31 Noise-Induced Hearing Loss

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INTRODUCTION AND BACKGROUND

Noise has been a universal problem from the time man first put hammer to stone. Our lives have not gotten any quieter. On-the-job hearing loss due to noise is the most common form of occupational injury in the United States. Estimates by the National Institute for Occupational Safety and Health (NIOSH) in the early 1980s estimated that about four million workers were exposed to noise in excess of 85 dBA (NIOSH, 1998). NIOSH calculates that a lifetime exposure to noise in excess of 85 dBA will cause an excess risk of 15% of those workers suffering a disabling hearing loss. With exposure to 90 dBA over a working lifetime, the estimated risk almost doubles to 29%. Current thinking is that every ear is vulnerable to noise — given enough acoustic energy at the right frequency for enough time.

NIOSH (1988) identified noise-induced hearing loss as one of its top ten priorities. In the 1990s, it was again identified as a major public health priority by ASHA (Cherow, 1991). Based on testimony from industry, private organizations, and governmental agencies, the National Occupational Research Agenda (NIOSH, 1996) identified noise-induced hearing loss as a priority area for research.

Hearing loss is common in the general population and appears to be becoming more so (Ries, 1985). With the introduction of high-power audio systems, boom boxes, and car stereos, noise-induced hearing loss is here to stay. Civilian and military firearms use is an important contributor to loss of hearing (Ylikoski, 1994; Prosser et al., 1988).

It is well known among practicing audiologists that all humans are not equally susceptible to the damaging effects of noise. Noise researchers know that noise susceptibility in experimental animals is quite variable (e.g., Cody and Robertson, 1983). The inbred mouse, while still variable from one mouse to another, appears to be less variable within strains. Between strains, the mouse shows great differences in susceptibility to noise as well as differences in prebycusis.

The goal of this chapter is to review mouse noise-induced hearing loss research and speculate about organizing principles. Most noise-induced hearing loss research has not been done in the mouse, but rather in the chinchilla, guinea pig, cat, and even rabbit. In the past 10 years, there has been a renaissance in noise studies in mice. It is believed that mechanisms of noise-induced hearing loss cross species, but exceptions will be noted. For additional background information, the reader is referred a review of the literature on physiological effects of noise-induced hearing loss by Borg, Canlon, and Engstrom (1995).

Equal Energy Hypothesis

The equal energy hypothesis (EEH) was first proposed by Eldred et al. (1955) and elaborated later by Ward et al. (1958). This hypothesis implies that the ear acts as an integrator of acoustical energy. Hearing loss is equal to the product of accumulated time and acoustic intensity. In addition, time and intensity may be traded. By doubling the exposure time, the noise dose is increased by 3 dB (because decibels are log units, 3 dB represents a doubling of energy; e.g., see Vernon, Katz, and Meikle, 1976). In recent years, the EEH has been shown to be a reasonable first approximation within certain limits of times and energies. However, Henderson's group (Henderson et al., 1991,

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Danielson et al., 1991) has shown that once a specific sound level is exceeded, equal energy no longer holds. This appears to be the point at which acoustic energy stops damaging the neural elements of the cochlea, and mechanical damage is being done to the cochlear partition. The equal energy limits for time remain unknown. However, when you begin to think about doubling time, you soon realize that it does not take many doublings to exceed the lifespan of the subject. Thus, time is bounded by the lifespan of the organism. Extremely short, high-level sounds (i.e., impacts and impulses) do not appear to obey the EEH (Henderson et al., 1991).

The EEH has been incorporated into U.S. occupational hearing conservation regulations. However, rather than utilizing 3 dB as the doubling and halving factor for time, 5 dB is used, under the premise that most workers are not exposed to continuous noise over an entire work shift. Rest breaks from sound appear to be more beneficial than one would predict based on the EEH (Price, 1974). For example, Clark et al. (1987) exposed two groups of chinchillas to a 95-dB octave band of noise centered on 500 Hz. A periodic rest group was exposed round-the-clock to 15 minutes of noise and 45 minutes quiet; a continuous group was exposed to 6 hours of continuous noise and 18 hours of quiet. When compared, the periodic rest group had less permanent threshold shift and less cochlear damage than the continuous group, although both groups had equal energy exposures. The effect of rest seems to begin almost immediately after the cessation of the sound (Price, 1976).

Conditioning

In some species, daily exposure to loud sound seems to protect the ear from a later, intense sound (Henderson et al., 1996a; Canlon et al., 1988; Canlon and Dagli, 1996). This is the so-called conditioning or toughening (or more recently, priming) effect which can provide 10 to 20 dB of protection against noise. Although demonstrated in the chinchilla, rabbit, gerbil, and guinea pig, some accounts have not been able to demonstrate the conditioning effect in mice (Fowler et al., 1995). In a very complex report, Fowler et al. (1995) exposed groups of CBA/Ca mice to a number of different conditioning paradigms (Table 31.1). They then exposed the mice to a traumatic noise of either 107, 110, or 117 dB for either 6 or 24 hours. They were not able to demonstrate any protective effect due to any of the pre-exposures.

From our experience, the spectral frequency of these exposures may have been too low to damage the mouse ear (R.R. Davis et al., 1994; Fay, 1988; see also Figure 31.2). On the other hand, in the chinchilla, low-frequency conditioning exposures are much more protective for the ear than high-frequency conditioning exposures (Henderson et al., 1996a). A recent article by Yoshida et al. (2000) has demonstrated conditioning in the CBA/CaJ mouse to an 81 dB (for one week) or 89 dB (for 15 minutes) conditioning stimulus consisting of 8-16 kHz octave-band noise. The traumatic noise exposure was 2 hours at 100 dB. The long-term conditioning exposure narrowed the range of frequencies affected by the trauma, while the short-term conditioning reduced peak PTS by 22 dB. This conditioning result is qualitatively different from that seen in other species. Conditioning

TABLE 31.1
Exposure Paradigm and Exposure Levels Used to Attempt Auditory Conditioning in CBA/Ca Mice

Exposure Paradigm	Exposure Level
4.5 kHz centered narrow-band noise 6 h/day for 10 days	86, 91, or 96 dB
Continuous noise 24 h/day for 24 days	80 or 86 dB
1-kHz tone for 10 days	75 dB

From Fowler et al., 1995. With permission.

may be a fruitful area to look at comparative species differences, and the mouse may be a valuable animal model to examine the mechanisms underlying conditioning.

Ohlemiller et al. (1999a; b) have produced a series of papers in C57BL/6J (B6) mice demonstrating hydroxyl free radicals are generated after a 110-dB broadband noise exposure for 1 hour. There is some evidence in chinchillas that points to the basis of conditioning as increases in levels of a protective enzyme (catalase) and scavenger (glutathione) to protect against oxygen free radicals (Jacono et al., 1998). Mice have the same enzyme and scavenger present in the ear and should condition equally well.

Along the same lines, Willott and Turner (1999) have demonstrated a reduction in age-related hearing loss in B6 and DBA/2J strains of mice to a low-level noise exposure (broadband noise bursts, 70 dB 12 h/day; see Chapter 14). These inbred mice strains possess the *Ahl* gene, which accelerates presbycusis (see Chapter 28). Assuming conditioning is acting upon oxygen free radical metabolism in the cochlea, the procedure described by Willott and Turner may be modifying oxygen free radical metabolism affecting aging in the ear.

TTS vs. PTS

The relationship between temporary threshold shift (TTS) and permanent threshold shift (PTS) to noise exposure is still being actively debated. (In some reports, TTS is referred to as *combined threshold shift* or CTS — having both temporary and permanent components.) TTS is an immediate post-exposure increase in auditory threshold which resolves over time, to either pre-exposure threshold levels or to a PTS. PTS is defined as a post-exposure increase in auditory threshold that remains relatively stable over time. The debate centers around whether TTS is an early, reversible form of PTS; or whether TTS and PTS are two separate processes. Most recent evidence points to two separate processes, although the U.S. occupational noise regulations are based on TTS being a predictor of PTS. There is evidence to support the view that TTS may be related to swelling of the VIIIth nerve fibers in the cochlea, perhaps due to neurotransmitter excitotoxicity (Puel et al., 1996), or it may be due to changes in hair cell or stereocilia stiffness (Husbands et al., 1999; Saunders et al., 1986). PTS appears to be due to cell death due to oxygen free-radical production, calcium influx, or mechanical disruption of the hair cell. Much effort and many resources have been expended on the TTS vs. PTS question over the past 40 years. It is a problem awaiting a clever new tool or technique. Recently, Bohne and colleagues (Bohne et al., 1999; Nordmann et al., 2000) have developed a technique in chinchillas allowing for temporal snapshots of cochlear changes in the same subject. This technique uses fixation of one ear immediately after exposure, and recovery and fixation of the second ear a number of days later. This procedure may be of use in teasing out differences between TTS and PTS and may be adaptable to the mouse.

DISADVANTAGES OF THE MOUSE IN STUDYING NIHL

Each species has its advantages and disadvantages as a model of noise-induced hearing loss (NIHL). The major disadvantage of using the mouse for NIHL is the frequency range of its audiogram. First, as the acoustic stimulus climbs above the frequency range of human audition, it becomes difficult to troubleshoot exposure and measurement systems. The experimenter becomes more dependent on test equipment and microphones to solve problems.

Second, the physics of the high-frequency acoustic wave become more complex above 10 to 15 kHz. As the acoustic wavelength becomes shorter, it becomes possible for reflecting sound waves to interfere both constructively and destructively with the main wavefront, producing standing waves (Appendix A).

Third, most equipment readily available off-the-shelf for measuring the auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOE) is designed to be used on humans. This limits the upper test frequency to 8 kHz (but see Chapter 4).

Finally, calibration at these frequencies becomes a challenge, often with two consecutive measures differing by many dB. Noise bands are easier to calibrate than pure tones, so noise exposures are usually more consistent, while calibrating ABR sources can be frustrating. A statistical approach should be taken. Measurements should be made often enough to get a good sample so that it can be assumed that the mean (or median or mode) is the normal situation. Moreover, the measuring method should be standardized. Five-dB steps are about the smallest ABR increment possible at the higher frequencies. In noise experiments, threshold shifts rather than absolute thresholds are usually the data of interest.

MEASURING NIHL

Generally, noise-induced hearing loss is measured by the ABR, although the cochlear microphonic or N_1 can also be used. The advantage of the ABR is that an animal may be measured a number of times, and this technique is discussed in detail in Chapters 4 and 38. Mice are typically anesthetized in order to obtain clear recordings. Restraint without anesthesia works well in chinchillas and rabbits with indwelling electrodes (Snyder and Salvi, 1994) and reduces complications due to multiple sessions of anesthesia; however this method has proven difficult with mice. Anesthetized mice should have their body temperatures externally supported by a heating pad.

We test using tone pips at 8, 16, and 32 kHz and a click. The 0.1-ms click results seem to be highly correlated with the results from the 8-kHz tone. Tones are tested with an envelope of 1-ms rise time, 1-ms decay time, and 1-ms steady state. The ABR is obtained by averaging 128 to 1024 presentations of the stimulus at 31 presentations per second. We have found that the most noise damage occurs at 16 kHz and have debated omitting other test frequencies to reduce test time but so far continue testing all frequencies. It is possible that maximum damage may occur at different frequencies in other mouse strains.

THRESHOLD CHANGES

In our experience, 1 week after a noise exposure, the mouse still shows a small amount of TTS. By 9 days any PTS predominates. We have seen a 40-dB TTS at 16 kHz measured at day 1 resolve to no measurable PTS at day 30 post-exposure (R.R. Davis et al., 1994). Because the ABR tends to be a rather crude measure of hearing loss, one cannot say that there was *no* PTS, only that none was detectible.

THE EXPOSURE ACOUSTIC STIMULUS

The damaging stimuli most commonly used for mice are tones, octave bands of noise, or broadband noise. Our experience is that exposures in which the stimulus energy is low in the 16-kHz region do not produce significant PTS (Figure 31.2) (R.R. Davis et al., 1994). Calibration of the exposure stimulus is paramount. The accompanying appendix (How We Do It) provides a tutorial for making and measuring sound.

When publishing exposure conditions, broadband exposures should include the spectrum of the exposure stimulus as well as the intensity. It is very difficult to replicate an exposure condition without a graphic description. It is helpful in the case of tonal exposures to indicate the amplitude of any harmonics. Anything unusual about the acoustic environment should be included in the methods section. For example, for long exposures, access to water is required. Sources of water can be water bottles, pieces of potato or apple, pans of water, etc. Each of these objects may interact with the sound field and could be important for replicating an exposure condition.

GENETICS OF NIHL SUSCEPTIBILITY

Although speculated in the human literature for a long time, the genetic basis of susceptibility to NIHL has only recently come under study, probably because an experimental animal model has become available only in the past 5 years. The discovery by Erway et al. (1993a) of a single recessive gene (*Ahl*) in the B6 mouse strain which increased the rate of presbycusis led to our current research. As discussed below and in Chapter 28, *Ahl* in the homozygous condition contributes to increased susceptibility to noise-induced hearing loss (Erway et al., 1996; Davis et al., 1999; Newlander et al., 1995).

Shone et al. (1991a) first showed differences in susceptibility to noise in B6 and CBA/Ca mice but hypothesized those differences were due to the underlying presbycusis. They exposed their mice to 101-dB broadband noise for 45 minutes. Their B6 mice were old enough so that they were already showing high-frequency hearing loss at pre-exposure testing.

Erway et al. (1996) noise exposed four groups of mice with four different genotypes prior to the detection of any presbycusis. C57BL/6J mice (B6) are homozygous for the *Ahl* gene (*Ahl/Ahl*). The CBA/CaJ inbred mouse strain (CB) have wild-type alleles in the corresponding position (+/+). A hybrid was generated from crossbreeding the CBA/CaJ and C57BL6J inbred strain (CB × B6), which are heterozygous at the *Ahl* allele (*Ahl/+*). Finally, a hybrid was generated from crossbreeding the inbred strain B6 with the inbred strain DBA/2J (B6 × DB). DBA/2J mice are known for their loss of hearing very early in life — earlier than even the B6. We hypothesized based on Erway et al. (1993a) that these hybrid offspring are homozygous for *Ahl* (*Ahl/Ahl*). Mice were exposed to 110 dB of broadband noise for 1 hour and tested at various times after exposure. There was a statistically significant difference between the two genotypes: the CB and CB×B6 strains showed no NIHL, and B6 and B6 × DB strains did show NIHL. This indicated that mice homozygous at the *Ahl* locus were more susceptible to noise than wild-type or heterozygotes.

Newlander et al. (1995) and Davis et al. (2001) were able to show in backcross mice that the *Ahl* gene followed Mendelian laws of genetics. In the parent generation, C57 mice were bred with CBA/CaJ mice. The resulting F1 generation mice (CB × B6) all have the *Ahl/+* genotype. These F1 hybrids are then back-bred to the inbred C57 stock. This backcross generation now had 50% of offspring with the *Ahl/+* genotype and 50% with the *Ahl/Ahl* genotype. Noise exposures were made in the backcross generation mice to define their phenotype. DNA markers for specific alleles defined their genotype. Newlander et al. (1995) were able to demonstrate a high level of predictability of noise susceptibility for certain DNA markers (presumably the *Ahl/Ahl* genotype). This is a strong logical argument that the changes seen in noise susceptibility are modulated by this gene. Due to hybrid vigor, these backcross mice required significantly greater noise exposures than the inbred strains (Table 31.2).

Davis et al. (1999) exposed groups of CB and B6 mice to increasing levels of noise (Table 31.2 and Figure 31.1) to produce a dose-response curve for the two strains. B6 mice were about 6 dB more sensitive to noise, and the slope of their dose-response curve was shallower than the dose-response curve for CB. It was speculated that the B6 mouse ears were being damaged through metabolic mechanisms, while the CB mouse ears were being damaged through direct acoustic trauma.

It now appears that the *Ahl* gene is present in a number of strains exhibiting early presbycusis (Q.Y. Zheng et al., 1999a; b). These strains include but are not limited to DBA/2J, BUB/BnJ, NOD/LtJ, A/J, and SKH2/J. Johnson and colleagues (see Chapter 28) mapped the *Ahl* gene to the inbred strains 129/ReJ, A/J, BALB/cByJ, BUB/BnJ, C57BRLtJ, DBA/2J, NOD/LtJ, SKH/2J, and STOCK760. They were able to demonstrate age-related hearing loss in 50% of offspring in a backcross generation, indicating a recessive gene.

Q.Y. Zheng et al. (1999b) cataloged 80 inbred mouse strains (see Chapter 28), and this should provide fertile ground for discovering other mouse strains that are highly susceptible to NIHL. For

TABLE 31.2
Exposure Parameters for Some Noise Studies in Mice

Authors	Strains	Stimuli[a]	Results	Notes
Davis et al., 1999	C57BL/6J and CBA/CaJ	BBN 1–30kHz 0, 98, 101, 104, 107, 110, 113, 116, and 119 dB, 1 h	Two noise dose-response curves	Exposed awake
Davis, Shiau, and Erway, 1994	CBA/CaJ × C57BL/6J and C57BL/6J	BBN 1–30kHz 110 dB, 1 h vs. BBN 7–24kHz, 110 dB, 1h	Low-freq. exposure produced TTS but not PTS; high-freq. produced equal TTS and significant PTS	Exposed awake
Henry, 1982b	CBA/J, AUS/sJ and SJL/J	Octave band 12–24 kHz, 124 dB, 5 min.	15–50 dB PTS depending on age and strain	Exposed awake, 3 inbred strains
Li and Borg, 1992a	CBA/Ca and C57BL/6J	BBN 2–7 kHz, 120 dB, 5 min	C57BL/6J more susceptible	Exposed anesthetized
Newlander et al., 1995; Davis et al., 2001	C57BL/6J × (C57BL/6J × CBA/J)	BBN 1–30 kHz 110 dB 8 h	Separated phenotypes into two groups	Exposed away, hybrid vigor
Ohlemiller, Wright, and Dugan, 1999	C57BL/6J	BBN 4–45 kHz, 110 dB 1h	Oxygen free radicals measured in perilymph	Exposed awake(?)
Shone et al., 1991a	CBA/Ca and C57BL/6	BBN 500–40 KHz, 101 dB, 45 min	30–35 dB TTS, 17 dB PTS in B6, 0 dB PTS in CB	Exposed awake, no hair cell differences between groups
Yanz et al., 1985	C56BL6J wild-type and albino	BBN 3.8–16 kHz, 125 dB, 10 min	20–52 dB PTS	Exposed awake, only measured to 8 kHz; no difference between albino and wild-type
Yoshida et al., 2000	CBA/CaJ and 129/SvEv	Octave band 8–16 kHz, 97 dB, 2 h; 100 dB, 2h; 103 dB, 2h; 103 dB, 8h; 106 dB, 8h	129/SvEv shows less damage than CBA/CaJ	Exposed awake measured via compound action pot and DPOE

[a] BBN = broadband noise.

example, it would be worthwhile to determine if NIHL susceptibility and age-related hearing loss are truly coupled. Of 19 strains that have late-onset hearing loss, only B6 has been studied for NIHL.

Yoshida et al. (2000) reported that the inbred strain 129/SvEv is much less sensitive to noise damage than the inbred strain CBA/CaJ. They exposed young mice to 97 to 106 dB for 2 to 8 hours (Table 31.2) and incorporated a slow onset of noise to prevent sensitive 129/SvEv mice from developing autogenic seizures. They were able to demonstrate that the differences between the strains were due to the inner ear and not middle ear transmission of vibration. This is a puzzling result because Johnson et al. (2000) listed a closely related substrain, 129/ReJ, as mapping the *Ahl* gene. Q.Y. Zheng et al. (1999b) also showed that 129/ReJ demonstrates early presbycusis. Yoshida et al. speculated that perhaps age-related hearing loss and susceptibility to noise-induced hearing loss may not be linked. Based on Newlander's data, this would require two closely linked genes on chromosome 10: one encoding susceptibility to AHL, the other encoding susceptibility to NIHL.

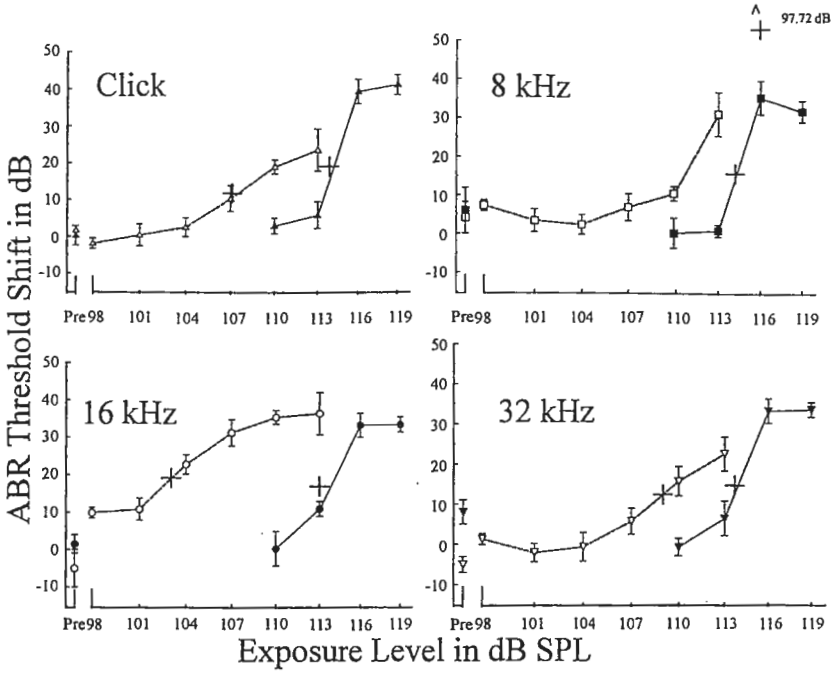

FIGURE 31.1 Dose-response curves for noise effects on ABR threshold. Open symbols are B6 data, filled symbols are CB data. Left-most points on all graphs are unexposed control levels. Each point represents the mean ABR threshold shift of one group of mice exposed to one noise level once, recorded 2 weeks after exposure. Each individual graph represents a different ABR test frequency. The cross represents the inflection point for each curve — the half effect point. Error bars indicate standard error. From Davis et al., 1999, with permission.

OTHER FACTORS AFFECTING NOISE SUSCEPTIBILITY

There are other agents that interact with noise to produce increased susceptibility to hearing loss. The following are some of the more commonly studied variables, a number of which have been studied in mice.

Sex

Although we routinely include sex as part of our analysis model, a statistically significant effect of gender has not yet been found in mice. However, others using chinchillas have seen a difference in susceptibility to noise between males and females, depending on the test frequency. McFadden et al. (1999d) showed a significant difference in auditory threshold between males and females prior to impulse noise exposure and a significant difference in hearing loss between sexes after exposure. The female chinchillas had about 10 dB more loss in the high frequencies and 5 dB less loss in the low frequencies than males.

Q.Y. Zheng et al. (1999b) statistically tested the thresholds measured in their mice and found no differences between males and females for any of the strains showing congenital or age-related hearing loss.

Although there are many confounding factors, human males tend to show hearing loss in excess of their female counterparts. They also tend to engage in noisier activities.

Anesthetized vs. Unanesthetized

There are advantages to using an anesthetized animal for noise-exposure studies. First, the stimulus can be introduced into the animal's ear canal with a minimum of variation. Sleeping animals do

not change orientation, cover ears, or locate acoustic nulls in their environment. Second, monaural exposures can be done with the unexposed ear serving as a within-subject control. Third, a major physiological or surgical manipulation may require the subject to be anesthetized. However, whenever the animal is anesthetized, a non-physiological subject is being used. Anesthetic agents can alter breathing, blood pressure, and the ability to maintain body temperature (J.N. Brown et al., 1988). There may be changes in middle ear muscle activity and ventilation. Also, neurological activity changes with reduction in protective mechanisms, including middle ear muscles. In guinea pigs, noise exposure during anesthesia leads to *less* TTS than in awake subjects (Hildesheimer et al., 1991). However, if body temperature is maintained, TTS is about 11 dB *greater* in the anesthetized guinea pigs. This may be an interesting area of inquiry for mouse researchers.

Body Temperature

Physiological systems operate best at normal body temperature for the species. Some animal data have indicated that lowering the subject's body temperature makes them less vulnerable to noise (e.g., Hildesheimer et al., 1991). There are also some human data indicating that working in hot environments increases vulnerability to noise (Pekkarinen, 1995).

Ototoxic Agents

It has been known for at least 20 years that the aminoglycoside antibiotics (i.e., kanamycin, neomycin, streptomycin, gentamicin, and amikacin) interact with noise in guinea pigs to increase noise-induced hearing loss (Brown et al., 1980; Brummett et al., 1992). It appears that asphyxiates such as carbon monoxide also act to increase the damaging effect of noise in rats (Young et al., 1987; Fechter et al., 1988b; Chen and Fechter, 1999; G.D. Chen et al., 1999). In rats and mice, industrial solvents such as toluene have been shown to interact with noise (Johnson and Canlon, 1994; Li, 1992b). Ototoxins that do not appear to interact with noise include loop-inhibiting diuretics (ethacrynic acid and furosemide; Vernon et al., 1977) and aspirin (sodium salicylate; Spongr et al., 1992).

Pigmentation

Conlee et al. (1986) and Creel (1980) have proposed that true albino rats and mice are more vulnerable to noise damage because they lack melanin in the stria vascularis of the cochlea. Some researchers have demonstrated a difference in hearing level between human races based on skin pigment. Members of dark-skinned races have greater hearing sensitivity than members of light-skinned races. Others discount this finding as a difference in recreational activity — suggesting that whites are more often involved in hunting and other noisy activities (Clark and Bohl, 1996). In probably the best test of this question to date, Yanz et al. (1985) exposed wild-type B6 and c-locus albino C57BL/6J(c^{2j}/c^{2j}) mice to 10 minutes of a 125-dB broadband noise. These inbred strains differed only in the absence or presence of tyrosinase. They found no difference between groups but only tested their mice to 8 kHz, which is not the most sensitive frequency for mice. Also, B6 mice tend to be sensitive to the traumatic effects of noise (Davis et al., 1999). A lower intensity exposure might have been able to separate the phenotypes. There are other, better models of albinism (e.g., CBA/J-$Tyr^{c\text{-}1J}$ strain) for hearing that might help to clarify the issue.

Until this controversy has been settled, it is probably better to use strains with the same amount of pigmentation for comparisons — either all albino or all pigmented. Care must be exercised in testing the effects of pigmentation in mice; although some strains are pigmented and others not, there are significant genetic and physiologic differences between inbred strains other than coat color.

Age

Numerous papers have been written on aging in the mouse auditory system (Chapters 24 and 28). The average laboratory mouse lives to about 2 years. Henry (1982b) exposed three mouse strains

(Table 31.2) to noise at 20, 180, and 360 days. He found strain differences in susceptibility to the damaging effects of the noise. In addition, the younger mice were more sensitive to the noise than the older animals. Results of coat color were opposite to that predicted for melanin: the albino strain was least sensitive to noise, the agouti was intermediate, and the black strain was the most sensitive to noise.

FUTURE DIRECTIONS

The mouse is the premier subject for mammalian genetic studies and will be the basis for numerous future projects investigating the role of genes and noise susceptibility. The National Institute on Deafness and Other Communication Disorders has encouraged the use of inbred mice to study diseases of the ear and hearing. Some interesting topics include the following.

Free-Radical Scavengers and Susceptibility

Free radicals have been implicated in hearing damage. Ototoxic and noise damage seem to be modulated, if not directly caused, by oxygen free radicals. It would be very useful to understand the mechanisms of free-radical generation, damage and repair, if any, in the ear. Very little is known about repair mechanisms in the organ of Corti.

One problem with hearing research in the mouse — and all animals for that matter — concerns the tools for detecting damage. The ABR, DPOE, behavioral audiogram, and even microscopy are all very gross techniques. Often, these tools do not agree well with one another. It would be very useful to have a much finer-grained tool for detecting cochlear changes. This would permit the study of noise exposures in the 80 to 95 dB range — the range normally encountered on the job. There is some evidence that the transient otoacoustic emission may be more useful for detecting organ of Corti changes. Histochemical and immunological techniques should be developed to probe subtle changes in hair cell physiology.

Age-related Hearing Loss and Noise Susceptibility

At this time, it appears that mice with an increased susceptibility to age-related hearing loss are also more susceptible to noise-induced hearing loss. This question needs to be explored in greater detail. Is an ear susceptible to presbycusis also more susceptible to noise, or just more vulnerable to *any* trauma? Is presbycusis a form of cumulative noise damage? Are some ears predestined to die early in life? How can susceptible ears be rescued? For this effort, it would be very useful to discover an inbred mouse strain that *exhibits presbycusis* but does not map for the *Ahl* gene. Yoshida et al. (2000) report that inbred strain 129/SvEv is not as susceptible to noise as CBA/CaJ.

Also, how do changes in the mitochondrial genome affect noise susceptibility?

Gene Therapy for Susceptible Individuals

If single genes can be associated with noise-induced hearing loss, it may be reasonable to contemplate the modification of these genes. Although the current costs of such procedures are prohibitive, one cannot predict what the future prices may be. These gene modifications should first be tried on mice susceptible to noise and presbycusis.

We are probably living in the golden age of ear research. We are beginning to understand how it is built and how it works. The mouse provides numerous models for abnormal development and functioning. The mouse also provides a unique model for learning about the pathology of the ear due to noise, genetics, and chemical agents.

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APPENDIX A: HOW WE DO IT

Making Noise

Procurement

The researcher in noise-induced hearing loss often finds the most suitable acoustic generating equipment at the professional audio store (i.e., the same place local rock-and-roll bands purchase or lease their equipment). That is because to obtain the levels of noise necessary, without distortion, requires many watts of electrical energy. Generally, a minimum audio amplifier will be 300 watts.

Speakers

The weakest link in the chain is speakers. Tweeters and super-tweeters are used to produce the frequencies necessary for mouse or rat exposures. The ideal speaker should respond linearly up to an output of about 130 dB, require less than 100 watts of drive, should be inexpensive, and should be available at the local electronics store. Unfortunately, this speaker has not yet been invented (or if it has, no one has told this author). Because of the physics of their high-frequency response, tweeters must have minimum mass. This implies thin diaphragms and thin wire voice coils. As the driving force increases, the thin wire starts to heat up like an incandescent lamp and eventually opens.

We have used a number of speakers. Our chinchilla work utilized Altec-Lansing 504 high-frequency drivers with horns. However, unless high-pass filtered, most of the noise energy is located in the frequencies below those effective for mouse hearing (Figure 31.2) (R.R. Davis et al., 1994). In fact, Altec-Lansing has discontinued this line of driver but does provide replacement voice coils. Radio Shack has the Realistic 1310B Supertweeter (Ft. Worth, TX), which currently costs about U.S. $20.00 each. They can be purchased at the local Radio Shack store or ordered in bulk from their toll-free number or Web page. We remove them from their plastic case and install them in a sheet of plywood that fits over the exposure chamber, four speakers per chamber. They are wired two in a series, then two series in parallel, so that the effective impedance is still 8 ohms. We utilize the factory-provided high-pass filter to keep DC and low frequencies off of the voice coils. Using an acoustic foam-lined exposure chamber (Davis and Franks, 1989), we can comfortably produce 116 dB for over 8 hours. With the foam out and a more reflective situation, we can easily produce over 120 dB. Going above that level requires some experimentation. We have found that placing heat sinks on the voice coil housing during exposure allows us a few more dB before speakers are damaged.

For one experiment, we were called upon to produce an octave band of noise centered on 20 kHz. We found that the Radio Shack supertweeters were not able to produce much effective output at these high frequencies. We resorted to using piezoelectric Motorola Bullet Supertweeters. We have not found them very useful for everyday use, but they are great at higher frequencies. Our impression is that the piezoelectrics do not appear to be as capable of surviving a short bout of being overdriven as voice-coil speakers. Motorola makes a piezoelectric driver/horn assembly that is rated at 400 watts (Powerline®). However, we have found that this driver still tops out at 110 dB, and more power does not result in more acoustic output. The piezoelectrics are very high impedance (2000 to 4000 ohms). This is often problematic for power amplifiers that are designed to drive 1 to

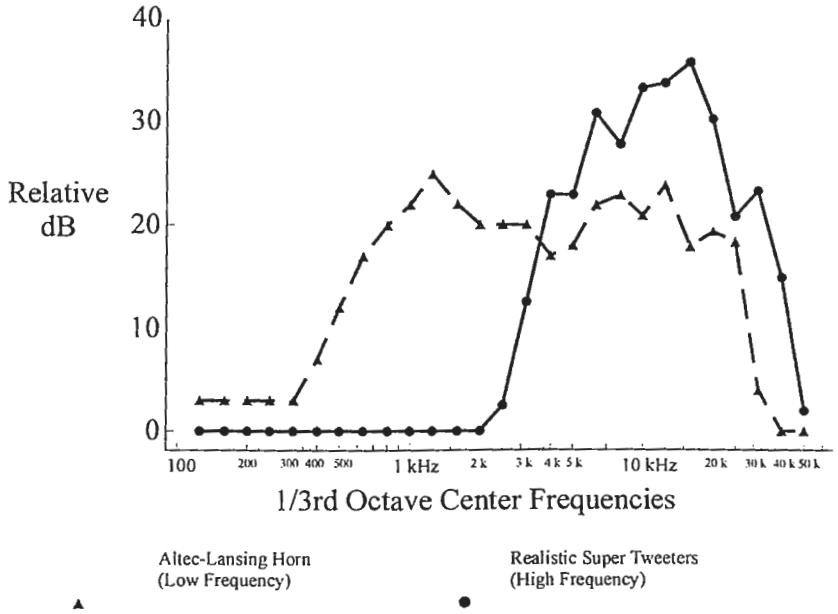

FIGURE 31.2 Relative comparison of noise spectra with Altec-Lansing 504 High Frequency Drivers (producing a broader, lower frequency) and Realistic 1310B Supertweeters (producing a narrower, higher frequency). Initial attempts at noise exposing CBA/CaJ and C57BL/6J strains with the Altec-Lansing speakers resulted in 40-dB TTS, which resolved into no measurable PTS. Mouse ears are sensitive to the acoustic energy present at 16 kHz. Our laboratory currently uses the Realistic Supertweeters in a four-speaker array wired in a series-parallel configuration to develop 8 ohms of load. The standard 5-kHz high-pass filter is maintained. (Adapted from Davis et al., 1994.)

16 ohms. Many power amplifiers are able to produce greater wattage the lower the load impedance, so they are unable to acceptably drive piezoelectrics.

Noise

Our signal source is an old General Radio 1382 Random Noise Generator. These can be purchased on the secondary equipment market (Tucker Electronics, Tucker, TX). We have had some experience with the Tucker Davis Technologies (Gainesville, FL) Noise Generators, along with programmable filters and headphone amplifiers as a noise source and they are viable.

We then control the signal with Wavetek turret attenuators in 1-dB steps. The signal is then passed into a Mackie 1202 pre-amplifier, usually utilized in the unity gain mode, although sometimes used to add a few dB of gain. We moved to the Mackie 1202 after we had a number of failures of our power amplifier. We decided that impedance mismatches in the signal path were causing problems. The Mackie, in effect, acts as an impedance match between the signal level equipment and the power amplifier.

The Physical Surround

At higher frequencies, the short-wavelength acoustic signal reflecting off of a flat, hard surface can result in standing waves. Standing waves result in nulls and peak intensity levels that can allow the subject to be exposed in unpredictable ways. Generally, an absorptive chamber should be used for exposure. This reduces standing waves. A broadband noise source is not as likely to generate noticeable standing waves as tonal sources.

Care should be taken to ensure that subjects are at least 1/4 wavelength from any reflective surface. Cages should be on short legs to boost them up from flooring material. Also, cages should be made of wires or tubing that is less than 1/4 wavelength in diameter. Trays to catch feces and urine should be avoided. We use plastic-backed absorbent paper to catch droppings. Monitoring microphones should be about the same height off the chamber floor as the mouse ears.

To reduce stress, apple or potato slices, or water bottles can be placed in the cage as sources of water. Although this reduces the homogeneity of the sound field, it may be required to maintain animal health.

Depending on the quality and quantity of the power available, an uninterruptable power source may be recommended. This will allow the exposure to continue even if the power should fail.

Pilot Studies

Although hours can be devoted to planning an exposure, the best way to discover errors in logic and hardware is to pilot test. We often pilot test exposure setups overnight without subjects. If a system will survive a 12- or 18-hour run, it will survive a shorter 2-, 4-, or 8-hour run the next day. The most likely element to fail is a speaker; thus, backups should be available.

A short pilot run with subjects, if possible, will indicate whether the exposures are producing the amount of TTS and PTS desired. Every effort should be taken to ensure that these pilot subjects are similar to one's study animals. It appears that every new strain requires some effort to generate acceptable levels of PTS. We usually start out with around a 110-dB 1-hour exposure and adjust from there. Usually, it is better to double the exposure (i.e., increase levels by +3 dB or double time) or quadruple the exposure (i.e., +6 dB or quadruple the time) as necessary.

Also, care should be taken not saturate the mouse's ear. For example, one is exposing two strains of mice to 125 dB for an hour; and upon completion, the TTS and PTS are similar in both groups. An additional group or two at some lower exposure intensities may reveal some interesting differences between the two strains. There is a level below which neither strain will show a PTS, and there is an upper level at which both strains will show maximum PTS. To demonstrate no differences between the strains, one must look at exposure levels between those extremes.

Handbook of Mouse Auditory Research

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