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Factors Affecting Hearing in Mice, Rats, and Other Laboratory Animals

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The auditory system of rodents and other animals is affected by numerous genetic and environmental variables. These include genes that cause hearing loss, exposure to noise that induces hearing loss, ameliorative effects of an augmented acoustic environment on hearing loss, and effects of background noise on arousal. An understanding of genetic and environmental influences on hearing and auditory behavior is important for those who provide, use, and care for laboratory animals.

Abbreviations: AAE, augmented acoustic environment; ABR, auditory brainstem response; ASR, acoustic startle response; AVCN, anteroventral cochlear nucleus; B6, C57BL/6J; D2, DBA/2J (inbred strain of mouse); PPI, prepulse inhibition; SPL, sound pressure level (relative to a 20-μPa standard)

Many types of research are affected by the neural processing and ultimate perception of auditory stimuli via the auditory system. The auditory system, in turn, is affected by numerous genetic and environmental variables. For example, people and animals can exhibit congenital or genetic progressive hearing loss, whereas the acoustic environment can affect the developing or adult auditory system. This review addresses the effect of genetic and acoustic factors on hearing in research animals (especially rodents, which represent the vast majority of research mammals) and particularly focuses on mice and the experience of the author and his colleagues. Indeed, more is known about genetic and other factors that affect hearing in mice than is known for any other nonhuman species.[38]

Assessing Auditory Function

The auditory brainstem-evoked response (ABR) has become the most widely used method of assessing hearing sensitivity in rats, mice, and most other laboratory animals.[6,7,14,18–20,23,26,27,42,46,47,49] The ABR is an electrophysiologic response (recorded via scalp electrodes) evoked in the inner ear and auditory brainstem by a series of rapidly repeated tone bursts or clicks. Stepwise reduction of the intensity of the acoustic stimuli enables identification of a threshold for detection of ABRs, which corresponds well to the behavioral threshold for hearing.[26] Threshold measurement by use of ABRs or other tests suggests that mice have good sensitivity to tones from about 2 to at least 80 kHz, with the actual range varying among strains.[7,8,12,15,23,49] Abnormally high ABR thresholds are indicative of hearing loss due to genetic causes, noise trauma, age-related cochlear degeneration, or other factors.

Behavioral tests such as the acoustic startle response (ASR) and prepulse inhibition (PPI) are used to quickly assess suprathreshold responses of rats and mice to sounds.[2,10,17,25,40,44,48] Behavioral techniques using learning paradigms provide accurate measures of hearing[8,12] but have not been used widely for screening because of the time required to obtain data. The ASR, a jerk-like motor reflex, is reliably elicited by intense bursts of noise or tones and is easily measured in rodents by use of movement-sensitive devices.[2,44] The ASR can be used as a measure of behavioral responsiveness to intense or unexpected sounds, but it is not a reliable indicator of threshold sensitivity.

PPI is a behavioral paradigm that has been widely used and well-studied for several decades using rodent and human subjects.[17,44] PPI occurs when a so-called prepulse stimulus, such as a moderately intense tone pip, is presented about 100 msec prior to an intense, startle-evoking stimulus. Although insufficiently intense to evoke a startle response, the prepulse causes the startle amplitude evoked by the subsequent startle stimulus to be reduced (or 'inhibited'). The degree to which startle amplitude is reduced (that is, the magnitude of PPI) serves as a measure of the behavioral salience of the prepulse. PPI, therefore, provides a convenient tool to evaluate the behavioral and psychophysical properties of moderately intense auditory stimuli and is practical for screening of large numbers of animals. Some of the studies presented in this review use PPI and the ASR as methods.

Hearing Loss-induced Plasticity

Auditory responses can be affected by both cochlear (peripheral) integrity and the physiologic properties of central pathways. In addition, peripheral hearing loss (genetic or noise-induced) also causes changes in central auditory physiology—the phenomenon of hearing loss-induced plasticity.[36,43,45] Briefly stated, the loss of high-frequency sensitivity induces changes in the auditory brainstem and cortex whereby processing of still-audible sounds becomes altered. In some cases, responses to still-audible sounds become stronger than normal after high-frequency hearing loss. This situation is manifested as a stronger PPI when the prepulse is comprised of still-audible frequencies[33,35,40,43] and an increase in the number of central neurons responding to those sounds.[39,45,47]

An extreme example of noise-induced exaggeration of auditory response to sound is 'acoustic priming' for audiogenic seizures.[13] Adolescent mice of strains like C57BL/6J (B6) that are not susceptible to audiogenic seizures can be made susceptible by creating temporary noise-induced hearing loss during development (for example, in the third week of life): several days after priming (administering an intense sound lasting tens of seconds) causes a stereotypical convulsive syndrome. Young DBA/2J (D2) mice, which have genetic high-frequency hearing loss, are also susceptible to audiogenic seizures during adolescence (without priming), before their hearing loss

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becomes severe.[34] Although ambient sounds in an animal facility are not likely to induce audiogenic seizures or cause priming, researchers using mice should nonetheless be aware of the phenomenon.

Genetic Hearing Loss

Several of the most commonly used inbred strains of mice exhibit progressive cochlear sensorineural hearing loss—degeneration of cochlear tissue including hair cells, spiral ganglion cells, supporting cells, and stria vascularis.[4,7,18,19,23,32,36,41,42,49] The typical pattern is for damage to begin in the basal end of the cochlea, which is responsible for high-frequency hearing; its damage therefore results in elevation of thresholds for high-frequency sounds. For example, B6 and BALB/c mice have normal or near-normal hearing when young but develop considerable high-frequency hearing loss by middle age (6 to 12 mo). By about 6 mo of age, mice of both strains exhibit some loss of high-frequency sensitivity (>20 kHz), and by age 1 y, hearing loss becomes severe.[9,14–16,20,21,24,25,31,46] Some losses at very high frequencies can be evident as early as 2 mo.[29,30] In addition, gender-associated differences in hearing loss have been noted in B6 mice, with the severity of loss accelerating in females at approximately 6 mo of age.[14,39] Thus, female mice older than 6 mo may have even more severe hearing loss than male mice of the same age. By contrast, in CBA strains, which maintain relatively good hearing as they age, old female mice may retain better hearing than males.[11,14]

Some inbred strains of mice exhibit very early hearing loss. For example, D2 mice show adolescent-onset hearing loss, as originally demonstrated 40 y ago by Ralls.[28] Subsequent studies showed that D2 mice probably never hear high frequencies (>25 kHz) well and between 1 and 2 mo of age develop severe loss of sensitivity to high and low frequencies.[33,34,45,47]

A number of other inbred strains of mice exhibit hearing loss at one age or another, but they have not been used as extensively in hearing research as have B6, BALB/c, and D2. A list of these strains can be found at http://www.jax.org/hmr/index.html ("Hearing Impairment in Mice"). Common strains of laboratory rats like Sprague-Dawley and Fischer 344 differ with respect to age-related hearing loss but do not show severe hearing loss as young adults.[8,27,32,37]

An appreciation of the hearing capacities of animal models is of obvious importance in many contexts, yet many researchers may be unaware of these influences, often to the detriment of their research. Indeed, in a few studies published in leading journals, the authors have concluded that a learning deficit occurred if an auditory stimulus was ineffective. The scientists and journal referees did not recognize that the mice were severely hearing impaired, and therefore the interpretation of the experiment was flawed.

Acoustic Environment

The acoustic environment within the animal facility provides the most likely potential influences on the auditory system, although diet, lighting, stress, pathogens, and other factors can also have an effect. Three ways in which sound can affect hearing and auditory behavior are via noise-induced hearing loss, amelioration of hearing loss by exposure to an augmented acoustic environment (AAE), and modulation of behavior by ongoing, ambient sound.

Noise-induced hearing loss. Experiments designed to induce hearing loss in mice or rats typically use noise levels well in excess of 100 dB sound pressure level (SPL), often for hours at a time.[3,5,41] Such levels would never be routine in a vivarium.[22] More unpredictable and potentially problematic are less intense noise levels maintained constantly over months, such as those from air conditioning, building construction, background music, and so forth. Routine ambient noise in the typical animal facility seems unlikely to cause noteworthy hearing loss, but the potential for excess noise should always be monitored. Obviously events such as fire alarm tests, which can result in a brief period of very intense noise, should be avoided. In addition, B6, BALB/c, and some other inbred strains of mice are genetically susceptible to noise-induced hearing loss.[3,41] The gene(s) causing AHL may be responsible for this vulnerability.

Effects of exposure to an AAE. A series of experiments augmented the acoustic environment of mice by exposing them at night to 12 h of repetitive bursts of noise at a moderate intensity (70 dB SPL) from speakers mounted above their cages. Such moderate noise levels do not cause hearing loss or any apparent negative health effects on mice, but actually lessen or slow age-related hearing loss in B6, BALB/c, D2, and other strain.[33,39,40,43,45,47] For example, when B6 or D2 mice were exposed nightly to an AAE, progressive hearing loss was reduced by 15 to 20 dB when they reached an age at which hearing loss would be severe at high frequencies.[39,45] The amelioration of hearing loss is a function of retention of cochlear hair cells, spiral ganglion cells, and neurons in the anteroventral cochlear nucleus (AVCN) and perhaps other central auditory structures.

The anatomical effects of AAE exposure are related to the frequency spectrum of the noise exposure with respect to the tonotopic organization of the auditory system. Tonotopic organization refers to the orderly topographic representation of sound frequency within the cochlea and many central auditory structures. For example, high-frequency sounds are processed in the basal portion of the mammalian cochlea and in the dorsal region of the AVCN.[39] In a recent study, D2 mice were exposed nightly to repetitive bursts of a high-frequency noise band of 70 dB SPL (high-frequency AAE).[47] At 55 d of age, when D2 mice exhibit severe hearing loss, AAE-treated mice exhibited less elevation of ABR thresholds for tone frequencies encompassed by the noise band (16 and 24 kHz) and had fewer missing outer hair cells in the corresponding tonotopic region of the cochlea. The AVCN of treated mice had larger neurons, more surviving neurons, and thicker neuropil than did those of untreated control mice—but only in the dorsal region, where the AAE spectrum is tonotopically represented.

An indicator of how AAE treatment may affect the AVCN is cytochrome oxidase, an enzyme involved in cellular activity and a marker of metabolic activity. In my laboratory, tissue sections from the AVCN of D2 mice that had been exposed to a high-frequency AAE for 2 mo were stained for cytochrome oxidase. Figure 1 presents representative sections from 4 AVCNs; magenta indicates positive staining, whereas tissue without staining is blue. The 2 control mice (Figure 1 A, B) exhibit little staining in the dorsal regions, compared with the 2 AAE-treated mice (Figure 1 C, D). This observation suggests that the high-frequency region of treated mice has greater metabolic activity than do the control mice. Because the mice were euthanized at least 2 h after the previous night's AAE treatment, cytochrome oxidase activity presumably represents baseline metabolism under conditions of normal ambient sound. These and previous findings[39,47] demonstrate the frequency specificity of AAE treatment effects, which probably results from increased afferent activity arising from AAE-evoked activation.

The possible AAE effects of ambient vivarium noise levels have not been determined systematically. However, these levels are likely to differ among facilities, raising the possibility of different degrees of hearing loss for the same inbred strains reared

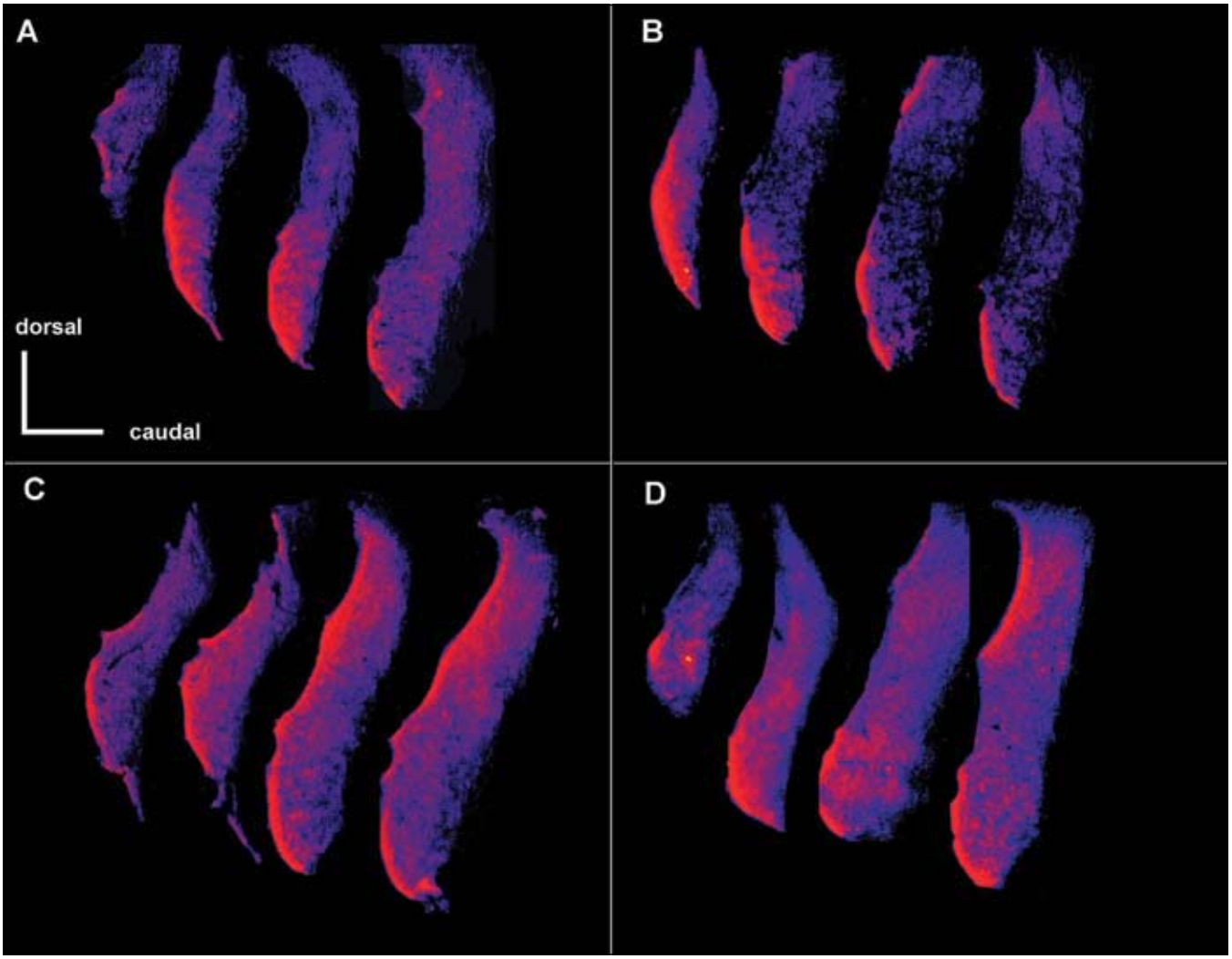

Figure 1. Cytochrome oxidase staining of AVCN sections. Magenta indicates positive staining; whereas tissue lacking staining is blue. The 2 control mice (A, B) exhibit little staining in the dorsal regions, compared with the 2 mice that exposed to an augmented acoustical environment (C, D). The mice were exposed for 12 h nightly to 70 dB SPL high-frequency noise bursts (the AAE). The left-hemisphere sections of 4 brains (2 cases from the AAE exposed group and 2 cases from the control group) were processed for cytochrome oxidase staining to visualize metabolic products. Tissue was stained for cytochrome oxidase. Sections were photographed at a magnification of ×40; Photoshop (Adobe, San Jose, CA) was used to convert the photographs to grayscale and then to pseudocolors that revealed the intensity of cytochrome oxidase staining.

and housed in different vivaria or even in different sections of the same facility.

Modulatory effects of ambient noise. Ongoing background noise modulates ASR amplitude in mice and rats.[1,2,17] In general, moderate levels tend to increase ASR amplitudes, whereas more intense levels of background noise tend to suppress ASRs. The increase in startle by moderate levels of background noise is thought to be due to arousal. These findings raise the possibility that over extended periods of time, ambient noise levels might increase arousal of research animals, perhaps inducing stress and its consequences. In contrast, suppression of ASRs by more intense background noise (for example, >70 dB SPL) may be the result of masking, which also could have consequences on behavior. For example, high-frequency vocalizations used by mice or rats for parenting and aggressive interactions might be interfered with, affecting behavior or reproduction in unknown ways. In my opinion, use of masking noises should be avoided in behavioral experiments.

Conclusions

Researchers doing studies that require normal auditory function (for example, some behavioral testing paradigms) should have a full understanding of the auditory capabilities of the animals and the potential effects of the acoustic environment. However, the many possible effects of background noise are difficult (if not impossible) to predict. Perhaps the best approach is to carefully measure and report the acoustic conditions in the vivarium and view this factor as a potential source of variance to be controlled.

Measurements of sound pressure levels in the animal facility should be made and provided to researchers or customers. These measurements should include sounds associated with ventilated caging and laminar flow hoods (blower fans). Measurements should be made using high-quality sound-measuring equipment capable of measuring high-frequency sounds (preferably as high as 80 to 100 kHz). In addition, measurements should be made within octave bands to characterize potential influences of high, middle, or low frequencies. Sound-measuring devices often have built-in octave-band filters; alternatively, an exterior adjustable band-pass filter can be used. Such data would help to identify potential acoustic problems within a facility and provide users with an empirical description of the acoustic environment in which the animals were raised and housed.

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