Marginal cell density in young and old CBA/J and CBA/CAJ mice

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MARGINAL CELL DENSITY IN YOUNG AND OLD CBA/J AND CBA/CAJ MICE

by

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Abstract: The goal of the present study was to compare aging characteristics of
the cochlear lateral wall in inbred mouse strains (CBA/J and CBA/CaJ) having
very different endocochlear potential (EP)-versus-age profiles to see which
anatomic differences might predict their EP differences.
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TABLE OF CONTENTS

ACKNOWLEDGEMENTS ................................................................. ii

LIST OF FIGURES ................................................................. 2

INTRODUCTION ................................................................. 3

  Generation of the EP ............................................................ 4
  Animal Models for study of strial presbycusis: Mongolian Gerbil ........ 5
  Animal Models for study of strial presbycusis: Inbred Mice .......... 6
  The Tyrp1^{Bh-it} allele ............................................................ 6
  BALB/cJ and C57BL/6J mice .................................................. 6
  C57BL/6-Tyr^{c2J} (B6 albino) mice ......................................... 7
  NOD.NON-H2^{Ab1}/LtJ (NOD.NON) mice ............................. 7
  CBA/J and CBA/CaJ mice .................................................... 7

METHODS .................................................................................. 9

RESULTS .................................................................................. 10

  General appearance of affected stria ....................................... 10

DISCUSSION .............................................................................. 16

  Comparison to other models ............................................... 17
  Genetic Factors ...................................................................... 19
  Clinical Implications ............................................................ 19

REFERENCES ............................................................................ 20
LIST OF FIGURES

FIGURE 1: Age in Months versus Basal Turn EP ................................................................. 8
FIGURE 2: Strial Profile from the Lower Base ................................................................. 10
FIGURE 3a: Strial Thickness by Location ................................................................. 11
FIGURE 3b: Strial Thickness by EP in the Upper Base ......................................... 11
FIGURE 4a: Marginal Cell Density by Location .......................................................... 12
FIGURE 4b: Marginal Cell Density by EP in the Upper Base ..................................... 13
FIGURE 5a: Ligament Thickness by Location ............................................................ 14
FIGURE 5b: Ligament Thickness by EP in the Upper Base ....................................... 14
FIGURE 6a: Capillary Density by Location ............................................................... 15
FIGURE 6b: Capillary Density by EP in the Upper Base ........................................... 16
FIGURE 7: Basal Turn EP versus Marginal Cell Density .......................................... 18
Introduction

Presbycusis or age-related hearing loss (ARHL) is defined as “the natural failure of hearing with advanced years, caused by degenerative changes in the internal ear” (Jennings and Jones, 2001). Age-related hearing loss is the most common form of hearing loss and one of the leading chronic health problems experienced by the elderly. More than 28 million people in the United States of America have a hearing loss. That number is expected to rise with the increasing number of elderly people. One figure projects that there will be close to 60 million Americans aged 65 and older by the year 2025 with this condition (Liu and Yan, 2007).

Schuknecht and Gacek (1993) studied 21 human temporal bones that met the criteria for presbycusis. Theirs and similar work advanced the concept of four independently arising pathologic types of presbycusis which include: sensory, neural, strial and cochlear conductive. Each type is characterized by a specific pattern of functional disturbance with hearing losses that are generally symmetrical and slowly progressive (Schuknecht et al., 1974; Schuknecht and Gacek, 1993). The present study focuses on strial presbycusis, which is associated with degeneration of the stria vascularis. This type, the essential feature of which is endocochlear potential (EP) decline, may appear by the third decade of life. According to Schuknecht, marginal cell anomalies seem to be one of the first signs of strial degeneration as they could be found in regions where strial capillaries, intermediate cells, and basal cells appeared normal (Schuknecht et al., 1974). Schuknecht suggested that strial presbycusis is clinically distinguishable from other types by a flat or slightly descending pure tone threshold audiometric pattern which is generally coupled with normal word discrimination scores. Gates et al. (Gates et al., 1999) studied genetic associations in age-related hearing thresholds and found a clear familial trend in hearing levels for sensory and strial presbycusis phenotypes. The heritability
estimate was greater for the strial phenotypes than the sensory and stronger in women than in men. These results support a genetic effect on inheritance.

*Generation of the EP*

The endocochlear potential is generated by the stria vascularis and provides the main driving force for sensory transduction (Wangemann, 2002). The stria vascularis is a three layered epithelium with tight junctions in the cells forming barriers along both margins (Jahnke, 1975). The endocochlear potential is a $K^+$ equilibrium potential generated by the $K^+$ channel $Kcnj10$ which is located in the intermediate cells of the stria vascularis in conjunction with a low $K^+$ concentration of intrastral fluid and a normally high $K^+$ concentration in the cytosol of intermediate cells. Strial marginal cells contribute to the endocochlear potential by supplying a low $K^+$ concentration in the intrastral fluid (Takeuchi et al., 2000).

All major cochlear cell types decrease in number with age, including strial cells (Ohlemiller and Frisina, 2008). However, not all individuals appear to age at the same rate. Ohlemiller and Frisina propose two general classes of cochlear pathology. The first is termed ‘biological-age synchronous’ (BAS) in which the aging of the cochlea should reflect the overall biological age of an individual. If a hearing loss does exist with this type, it would probably not stand out among the other limitations. Conversely, the ‘biological-age accelerated’ (BAA) class would suggest that an individual’s cochlea has aged beyond the expected point of degeneration for a person’s chronological age and may affect quality of life. Among other forms, strial presbycusis, which is suggested to manifest early in life (Schuknecht, 1974) may particularly adhere to this form.
Animal Models for study of strial presbycusis: Mongolian Gerbils

Although strial degeneration has been shown to correspond with hearing loss in humans, EP has never been measured in humans. Animal models can promote correlation of specific patterns of strial pathology with EP reduction. The earliest animal model shown to undergo age-related EP decline was the Mongolian gerbil. Schulte and Schmiedt (1992) found the Mongolian gerbil to exhibit age-related strial degeneration, wherein about half of tested animals showed a reduction in EP by 39 months followed by strial degenerations including marginal cell loss (Schulte and Schmiedt, 1992).

Gratton and Schulte (1995) described cochleae from 28 gerbils ranging in age from 3 to 36 months. A few younger animals (5-9 months old), showed small regions containing few or no capillaries at the extreme basal end of the stria, while some gerbils aged 33 months or older showed portions of normal strial vasculature. The remainder of the stria in old animals, however, contained degenerated regions where capillary loss and some atrophy of strial marginal cells were observed. The authors noted that regions containing capillary degeneration were not necessarily accompanied by atrophic changes in marginal cells (Gratton and Schulte, 1995).

Re-evaluation of material from Mongolian gerbils revealed results that favored Schuknecht’s suggestion that the initial changes were manifested in strial marginal cells, and the degeneration of the capillaries were a secondary occurrence. Spicer and Schulte (2005) examined strial atrophy in 30-36 months old gerbils by electron microscopy suggested that pathologic changes begin with loss of marginal cell secondary processes, followed by ablation of the primary processes (Spicer and Schulte, 2005).
Animal Models for study of strial presbycusis: Inbred Mice

Mouse models might be expected to hold advantages for study of strial presbycusis, since a variety of inbred strains are available.

*The* **Tyrp1*<sup>B-lt</sup>* allele  Mice known to have at least one copy of the *Tyrp1*<sup>B-lt</sup> allele (mouse chromosome 4) also show a normal EP early in life, followed by an EP reduction that is highly variable after 2-3 months of age (Cable et al., 1993). This is a very important finding as it identifies a specific gene defect that would result in EP reduction in the mouse model.

**BALB/cJ and C57BL/6J mice**  BALB/cJ (BALB) mice and C57BL/6J (B6) mice share multiple genes that promote progressive sensory cell and hearing loss (Ohlemiller, Lett & Gagnon, 2006). If strial pathology and EP decline are a broad effect of many hearing loss genes, one might expect to find age-related EP decline in both of these lines. The results revealed quite a different trend. Early in life, the BALB mice start with a lower EP as compared to the B6 mice. Additionally, the average EPs of some BALB mice show a further decline by 19 months of age. The two features of the cochlear lateral wall that best accounted these differences were marginal cell density and ligament thickness. These features were the only good predictors of both EP stability in B6 mice and EP decline in BALB mice. Another notable conclusion from this work is that microvascular changes including basement membrane thickening and capillary loss do not necessarily promote EP decline. These BALB results notably echo the findings of human and gerbil results that propose marginal cells as the initial degenerative structure.

BALB/cJ mice are albino, and thus produce no strial melanin. Previous studies (Riley, 1997; Schraermeyer and Heimann, 1999; Trachimowicz et al., 1981) show melanin to be a protective element against injury and some effects of aging in the skin and eye. It can then be
theorized that melanin may also provide the same protection to the cochlea against aging (Ohlemiller, 2009).

**C57BL/6-Tyr<sup>c<sup>-2J (B6 albino) mice** To explore the role of melanin in the aging stria, Ohlemiller and colleagues (Ohlemiller et al., 2009) compared the C57BL/6 J and C57BL/6-Tyr<sup>c<sup>-2J (B6 albino) mouse lines. B6 and B6 albino hearing loss and distribution of EP seems to progress identically up until 2 years of age. After this point, EPs in the old B6 albino mice are significantly depressed, unlike the B6 mice that showed EPs that remained stable. Only some of the B6 albino mice experienced this decline in EP. Structures that are most affected in the albino mice are the thickness of the stria and marginal cells. The albino line showed greater strial thinning than pigmented mice, which also correlates with fewer marginal cells. This suggests that a major function of melanin may be to promote marginal cell survival since marginal cell degeneration was seen in lines not containing melanin (BALB and B6 albinos).

**NOD.NON-H2<sup>nb1</sup>/LtJ mice** NOD.NON-H2<sup>nb1</sup>/LtJ (NOD.NON) mice show a rapidly progressive hearing loss early in life, one aspect of which is EP reduction after 6 months of age (Ohlemiller et al., 2008). EP decline was correlated with strial thickness in the lower base and apex of the cochlea. The NOD.NON mice show dramatic strial capillary degeneration as the first sign of abnormality. It may be that the form of strial presbycusis observed in the NOD.NON mice is not similar to what is seen with aging but could point to a form having a microvascular origin. It should be noted, again, that there is variability in the extent of EP reduction indicating and interaction between genetic and other factors.

**CBA/J and CBA/CaJ mice** Because of the similarities of their names and common origins, these two strains are often used interchangeably and assumed to be equivalent. Although these genetically similar strains are both considered to be ‘good hearing’ strains, one (CBA/CaJ)
shows age-related EP decline. Even though both strains show reasonably good hearing up to 26 months, after one year of age, the CBA/CaJ mice experience a more rapid decline in hearing.

Since hair cells and neurons are maintained in the CBA/CaJ mice as they age until the rapid decline, it has been argued that this line represents the most ‘pure’ model of strial presbycusis among mice (Ohlemiller, 2009). However, the anatomic correlates of age-associated EP decline in CBA/CaJ mice have not been determined. It is of considerable interest whether CBA/CaJ mice adhere to patterns like those demonstrated for BALB and B6 albino mice, or whether they will reveal new cellular correlates of EP reduction. Since the majority of models show marginal cells to be the initially affected cell type, my investigation also focused on marginal cells.
Methods

All procedures were approved by the Washington University Institutional Animal Care and Use Committee. The sample was comprised of ‘young’ (<4 months) and ‘old’ (18-27 months) CBA/J and CBA/CaJ mice. Sample sizes by age and strain for the main analyses were 8 young CBA/J, 12 old CBA/J, 7 young CBA/CaJ, and 13 old CBA/CaJ. These mice included both genders, and ranged from 1.8 to 27 months in age. All mice were derived from breeding pairs purchased from The Jackson Laboratory. All cochleae were previously histologically processed and sectioned in the mid-modiolar plane. The following areas of the cochlea were examined: lower base, upper base, and the lower apex. Metrics include strial thickness, spiral ligament thickness, capillary density, and marginal cell density. Strial thickness was measured orthogonal to the midpoint. Spiral ligament thickness was measured on an axis co-linear with the strial midline. Marginal cells were counted in an 80 µm linear segment of stria, centered at the midpoint. Unconnected capillary profiles were counted over the same 80 µm span. Metrics were obtained using a Nikon Optiphot™ light microscope using a 100x oil objective and a calibrated grid ocular. For each animal, measures were obtained from 10 sections spanning 200 microns through the modiolar core.

For each animal, ten estimates were averaged to obtain overall means for each of the four histological metrics. Statistical analyses included a two way analysis of variance (ANOVA) as well as an all pairwise multiple comparison procedure (Tukey Test). Tests yielding a p-value less than 0.05 were considered to be significant.
Results

General appearance of affected stria

Figure 2 shows a strial profile from the lower cochlear base in an old CBA/CaJ mouse having a low EP (69 mV). The normal range of EP in this and other strains is roughly 80-120 mV. It is apparent that the stria was quite variable in appearance, reflecting similar variability of the EP in the older CBA/CaJ mice. In the example shown, EP reduction coincides with strial thickening caused by edema.

Figure 2: Representative strial profile from the lower cochlear base. This mouse exhibited EP decline and intrastrial swelling. (Ohlemiller, unpublished)
Figure 3a. Average strial thickness measures taken at 3 different locations: lower base, upper base, and lower apex. Significant differences between groups are highlighted using brackets with corresponding significance levels.

Figure 3b. Scatter plot of strial thickness versus EP in the upper base. Points represent data for individual animals.
As shown in Figure 3a, strial thickness was similar at all locations in both strains. Although there was significant reduction in both strains, the extent of the reduction was greater in the old CBA/J mice.

Figure 4a demonstrates how both CBA/J and CBA/CaJ mouse strains lose a significant amount of marginal cells due to age. The old CBA/CaJ mice lose more marginal cells than old CBA/Js by a significant amount.

![Marginal Cells by Location](image)

*Figure 4a: This plot shows average marginal cell densities measured at 3 different locations: lower base, upper base, and lower apex. Significant differences between groups are highlighted using brackets with corresponding significance levels.*
Figure 4b: Above is a scatter plot of marginal cell density versus EP in the upper base. Points represent data for individual animals.

The spiral ligament appeared thinner in CBA/CaJ mice than CBA/J mice, independently of age (Figure 5a). There was some suggestion that the ligament may even thicken in old CBA/CaJ mice, counter to any trend in CBA/J.
Figure 5a. Average ligament thickness at 3 different locations: lower base, upper base, and lower apex. Significant differences between groups are highlighted using brackets with corresponding significance levels.

Figure 5b: Scatter plot of ligament thickness versus EP in the upper base. Points represent data for individual animals.
Capillary density in the strial profile is shown in Figure 6a. The appearance of capillaries lessens for both strains with age. However, old CBA/CaJs show significantly fewer capillaries than the old CBA/Js.

Figure 6a: This plot shows average capillary densities measured at 3 different locations: lower base, upper base, and lower apex. Significant differences between groups are highlighted using brackets with corresponding significance levels.
Figures 3b-6b compare strial thickness, marginal cell density, ligament thickness and capillary density with EP in the upper base with regard to data from individual animals. There was no suggestion of correlation with EP in any of the four groups. These figures reveal that the old CBA/CaJ mice show more variation in all four measures obtained.

**Discussion**

Recent work by Ohlemiller (2009) plotted age versus basal turn EP in both the CBA/J and CBA/CaJ strains. The first of many differences between these strains is that the EP declines as the CBA/CaJ mouse ages, while the EP stays stable with age in the CBA/J strain. By examining these two stains further, more differences emerged. All four measures obtained
showed significant differences between CBA/Js and CBA/CaJs. The overall profile for differences between these two strains is unique among inbred strains studied to date. Both the CBA/J and CBA/CaJ mice showed decreases in every measure with age, except for the ligament thickness. The CBA/CaJs begin with a significantly thinner stria which thins more as they age as compared to the CBA/J mice. Interestingly, the CBA/CaJ stria does not thin as much as the CBA/J stria even though the strial profile shows there to be fewer marginal cells in the older CBA/CaJs. The CBA/CaJ mouse line is also unique in that these mice showed capillary loss with age.

Comparison to other models

According to this study, marginal cells in the upper base are fewer throughout the lifespan in CBA/CaJs as compared to CBA/J mice. Although not a significant correlation, there seems to be the same trend in reduction in EP with the loss of marginal cells that is seen with BALB and B6 albino mice. As the stria becomes thinner in BALB and B6 albino mice, it is marginal cells that are most dramatically reduced in number (Ohlemiller et al., 2009). Plotting basal turn EP versus strial marginal cell density in both strains suggests a causative role for marginal cell pathology in strial presbycusis (Figure 7). In BALB and B6 albino mice, a linear correlation can be derived between EP and marginal cells. That relationship is not seen as clearly in CBA/CaJs because there are four differing metrics which suggest that there is a combination of factors to consider.
Even though old CBA/CaJ mice seem to resemble the old BALB mice in ligament thickness and marginal cell density, the same cannot be said for capillary density. In no case does capillary density predict EP. BALB and B6 albino mice did not experience a significant loss of capillaries even though the EP decreased. There is, however, a significant loss of capillaries in the CBA/CaJ mouse line. Since the data show that no one variable predicts the EP for any metric, this may suggest a separate process is taking place in the CBA/CaJs that makes them a unique and more complicated model. The lack of a correlation between the EP and any one of the four metrics in CBA/CaJ mice may suggest that a combination of factors must be taken into account in predicting the EP. This would require multivariate analyses.

Thickening of the stria, as observed in older CBA/CaJ mice, is an unusual finding. A variety of cell loss, including marginal cell loss, should result in a thinner stria, as has been reported (Ohlemiller et al., 2006, 2009). Apparent strial thickening in old CBA/CaJ could be explained by the formation of fluid filled spaces between the cells in the stria, a tendency suggested in Figure 2.
Genetic Factors

CBA/CaJs resemble BALBs with regard to EP decline, marginal cell loss and thinning of the ligament. This resemblance could imply that BALBs and CBA/CaJ mice share alleles at one or more loci that promote these traits. In the case of B6 albino mice, lack of strial melanin was shown to promote marginal cell loss (Ohlemiller et al., 2009). Although albinism was not a factor in the present study, other pigment-related factors could similarly promote EP marginal cell loss and EP reduction. One is Agouti (A), for which CBA/CaJ are carriers (Ohlemiller, 2009). However, CBA/J mice also carry this allele, and further, share alleles at all major coat color loci.

In summary, it is likely that CBA/CaJ mice carry a number of alleles of genes that work together to create their strial aging profile.

Clinical Implications

The causes of strial presbycusis have proven resistant to study in humans. Mice hold advantages for the study of genetic aspects of disease; thus the identification of mouse strains that usefully model aspects of strial presbycusis is an important advancement. If, as has been suggested, CBA/CaJ mice represent a mouse model of ‘pure’ strial presbycusis, this model may hold special advantages for discovering strial presbycusis genes in humans.
References


