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The relationship between vulnerability to temporary versus permanent threshold shifts in BALB, CBA/J and B6 inbred mice

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THE RELATIONSHIP BETWEEN VULNERABILITY TO TEMPORARY VERSUS PERMANENT THRESHOLD SHIFTS IN BALB, CBA/J AND B6 INBRED MICE

by

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Abstract: This study examines the dose-response relationship between TTS and PTS. Results indicate that susceptibility to TTS does not predict susceptibility to PTS and that long standing principles such as the equal energy hypothesis and early vulnerability period do not apply at low levels of noise.

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Excluding the effects of aging, noise-related hearing loss is the single most common neurodegenerative clinical condition (Lynch & Kil, 2005). Blast-related injuries and related hearing disorders (e.g., hearing loss, tinnitus, central auditory processing disorders) also constitute common health issues in veterans (Fausti, Wilmington, Helt, Helt, & Konrad-Martin, 2005). Accordingly, cochlear injury due to noise exposure has been the subject of intensive research since the end of World War II, when new innovations for signal and sound measurement became readily available (Glorig, 1980).

The effects of loud noise exposure on the cochlea are generally physiologically defined in terms of their effects on thresholds. Noise-induced hearing loss may be classified either temporary threshold shifts (TTS) or permanent threshold shifts (PTS) depending on whether hearing thresholds eventually statistically recover or remain significantly elevated at one or more test frequencies. TTS and PTS have somewhat different frequency distributions and anatomic substrates. More importantly for this study, TTS has remained of interest for its potential ability to identify conditions or individuals most likely to manifest PTS. It is the predictive ability of TTS that formed the basis of this work.

Potential Utility of TTS in Predicting PTS

Much of what we know about PTS derives from work in animals (see below), where PTS can be intentionally inflicted in a highly controlled manner (Clark, 1991). Nevertheless, this does not completely obviate the need to examine, when possible, the correlates and predictors of human PTS using human models. Since it is unethical to deliberately induce PTS in humans, researchers must find other ways to study this phenomenon. The only way to study PTS in humans ethically has been to find populations that may have sustained PTS over years of

working in noisy environments through exposures that could be well-characterized. In 1983, the Occupational Safety and Health Agency (OSHA) began mandating the use of hearing protective devices (HPDs) in settings where the time-weighted average exceeded 85 dBA (Ohlemiller, 2012). This vital development, however, carried the 'downside' that such retrospective population studies must be carried out prior to OSHA regulations or in countries lacking such regulations. Another strategy has been to study military populations where dangerous exposures cannot be prevented (Attias et al., 1994; Kopke et al., 2001; LePrell et al., 2011). An appealing alternative has been to use TTS as 1) a predictor of PTS risk, or 2) a metric for therapeutic efficacy. In the former instance, one might measure individual TTS accumulation over the course of a day on a noisy job, then compare inter-individual daily threshold shifts with multi-year PTS trends. In the latter case, one might intentionally inflict a mild TTS in the laboratory, then test the ability of some compound (e.g., an antioxidant) to reduce the amount of TTS. This is then taken as proxy evidence for protection against PTS. For example, in 2012, Le Prell and colleagues applied a moderate music exposure that reliably induced TTS in human subjects. They argued that the development of a TTS inducing protocol was critical for the study of otoprotective agents. Otoprotectants such as Ebselen, magnesium, N-acetylcysteine and vitamin B12 have been tested in humans to assess their otoprotective qualities by demonstrating their ability to reduce TTS rather than PTS (Attias, Sapir, Bresloff, Reshef-Haran, & Ising, 2004; Quaranta, Scaringi, Bartoli, Margarito, & Quaranta, 2004; Kramer et al., 2006; Lin et al., 2010; Le Prell et al., 2011; Lindblad, Rosenhall, Olofsson, & Hagerman, 2011).

Characteristics of TTS versus CTS and PTS

Decades of human and animal studies have compared the effects of TTS versus PTS. What TTS is decidedly NOT is the acute part of a PTS. At the end of a PTS exposure, there will occur some amount of threshold recovery. After a period ranging 10-30 days, thresholds appear to settle to a stable residual threshold shift. The acute phase has been termed the compound threshold shift (CTS, Miller, 1963), and likely reflects partial repair to cell types (e.g., OHCs) that also show some loss for the same exposure. In addition, the time constant of recovery differs between TTS and CTS (e.g., Mills 1973), suggesting that different processes are involved. TTS characteristics are also distinct from PTS. Anatomically, TTS involves injury that is associated with reversible changes in cells that might instead die in PTS. TTS injury is extends more basally while PTS injury is concentrated at the center frequency of the noise exposure. The cellular substrate of TTS has been asserted to be reversible excitotoxic injury to nerve fibers (Kujawa & Liberman, 2009), reversible damage to stereociliary bundles, and reversible structural changes to the organ of Corti (Nordman, Bohne, & Harding, 2000). Buckling of the pillar cells and resulting uncoupling of the stereocilia from the tectorial membrane have been postulated to protect from PTS. Although hair cell function may return to normal in TTS, a recent much-discussed finding is diffuse neuronal loss that seems to occur even without loss of inner hair cells (Kujawa & Liberman, 2009). Retraction of nerve terminals and loss of synaptic ribbons suggest irreversible damage is done to the spiral ganglion cells even when hearing thresholds only show a temporary shift. Thus, the long term correlates of TTS have effectively been re-defined in a manner that places noise exposure as a frequent cause of neural presbycusis. This pathology may have no effect on thresholds, and is not evaluated in the threshold-based experiments described here.

PTS is most often and most importantly marked by the loss and damage of hair cells and their stereocilia (Wang, Hirose, & Liberman, 2002; Liberman & Dodds, 1984). Noise exposure at high enough levels can cause damage to almost all of the structures of the cochlea but other notable physiologic changes that are associated with PTS are neuronal loss, stereocilia damage, supporting cell collapse, loss of fibrocytes in the limbus and lateral wall, strial degeneration and strial shrinkage (Wang et al., 2002).

The early vulnerability window for PTS

It is widely accepted that young-but-sexually-mature animals are more susceptible to noise-induced hearing loss than older animals of the same strain (e.g. Saunders & Chen, 1985; Ohlemiller, Wright, & Heidbreder, 2000). One of the histologic correlates of this increased vulnerability in young animals is greater loss of outer hair cells in young adult animals compared to older animals. Thus, the 'locus' of early vulnerability includes the organ of Corti, although other sites such as the lateral wall cannot be ruled out. The early vulnerability window in mice has been shown to extend to about 4 months of age, with a 'peak' in vulnerability between 1 and 2 months (Henry, 1982; Henry, 1983). Presently it is not clear whether TTS shows a similar early vulnerability window. The finding of such a window—one goal of the present study—would constitute evidence that TTS vulnerability is systematically related to PTS vulnerability, independent of age.

Genetics of PTS

The level of noise that induces PTS varies by strain, that is, genetic composition. When compared to CBA/Ca and C57BL/6J (B6), BALBs showed the highest noise vulnerability in both young and old animals (Ohlemiller, Wright, & Heidbreder, 2000). For ages beyond the early vulnerability window, B6 mice appear more vulnerable to noise than CBA/CaJ and CBA/J mice, largely due to the effects of the *Ahl* allele of Cadherin 23 (Shone, Altschuler, Miller, & Nuttall, 1991; Li 1992; Erway, Shiau, Davis, & Krieg, 1996). According to Johnson, Zheng, & Erway (2000), BALBs, like B6s carry the Ahl allele. Both CBA/CaJ and CBA/J mice have historically been applied as 'good hearing' and noise-resistant models. However, Ohlemiller, Rybak Rice, Rellinger, & Ortmann (2011) reported striking and unexpected noise vulnerability in young CBA/Js, so that a revised ordering by vulnerability appears as follows: During the early window, CBA/J are roughly equivalent to BALB, which are more vulnerable than CBA/CaJ and B6 mice. When mice reach 6 months of age (after the early window) the order most-to-least noise vulnerable changes to BALB, B6, CBA/J and CBA/CaJ (Ohlemiller et al., 2011). BALB mice remain vulnerable throughout the aging process while CBA/J mice are extremely vulnerable only when they are young. The genes and age-dependent processes that impart differential noise vulnerability to inbred mice are not well understood. The examples found in mice partly serve to illustrate the principle of inter-individual variability in noise vulnerability, which could be relevant to human studies. Notably, these examples have been explored only at high noise levels, and it has simply been presumed that they would apply under a wide range of exposure conditions.

Purpose of the Current Study

The goal of this study is to test whether TTS-susceptible individuals also more PTS-susceptible. We address this question by comparing TTS sensitivity in individuals *known* to possess very different PTS sensitivities. This approach is based on data from previous studies in B6, CBA, and BALB inbred mice. The previous studies (Davis, Cheever, Krieg, & Erway, 1999; Ohlemiller et al., 2000) used a dose-response paradigm to compare the PTS vulnerability of young (1-2 months) and older (5-7 months) mice. In those studies, the independent variable was duration of a broadband noise stimulus fixed at 110 dB SPL. Here we compare the same inbred strains and ages, yet we expand the paradigm to derive both TTS and PTS exposure thresholds. Since the PTS exposure threshold varies by both strain and age, the expected variation in PTS threshold allowed us to make predictions about variation in TTS threshold. If the processes that underlie TTS extensively overlap with those that cause PTS, then TTS and PTS are predicted to appear with a fixed relation in a dose-response assessment. That is, since BALB and B6 mice appeared more vulnerable to PTS than CBA mice in the previous studies, they should also appear more vulnerable to TTS, and their dose-response relations for both TTS and PTS should be left-shifted on the noise intensity axis compared to CBA mice. In adapting the previous methods to a TTS dose-response paradigm, one additional change was made whereby the independent variable was changed to noise level and the exposure intensity was fixed at 2 hours. This change was made to allow the present study to also address the question whether all dose-response paradigms yield the same relationships. That is, does the same relative noise vulnerability by strain and age appear if we vary the duration of intense noise versus noise intensity? Does the threshold noise energy for eliciting PTS at high noise levels predict PTS thresholds at low noise levels? A literature review suggested to us that previous

studies comparing relative noise vulnerabilities or establishing the principles of PTS acquisition were carried out solely at high noise levels.

Methods

Animals

C57BL/6J (B6), CBA/J (henceforth CBA) and BALB/cJ (BALB) mice were raised in-house at the WUSM/CID Animal facility. All mice were descendents of animals originally purchased from the Jackson Laboratory. Mice were in the age window of 1-2 months ('young') or 5-7 months ('old') when they were tested or noise-exposed. Included were 20 young B6 and 24 old B6; 32 young CBA and 19 old CBA; 26 young BALB and 23 old BALB. Each experimental group included both male and female mice. Those with abnormally elevated thresholds at the time of baseline testing (>10 dB above norms at any frequency less than 40 kHz) were not included in the study. After testing, mice were sacrificed and their cochleae were harvested for histopathology. The procedures used in this project were approved by the Washington University School of Medicine Animal Studies Committee.

ABR Recording Protocol

For ABR recording, animals were anesthetized with a mixture of ketamine and xylazine (80/15 mg/kg) injected intraperitoneally. Anesthetized mice were placed in a prone position on a platform with their right ear 7 cm from an ES-1 freefield speaker (Tucker-Davis Technologies).

All ABR recording was done using the BioSig 32 program and TDT hardware and software. Subdermal platinum electrodes were placed behind the right pinna (active) on the vertex (reference), and on the back (ground). A rectal probe was used to monitor temperature which was maintained at 37 ± 2 °C using a DC current based isothermal pad (FHC). Tonebursts 5 ms in duration were presented in descending order using a 5 dB minimum step size until wave I of the ABR could no longer be discerned. Tone intensity was then increased in 5 dB steps until wave I reappeared to verify threshold.

Baseline auditory brainstem response tests (ABRs) were obtained at 5, 10, 20, 28.3 and 40 kHz 2-7 days prior to noise. One to three hours after noise exposure, ABR testing was repeated. If a shift of 10 dB or more was recorded at any two frequencies, the animal was considered to have a threshold shift for purposes of defining TTS or PTS. Mice were tested again 7-10 days after noise to differentiate TTS vs. PTS. If thresholds remained elevated at least 10 dB above baseline at any two frequencies, mice were considered to have permanent threshold shift. If all thresholds returned to within 5 dB of baseline, shifts were classified as temporary. Thresholds at 40 kHz were tested but not included in threshold shift criteria because of the early effects of the *Ahl* allele on this frequency. We also did not want the exaggerated noise vulnerability of the deep cochlear base of mice to overly influence determinations of threshold shift.

Noise Exposure

Mice were exposed fully awake to 8-16 kHz octave band noise for two hours. Each animal was exposed once, while exposure levels across animals varied from 80 to 98 dB SPL. Noise exposure was performed in an Industrial Acoustics double walled sound booth. Up to two 30 x 19 x 13 cm plastic-lined reverberant cages containing one or two mice were suspended 50 cm

below an exponential horn (Selenium Corneta HM4750-SLF). Cages were lined with chicken wire to reduce the ability of the mice to huddle against the sides of the enclosure. Sound level was monitored by Brüel & Kjæl Type 2203 Precision Sound Level Meter. Noise was generated digitally using custom Labview routines, then presented using a TDT RZ6 in combination with a Crown power amplifier.

Analysis

For each strain, age group, and noise intensity, the number of animals that met criteria for TTS or PTS as a fraction of the total number of animals tested (typically 3-5 per group) was calculated. Thus, the ABR threshold data for each age and strain were converted to a sigmoidal function that varied between 0 and 1.0. This relation was then fitted to a 4-parameter logistic function (Ohlemiller et al., 2000, 2011) using Sigmastat. For each strain, age, and condition (TTS vs PTS), the fitted function was then solved for Y = 0.9, and the corresponding X value was considered the 'threshold exposure' for TTS/PTS for that strain/age. Data were padded at the extremes as needed with an extra '0' or '1.0' to stabilize the fitting algorithm (That is, each fitted function included two '0' points and two '1.0' points.). For each strain and age, the relation for TTS and PTS constituted 3 phases: a 'no threshold shift' range, a variable range, and a range wherein all animals met criteria for either TTS or PTS. At the TTS/PTS transition where all animals showed either TTS or PTS, a TTS value of 1.0 was entered for the purpose of logistic fits to the TTS data. Subsequent analyses of derived curve-fit data included 1) ranking of animals/ages by PTS vulnerability; 2) Comparison of current PTS threshold data with previous duration-based estimates based on equal power (Ohlemiller et al., 2000); and 3) Comparison of derived TTS threshold exposure levels with PTS threshold exposure levels.

Results

Baseline thresholds were similar within strain and age groups and consistent with previous data (Ohlemiller et al., 2000) (Figure 1). Old B6 are known to have high frequency hearing loss with age, as a group, their baselines were elevated at 40 kHz. This had no effect on the analysis because 40 kHz was not taken into account for threshold shifts.

Average threshold shifts were calculated using the intensity just above the TTS and PTS thresholds (Figure 2). PTS shifts were large (around 70 dB) for the younger animals while TTS shifts were minimal (around 40 dB). For the older animals, both TTS and PTS shifts were large (50-60 dB).

Figure 3 shows example of fitted logistic curve. The logistic fits take arbitrary data points and fit them to a four parameter function. These curves produced exact fits to the data in much of the current data. Old B6s were not examined in any of the following analysis because of their incomplete and inconsistent results. The subject groups examined in the analysis are young BALB, old BALB, young B6, young CBA/J and old CBA/J.

Based on the curve fit calculations described above, TTS thresholds were determined to be 91 dB for old and young BALBs, 88 dB for young B6 and young CBA/J, and 94 dB for old CBA/J. PTS thresholds are 93 dB for old BALBs, 97 dB for young BALBs, 94 dB for young B6s, 98 dB for young CBA/Js and 96 dB for old CBA/Js (Table 1).

TTS Susceptibility as a Predictor of PTS Susceptibility

The first relation we examined using curve-fit-derive TTS and PTS thresholds was the germinal idea for this project. Both historically and currently, the TTS sensitivity of any individual has been proffered as a proxy measure for PTS sensitivity. For this reason, derived TTS and PTS thresholds were compared to determine if there appeared any consistent relationship between TTS and PTS exposure thresholds, independent of mouse strain and age (Figure 4). If TTS susceptibility is a good predictor of PTS susceptibility, the relationship between TTS and PTS thresholds should be orderly, potentially lying just above the Y = X diagonal. All data do fall above the diagonal, indicating that PTS thresholds are generally higher than TTS thresholds. However, the distance from each data point to the diagonal varies widely (2-10 dB), indicating that the 'dynamic range' between exposures that yield TTS versus PTS is highly variable with age and genetic background.

PTS Susceptibility using Different Paradigms

The data from this project allow a first-ever comparison of PTS threshold exposure metrics for the same inbred mouse strains and age groups. Ohlemiller et al. (2000, 2011) derived PTS thresholds by varying the duration of an intense noise (110 dB SPL) spectrally similar to that used here. That permitted a comparison of the previous and present data with regard to relative noise vulnerability with mouse strain and age, with the expectation that both low and high noise level-based PTS threshold should yield the same results. There are several way such a comparison may be made. First, using the current and previous data, subject groups were ranked from highest to lowest susceptibility to PTS. Figure 5 shows this comparison graphically, comparing the current ranking on the X axis and the previous ranking (Ohlemiller et al., 2000,

2011) on the Y axis. Examination of this graph reveals no systematic relation of the rankings obtained by the two methods.

Another way to compare the previous and present paradigms is to take the PTS exposure thresholds obtained by Ohlemiller et al. (2000, 2011) and convert them to an equal energy exposure having a duration of 2 hours. If equal energy exposures yield the same injury and if PTS exposure thresholds obtained using low and high level noise exposures are the same, then the current estimates of PTS threshold and those obtained by converting the data of Ohlemiller et al. should be the same. Figure 6 and Table 2 show actual and predicted thresholds. Predicted intensity-based PTS thresholds do not match the thresholds found in this study. Predicted thresholds were higher than actual thresholds for old BALBs, old CBA/Js and young B6s. Actual thresholds were higher than predicted thresholds for young CBA/Js and young BALBs. The differences between the groups were greater than 6 dB for 5 of 6 groups.

Yet another indirect comparison that can be made between the previous (Ohlemiller et al., 2000, 2011) data is to compare both TTS and PTS vulnerability in young versus old animals. The earlier PTS data robustly indicate that younger animals (those within the early vulnerability window) are more vulnerable. There are no viable TTS comparisons, so the present study marks the first examination of this question. Again, if TTS sensitivity is informative about PTS sensitivity, then we may expect that younger animals should be more vulnerable to both TTS and PTS. This applies, however, only if the relations established at high exposure levels (Ohlemiller et al., 2000, 2011) apply to low exposure levels. Figure 7 examines these predictions graphically. The simple prediction is that all the data should lie above the Y = X diagonal. Young CBA/Js appear more susceptible than old CBA/Js to TTS and lay above the diagonal. However, BALBs appear equally susceptible to TTS in the young and old groups, and lay

directly on the diagonal. By contrast with expectation, old B6s appear more susceptible than young B6s to TTS. For PTS, older BALB and CBA/Js appear more susceptible than younger animals, again in direct contrast to expectation.

Possible Relation between PTS thresholds and Noise Level Eliciting EP Reduction

The lack of patterns in the present data might be taken to simply indicate that the results incorporate too much variability, so that previous trends are simply obscured. While that is possible, it is worthwhile to point out a way that the new date fit with another data set obtained using the same mouse strains, ages, and noise exposure protocol. Ohlemiller and Gagnon (2007) showed that a 2 hr broadband exposure at 110 dB SPL causes endocochlear potential (EP) reduction in a manner that depends on mouse strain. More recent unpublished data extend this finding to low noise levels, and make it clear that there exist both strain and age effects in EP reduction. Figure 8 shows the relationship between noise intensity and EP level for young and older BALB and CBA/J mice, and indicate the intensity range where the current PTS thresholds lie. The lowest noise intensities associated with notable EP reduction appear quite close to the PTS thresholds obtained in this study. These data suggest a relation between the processes that underlie EP reduction and PTS.

Discussion

There is an immense body of data describing characterizing the relation between TTS and PTS, and constructing the principles that govern the appearance of PTS (e.g., Miller, 1963;

Taylor, Pearson, Mair, & Burns, 1964; Mills, 1973; Henry, 1982). The current data suggest that some established principles and relations only hold for high level noise exposures. Susceptibility to low levels of noise may be governed by a different set of principles. Some caveats apply such as the fact that real world noise exposure is not Gaussian noise or that mice may not be a universal model to represent other species or that narrow band noise (8-16 kHz) was used in the current study and in the previous Ohlemiller et al. (2010, 2011) studies, 10 kHz centered noise was used.

TTS Susceptibility as a Predictor of PTS Susceptibility

TTS susceptibility as a predictor of PTS susceptibility is the original question that started this project. The present data indicate that susceptibility to TTS does not predict susceptibility to PTS in the sense that PTS thresholds can be assumed to lie at a particular location on the exposure continuum based on TTS thresholds. The points on the graph in Figure 4 fall within a wide range above the diagonal (2-10 dB), indicating that TTS and PTS are very different injuries. The effects of differing time constants, different recovery time and different anatomic effects are evident in this data.

Different Results at Low and High Noise Levels

When establishing the threshold noise exposure for PTS, our results argue that using high levels of noise (110 dB) and varying the intensity does not effect the same change as varying the amount of fixed-duration low-level noise. The simplest way we examined this was to compare

ranking of mice by strain and age in terms of noise vulnerability, as determined by the present and previous methods. These gave completely different results.

PTS Prediction based on Equal Energy Hypothesis We extended the comparison of high and low exposure levels by comparing predicted and calculated PTS thresholds, based on the equal energy hypothesis (EEH). According to this hypothesis (Ward, Duvall, Santi, & Turner, 1981; Ward & Turner, 1982) noise exposures of equal energy will cause the same amount of damage and hearing loss. The current data do not support this hypothesis, in that the PTS thresholds predicted from the previous studies of Ohlemiller et al. (2000, 2011) using high noise levels differed widely from the results actually obtained. Consideration of previous work supporting the EEH (e.g., Ward et al., 1981; Ward & Turner, 1982) reveals that the exposures that were compared typically were all at high noise levels. The application of a dose-response paradigm at low noise levels is a novel feature of the current approach that may reveal for the first time that high and low level noise exposures simply engage different mechanisms. A bit of thought reveals why this might be the case. A good analogy might be the ability of a building to withstand a magnitude 4.0 earthquake versus a magnitude 8.0 earthquake. We would not intuitively expect a brief 8.0 quake on the Richter scale to impart the same amount and type of damage as a longer 4.0 quake. The two degrees of severity will clearly drive building materials in different tolerance ranges. Noise injury is likewise often parsed into hypothetical metabolic and mechanical components, which are presumed to predominate at low and high noise levels, respectively (Ohlemiller, 2008, 2012). Low and high levels of noise will engage these processes differently, and in a way that cannot be predicted by simply extrapolating from either range.

Early Vulnerability Period to Noise The early vulnerability period for PTS is well established and supported by a number of studies (e.g., Henry, 1982b; Henry, 1983; Saunders &

Chen, 1985; Ohlemiller et al., 2000), and is the subject of much speculation as to its physical basis and relevance to humans. A review of the literature, however, reveals that the early vulnerability period, like the EEH, is supported largely by studies applying noise at high levels. We observed little evidence of enhanced noise vulnerability in younger mice expected to be at peak vulnerability. Concerns for human hearing based on the early window exhibited by animals stems from direct extrapolation of the earlier high-level noise results. It may be that this window should be re-examined.

Limitations and Future Research

The data presented here are not complete. Because of limits on time and animals available at the appropriate ages, all groups did not include the optimum number of participants. Generally speaking, most TTS and PTS dose-response curves were nevertheless well-behaved and well-fit by a logistic function. The one exception was the case of old B6 mice, for which the dose-response relation appeared non-monotonic. Future studies should fill out the gaps in the current data and confirm the present findings.

Indications for human studies

Although the present findings are tentative, they hold considerable implications for how noise vulnerability is characterized in both laboratory and clinical settings. Firstly, the variability of the relation between TTS and PTS suggests that the physico-chemical relation between these may vary with subject. It also suggests that the safety margin for imposing a TTS as a clinical method may be variable, and potentially narrow for some individuals.

Findings from the current study are also relevant to virtually all applications that assume the principles derived from high level exposures can be extrapolated to low levels. One 'rule' that does not seem to apply across domains is the equal energy hypothesis. Also affected are such accepted principles as the early vulnerability window. Finally the present results are relevant to the entire predominant paradigm of comparing animal groups that vary by genetics or by treatment. Such studies have essentially applied high levels of noise, and then imply in their discussion that the findings are relevant to 'more human-like' low level exposure conditions.

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Fig. 1. Average baseline thresholds for each age and strain. All fell within normal limits based on previous data.

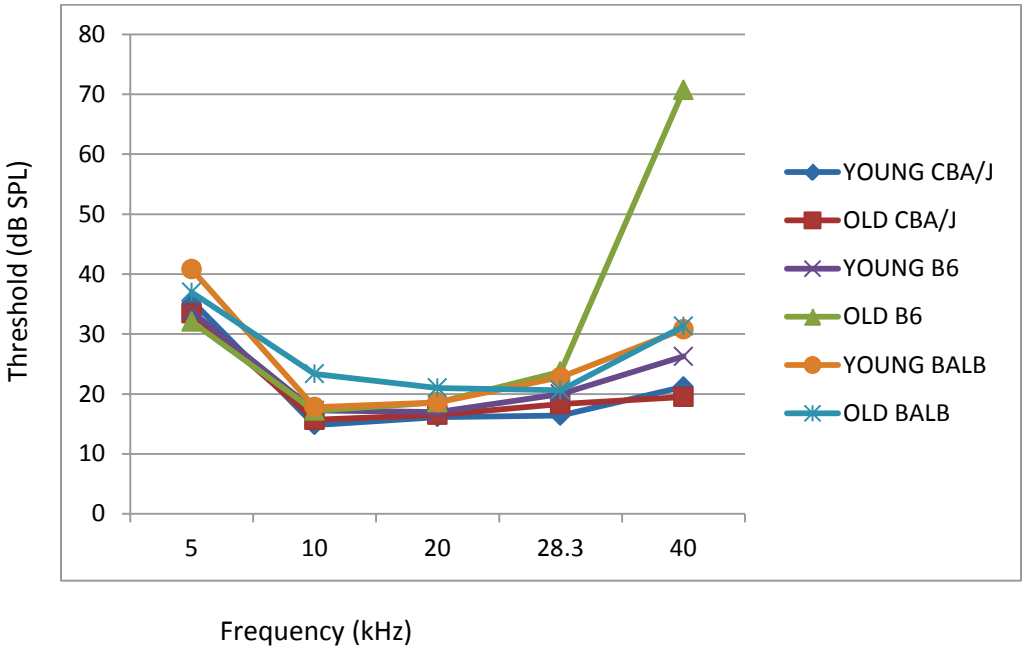

Fig. 2. Average threshold shifts (+/- standard deviation) by strain and age. Young animals are in the top row and old animals are in the bottom row. Old animals experienced larger TTSs than young animals.

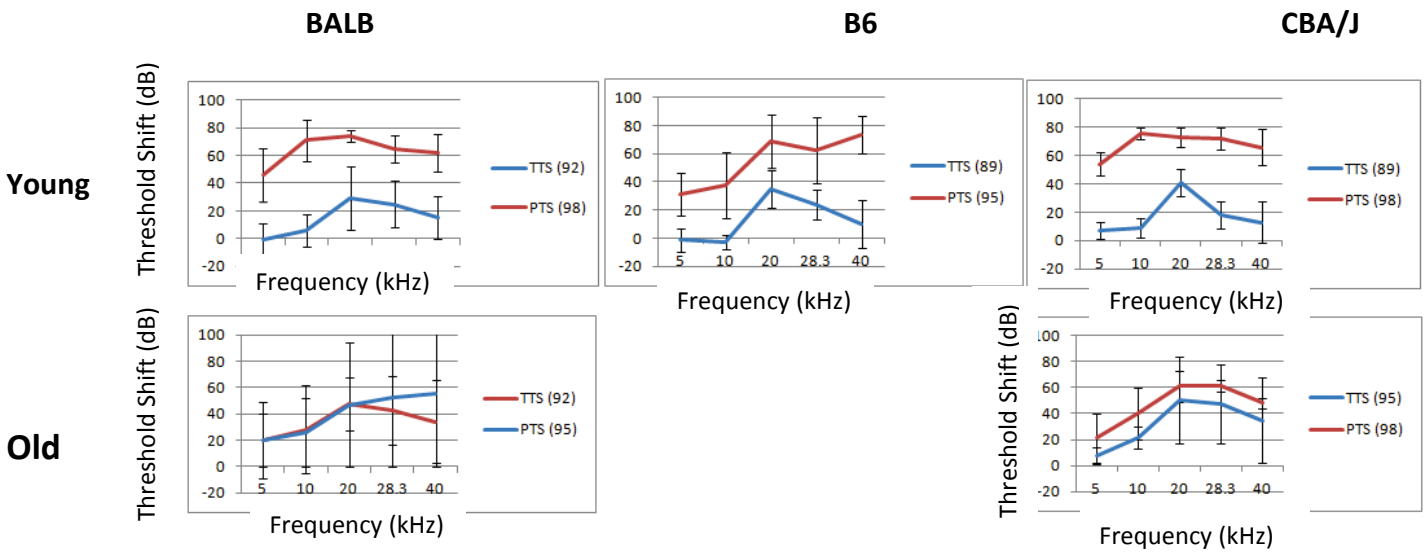

Fig. 3. Probability of threshold shift for old BALB mice as calculated by logistic curve fit

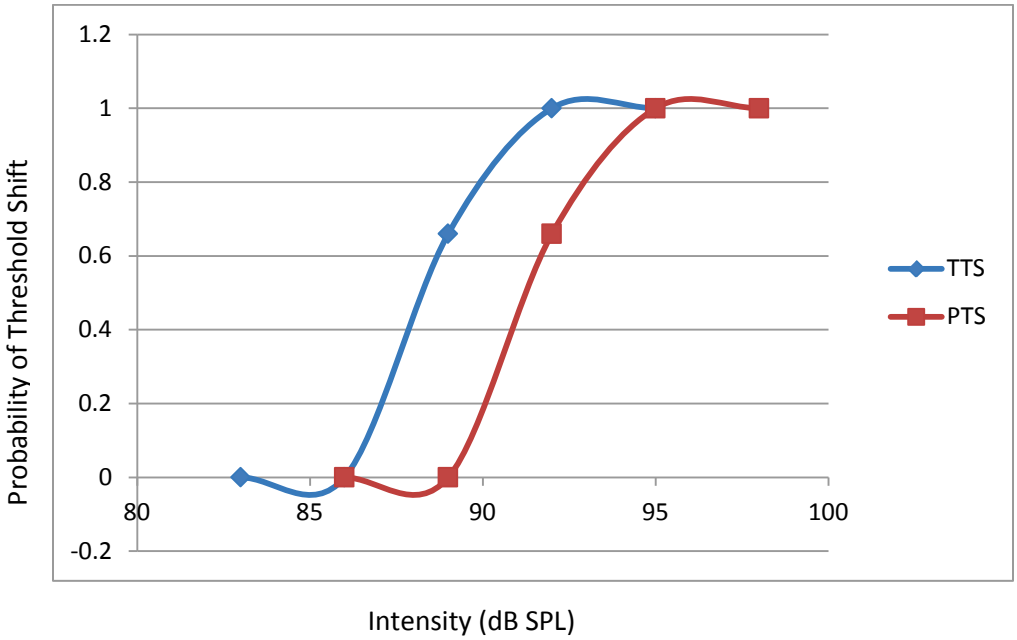

Fig. 4. Susceptibility to TTS vs. Susceptibility to PTS. TTS thresholds were compared to PTS thresholds for each age and strain. It is evident that susceptibility to TTS does not predict susceptibility to PTS.

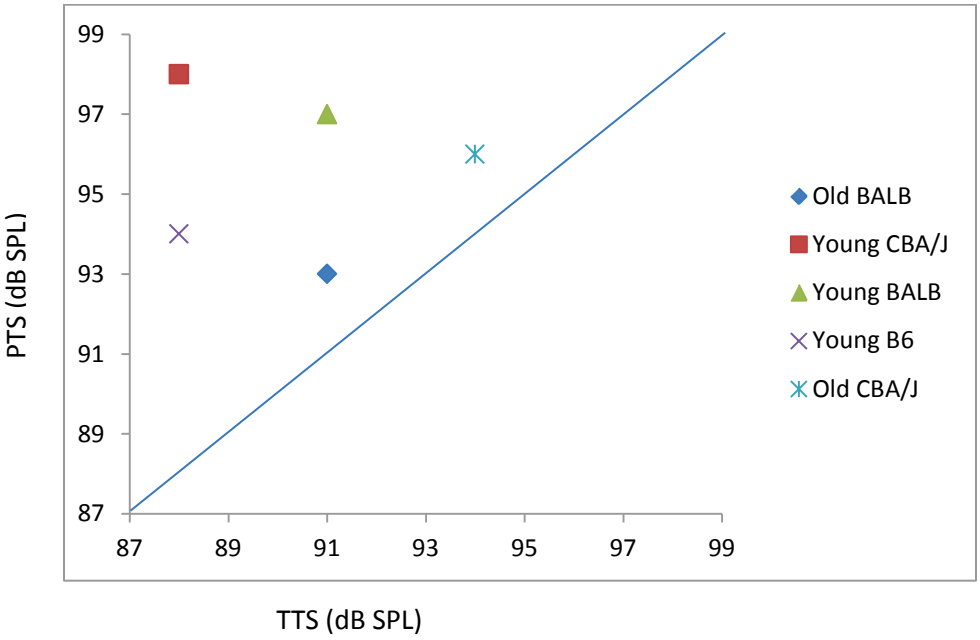

Fig. 5. Rank of PTS Susceptibility. Subject groups were ranked from most to least susceptible using data from the current study (Rank by Intensity) and data from Ohlemiller, 2000, (Rank by Duration) which exposed mice to different durations of 110 dB noise. Except for old CBA/Js, rank by intensity did not predict rank by duration.

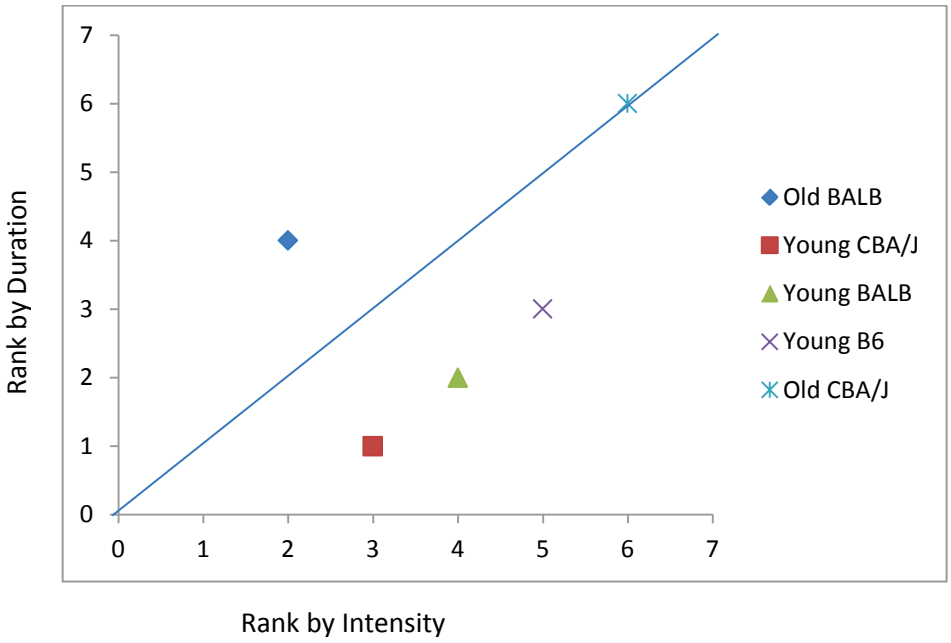

Fig. 6. Actual and Predicted PTS Thresholds. PTS thresholds from current data are compared to PTS thresholds predicted based on the equal energy hypothesis from Ohlemiller (2000) data.

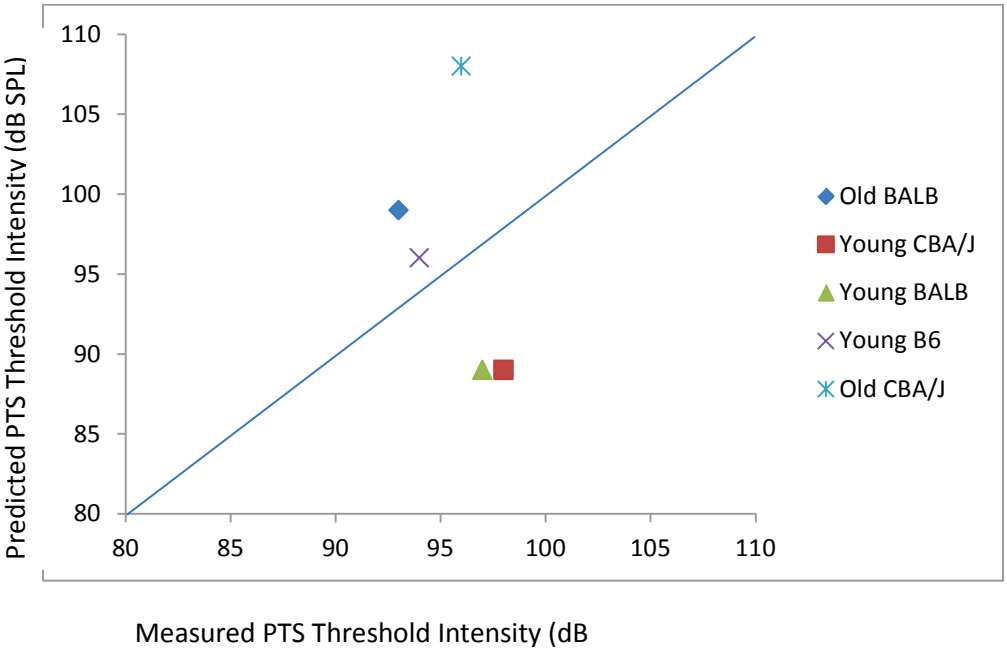

Fig. 7. Young vs. Old Comparison. Young and old animals were compared to examine interspecies differences by age. 4 of 5 conditions showed no early vulnerability period.

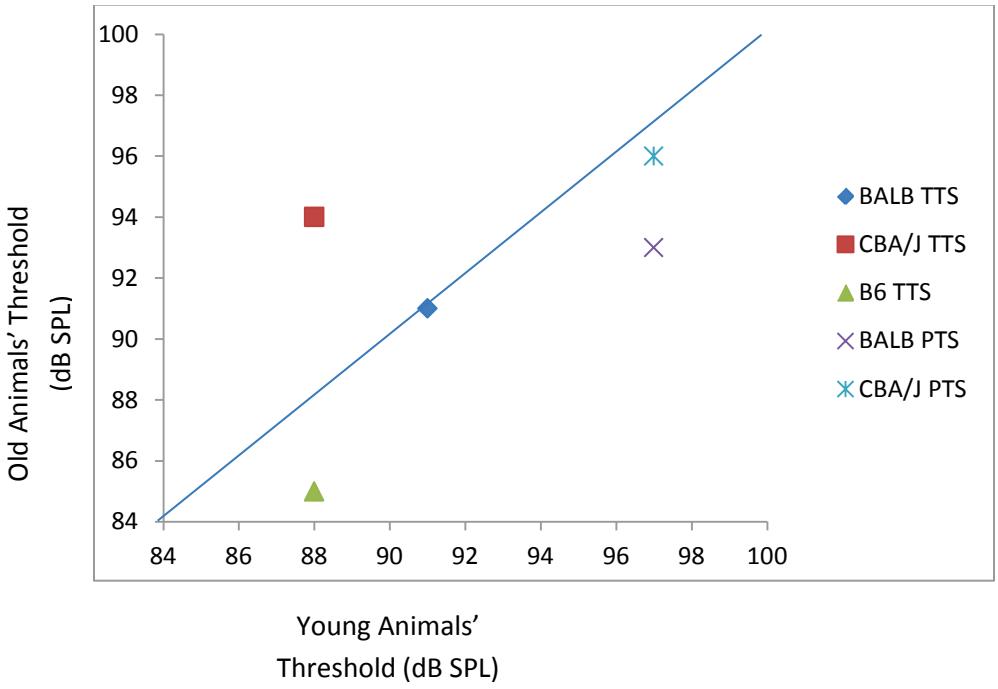

Fig. 8. PTS Intensity coincides with Noise Level that causes EP Reduction. Ongoing studies into electrocochlear potential (EP) reduction have shown that EP drops at the same intensity of the PTS found in this study (Ohlemiller, unpublished).

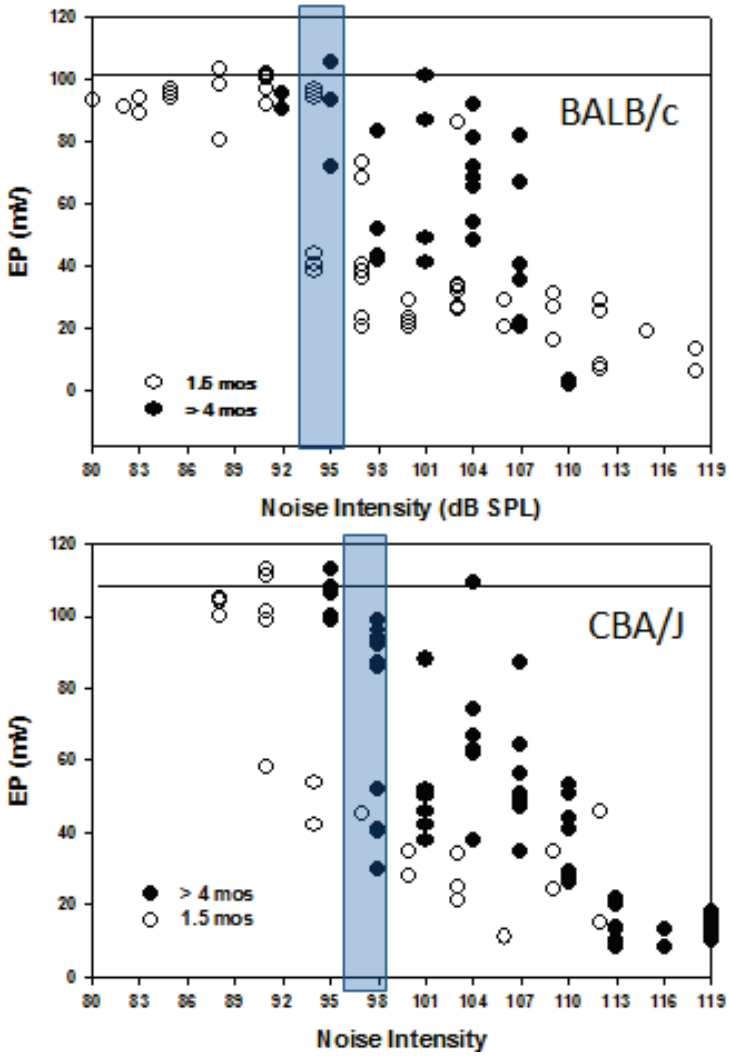

Table 1. TTS and PTS thresholds for each strain and age as determined by curve fit calculations.

Mice	**TTS (dB SPL)**	**PTS (dB SPL)**
Old BALB	91	93
Young BALB	91	97
Young B6	88	94
Old CBA/J	94	96
Young CBA/J	88	98

Table 2. Actual and Predicted PTS Thresholds. PTS thresholds from current data are compared to PTS thresholds predicted based on the equal energy hypothesis from Ohlemiller (2000, 2011) data.

	Actual Thresholds (dB SPL)	Predicted Thresholds (dB SPL)
Old BALB	93	99
Young CBA/J	98	89
Young BALB	97	89
Young B6	94	96
Old CBA/J	96	108