J, RIIIs/J, SJL/J, SM/J, SWR/J], **(B)** mild severity high-frequency hearing loss strains [AKXL17a/TyJ, AXB12/PgnJ, BALB/CbyJ, BXA1/PgnJ, BXA16/PgnJ, BXA4/PgnJ, BXA7/PgnJ, BXD1/TyJ, BXD14/TyJ, BXD15/TyJ, BXD18/TyJ, BXD5/TyJ, BXD6/TyJ, BXD70/RwwJ, BXD75/RwwJ, BXH10/TyJ, BXH6/TyJ, BXH8/TyJ, C58 /J, CXB13/HiAJ, LG/J, SEA/GnJ], **(C)** moderate severity high-frequency hearing loss strains [129X1/SvJ, AXB13/PgnJ, BXD11/TyJ, BXD2/TyJ, BXD29/TyJ, BXD34/TyJ, BXD50/RwwJ, BXD55/RwwJ, BXD73/RwwJ, BXD8/TyJ, BXD84/RwwJ, BXD9/TyJ, BXH19/TyJ, C57BLKS/J, CXB9/HiAJ], **(D)** severe high-frequency hearing loss strains [AXB15/PgnJ, AXB19/PgnJ, AXB19a/PgnJ, AXB19b/PgnJ, AXB5/PgnJ, BXA25/PgnJ, BXD12/TyJ, BXD20/TyJ, BXD32/TyJ, DBA/2J, MA/MyJ, NOD/ShiLtJ], **(E)** flat-frequency hearing loss strains, and **(F)** high and low frequency hearing loss strains [AKR/J, C57L/J, I/LnJ, LP/J] indicated by solid shapes/lines and notch-type hearing loss strains [BXD21/TyJ, BXD38/TyJ, BXD42/TyJ, CE/J] indicated by clear shapes/dotted lines.

Figure 2. Inbred strains of mice show distinct patterns of noise sensitivity.
Noise sensitivity for each inbred mouse strain is represented by PTS values, which are shown in audiogram format. PTS values (in dB) are plotted as a function of auditory stimulus frequency (in kHz). The following patterns of noise-sensitivity were observed: **(A)** noise resistant [AXB24/PgnJ, BXA1/PgnJ, BXA13/PgnJ, BXA24/PgnJ, BXD31/TyJ, BXD84/RwwJ, BXH6/TyJ, BXH7/TyJ, C3H/HeJ, I/LnJ, NZB/BinJ, NZW/LacJ, SJL/J, SM/J], **(B)** high-frequency sensitivity, **(C-D)** broad-frequency sensitivity, **(E)** multiple peak sensitivity, **(F)** mild severity middle-frequency sensitivity, **(G-H)** moderate severity middle-frequency sensitivity, **(I-L)** severe middle-frequency sensitivity, **(M-N)** notch-type sensitivity, and **(O-P)** sloping sensitivity. The 16 strains that were categorized as having a "ceiling effect" as described in the text were not included in this figure but are included in Supplemental Figure 2.

Figure 3. Noise sensitivity vs baseline hearing at 16 kHz stimulus frequency.
For each strain, the permanent threshold shift value (dB) is plotted against the baseline hearing threshold (dB SPL) at a stimulus frequency of 16 kHz. All strain names are abbreviated for figure clarity.

Supporting Information Legends

Supplementary Table 1. Baseline Hearing Function and Noise-Sensitivities for 100 HMDP Strains.
Mean ABR thresholds in dB SPL both before (PRE) and after (POST) noise exposure at the stimulus frequencies listed (4, 8, 12, 16, 24, and 32 KHz) are shown with corresponding standard deviations (SD) and sample sizes (N) tested in each condition. Overall baseline hearing pattern is also shown for each strain. PTS values in dB are shown at the stimulus frequencies listed (4, 8, 12, 16, 24, and 32 KHz) along with the overall noise-sensitivity pattern. Classical inbred strains and recombinant inbred strains (blue) are listed in alphabetical order. Grades of hearing impairment and grades of noise-sensitivity are indicated by the following color scheme: mild (green), moderate (orange), severe (red). "Data not collected" (not assessed) indicates that no hearing data was collected at that particular frequency. Grey-colored boxes indicate that PTS calculations at that particular frequency were excluded due to either absent PRE/POST data or due to presence of a ceiling effect described in the methods.

Supplemental Figure 1. Baseline hearing function of inbred mouse strains.

Baseline hearing function for each of the 100 inbred mouse strains prior to noise exposure is shown in audiogram format. Strains are arranged in alphabetical order. Error bars +/- 1 SE.

Supplemental Figure 2. Noise sensitivity of inbred mouse strains.
Noise sensitivity for each of the 100 inbred mouse strains is represented by permanent threshold shifts (dB) shown in audiogram format as a function of auditory stimulus frequency (in kHz), with fewer strains per graph to improve clarity. Strains are arranged in alphabetical order. Included are the 16 strains categorized as having a "ceiling effect", in which baseline thresholds were near the upper limit of testing such that interpretation of subsequently calculated PTS values was difficult. Strain A/J was excluded entirely from PTS calculations because baseline hearing was completely absent. Frequencies affected by a ceiling effect are denoted by *.

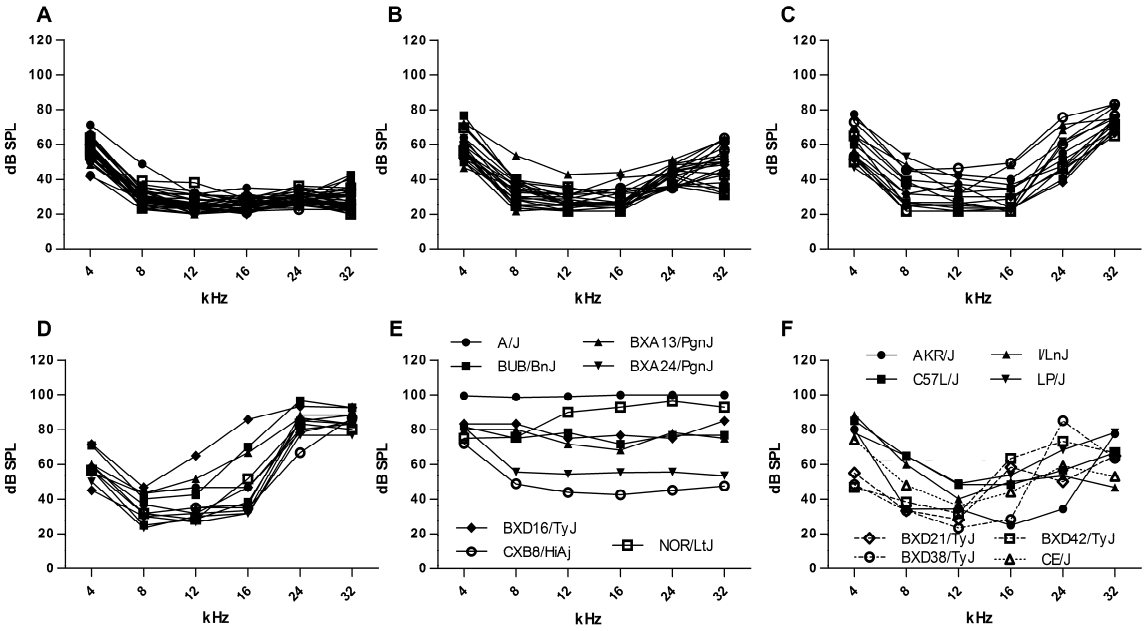
Supplemental Figure 3. Noise sensitivity vs baseline hearing at 4 kHz stimulus frequency.
For each strain, the permanent threshold shift value (dB) is plotted against the baseline hearing threshold (dB SPL) at a stimulus frequency of 4 kHz. All strain names are abbreviated for figure clarity.

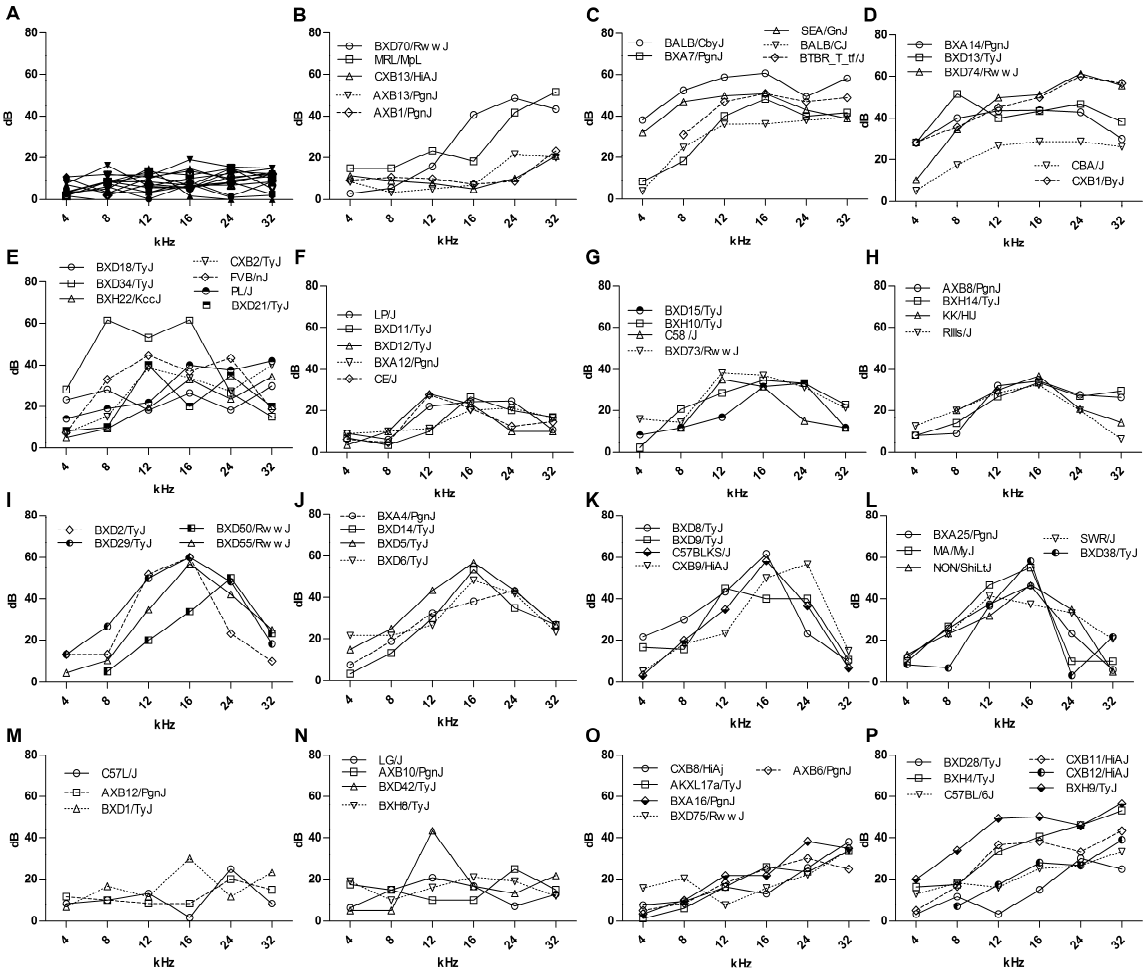
Supplemental Figure 4. Noise sensitivity vs baseline hearing at 8 kHz stimulus frequency.
For each strain, the permanent threshold shift value (dB) is plotted against the baseline hearing threshold (dB SPL) at a stimulus frequency of 8 kHz. All strain names are abbreviated for figure clarity.

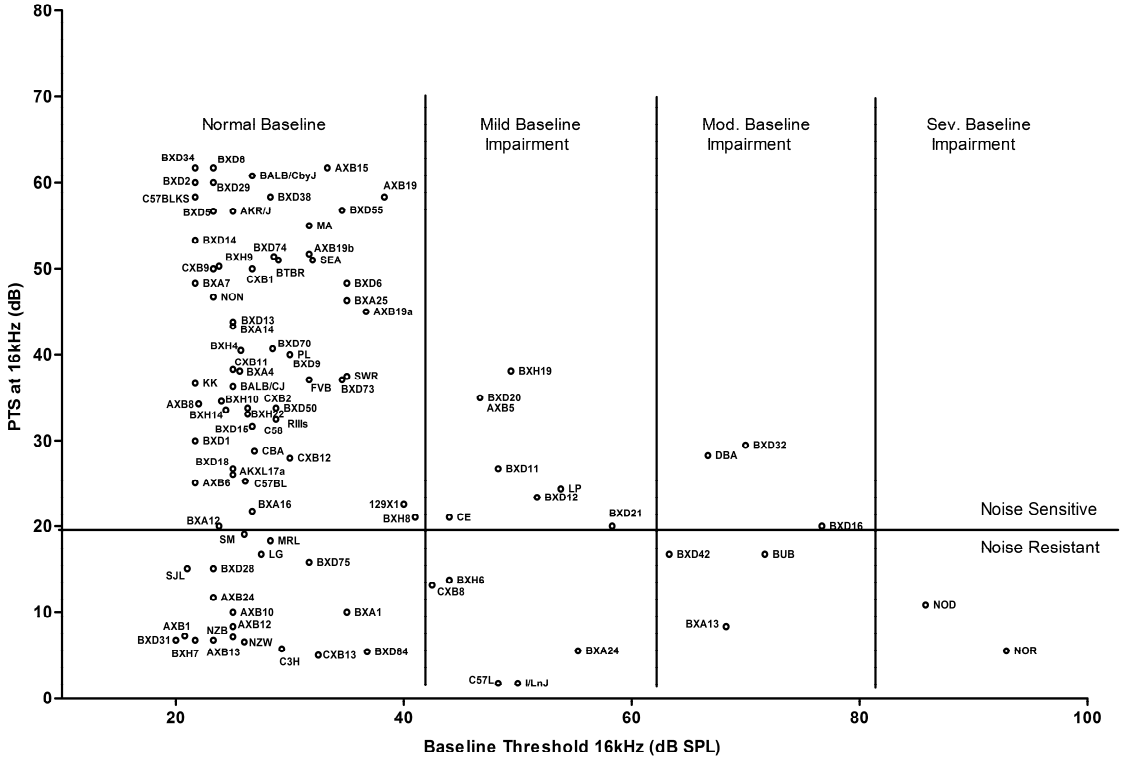
Supplemental Figure 5. Noise sensitivity vs baseline hearing at 12 kHz stimulus frequency.
For each strain, the permanent threshold shift value (dB) is plotted against the baseline hearing threshold (dB SPL) at a stimulus frequency of 12 kHz. All strain names are abbreviated for figure clarity.

Supplemental Figure 6. Noise sensitivity vs baseline hearing at 24 kHz stimulus frequency.
For each strain, the permanent threshold shift value (dB) is plotted against the baseline hearing threshold (dB SPL) at a stimulus frequency of 24 kHz. All strain names are abbreviated for figure clarity.

Supplemental Figure 7. Noise sensitivity vs baseline hearing at 32 kHz stimulus frequency.
For each strain, the permanent threshold shift value (dB) is plotted against the baseline hearing threshold (dB SPL) at a stimulus frequency of 32 kHz. All strain names are abbreviated for figure clarity.







Large-scale Phenotyping of Noise-Induced Hearing Loss in 100 Strains of Mice: Highlights

- We conducted a superficial screening study for hearing function in 100 inbred strains of mice.
- Several distinct patterns of baseline hearing impairment are observed, and possible avenues of research are discussed.
- We also characterize the sensitivity of the same 100 strains to damaging levels of noise.
- Several distinct patterns of noise-sensitivity are observed, and possible avenues of research are discussed.
- The combined dataset is made available for general use.