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Age-related auditory pathology in the CBA/J mouse

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Abstract

Commercially obtained aged male CBA/J mice presented a complex pattern of hearing loss and morphological changes. A significant threshold shift in auditory brainstem responses (ABR) occurred at 3 months of age at 4 kHz without apparent loss of hair cells, rising slowly at later ages accompanied by loss of apical hair cells. A delayed high-frequency deficit started at 24 kHz around the age of 12 months. At 20 to 26 months, threshold shifts at 12 and 24 kHz and the accompanying hair cell loss at the base of the cochlea were highly variable with some animals appearing almost normal and others showing large deficits. Spiral ganglion cells degenerated by 18 months in all regions of the cochlea, with cell density reduced by approximately 25%. There was no degeneration of the stria vascularis and the endocochlear potential remained stable from 3 to 25 months of age regardless of whether the animals had normal or highly elevated ABR thresholds. The slow high frequency hearing loss combined with a modest reduction of ganglion cell density and an unchanged endocochlear potential suggest sensorineural presbycusis. The superimposed early hearing loss at low frequencies, which is not seen in animals bred in-house, may complicate the use of these animals as a presbycusis model.

Keywords

Age-related hearing loss; presbycusis; hair cells; stria vascularis; endocochlear potential; spiral ganglion neurons

1. Introduction

Presbycusis, or age-related hearing loss, is multifactorial, combining genetic predispositions and the aging process with a plethora of lifetime insults to the auditory organ. These insults can include diseases that impact the inner ear (e. g. diabetes, vascular disorders), life style (e. g. smoking), exposure to excessive noise, ototoxic drugs. Such confounding factors lead to an exceedingly complex pathology of presbycusis and raise the question of an appropriate model to study age-related hearing loss. Schuknecht (Schuknecht, 1964) made one of the first attempts to classify the etiology of presbycusis, based on the changes in the temporal bones of aged humans and cats, dividing it roughly into four distinct types: (1) sensory, with hair cell loss and subsequent neural degeneration; (2) neural, with primary degeneration of cochlear neurons; (3) metabolic, with atrophy of the stria vascularis and a flat pure-tone audiogram; (4) mechanical, with changes in the basilar membrane affecting its properties and function. In

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sensory presbycusis, loss of outer hair cells begins in the basal region of the cochlea, leading to high-frequency hearing loss (Johnsson et al., 1967; Johnsson et al., 1972). Because of the intimate connection between loss of hair cells and their neurons, the term “sensorineural” presbycusis describes the high frequency hearing impairment resulting from loss of hair cells and degeneration of spiral ganglion cells without major changes in the function of the stria vascularis and other accessory structures of the inner ear.

The different patterns of age-related hearing loss are represented to some degree in various animal models. In the Fischer 344 rat, the underlying pathology has been linked to a combination of loss of hair cells and spiral ganglion cells (Keithley et al., 1982; Keithley et al., 1992). Mongolian gerbils are considered a model for strial presbycusis (Mills et al., 1990) associated with strial atrophy and a decreased endocochlear potential (Schmiedt, 1996; Gratton et al., 1996). Decreased cochlear blood flow (Prazma et al., 1990), degeneration of spiral ganglion cells (Keithley et al., 1989; Suryadevara et al., 2001), and fibrocyte vacuolization and interstitial edema in the spiral ligament are also observed in gerbils (Spicer et al., 2002).

Mice offer unique opportunities for studying the cellular and genetic bases of age-related hearing loss. Different strains show widely different patterns of age-related changes and in some of these strains distinct loci have been associated with accelerated hearing loss. CBA/J exhibit minimal hearing loss by 18 months of age but others such as C57BL/6, BALB/cJ and DBA/2J show significant deficits already between three and eight months of age (Li et al., 1991; Li et al., 1994; Spongr et al., 1997). Such strains may not reflect a normal biological aging process since the accelerated hearing loss correlates with the presence of pre-disposing genetic variants, including an *ahl* locus on chromosome 10, the *Cdh23^{ahl}* allele (Johnson et al., 1997; Johnson et al., 2000; Noben-Trauth et al., 2003). In contrast, the CBA/J mouse carries an *ahl*-resistant allele and is not prone to premature hearing loss. Furthermore, with a mean life span of 22 to 25 months, CBA/J mice stay relatively free of disease until at least 20 to 22 months (Smith et al., 1973; Bronson, 1990).

“CBA” mice have been tested for some aspects of age-related hearing loss in earlier studies (Li et al., 1991; Li et al., 1994; Spongr et al., 1997), but past results may not pertain to currently available mouse colonies. Some studies combine CBA/J with CBA/CaJ (Spongr et al., 1997) while others (e. g., Henry and Chole, 1980) date back several decades. CBA/J and the more widely used CBA/CaJ strains diverged over 70 years ago, sufficient time for them to acquire different aging and hearing characteristics. Supporting this notion, Ohlemiller and Gagnon (2007a) recently pointed out that CBA/CaJ mice lose hearing more rapidly than CBA/J, and show a reduction of their endocochlear potential.

The availability of aged mice through commercial breeders under the auspices of the National Institutes of Aging should greatly facilitate research into presbycusis and obviate the need for the maintenance of extensive breeding colonies. In this study, we investigate commercial CBA/J mice in detail in order to characterize the age-related pathology by measuring auditory thresholds, loss of hair cells and degeneration of spiral ganglion cells along the cochlear spiral. In addition, we assess the morphology of the spiral ligament and stria vascularis and monitor the endocochlear potential.

2. Materials and Methods

2.1 Animals

Male CBA/J mice were purchased from Harlan Sprague-Dawley Co. (Indianapolis, IN) through the National Institute on Aging a few weeks prior to the intended age for the experiments. The animals were kept at $22 \pm 1^\circ\text{C}$ under a 12h:12h light-dark cycle, and had free access to water

and a regular mouse diet (Purina 5025, St. Louis, MO). They were acclimated for at least one week before experimental procedures were performed. Animal care was under the supervision of the University of Michigan's Unit for Laboratory Animal Medicine and all experimental protocols were approved by the University of Michigan Committee on Use and Care of Animals.

2.2 Middle ear assessment

Visual inspection of the tympanic membrane for middle ear infections was performed through otoscopic examination at the time of each physiological measurement and again at the time of euthanasia. Animals with middle ear infections were removed from the study.

2.3 Auditory brainstem response (ABR)

Mice were anesthetized with an intra-peritoneal injection of xylazine (7 mg/kg), ketamine (65 mg/kg), and acepromizine (2 mg/kg). Animals were placed in a sound-isolated and electrically-shielded booth (Acoustic Systems, Austin, TX) and body temperatures were monitored and maintained near 37°C with a warm water heating pad. Acoustic stimuli were delivered monaurally to the left ear via a Beyer earphone mated to a customized plastic speculum inserted into the ear canal. Sub-dermal needle electrodes were inserted at the vertex of each animal skull, under the left ear and under the right ear (ground). ABRs were measured at 4, 12, 24 and 48 kHz. Tucker Davis Technology (TDT) System II (Tucker-Davis, Alachua, FL) and SigGen/Biosig software were used to present the stimuli (15 ms duration tone bursts with 1 ms rise-fall time) and record the response. During the study TDT System III hardware became available and was used for measurements at 48 kHz on a subgroup of animals. The sound driver was calibrated with a B&K 1/8 inch condenser microphone (cartridge type 4138) in a volume approximating that of a mouse ear canal, and a Stanford Research Systems lock-in amplifier (model SR530). Up to 1024 responses were averaged for each stimulus level. Thresholds were determined for each frequency by reducing the sound intensity in 10 dB increments and then in 5 dB steps near the threshold. Thresholds were estimated to lie between the lowest stimulus level where an ABR response in wave V was observed and the next lowest level without response.

2.4 Endocochlear potential

Mice were anesthetized with ketamine (65 mg/kg), xylazine (3.5 mg/kg), acepromazine (2 mg/kg), and received additional glycopyrrolate (0.2 mg/kg). Body temperature was maintained through the use of heating pads and heat lamps. The animals were placed into a head holder, the pinna of the left ear was removed and soft tissue was dissected away from the auditory bulla. The ossicles and tympanic membrane were disconnected and some of the bony wall of the bulla was removed to allow a clear view of the cochlea. A small opening was made in the otic capsule over the stria vascularis for penetration with a glass micropipette (filled with 150 mM KCl) into the scala media, inserted with a hydraulic microdrive (David Kopf Instruments, Tujunga, CA). The electrode signals were amplified by a capacity-compensated DC preamplifier and recorded using Tucker-Davis hardware and chart recorder software written in-house. Animals were euthanized at the end of the recording.

2.5 Surface preparations of the organ of Corti

The left temporal bones were removed immediately after euthanasia of the mice and were intrasclerally perfused with 4% paraformaldehyde in phosphate-buffered saline, pH 7.4 (PBS), and kept in this medium overnight at 4°C. Cochleae were then rinsed in PBS and decalcified in 5% sodium EDTA (adjusted with HCl to pH 7.4) for 24 hr at room temperature. Following decalcification the softened otic capsule, stria vascularis, Reissner's membrane, and tectorial membrane were removed. The remaining tissue (still including the modiolus) was

permeabilized with 0.3% Triton X-100 in PBS for 30 min and stained for actin with rhodamine phalloidin at a dilution of 1:100 for 60 min. After several rinses with PBS, the epithelium of the organ of Corti (base to apex) was lifted off the modiolus in three sections, corresponding to apical, middle and basal portions of the cochlea. The hook region was generally not recovered. The sections were mounted on a slide with anti-fade mounting media and observed with a Zeiss Axioplan microscope and imaged with a Zeiss laser confocal microscope (Zeiss LSM 510; Carl Zeiss Microimaging Inc., Thornwood, NY).

2.6 Hair cell counts

Hair cells of surface preparations of the organ of Corti were counted on a Leitz Orthoplan upright microscope equipped for epifluorescence, using a 50x oil immersion objective. The right objective had a 0.19 mm calibrated scale imposed on the field for reference and the rows of inner and outer hair cells were oriented longitudinally within each 0.19 mm frame. Beginning at the apex and moving down the cochlear spiral to the base, each successive 0.19 mm field was evaluated for the presence or absence of hair cells. Manual count data were entered into a computer program (KHRI Cytocochleogram, version 3.0.6) and compared to a data base of normal CBA/J mouse cochleae. The percentage of missing hair cells was calculated for each row and plotted as a function of distance (in mm) from the apical end of the surface preparation.

2.7 Spiral ganglion cell counts

Temporal bones were removed after vascular perfusion of anesthetized mice with a fixative containing 4% paraformaldehyde in phosphate buffer, pH 7.4. Cochleae were placed in 5% EDTA for decalcification for 24 hr at room temperature. Afterward, the cochleae were dehydrated and then embedded in JB-4 Plus, a glycol methacrylate plastic. Modiolar sections of 5- μ m thickness were cut, rehydrated, dipped in Paragon for one minute, dehydrated in graded strengths of ethanol, then dipped in xylene, and finally coverslipped with Permount mounting medium.

Every second plastic section was placed under the microscope and the most basal profile of the Rosenthal's canal was acquired at a low (2.5 to 10x) magnification and its outline was circled to determine its total area using the MetaMorph® imaging system (Molecular Devices, Sunnyvale, CA). Magnification was then increased to 16 to 25x and all spiral ganglion cells were counted. The spiral ganglion cell density was then calculated by dividing the number of spiral ganglion cells by the area measured. Sections from animals of different ages were matched for their location in the cochlea. A total of six sections for each animal were assessed by a blinded observer.

2.8 Morphometry of spiral ligament and stria

Plastic sections were prepared as described for ganglion cell counts. Images at 10 and 25x magnification were taken of three different regions of the cochlea from representative sections. The first region (apex) corresponded to the cross section of the cochlear spiral at 0 to 25% distance from the apex; the second region (middle) to a 40 to 50% distance; and the third region (basal) to a 70 to 80% distance from the apex. At least 4 sections from each cochlea were analyzed by an observer blinded to the age of the animals. The combined area of the spiral ligament and stria vascularis was determined from images at 10x magnification, the stria area alone from 25x images using the MetaMorph® imaging system. The area of the spiral ligament was calculated by subtracting the stria area from the combined area.

2.9 Statistical analysis

Data given in text and legends are mean $\pm$ s.d. Data were statistically evaluated by Student's *t*-test and by analyses of variance with Student-Newman-Keuls test for significance ($p < 0.05$) using Primer of Biostatistics software (McGraw-Hill Software, New York, NY).

3. Results

3.1 General condition of aging CBA/J mice

Body weight of the animals increased rapidly from 6 weeks (24.7 ± 2.3 g; $n = 30$) to 3 mo (30.5 ± 2.0 g; $n = 16$) and further to 8 mo (33.9 ± 3.4 g; $n = 17$). Then it remained relatively stable until 18 mo (36.8 ± 5.2 g; $n = 49$) and declined thereafter (30.0 ± 2.8 g at 23 to 25 mo; $n = 25$). Middle ear infections were no problem in younger mice. At 18 months and older about 10% of the animals had to be excluded from the study. Mice at all ages tolerated the anesthesia well.

3.2 Progressive frequency-dependent threshold shifts

Auditory thresholds were determined by stimulus-evoked auditory brain stem responses (ABR, fig. 1). A notable and consistent feature was an early elevation of thresholds at 4 kHz. This rise was already evident at an age of 3 months and slowly progressed to an almost complete loss of response to sound at later ages (fig. 1, 4 kHz).

In contrast to this pattern, thresholds at higher frequencies remained initially low and showed only a slow rise beginning at around 12 mo of age. They were significantly elevated at 12 months for 24 kHz and at 18 months for 12 kHz ($p < 0.05$). After 18 months, high-frequency thresholds from 12 kHz to 48 kHz suggested a bimodal distribution. Some animals maintained almost normal thresholds near or below 40 dB SPL while others had significantly elevated thresholds of 60 dB SPL and above. Even at 25 and 26 months of age some animals maintained near-normal thresholds; however, at 26 months six of ten animals showed no response at 100 dB, the maximum intensity delivered by the system at 24 kHz.

The unusually early 4-kHz rise in these commercial animals prompted us to compare thresholds to those of mice raised in-house. Parents were taken from the same strain and supplier and offspring was bred in our animal facilities. At 3 months of age, thresholds of in-house bred animals were significantly lower at 4 kHz (47 ± 8 dB) than in commercial animals (62 ± 8 dB). Thresholds at 6 kHz averaged 36 ± 6 dB. ABR thresholds at higher frequencies did not differ: in-house bred animals showed 26 ± 3 dB at 12 kHz, 25 ± 2 dB at 24 kHz, and 28 ± 5 dB at 48 kHz ($n = 6$) while commercial animals (see fig. 1) were recorded at 27 ± 6 dB at 12 kHz, 30 ± 7 dB at 24 kHz, and 31 ± 7 dB at 48 kHz ($n = 13$).

3.3 Hair cell loss

To determine whether the functional deficits corresponded to the extent and the localization of hair cell loss, cochleae were processed for surface preparations and quantitative hair cell counts following the ABR measurements. At the apex, surface preparations showed regular outlines of one row of inner hair cells and three rows of outer hair cells at 3 months. At 8 months, only few irregularities were apparent (fig. 2 Apex). Beginning at 12 months, however, loss of hair cells set in (fig. 3), progressing to a complete loss by the age of 20 months and later (fig. 2 Apex, fig. 3).

In the basal region of the cochlea hair cells were well preserved by 8 months and loss was minimal at 12 or even 18 months (fig. 2 Base, fig. 3). Thereafter, morphology became highly variable and ranged from scattered loss to almost complete obliteration. For example, an animal that had exhibited low thresholds at 26 months also showed well preserved hair cells (fig. 2

Base, 26 months upper panel). In contrast, an animal with no response to sound stimuli at high frequencies had essentially lost all hair cells (fig. 2 Base, 26 months lower right panel).

The visual impressions were confirmed in quantitative hair cell counts of the entire length of the cochlea (fig. 3). At the age of 3 months no loss of hair cells was recorded. Animals at 12 and 18 months of age began showing deficits of outer hair cells at the apical region of the cochlea, but maintained a full complement of cells in the base, consistent with low ABR thresholds at 12 and 24 kHz.

Up to 18 months these cytochleograms were highly similar between individuals of the same age. At later ages, hair cell loss in the base varied as already suggested by the samples presented in fig.2, but destruction in the apex was consistent. A mouse with a near-normal threshold of 29 dB SPL at 24 kHz had almost no hair cell loss in the basal region but complete loss in the apex (25 mo, No. 1). Massive hair cell loss in both apex and base was seen in an animal with a threshold of 74 dB SPL at 24 kHz (25 mo, No. 2). In animals at this age, the middle region at about 3 to 4 mm distance from the apex showed the best-preserved hair cells.

3.4 Degeneration of spiral ganglion cells

Type 1 spiral ganglion cells with granular cytoplasm and a large spherical nucleus (fig. 4-1, 3 mo, arrowhead) as well as Schwann cells (fig. 4-1, 3 mo; arrow = nucleus) with characteristic myelin sheaths were apparent in the sections. At 18 months of age the density of type 1 cells in Rosenthal's canal had significantly decreased but then remained constant to 23 months (fig. 4-1, 23 mo). Changes were apparent in all regions of the cochlea (fig. 4-2).

3.5 Morphology of the stria vascularis and spiral ligament

Lateral wall tissues underwent some morphological changes with aging (fig. 5). Sporadic hyperpigmentation occurred in the stria vascularis and vacuolization in both stria and spiral ligament (arrow) appeared in animals between the ages of 18 to 23 mo. There was, however, no obvious atrophy of stria vascularis and no apparent degeneration of marginal cells in aged animals. The stria area remained unchanged in the apical and middle regions of the cochlea and was somewhat increased in old animals in the basal turn, possibly due to the increased vacuolization (fig. 5-2). The area of the spiral ligament did not vary with age.

3.6 The endocochlear potential (EP) is stable with aging

The EP values determined from the basal turn of scala media ranged from about 70 mV to 110 mV (fig. 6), in good agreement with previous studies in this and other mouse strains (Sadanaga and Morimitsu, 1995; Wu and Marcus, 2003; Ohlemiller and Gagnon, 2007b). There was no decline with age and EP remained high even in animals with severe elevations in ABR threshold.

4. Discussion

Commercially available aged CBA/J mice are a potentially valuable and convenient resource for studies of age-related hearing loss. Two important questions arise in this context: which type of presbycusis is seen in these animals and how do they differ (if at all) from previously studied strains and from mice that were reared under controlled conditions in non-commercial breeding facilities? The sum of the physiological and morphological data suggests that these CBA/J mice represent a model for sensorineural presbycusis, but some caveats apply.

Progressive high-frequency threshold shifts and hair cell loss

ABR thresholds in 6-week old animals fall within the range seen in several strains of mice such as CBA/J, CBA/CaJ, C57, or BALB in other studies (Ohlemiller et al., 2000; Tadros et al.,

2007; Henry, 2004). A high-frequency hearing loss develops slowly and increased ABR thresholds at 24 kHz appear in some mice at 12 months of age while thresholds at 12 kHz remain largely normal until 22–24 months. The proportion of animals with high-frequency hearing loss increases with age, but some animals still maintain excellent thresholds even at 48 kHz until 26 months. Such different degrees of presbycusis were also observed by Tadros et al. (2007) in locally raised CBA/CaJ mice although the range of thresholds of our animals from near-normal to unresponsive (>100 dB) seems to exceed that of earlier studies.

We should note, however, that direct comparisons to earlier reports can only be made with caution. While our study uses male mice, others have mixed the sexes even though patterns of hearing loss differ between male and female mice (Henry, 2004); others have combined strains (CBA/Ca and CBA/J) in the same investigation; and different tone stimuli, ABR recording frequencies, and ABR stimuli further contribute to confound any quantitative comparison.

The high-frequency deficits seem well reflected in loss of hair cells in the base of the cochlea. Until about 18 months of age, appearance of hair cells is normal. At later ages there is considerable variation. The spotty losses in animals with good hearing and the complete destruction of hair cells in animals with high threshold shifts suggest hair cell loss as a cause of the observed presbycusis.

Low-frequency threshold shift and hair cell loss

While the foregoing pattern is consistent with a sensory presbycusis, the early threshold elevation at 4 kHz apparent at 3 months is not and is at odds with other reports on hearing in young animals. Although CBA/CaJ mice (Tadros et al., 2007) lose hearing more rapidly than CBA/J (Ohlemiller and Gagnon, 2007a), they maintain lower thresholds at 3 to 12 months of age. Even the C57BL/6 and BALB/c strains, which are frequently used as models for accelerated hearing loss, maintain lower thresholds at this frequency as young adults (5–7 months old: Ohlemiller et al., 2000; 10–12 months old: Willott et al., 1998) than the mice examined here. Since the animals in the quoted studies were derived from breeding colonies associated with the respective laboratories, the provenance of the mice was suspected to confound the data of the commercial animals. This notion is supported by data on young (3 months old) offspring from commercially obtained parents but bred and raised in our own animal facilities. These animals indeed had low-frequency thresholds comparable to those seen in the reports mentioned above. The discrepancy between commercially obtained and in-house bred animals only affected the lowest frequency tested while thresholds at 12 to 48 kHz were similar to other studies.

An oddity of this early 4 kHz-shift is the lack of accompanying hair cell loss. Although apical loss starts earlier than basal loss, there is no indication of any hair cell deficits in the apex at 3 months of age. A more subtle pathology, for example, involving stereocilia or synaptic contacts must be considered. However, since the conditions of housing and shipping of commercial animals are beyond the control of the investigators detailed examinations into this phenomenon may not be fruitful.

The subsequent pattern of apical hair cell loss, on the other hand, is in general agreement with the study by Spongr et al. (1997) who observed little hair cell loss until 18 months of age and then found destruction predominately in the apical outer hair cells and little loss towards the base. Our data differ quantitatively but Spongr's evaluation mixes the CBA/J and CBA/Ca strains from different commercial sources for hair cell counts. Nevertheless, the studies agree that the middle range of the cochlea remains least affected.

Degeneration of spiral ganglion cells

Spiral ganglion cells degenerate in presbycusis in many species including human, rat, gerbil and mouse, mostly in response to hair cell loss (Schuknecht, 1964; Keithley et al., 1989; Keithley et al., 1992; Saitoh et al., 1994). The decrease in cell density in CBA/J is apparent at 18 months, a time when outer hair cell loss also becomes evident and the loss is of similar magnitude (20 – 25%) as reported by Willott et al. (1991). However, there is only modest inner hair cell loss in the basal turn, hence it is possible that spiral ganglion cell loss is not subsequent to hair cell loss in this strain. Clearly, there is a “neural” contribution to presbycusis in these CBA/J mice.

Stable endocochlear potential

Important for a classification of the observed pathology is a consideration of the stria vascularis. Ruling out a strial origin of the age-related hearing loss is the fact that the endocochlear potential is stable up to the oldest ages. Furthermore, only a very mild pathology of stria and spiral ligament is observed, and the appearance of hyperpigmentation and some vacuolization remain without consequence for strial function. In this regard, the CBA/J mouse stands in contrast with a model like the gerbil where strial atrophy accompanies a decline in EP with age (Schmiedt, 1996; Gratton et al., 1996). Mouse strains, however, also differ in the stability of the EP. In BALB/cJ and CBA/CaJ mice, the EP progressively declines with age, while C57/BL and CBA/J mice largely maintain their EP (Ohlemiller et al., 2006). The EP in the CBA/J mouse in our study is also independent of age, commensurate with the largely normal appearance of stria vascularis. Importantly, the EP is maintained in animals that show high ABR threshold shifts.

Conclusion

The commercially available CBA/J strain demonstrates sensorineural presbycusis with high-frequency hearing loss accompanied by hair cell loss, some neuronal degeneration, and a stable EP. The superimposed early (probably artifactual) threshold shift at 4 kHz is a potentially confounding factor and complicates, for example, the analysis of the apex for comparison with basal turn events. Despite this limitation, these animals may be useful tools to examine sensorineural presbycusis in a mouse model.

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Non-standard abbreviations used

ABR, Auditory brain stem evoked response; EP, Endocochlear potential; IHC, Inner hair cell; OHC, Outer hair cell; PBS, phosphate-buffered saline.

References

- Bronson, RT. Rate of occurrence of lesions in 20 inbred and hybrid genotypes of rats and mice sacrificed at 6 month intervals during the first years of life.. In: Harrison Caldwell, DE., editor. Genetic Effects on Aging II. The Telford Press; NJ: 1990. p. 279-357.
- Gratton MA, Schmiedt RA, Schulte BA. Age-related decreases in endocochlear potential are associated with vascular abnormalities in the stria vascularis. *Hear. Res* 1996;102:181–190. [PubMed: 8951461]
- Johnson KR, Zheng QY, Erway LC. A major gene affecting age-related hearing loss is common to at least ten inbred strains of mice. *Genomics* 2000;70:171–180. [PubMed: 11112345]

- Johnson KR, Erway LC, Cook SA, Willott JF, Zheng QY. A major gene affecting age-related hearing loss in C57BL/6J mice. *Hear. Res* 1997;114:83–92. [PubMed: 9447922]
- Johnsson LG, Hawkins JE Jr. Otolithic membranes of the saccule and utricle in man. *Science* 1967;157:1454–1456. [PubMed: 5341324]
- Johnsson LG, Hawkins JE Jr. Sensory and neural degeneration with aging, as seen in microdissections of the human inner ear. *Ann. Oto. Rhinol. Laryn* 1972;81:179–193.
- Keithley EM, Feldman ML. Hair cell counts in an age-graded series of rat cochleas. *Hear. Res* 1982;8:249–262. [PubMed: 7153180]
- Keithley EM, Ryan AF, Woolf NK. Spiral ganglion cell density in young and old gerbils. *Hear. Res* 1989;38:125–133. [PubMed: 2708154]
- Keithley EM, Ryan AF, Feldman ML. Cochlear degeneration in aged rats of four strains. *Hear. Res* 1992;59:171–178. [PubMed: 1618708]
- Li HS, Borg E. Age-related loss of auditory sensitivity in two mouse genotypes. *Acta Oto-laryngol* 1991;111:827–8234.
- Li HS, Hultcrantz M. Age-related degeneration of the organ of Corti in two genotypes of mice. *ORL* 1994;56:61–67. [PubMed: 8177586]
- Mills JH, Schmiedt RA, Kulish LF. Age-related changes in auditory potentials of Mongolian gerbil. *Hear. Res* 1990;46:201–210. [PubMed: 2394633]
- Noben-Trauth K, Zheng QY, Johnson KR. Association of cadherin 23 with polygenic inheritance and genetic modification of sensorineural hearing loss. *Nat. Genet* 2003;35:21–23. [PubMed: 12910270]
- 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. *Hear Res* 2000;149:239–247. [PubMed: 11033262]
- Ohlemiller KK, Lett JM, Gagnon PM. Cellular correlates of age-related endocochlear potential reduction in a mouse model. *Hear. Res* 2006;220:10–26. [PubMed: 16901664]
- Ohlemiller KK, Gagnon PM. Divergence of hearing sensitivity and endocochlear potential (EP) in CBA/J and CBA/CaJ mice after 12 months of age. *Assoc. Res. Otolaryngol. Abstr* 2007a;454
- Ohlemiller KK, Gagnon PM. Genetic dependence of cochlear cells and structures injured by noise. *Hear Res* 2007b;224:34–50. [PubMed: 17175124]
- Prazma J, Carrasco VN, Butler B, Waters G, Anderson T, Pillsbury HC. Cochlear microcirculation in young and old gerbils. *Arch. Otolaryngol* 1990;116:932–936.
- Sadanaga M, Morimitsu T. Development of endocochlear potential and its negative component in mouse cochlea. *Hear Res* 1995;89:155–161. [PubMed: 8600121]
- Saitoh Y, Hosokawa M, Shimada A, Watanabe Y, Yasuda N, Takeda T, Murakami Y. Age-related hearing impairment in senescence-accelerated mouse (SAM). *Hear. Res* 1994;75:27–37. [PubMed: 8071152]
- Schmiedt RA. Effects of aging on potassium homeostasis and the endocochlear potential in the gerbil cochlea. *Hear. Res* 1996;102:125–132. [PubMed: 8951457]
- Schuknecht HF. Further Observations on the Pathology of Presbycusis. *Arch Otolaryngol* 1964;80:369–382. [PubMed: 14198699]
- Smith GS, Walford RL, Mickey MR. Lifespan and incidence of cancer and other diseases in selected long-lived inbred mice and their F 1 hybrids. *J. Natl. Cancer I* 1973;50:1195–1213.
- Spicer SS, Schulte BA. Spiral ligament pathology in quiet-aged gerbils. *Hear. Res* 2002;172:172–185. [PubMed: 12361880]
- Spongr VP, Flood DG, Frisina RD, Salvi RJ. Quantitative measures of hair cell loss in CBA and C57BL/6 mice throughout their life spans. *J. Acoust. Soc. Am* 1997;101:3546–3553. [PubMed: 9193043]
- Suryadevara AC, Schulte BA, Schmiedt RA, Slepecky NB. Auditory nerve fibers in young and quiet-aged gerbils: morphometric correlations with endocochlear potential. *Hear. Res* 2001;161:45–53. [PubMed: 11744280]
- Tadros SF, D'Souza M, Zettel ML, Zhu X, Lynch-Erhardt M, Frisina RD. Serotonin 2B receptor: upregulated with age and hearing loss in mouse auditory system. *Neurobiol Aging* 2007;28:1112–1123. [PubMed: 16822592]

- Willott JF, Parham K, Hunter KP. Comparison of the auditory sensitivity of neurons in the cochlear nucleus and inferior colliculus of young and aging C57BL/6J and CBA/J mice. *Hear. Res* 1991;53:78–94. [PubMed: 2066290]
- Willott JF, Turner JG, Carlson S, Ding D, Seegers Bross L, Falls WA. The BALB/c mouse as an animal model for progressive sensorineural hearing loss. *Hear Res* 1998;115:162–174. [PubMed: 9472745]
- Wu T, Marcus DC. Age-related changes in cochlear endolymphatic potassium and potential in CD-1 and CBA/CaJ mice. *J Assoc Res Otolaryngol* 2003;4:353–362. [PubMed: 14690053]

Figure 1. Auditory thresholds increase with aging in commercial CBA/J mice

Early elevations in ABR thresholds at 4 kHz were followed by gradually increasing thresholds at higher frequencies, reaching significance ($p < 0.05$) at 12 mo (24 kHz) and 18 mo (12 kHz). Each circle represents an individual animal. $n = 22$ at 1.5 mo; $n = 16$ at 3 mo; $n = 10$ at 8 mo; $n = 13$ at 12 mo; $n = 17$ at 18 mo; $n = 7$ at 20 mo; $n = 8$ at 23 mo; $n = 8$ at 25 mo; and $n = 10$ at 26 mo.

The wide range of high-frequency responses from near-normal to severely impaired was confirmed for 23-mo old mice at 48 kHz when updated equipment was acquired; $n = 16$.

Figure 2. Hair cell loss

Surface preparations were stained with rhodamine phalloidin for actin. Four mice in each age group were evaluated and representative samples are shown.

Apex: A normal pattern of hair cells is evident at 3 mo, minor changes appear at 8 mo, and later ages show complete destruction of hair cells.

Base: A normal pattern persists until 18 mo, after which time hair cell loss becomes highly variable, as evident in two samples from 26-mo old mice.

Figure 3. Quantification of hair cell loss by cytochrome C

For each age, hair cell loss in 5 to 6 cytochrome C was quantified and representative samples are shown. Early hair cell loss was evident at 12 mo in the very apex of the cochlea. Loss of basal hair cells developed later with scattered loss at 18 mo and more severe loss at 25 mo. The inserted table lists corresponding ABR thresholds.

Figure 4. Degeneration of spiral ganglion cells

4-1: Type 1 cells of young animals exhibited granular cytoplasm and large spherical nuclei (3 mo, arrowhead), and well defined Schwann cell nuclei (3 mo, arrow). At 23 mo of age, the density of spiral ganglion cells decreased. Samples shown are from the basal region of the cochlea.

4-2: Quantification of cell density showed significant changes in all three regions of the cochlea in animals at 18–23 mo ($p < 0.05$). Bars indicate means $\pm$ s.d. at 3 mo ($n = 6$ animals) and 18 – 23 mo ($n = 6$). Six sections were counted and averaged to obtain a value from one animal.

5-1

5-2

Figure 5. Morphology of stria vascularis and spiral ligament

5-1: Specimens from the basal region show hyperpigmentation and vacuolization in the stria vascularis but morphometry did not indicate any atrophy. In the spiral ligament, vacuolization of fibrocytes was observed (arrow).

5-2: Quantification of area only showed an age-dependent change in the basal stria at 23 mo ($p < 0.05$). Bars indicate means $\pm$ s.d. at 3 mo ($n = 6$ animals) and 18 – 23 mo ($n = 6$). Six sections were counted and averaged to obtain a value from one animal.

Figure 6. Endocochlear potential does not decline with aging

The EP was measured 3 mo ($n = 4$), 18–20 mo ($n = 5$) and 25 mo ($n = 5$) and plotted against threshold shift at 24 kHz. EP was within normal range for both aged and young CBA/J mice and remained unaffected by the magnitude of hearing loss at 24 kHz. Each point indicates an individual animal.