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Divergence of noise vulnerability in cochleae of young CBA/J and CBA/CaJ mice

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Abstract

CBA/CaJ and CBA/J inbred mouse strains appear relatively resistant to age- and noise-related cochlear pathology, and constitute the predominant ‘good hearing’ control strains in mouse studies of hearing and deafness. These strains have often been treated as nearly equivalent in their hearing characteristics, and have even been mixed in some studies. Nevertheless, we recently showed that their trajectories with regard to age-associated cochlear pathology diverge after one year of age (Ohlemiller et al., 2010 *J. Assoc. Res. Otolaryngol.* in press). We also recently reported that they show quite different susceptibility to cochlear noise injury during the ‘sensitive period’ of heightened vulnerability to noise common to many models, CBA/J being far more vulnerable than CBA/CaJ (Fernandez et al., 2010 *J. Assoc. Res. Otolaryngol.* 11:235–244). Here we explore this relation in a side-by-side comparison of the effect of varying noise exposure duration in young (6 week) and older (6 month) CBA/J and CBA/CaJ mice, and in F1 hybrids formed from these. Both the extent of permanent noise-induced threshold shifts (NIPTS) and the probability of a defined NIPTS were determined as exposure to intense broadband noise (4–45 kHz, 110 dB SPL) increased by factors of two from 7 seconds to 4 hours. At 6 months of age the two strains appeared very similar by both measures. At 6 weeks of age, however, both the extent and probability of NIPTS grew much more rapidly with noise duration in CBA/J than in CBA/CaJ. The ‘threshold’ exposure duration for NIPTS was <1.0 minute in CBA/J versus >4.0 minutes in CBA/CaJ. F1 hybrid mice showed both NIPTS and hair cell loss similar to that in CBA/J. This suggests that dominant-acting alleles at unknown loci distinguish CBA/J from CBA/CaJ. These loci have novel effects on hearing phenotype, as they come into play only during the sensitive period, and may encode factors that demarcate this period. Since the cochlear cells whose fragility defines the early window appear to be hair cells, these loci may principally impact the mechanical or metabolic resiliency of hair cells or the organ of Corti.

Keywords

Noise-induced permanent threshold shifts; noise-induced hearing loss; hair cells; development; susceptibility; genetics

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INTRODUCTION

Variation in the rate of acquired hearing loss in human populations must in part reflect allelic variation in genes that impact the integrity or resiliency of cochlear sensory cells. Accordingly, loci have been identified that modulate cochlear injury associated with aging (Willott, 1991; Erway et al., 1993; Van Eyken et al., 2007; Ohlemiller and Frisina, 2008; Rodriguez-Paris et al., 2008), noise (Erway and Willott, 1996; Ohlemiller, 2006, 2008; Konings et al., 2009; Pawelczyk et al., 2009), and ototoxins (Forge and Schacht, 2000; Rybak, 2007; Perletti et al., 2008) in humans and animals. Susceptibility to noise and ototoxins is not constant throughout life, but depends on age, such that young humans and animals appear especially vulnerable (Stanek et al., 1977; Bernard, 1981; Henry, 1982b; Saunders and Chen, 1982; Henry, 1983; Henley and Rybak, 1995; Li and Steyger, 2009). The physiologic basis of this 'sensitive period' (alternately termed 'critical period' or 'early window'), is not well understood, but probably differs for noise versus ototoxins.

Mouse models have been invaluable to our understanding of just how strongly genetically influenced are some processes that might otherwise be taken to be qualitatively homogeneous across mammals. For example, using mice it has become clear that, for a given noise exposure, the endocochlear potential (EP) may or may not be transiently—or permanently—depressed (Ohlemiller and Gagnon, 2007; Ohlemiller et al., 2010b; Ohlemiller et al., 2010c), or a particular preconditioning treatment may or may not be protective (Gagnon et al., 2007), depending on genetic background. Such observations warn against overgeneralization of findings from any one study, model, or species. Variation across inbred mouse strains with respect to vulnerability to noise (Henry, 1982a; Li, 1992; Erway and Willott, 1996; Ohlemiller et al., 2000; Yoshida et al., 2000) and ototoxins (Saunders and Chen, 1982; Wu et al., 2001) suggests that many genes governing acquired hearing loss remain to be identified. Mouse studies have typically been anchored using 'good hearing', noise resistant, standard strains that prominently include CBA/CaJ and CBA/J mice (Henry and McGinn, 1992). These strains share a common origin and a similar name, and have been applied nearly interchangeably to issues of hearing and deafness. Both derive from a cross between Bagg albino and DBA mice in 1920 (Fox et al., 1997), but in fact began divergent paths in 1933, and are now distinguished by over 2,200 polymorphisms (Bult et al., 2008). We recently demonstrated that after one year of age CBA/CaJ and CBA/J cochleae diverge qualitatively, such that only CBA/CaJ show significant EP decline, stria marginal cell loss, and spiral ligament outer sulcus cell/root cell loss (Ohlemiller et al., 2010a). Nevertheless, in a concurrent study we were ourselves caught up by the assumption that CBA/CaJ and CBA/J cochleae respond similarly to noise exposure. While searching for a subclinical noise exposure that could be combined with subtoxic kanamycin in one month old CBA/J mice (Fernandez et al., 2010), we found that as little as 30 s of broadband noise at 110 dB SPL caused severe noise-induced permanent threshold shifts (NIPTS) and moderate hair cell loss. Using the same type of noise, we had previously reported (Ohlemiller et al., 2000) and later confirmed (Ortmann et al., 2004) that the exposure 'threshold' for NIPTS in 6 week old CBA/CaJ mice was ~4.0 minutes. Our assumptions going into the kanamycin work were based on observations of similar injury to the cochlear lateral wall (Hirose and Liberman, 2003; Ohlemiller and Gagnon, 2007) and impressions of NIPTS in older mice of the two strains. At that point we followed our own advice about assumptions of similarity, and undertook a systematic study comparing noise vulnerability in young (6 week) and older (6 month) CBA/J and CBA/CaJ mice. Here we present those results, showing that only within the sensitive period do the two strains differ with respect to noise vulnerability, manifested in the form of a much lower exposure threshold for NIPTS and greater hair cell loss in CBA/J mice. The noise injury phenotype of F1 hybrid mice formed from the two strains strongly resembles that of CBA/J, suggesting that dominant-acting alleles at only one or a few loci establish the CBA/J phenotype. The genes involved

are remarkable in that their influence is observed during the sensitive period. Thus they may encode factors that define this period.

METHODS

Our overall approach was to stratify CBA/J and CBA/CaJ inbred mice into two age groups (6 weeks and 6 months), then to expose the mice once to loud broadband noise of varying duration. The data were analyzed to compute the growth of both the *severity* and *probability* of noise-induced permanent threshold shifts (NIPTS) as a function of age, strain, and noise duration. Auditory sensitivity as monitored by auditory brainstem recording (ABR) was determined prior to noise, and then two weeks after exposure, to establish the severity of NIPTS after transient threshold shifts had resolved. Dose-response experiments established that an exposure duration of 1.88 min caused NIPTS in CBA/J but not in CBA/CaJ. Therefore, as an initial approach to the genetic basis of this difference, F1 hybrids were bred from the two strains and exposed for 1.88 min. After two weeks, these were tested for NIPTS, and sample cochleae from these and inbred mice receiving the same exposure were evaluated for hair cell survival. All animal procedures were approved by the Washington University Institutional Animal Care and Use Committee.

Animals

Our study encompassed 57 CBA/J mice, 67 CBA/CaJ mice, and 11 (CBA/CaJ $\times$ CBA/J) F1 hybrid mice of either gender. Inbred mice were purchased from The Jackson Laboratory (JAX) or bred from these. All mice were either 6 weeks or 6 months of age at time of noise exposure. These two ages were chosen because they correspond to those examined in previous work. They are also intended to lie within, and well outside of, the sensitive period for mice as demonstrated by Henry (Henry, 1982b, 1982a). Noise vulnerability in CBA/J and CBA/CaJ mice appears to peak around one month of age, then to progressively decrease to a posited 'stable' adult level by about 4 months (Kujawa and Liberman, 2006). Sample sizes by inbred strain, age group, and exposure duration are given in Table I.

Noise exposure

Broadband noise (4–45 kHz, 110 dB SPL) was produced and filtered with General Radio 1310 generators and Krohn-Hite 3550 filters, respectively. The spectral shape of the noise was as previously published (Ohlemiller et al., 1999). Two animals at a time were placed in a wired cage suspended between four speakers at 0, 90, 180, and 270 degrees azimuth in a single-walled sound-proof booth with foam treatment (Industrial Acoustics, Bronx, NY). The cage was rotated at 0.013 Hz during the exposure to achieve a homogeneous sound field. The duration of exposure varied in multiples of two from 7 seconds to 4 hours (.117 min–240 min; See Figs. 4–5). Exposure durations technically entailing fractions of seconds were rounded to the nearest second.

ABR recording

ABR testing was performed using Tucker-Davis Technologies (TDT) System II hardware and software. Animals were anesthetized with a solution of ketamine and xylazine (80/15 mg/kg, i.p.) and positioned dorsally in a custom headholder. Subdermal platinum needle electrodes (Grass) were placed in the mid-back (ground), behind the right pinna (active), and at the vertex (reference). Body temperature was monitored throughout testing using a rectal probe, and maintained at $37.5 \pm 1.0^\circ\text{C}$ using a DC current-based isothermal pad (FHC). The right ear of each mouse was stimulated in freefield using a TDT ES-1 speaker placed at 7 cm distance along the interaural axis. Stimuli were 5 ms tonebursts (1000 repetitions, 20/s, 1.0 ms rise/fall time) at frequencies of 5, 10, 20, 28.3, and 40 kHz. To eliminate contributions to the ABR by the unstimulated ear, the left external meatus was compressed using a spring-

loaded clip. Responses were amplified $\times 100,000$ and filtered at 100–10,000 Hz. Thresholds were taken to be the lowest sound level for which Wave I could be identified, using a 5 dB minimum step size. Where appropriate (Fig. 6), data were analyzed by 2 way ANOVA (threshold by group, frequency), followed by Bonferroni multiple comparisons tests.

Noise dose-response analysis

Two different approaches were taken to analyze the growth of cochlear noise injury with exposure duration. The first approach was to generate for each age, strain, and noise duration a metric for the overall NIPTS, based roughly on the area under the NIPTS-versus-frequency curve. For a given group and exposure duration, the average NIPTS shown in Figures 2 and 3 was summed across all ABR test frequencies and the resulting value was plotted versus exposure duration (Total NIPTS in dB, Fig. 4). The second approach was to compute for each strain, age group, and exposure duration the dose-response relation for the probability of NIPTS, including the minimum exposure required. Dose-response paradigms have been applied in many studies, with the 'dose' varying in intensity and/or duration, and the NIPTS 'response' defined different ways (Spoendlin, 1971; Spoendlin and Brun, 1973; Bohne and Clark, 1982; Fredelius et al., 1987; Kaltenbach et al., 1992; Clark and Pickles, 1996; Davis et al., 1999). Similar to our previous application of the same method (Ohlemiller et al., 2000), NIPTS in any animal was defined as an increase in threshold of at least 10 dB at two or more test frequencies. Since we wished to compare our current data with probability estimates we derived previously (Table II), threshold shifts at 28.3 kHz were not included in this analysis. The probability of NIPTS was then determined based on the proportion of animals tested that met the criterion for NIPTS two weeks after exposure. Except as noted in Table I, the number of animals per condition typically began at four, and then was expanded to 8–9 animals for conditions found to lie on the dynamic portion of the curve. As in our previous paper, probability-of-NIPTS data were then fit to a symmetrical logistic equation of the form

$$y = \{(a - b) / (1 + [x/c]^b)\} + d \quad \text{Eqn. 1}$$

where y is the proportion of animals exhibiting NIPTS, x is exposure duration in minutes and a , b , c , and d are fitting parameters (SigmaStat). The fitted function solved for x at $y = 0.9$ was considered the threshold exposure for NIPTS. The assignment of subject outcomes to categories, followed by logistic or probit modeling, has been applied to problems as diverse as munitions testing and determination of LD50 (Finney, 1985). This approach was chosen because of its economy for analyses. Because y values must lie between 0.0 and 1.0, key descriptors of the relation do not have to be obtained by extrapolation. Also, two-category data reduce data variance because they follow a binomial distribution which, for small samples and probabilities other than 0.5, has a reduced variance. Finally, the requirement of threshold increases at two test frequencies formed a logical 'and' gate, which further decreases variance.

Sample preparation and histology

One cochlea from each of 17 six week old mice (6 CBA/CaJ, 5 CBA/J, 6 F1) that had been exposed to 1.88 min of noise were prepared for hair cell counts after recording. The remaining cochlea of 9 mice from the same treatment groups ($n=3/\text{group}$) was sectioned in the mid-modiolar plane for qualitative light microscopy. For sacrifice, animals were overdosed using Pentobarbital and perfused transcardially with cold fixative containing 2.0% paraformaldehyde and 2.0% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). Each cochlea was rapidly isolated, immersed in the same fixative, and the stapes was removed. Complete infiltration of the cochlea by fixative was facilitated by drilling a small hole at the apex of the cochlear capsule, and gently circulating the fixative over the cochlea using a

transfer pipet. After decalcification in sodium EDTA for 72 hours, cochleas were post-fixed in buffered 1% osmium tetroxide, dehydrated in an ascending acetone series, and embedded in Epon. Cochleae taken for light microscopy were sectioned at 4 μm in the mid-modiolar plane, and toluidine blue-stained sections were examined conventionally. Cochleae taken for hair cell counts were dissected using fine blades into half-turn segments, immersed in mineral oil in a depression slide, and examined as surface preparations by Nomarski optics using a 20 $\times$ oil objective and a calibrated grid ocular. The percent outer hair cells (OHCs) and inner hair cells (IHCs) missing (as judged by the absence of nuclei) was estimated in contiguous 200 μm segments, and data were recorded separately by cell type as a function of distance from the basal tip. For each hair cell type, distance versus percent present was plotted as a function of frequency based on Muller et al. (Muller et al., 2005). Hair cell survival differences by group were analyzed by 2 way ANOVA (cell loss by group, cochlear place), followed by Bonferroni multiple comparisons tests.

RESULTS

Consistent with the literature for CBA/J and CBA/CaJ (e.g., Henry and McGinn, 1992; Zheng et al., 1999), initial thresholds in inbred and F1 mice were normal at both ages examined and showed little variation (Fig. 1). Mean NIPTS generally grew with exposure duration, yet grew more rapidly in young CBA/J than in young CBA/CaJ (Fig. 2). At the longest exposure durations examined, NIPTS was greater in CBA/J than in CBA/CaJ at most frequencies, even when comparing .94 min of noise in CBA/J with 30 min in CBA/CaJ. The growth of NIPTS in older mice (Fig. 3) was more similar in the two strains. In general, there was less NIPTS at lower frequencies. Larger shifts at higher frequencies were on the order of 40–50 dB at the maximum exposure duration examined (240 min).

Comparison of NIPTS growth metrics

Figure 4 examines the overall growth of NIPTS with exposure duration by age group and strain based on the sum of threshold shifts across frequencies (Total NIPTS in dB). As predicted by Figure 2, NIPTS in young mice grows more abruptly for shorter exposure durations in CBA/J than in CBA/CaJ (Fig. 4A). For CBA/Js, threshold shifts markedly begin to accumulate for exposures as brief as .47 minutes, while CBA/CaJs require exposures between 3.75 and 7.5 minutes. Maximal levels of NIPTS in CBA/Js are reached by one minute of exposure, and appear greater in CBA/J despite the fact that some CBA/CaJs were exposed for durations over 30 times longer. By contrast with young animals, the growth of NIPTS in older mice was very similar for the two strains (Fig. 4B), showing a shallow rise with exposure beginning by 60 minutes. Maximal summed threshold shifts were also similar in the two strains.

Figure 5 compares the growth of the probability of NIPTS across groups, along with logistic curve fits. Recapitulating the essential points of Figure 4, curves for young CBA/J and CBA/CaJ mice are quite different, while those for older mice largely overlap. The probability of NIPTS in young CBA/J mice approaches 1.0 for durations as short as 1.0 minute, compared to >7.0 minutes for CBA/CaJ. While the findings in Figures 3 and 4 may seem at odds with our recent report of severe threshold shifts in CBA/J exposure for only 30 seconds (Fernandez et al., 2010), those results were obtained for mice exposed at only 30 days of age, at which time noise vulnerability appears highly dynamic. For older mice, the curves approach 1.0 by about one hour. Calculated ‘threshold’ exposure durations for our present and previous studies are listed in Table II. Although the present estimates for young and older CBA/CaJ mice yield somewhat longer threshold durations than we have previously found, the table supports stability of the analysis method, and reinforces both similarity of noise susceptibility in older CBA/J and CBA/CaJ and stark differences for young CBA/J and CBA/CaJ.

Susceptibility of young F1 hybrid mice

The foregoing suggests that CBA/J and CBA/CaJ mice harbor different alleles at unknown loci that impart greater noise susceptibility to CBA/J, yet only in young mice. The simplest test of the principles by which these gene(s) operate is to examine F1 hybrids formed from the two strains. Accordingly, we measured NIPTS in young F1 mice exposed for 1.88 min, a duration that caused NIPTS in young CBA/J, but not in young CBA/CaJ (Figs. 2,5). Figure 6 shows that the resulting NIPTS in the F1s much more closely resembles CBA/J than CBA/CaJ. Thresholds in F1s and CBA/Js did not differ significantly from each other, but both differed significantly from CBA/CaJ ($p < .001$, 2 way ANOVA). This is consistent with the assertion that the heightened noise susceptibility of CBA/Js reflects dominant action of alleles at one or more loci.

Hair cell loss in young inbred and F1 mice

We previously showed that the heightened noise vulnerability of young CBA/J mice is associated with increased outer hair cell (OHC) loss (Fernandez et al., 2010). We therefore compared hair cell loss in the young CBA/J, CBA/CaJ, and F1 hybrid mice exposed for 1.88 minutes (Fig. 7). Consistent with the earlier work, the young CBA/J mice showed OHC loss extending over most of the cochlear basal turn. F1 mice showed more restricted loss, but much more clearly resembled CBA/J mice than CBA/CaJ mice. CBA/CaJ mice were not completely spared OHC loss, and probably would have shown NIPTS for some frequencies above 40 kHz. All three groups showed inner hair cell losses confined to the deep base. Although the hair cell results more clearly suggest the effects of modifying genes than did the accompanying NIPTS (since F1 hair cell loss was somewhat intermediate), they likewise support a significant role for dominant-acting alleles in CBA/J at one or more loci.

Qualitative examination of mid-modiolar sections from CBA/J, CBA/CaJ, and F1 mice further suggested moderate loss of spiral ganglion cells in the lower base and hook region in all three groups. This may reflect common patterns of IHC loss and injury in this region. No other clear effects of noise on the organ of Corti, lateral wall, or spiral limbus distinguished the three groups.

DISCUSSION

We have uncovered surprising differences in noise susceptibility in CBA/J and CBA/CaJ, two 'good hearing' inbred mouse strains sometimes treated interchangeably in hearing studies. These differences are manifested only during the early sensitive period to noise, and may reflect the action of a small set of genes that establish or modulate this period. We do not yet know whether the peculiar vulnerability to noise of young CBA/J extends to ototoxicity. They are reportedly more vulnerable to kanamycin than are C57BL/6 mice (Wu et al., 2001), but there has been no direct comparison with CBA/CaJ. Nevertheless, since the basis of the sensitive period for noise is poorly understood, characterization of the 'sensitizing genes' carried by CBA/J may help us to understand what factors this period reflects. Moreover, human homologs of these genes may represent candidate genes for enhanced risk of early NIPTS, or for delayed injury that presents as presbycusis.

Sensitive period for cochlear injury by noise and ototoxins

Heightened vulnerability early in life has been shown for both noise and ototoxins in a variety of animal models (Falk et al., 1974; Price, 1976; Stanek et al., 1977; Saunders and Chen, 1982; Pujol, 1992; Rybalko and Syka, 2001). In mice, the sensitive period for ototoxicity ends by one month of age (Henry et al., 1981), roughly at sexual maturity and somewhat after cochlear sound responses become fully adult-like. While there may be some variability by strain, the window for NIPTS in mice may exceed four months, well into

adulthood (Henry, 1982b, 1982a). Findings in mice are in line with characterizations of other mammals such as rats and hamsters suggesting different timeframes and somewhat non-overlapping mechanisms for early susceptibility to noise versus ototoxins (Saunders and Chen, 1982; Pujol, 1992). Heightened ototoxic injury may be more closely tied to metabolic characteristics of the immature cochlea (e.g., Zelck et al., 1993; Whitton et al., 1999), while the underpinnings of the longer sensitive period for noise remain unclear. Although it is difficult to superimpose findings in altricial mammals (such as rodents) onto precocial mammals as are humans, studies suggest that neonates and children are particularly vulnerable to both noise and ototoxins (Bernard, 1981; Henry et al., 1981; Henley and Rybak, 1995; Kent et al., 2002; Li and Steyger, 2009). Moreover, recent years have seen increasing interest in potential delayed and long term effects of prenatal and early postnatal environments in both animals and humans (Kujawa and Liberman, 2006; Ohlemiller, 2008; Kujawa and Liberman, 2009). Thus the injury processes that define the sensitive period in animals are likely relevant to human early acquired hearing loss, and potentially to delayed onset hearing loss.

The sensitive period for NIPTS in mice has been examined in detail only for CBA/J (Henry, 1982b, 1983), but appears similar in CBA/CaJ (Kujawa and Liberman, 2006). In both strains, NIPTS from a given exposure actually increases over the first month of life, presumably paralleling the development of adult-like hearing sensitivity. The fact that the differences in noise vulnerability seem to impact the ‘amplitude’, not the duration, of the sensitive period in CBA/J and CBA/CaJ suggests that the characteristics of one or more gene products differ, yet the underlying genes are expressed following the same timeframe. It is worth noting that our experimental paradigm did not necessarily address the same questions as would have a paradigm involving systematic changes in the intensity of noise fixed in duration. While both approaches would probably uncover the same strain disparities, the latter might reveal additional differences in mechanical tolerance of the inner ear of CBA/J versus CBA/CaJ (e.g., Davis et al., 1999).

Targets of noise during the sensitive period

Both permanent threshold shifts (PTS) and purely temporary threshold shifts (TTS) are increased during the sensitive period (Pujol, 1992). Accordingly, it may be a common target of PTS and TTS noise that limits the resiliency of the inner ear during this period. Depending upon the severity of the exposure, the cochlear targets of PTS noise include hair cells, afferent neurons, and lateral wall (Wang et al., 2002; Ohlemiller, 2008), while TTS noise may principally target the organ of Corti and inner hair cell afferent synapses (Nordmann et al., 2000; Kujawa and Liberman, 2009). TTS-related changes in hair cells and the organ of Corti may be distinguished from PTS-related changes only by degree, being limited to reversibly altered spatial relationships and reversible injury to hair cell stereocilia. By contrast, PTS appears principally associated with permanent hair cell impairment or loss. In keeping with this, the sensitive period appears associated with exacerbated outer hair cell loss both in mice (Ohlemiller et al., 2000; Fernandez et al., 2010) and other models (Falk et al., 1974; Pujol, 1992; Freeman et al., 1999). Notably, in displaying CBA/J-like noise vulnerability the F1 mice in our study also displayed CBA/J-like OHC loss. Thus we may posit that the gene(s) that confer enhanced noise injury to young CBA/J exert their influence via OHC protective, repair, or survival mechanisms.

Genetic contributions

A host of human and animal data implicate specific genes in noise vulnerability, including genes involved in stress responses (Ohlemiller, 2006, 2008; Konings et al., 2009; Pawelczyk et al., 2009). Few, however, show effects tied to particular ages. We previously identified BALB/c mice as an especially noise-vulnerable strain irrespective of age (Ohlemiller et al.,

2000). In that study, the ‘threshold exposure duration’ for NIPTS in BALBs at 1–2 months of age was estimated at .97 minutes, close to our .90 minute estimate for CBA/J. In the previous comparison of CBA/CaJ, C57BL/6J (B6), and BALB/cJ (BALB) mice, we noted a change in the order of noise susceptibility between 1–2 months and 6 months. Juxtaposed with the new data, the order by decreasing noise susceptibility in young mice becomes CBA/J $\approx$ BALB > CBA/CaJ $\approx$ B6. A much earlier study by Henry (Henry, 1984) also indicated that young CBA/J mice are more sensitive to noise than are young B6 mice. By 6 months, the order of strains by vulnerability becomes BALB > B6 > CBA/J $\approx$ CBA/CaJ. Thus there appear to be three distinct phenotypes among four strains, whereby CBA/J mice are especially vulnerable only when young, B6 mice are relatively vulnerable only at the older time point, and BALB mice appear consistently vulnerable. The shift with age in B6 ostensibly reflects the influence of the *Cdh23^{ahl}* allele (Davis et al., 2001), which does not seem to influence NIPTS in young animals (Ohlemiller et al., 2000). While BALBs also carry *Cdh23^{ahl}* (Johnson et al., 2000), they likely carry alleles at one or more additional loci that contribute to noise injury both early in life and later. It is possible that one or more ‘early’ NIPTS susceptibility alleles in CBA/J and BALB overlap.

CBA/J versus CBA/CaJ

CBA/J and CBA/CaJ cochleae differ both qualitatively and quantitatively in the effects of aging, wherein CBA/CaJ appear more affected (Ohlemiller et al., 2010a), and now in the effects of noise, wherein young CBA/J are more affected. Since more than 80 years of independent mutation separate these strains (Fox et al., 1997), it should not be surprising that they differ in a host of characteristics. It may ultimately prove highly useful that they uniquely model different pathologies of interest to auditory researchers, yet it is clear that the choice between these for a given study matters. As a corollary it should not be assumed that other CBA substrains are equivalent, nor substrains of other commonly used strains. Whether the topic is vulnerability to noise or ototoxins, aging characteristics, or the efficacy of a particular drug or preconditioning paradigm, broad declarations about what ‘CBA’ mice do—let alone what ‘mice’ do—should be avoided. This variety is both the burden and beauty of the mouse strains we may apply to the origins of human hearing loss.

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References

- Bernard PA. Freedom from ototoxicity in aminoglycoside treated neonates: A mistaken notion. *Laryngoscope*. 1981; 91:1985–1994. [PubMed: 7321720]
- Bohne, BA.; Clark, WW. Growth of hearing loss and cochlear lesion with increasing duration of noise exposure. In: Hamernik, RP.; Henderson, D.; Salvi, R., editors. *New Perspectives on Noise-Induced Hearing Loss*. New York: Raven Press; 1982. p. 283-302.
- Bult CJ, Eppig JT, Kadin JA, Richardson JE, Blake JA. The Mouse Genome Database (MGD): mouse biology and model systems. *Nucleic Acids Research*. 2008; 36:D724–D728. [PubMed: 18158299]
- Clark JA, Pickles JO. The effects of moderate and low levels of acoustic overstimulation on stereocilia and their tip links in the guinea pig. *Hearing Res*. 1996; 99:119–128.
- Davis RR, Cheever ML, Krieg EF, Erway LC. Quantitative measure of genetic differences in susceptibility to noise-induced hearing loss in two strains of mice. *Hearing Res*. 1999; 134:9–15.
- Davis RR, Newlander JK, Ling X-B, Cortopassi GA, Krieg EF, Erway LC. Genetic basis for susceptibility to noise-induced hearing loss in mice. *Hearing Res*. 2001; 155:82–90.
- Erway, LC.; Willott, JF. Genetic Susceptibility to Noise-Induced Hearing Loss. In: Axelsson, A., editor. *Scientific Basis of Noise-Induced Hearing Loss*. New York: Thieme; 1996. p. 56-64.

- Erway, Lc; Willott, JF.; Archer, JR.; Harrison, DE. Genetics of age-related hearing loss in mice: I. Inbred and F1 hybrid strains. *Hearing Res.* 1993; 65:125–132.
- Falk SA, Cook RO, Haseman JK, Sanders GM. Noise-induced inner ear damage in newborn and adult guinea pigs. *Laryngoscope.* 1974; 84:444–453. [PubMed: 4592286]
- Fernandez EA, Ohlemiller KK, Gagnon PM, Clark WW. Protection against noise-induced hearing loss in young CBA/J mice by low-dose kanamycin. *J. Assoc. Res. Otolaryngol.* 2010; 11:235–244. [PubMed: 20094753]
- Finney DJ. The median lethal dose and its estimation. *Archives of Toxicology.* 1985; 56:215–218. [PubMed: 3994505]
- Forge A, Schacht J. Aminoglycoside antibiotics. *Audiol. Neuro-Otol.* 2000; 5:3–22.
- Fox, RR.; Witham, BA.; Neleski, LA., editors. Handbook of genetically standardized JAX mice. Bar Harbor ME: The Jackson Laboratory; 1997.
- Fredelius L, Johansson B, Bagger-Sjoberg D, Wersall J. Qualitative and quantitative changes in the guinea pig organ of Corti after pure tone acoustic overstimulation. *Hearing Res.* 1987; 30:157–168.
- Freeman S, Khvoles R, Cherny L, Sohmer H. Effect of long-term noise exposure on the developing and developed ear in the rat. *Audiol. Neuro-Otol.* 1999; 4:207–218.
- Gagnon PM, Simmons DD, Bao J, Lei D, Ortmann A, J, Ohlemiller KK. Temporal and genetic influences on protection against noise-induced hearing loss by hypoxic preconditioning in mice. *Hearing Res.* 2007; 226:79–91.
- Henley C, M, Rybak LP. Ototoxicity in developing animals. *Brain Research Reviews.* 1995; 20:68–90. [PubMed: 7711768]
- Henry KR. Influence of genotype and age on noise-induced auditory losses. *Behav. Genet.* 1982a; 12:563–573. [PubMed: 7183323]
- Henry KR. Age-related changes in sensitivity of the postpubertal ear to acoustic trauma. *Hearing Res.* 1982b; 8:285–294.
- Henry KR. Lifelong susceptibility to acoustic trauma: Changing patterns of cochlear damage over the life span of the mouse. *Audiology.* 1983; 22:372–383. [PubMed: 6615342]
- Henry KR. Noise and the young mouse: Genotype modifies the sensitive period for effects on cochlear physiology and audiogenic seizures. *Behav. Neurosci.* 1984; 98:1073–1082. [PubMed: 6508912]
- Henry KR, McGinn MD. The mouse as a model for human audition. A review of literature. *Audiology.* 1992; 31:181–189. [PubMed: 1444929]
- Henry KR, Chole RA, McGinn MD, Frush DP. Increased Ototoxicity in Both Young and Old Mice. *Arch. Otolaryngol.* 1981; 107:92–95. [PubMed: 7469897]
- Hirose K, Liberman MC. Lateral wall histopathology and endocochlear potential in the noise-damaged mouse cochlea. *J. Assoc. Res. Otolaryngol.* 2003; 4:339–352. [PubMed: 14690052]
- Johnson KR, Zheng QY, Erway LC. A major gene affecting age-related hearing loss is common to at least 10 inbred strains of mice. *Genomics.* 2000; 70:171–180. [PubMed: 11112345]
- Kaltenbach JA, Schmidt RN, Kaplan CR. Tone-induced stereocilia lesions as a function of exposure level and duration in the hamster cochlea. *Hearing Res.* 1992; 60:205–215.
- Kent WDT, Tan AKW, Clarke MC, Bardell T. Excessive noise levels in the neonatal ICU: Potential effects on auditory system development. *Journal of Otolaryngology.* 2002; 31:355–360. [PubMed: 12593547]
- Konings A, Van Laer L, Van Camp G. Genetic studies on noise-induced hearing loss: A review. *Ear Hear.* 2009; 30:151–159. [PubMed: 19194285]
- Kujawa SG, Liberman MC. Acceleration of age-related hearing loss by early noise: Evidence of a misspent youth. *J. Neurosci.* 2006; 26:2115–2123. [PubMed: 16481444]
- Kujawa SG, Liberman MC. Adding insult to injury: Cochlear nerve degeneration after 'temporary' noise-induced hearing loss. *J. Neurosci.* 2009; 29:14077–14085. [PubMed: 19906956]
- Li H, Steyger PS. Synergistic ototoxicity due to noise exposure and aminoglycoside antibiotics. *Noise & Health.* 2009; 11:26–32. [PubMed: 19265251]
- Li HS. Influence of genotype and age on acute acoustic trauma and recovery in CBA/Ca and C57BL/6J mice. *Acta Oto-Laryngol.* 1992; 112:956–967.

- Muller M, von Hunerbein K, Hoidis S, Smolders JWT. A physiological place-frequency map of the cochlea in the CBA/J mouse. *Hearing Res.* 2005; 202:63–73.
- Nordmann AS, Bohne BA, Harding GW. Histopathological differences between temporary and permanent threshold shift. *Hearing Res.* 2000; 139:13–30.
- Ohlemiller KK. Contributions of mouse models to understanding of age- and noise-related hearing loss. *Brain Res.* 2006; 1091:89–102. [PubMed: 16631134]
- Ohlemiller KK. Recent findings and emerging questions in cochlear noise injury. *Hearing Res.* 2008; 245:5–17.
- Ohlemiller KK, Gagnon PM. Genetic dependence of cochlear cells and structures injured by noise. *Hearing Res.* 2007; 224:34–50.
- Ohlemiller, KK.; Frisina, RD. Age-related hearing loss and its cellular and molecular bases. In: Schacht, J.; Popper, AN.; Fay, RR., editors. *Auditory Trauma, Protection, and Repair*. New York: Springer; 2008. p. 145-194.
- Ohlemiller KK, Wright JS, Dugan LL. Early elevation of cochlear reactive oxygen species following noise exposure. *Audiology & NeuroOtolology.* 1999; 4:229–236.
- Ohlemiller KK, Wright JS, Heidbreder AF. Vulnerability to noise-induced hearing loss in 'middle-aged' and young adult mice: A dose-response approach in CBA, C57BL, and BALB inbred strains. *Hearing Res.* 2000; 149:239–247.
- Ohlemiller KK, Dahl AR, Gagnon PM. Divergent aging characteristics in CBA/J and CBA/CaJ mouse cochleae. *J. Assoc. Res. Otolaryngol.* 2010a (in press).
- Ohlemiller KK, Rosen AD, Gagnon PM. A major effect QTL on chromosome 18 for noise injury to the mouse cochlear lateral wall. *Hearing Res.* 2010b; 260:47–53.
- Ohlemiller KK, Rosen AR, Rellinger EA, Montgomery SC, Gagnon PM. Different cellular and genetic basis of noise-related endocochlear potential reduction in CBA/J and BALB/cJ mice. *J. Assoc. Res. Otolaryngol.* 2010c (in press).
- Ortmann AJ, Faulkner KF, Gagnon PM, Ohlemiller KK. Removal of the Ahl allele from the C57BL/6 background does not improve noise resistance. *Abstr., Assn. Res. Otolaryngol.* 2004; 27:168.
- Pawelczyk M, Van Laer L, Franssen E, Rajkowska E, Konings A, Carlsson PI, Borg E, Sliwiska-Kowalska M. Analysis of gene polymorphisms associated with K⁺ ion circulation in the inner ear of patients susceptible and resistant to noise-induced hearing loss. *Ann. Hum. Genet.* 2009; 73:411–421. [PubMed: 19523148]
- Perletti G, Vral A, Patrosso MC, Marras E, Ceriani I, Willems P, Fasano M, Magri V. Prevention and modulation of aminoglycoside ototoxicity (Review). *Mol. Med. Rep.* 2008; 1:3–13.
- Price GR. Age as a factor in susceptibility to hearing loss: Young versus adult ears. *J. Acoust. Soc. Am.* 1976; 60:886–892.
- Pujol, R. Sensitive Developmental Period and Acoustic Trauma: Facts and Hypotheses. In: Dancer, AL., editor. *Noise-Induced Hearing Loss*. St. Louis: Mosby; 1992. p. 196-203.
- Rodriguez-Paris J, Ballay C, Inserra M, Stidham K, Colen T, Roberson J, Gardner P, Schrijver I. Genetic analysis of presbycusis by arrayed primer extension. *Ann. Clin. Lab. Sci.* 2008; 38:352–360. [PubMed: 18988928]
- Rybak LP. Mechanisms of cisplatin ototoxicity and progress in otoprotection. *Curr. Opin. Otolaryngol. Head Neck Surg.* 2007; 15:364–369. [PubMed: 17823555]
- Rybalko N, Syka J. Susceptibility to noise exposure during postnatal development in rats. *Hearing Res.* 2001; 155:32–40.
- Saunders JC, Chen C-S. Sensitive periods of susceptibility to auditory trauma in mammals. *Environ. Health Persp.* 1982; 44:63–66.
- Spoendlin H. Primary structural changes in the organ of Corti after acoustic overstimulation. *Acta Otolaryngol.* 1971; 71:166–176.
- Spoendlin H, Brun JP. Relation of structural damage to exposure time and intensity in acoustic trauma. *Acta Otolaryngol.* 1973; 75:220–226. [PubMed: 4571032]
- Stanek R, Bock G, R, Goran ML, Saunders JC. Age dependent susceptibility to auditory trauma in the hamster: Behavioral and electrophysiologic consequences. *Trans. Am. Acad. Ophthalmol. Otolaryngol.* 1977; 84:465–472.

- Van Eyken E, Van Camp G, Van Laer L. The complexity of age-related hearing impairment: contributing environmental and genetic factors. *Audiol. Neurotol.* 2007; 12:345–358.
- Wang Y, Hirose K, Liberman MC. Dynamics of noise-induced cellular injury and repair in the mouse cochlea. *J. Assoc. Res. Otolaryngol.* 2002; 3:248–268. [PubMed: 12382101]
- Whitlon DS, Wright LS, Nelson SA, Szakaly R, Siegel FL. Maturation of cochlear glutathione-S-transferases correlates with the end of the sensitive period for ototoxicity. *Hearing Res.* 1999; 137:43–50.
- Willott, JF. *Aging and the Auditory System: Anatomy, Physiology, and Psychophysics.* San Diego: Singular Publishing Group; 1991.
- Wu W-J, Sha S, McLaren JD, Kawamoto K, Raphael Y, Schacht J. Aminoglycoside ototoxicity in adult CBA, C57BL, and BALB mice and the Sprague-Dawley rat. *Hearing Res.* 2001; 158:165–178.
- Yoshida N, Hequembourg SJ, Atencio CA, Rosowski JJ, Liberman MC. Acoustic injury in mice: 129/SvEv is exceptionally resistant to noise-induced hearing loss. *Hearing Res.* 2000; 141:97–106.
- Zelck U, Nowak R, Karnstedt U, Koitschev A, Kacker N. Specific activities of antioxidant enzymes in the cochlea of guinea pigs at different stages of development. *Eur. Arch. Oto-Rhino- Laryngol.* 1993; 250:218–219.
- Zheng QY, Johnson KH, Erway LC. Assessment of hearing in 80 inbred strains of mice by ABR threshold analysis. *Hearing Res.* 1999; 130:94–107.

Figure 1. Mean($\pm$ SD) ABR thresholds prior to noise exposure for all mice examined at 6 wks (**A**) and 6 months (**B**).

Figure 2.

Mean(+SD) noise-induced permanent threshold shifts (NIPTS, in dB) for 6 wk old CBA/J mice (**A**) and CBA/CaJ mice (**B**) determined 2 wks after exposure to noise of the indicated durations. All noise was broadband (4–45 kHz) at 110 dB SPL.

Figure 3. Mean(+SD) noise-induced permanent threshold shifts (NIPTS, in dB) for 6 month old CBA/J mice (A) and CBA/CaJ mice (B) determined 2 wks after exposure to noise of the indicated durations. All noise was broadband (4–45 kHz) at 110 dB SPL.

Figure 4.

Total threshold shift in dB for CBA/J and CBA/CaJ mice examined at 6 wks (**A**) and 6 months (**B**) versus noise exposure duration. For each strain and age group, the average NIPTS was summed across all test frequencies, so that each trace in Figures 2 and 3 was collapsed to a single value. CBA/J and CBA/CaJ differ sharply at 6 wks, but extensively overlap at 6 months.

Figure 5.

Probability of criterion NIPTS versus noise exposure duration for CBA/J and CBA/CaJ mice examined at 6 wks and 6 months. For each strain, age group, and noise exposure duration, the proportion of mice meeting criteria for NIPTS was calculated (see Methods). Lines are fits of Eqn. 1. CBA/J and CBA/CaJ differ sharply at 6 wks, but negligibly at 6 months.

Figure 6. Mean(+SD) noise-induced permanent threshold shifts (NIPTS, in dB) for 6 wk old CBA/J, CBA/CaJ, and (CBA/CaJ $\times$ CBA/J) F1 hybrid mice determined 2 wks after exposure to 1.88 min of broadband noise (4–45 kHz, 110 dB SPL). The extent of NIPTS was statistically indistinguishable between CBA/J and F1 mice, but both of these showed an overall significant difference versus CBA/CaJ.

Figure 7.

Mean(–SD) cytochleograms for 6 wk old CBA/J, CBA/CaJ, and (CBA/CaJ × CBA/J) F1 hybrid mice examined 2 wks after exposure to 1.88 min of broadband noise (see Fig. 6). Horizontal bar indicates specific locations where OHC counts in CBA/J and F1 mice differed significantly ($p < 0.05$) from CBA/CaJ. Horizontal dots indicate significant difference between CBA/J and F1 ($p < 0.05$, Bonferroni multiple comparisons test). IHC counts did not differ by group.

Table 1

Number of animals tested and proportion with NIPTS

							Minutes exposure duration											
Strain	Age		.117	.23	0.47	0.94	1.88	3.75	7.5	15	30	60	120	240				
CBA/J	6 wk	NIPTS / n exposed	0/4	0/5	4/8	7/8	5/5	5/5										
		Proportion	0	0	0.5	0.88	1	1										
CBA/J	6 mo	NIPTS / n exposed								0/3	0/4	5/7	4/4	7/8				
		Proportion								0	0	0.71	1	0.88				
CBA/Cal	6 wk	NIPTS / n exposed		0/4	0/4	0/3	0/7	0/4	7/9	4/4	5/5							
		Proportion		0	0	0	0	0	0.78	1	1							
CBA/Cal	6 mo	NIPTS / n exposed								0/4	1/4	5/7	7/7	4/4				
		Proportion								0	0.25	.71	1	1				

Table II

Threshold exposure duration for NIPTS (Minutes) *

	6 wk CBA/J	6 wk CBA/CaJ	6 mo CBA/J	6 mo CBA/CaJ
Ohlemiller et al., 2000		3.43		63.00
Ortmann et al., 2004		4.01		63.22
Present study	0.90	8.37	67.29	86.19

* Fitted Eqn. 1 solved for $y = .9$