Abstract

The goal of the present chapter is to present some novel findings and insights related to biologically-based changes in the auditory inner ear and brain that might underlie some of the performance limitations of contemporary hearing aids designed for elderly clients. Specifically, at the cochlear level, age-related cell loss occurs for both sensory hair cells, supporting cells and auditory nerve fibers, resulting in a loss of auditory sensitivity. Peripheral cell loss can induce plastic changes in the parts of the brain used for hearing, due to declining inputs from the ear with age. Aging can also directly affect the brain, resulting in declines in the efferent feedback system from the brainstem to the cochlear hair cells, which can even start in middle age in humans and lab animals. Aging can also result in auditory temporal processing deficits at the level of the brainstem, which are related to suprathreshold speech comprehension deficits in aged listeners. There is also new evidence indicating that in old mice with hearing loss, there are gene expression alterations in the cochlea and central auditory system involving changes in key neurotransmitter systems, cell death (apoptosis) pathways and ionic regulation systems. These findings provide essential incremental information concerning possible therapeutic targets for future biomedical interventions. Clinical trials utilizing translational medicine approaches are years off and, of necessity, will require extensive experimentation and proof-of-concept in mammalian animal models. In the meantime, as new aspects of how biological changes can affect mammalian complex sound processing in realistic acoustic environments, improvements in the design of hearing aids and cochlear implants targeted for our older listeners can be achieved.

Why don’t Hearing Aids Work with many Aged Listeners?

Often times, even when aged listeners with hearing loss are fitted with high performance hearing aids, they are less than satisfied with the results, and may return their hearing aid after the trial period. They frequently share that they do pretty well in quiet, yet in background noise suffer limited speech comprehension. On the other hand, it may very well be the case that the hearing aid is compensating well for reduced auditory sensitivity and fairly accurately controlling the output of the hearing aid with smart automatic gain control features. There may even be some fairly sophisticated background noise suppression algorithms as part of the hearing aid circuitry. However, a major reason for this less-than-satisfactory outcome is that there are aging changes in the cochlea and parts of the brain used for hearing (decentral auditory system) that are not easily compensated for in the design of the hearing aid, and these age-related hearing deficits manifest themselves at primarily supra-threshold levels. The goal of the present chapter is to provide some new insights and findings regarding the neural and molecular genetic age changes in the auditory inner ear and brain that may possibly be responsible for some of the limitations of contemporary hearing aids for elderly clients.
Is it all about Hair Cell Loss? – No, but …

It has been known for some time that loss of cochlear hair cells with age contributes to elevated thresholds, and likely contributes to complex sound processing problems, including coding of speech (e.g., Willott 1991; Spongr et al. 1997; Frisina et al. 2001). The more severe the peripheral hearing loss, as reflected in elevation of pure-tone thresholds and speech thresholds in quiet, the more important it is as a determinant of perception of complex sounds. However, if an aged listener has a relatively mild hearing loss, other cochlear age changes and/or deficits in the central auditory system can significantly impair speech/language perception in complex acoustic environments.

Timing is everything!

Temporal processing capacity of the auditory system plays an essential role in speech perception, or vocalization coding in mammals (Pichora-Fuller 2003; Grose et al. 2006). Much evidence indicates that auditory temporal processing declines with age in human listeners, either in the presence of peripheral hearing loss, or in cases where auditory sensitivity is fairly good or even within normal limits (Fitzgibbons and Gordon-Salant 1996; Helfer and Vargo 2009). Not surprisingly, additional reports demonstrate links between auditory temporal processing problems and deficits in speech processing, in background noise in middle age listeners and in the aged (Gordon-Salant and Fitzgibbons 2001; Pichora-Fuller and Souza 2003; Pichora-Fuller et al. 2007).

Feedback Loop from the Brain to the Ear Declines, Starting in Middle Age

Reduced efferent feedback control from the central auditory system to the cochlear hair cells is another type of deficit of the aging auditory system that can interfere with normal sound processing. This aspect of age-related hearing loss – presbycusis – was actually discovered in groups of subjects of different ages who had audiograms that fell within the normal, young adult range (Kim et al., 2002). Soon after that finding in humans, this decline in efferent feedback was uncovered in the CBA mouse strain, a type of mouse that loses its hearing slowly over time (Jacobson et al., 2003; Varghese et al., 2005).

Gene Discovery in the Aging Auditory System

Gene Expression Experiment

General Hypothesis: Gene expression changes occur in the ear and the brain in presbycusis.

Experimental Subjects: CBA mice – slow progressive hearing loss with age, characteristic of most hu-

Table 1: Four age groups derived from functional hearing abilities based on DPOAE amplitudes and ABR thresholds in CBA mice. Adapted from D’Souza et al. (2008).

<table>
<thead>
<tr>
<th>Group</th>
<th>No of mice</th>
<th>No of chips (1 chip/mouse)</th>
<th>Age-months ±range</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young, good hearing</td>
<td>8</td>
<td>8</td>
<td>3.5± 0.4</td>
<td>Male = 5, Female = 4</td>
</tr>
<tr>
<td>Middle aged, good hearing</td>
<td>17</td>
<td>17</td>
<td>12.3±1.5</td>
<td>Male = 8, Female = 9</td>
</tr>
<tr>
<td>Mild presbycusis</td>
<td>9</td>
<td>9</td>
<td>27.7±3.4</td>
<td>Male = 4, Female = 5</td>
</tr>
<tr>
<td>Severe presbycusis</td>
<td>6</td>
<td>6</td>
<td>30.6±1.9</td>
<td>Male = 2, Female = 4</td>
</tr>
</tbody>
</table>
mans. Four subject groups were utilized: Young adults with good hearing, middle aged mice with good hearing, old mice with mild presbycusis, and old mice with severe presbycusis, as described in more detail in Table 1.

**Tissue:** Cochlear tissue was harvested from the organ of Corti and lateral wall (including the stria vascularis); and from the inferior colliculus (auditory midbrain). A strength of this investigation was that RNA samples from individual mice were processed on individual microarrays, i.e., 1 microarray for each subject’s cochlea (the tissue from each cochlea of a particular mouse were combined), and 1 microarray for each inferior colliculus.

**Investigative Tool:** The Affymetrix murine GeneChip was utilized, where one chip analyzes 22,600 gene probes for each sample, from each mouse.

**Project Strengths:** Number of replicates, N=40 for the cochlea, and N = 40 for the auditory midbrain, which strengthened the statistical analysis. This one chip-one mouse experimental design allows for exploration of the biological phenotype variance from mouse to mouse.

**Gene Expression Changes Found in the Aging Cochlea**

Gamma aminobutyric acid (GABA) is the primary inhibitory neurotransmitter of the auditory system, and a key neurotransmitter of the efferent feedback system from the central auditory system to the cochlea. D’Souza et al. (2008) discovered that several aspects of the receptor distribution for GABA in the cochlea showed significant declines with age. An interesting aspect of these age-related declines were that they began appearing in middle aged mice, despite the fact that the samples came from mice who had fairly good peripheral auditory sensitivity, as reflected in auditory brainstem response (ABR) thresholds and otoacoustic emission amplitudes, which reflect the health and well being of the cochlear outer hair cell system.

Aquaporin proteins comprise the primary cellular membrane channels for regulation of water in mammalian cells, including cells of the cochlea. Christensen et al. (2009) reported that aquaporin channels in the cochlea show a significant down-regulation of gene expression in the mouse cochlea with age and hearing loss. Impairment of these channel proteins with age could disrupt intracellular water regulation, and therefore degrade normal intracellular ionic concentrations and membrane transport. These age-linked disruptions could then interfere with normal sound transduction and cochlear hair cell physiological coding.

In contrast to certain GABA receptors and aquaporin channel proteins, which decline with age and hearing loss, Tadros et al. (2008) discovered that there is a noteworthy upregulation of certain cochlear apoptotic (programmed cell death) pathways with age and hearing loss in mice. In particular, the following key elements of the cell cycle pathways are upregulated: Atf3 – activating transcription factor3; Bcl2 – B-cell leukemia/lymphoma2; Bcl2l1 – Bcl2-like1protein; and Casp4 - caspase4 apoptosis-related cysteine protease 4. It is interesting to note that in the middle aged mice with relatively good hearing, these apoptotic elements are either at the baseline level (baseline in this experiment was the gene expression level found in the young adult mice) or are slightly down-regulated. Generally, these apoptotic elements were not significantly upregulated until the mice reached the oldest age and had a significant hearing loss.

**Gene Expression is Altered in the Aging Auditory Brain**

Overall, age can affect the cochlea directly, or have a direct impact on the central auditory system. In addition, there may be plastic changes in the auditory brain resulting from loss of cochlear outputs with age, sometimes referred to as a peripherally-induced central effect (Frisina et al., 2001). In the present context, age or hearing loss-related gene expression changes in the central auditory system can be a combination of direct aging effects, or due to a loss of peripheral inputs.

Glutamate is the primary excitatory neurotransmitter of the auditory system, both for the hair cell/auditory nerve cochlear synapses and for nerve cells of the central auditory system. Tadros et al. (2007a) discovered that a key enzyme and important glutamate transporter changed their gene expression patterns with age and hearing loss in the mouse auditory midbrain (inferior colliculus). Specifically, Pycs plays a role in converting glutamate to proline in nerve cells of the central auditory system. A Pycs deficiency (gene expression down-regulation) in old age may lead to higher levels of glutamate, making the auditory midbrain nerve cells more susceptible to a glutamate, overstimulation toxicity. In conjunction with this undesirable outcome, proline deficiencies in the auditory midbrain could lead to a loss of the normally neuroprotective benefits of proline. The other major change in gene expression with age and hearing loss in the glutamate family was for Slc1a3. Slc1a3 is a glutamate
transporter that normally removes glutamate from nerve cells when the intracellular levels become too high. The Slc1a3 gene upregulation in the oldest mice with the greatest amount of hearing loss may reflect a cellular compensatory mechanism to protect auditory midbrain nerve cells against age-related glutamate or calcium excitotoxicity.

Serotonin is a key neuromodulator in the mammalian brain, including the central auditory system at the level of the auditory midbrain. For example, Hurley and Pollak (1999) have provided convincing evidence that serotonin can markedly affect complex sound processing in nerve cells of the mammalian inferior colliculus. Tadros et al. (2007b) explored the gene expression changes of members of the serotonin family in the aging CBA mouse. They found a significant upregulation of serotonin receptors with age and hearing loss in the inferior colliculus, at both gene expression (PCR) and protein expression (immunocytochemistry) levels. This upregulation of serotonin receptors could help compensate for declines in serotonin itself with age, in order to help stabilize the complex sound processing capabilities of auditory midbrain nerve cells in old mammals. However, one complicating factor of increasing the number of serotonin receptors is that under certain conditions, increased influx of serotonin can stimulate activation of an intracellular messenger called IP3, which in turn could induce intracellular Ca++ toxicity (unwanted buildup of calcium ions) in auditory nerve cells of the inferior colliculus. Consistent with the notion of a possible Ca++ buildup with age, Zettel et al. (1997, 2001) previously reported an upregulation of calretinin in the CBA mouse auditory midbrain with age. Calretinin is one of the key calcium-binding proteins, or intracellular calcium regulators in the brain. This upregulation turned out to be activity dependent, as it was not present in old CBA mice that were deafened artificially, nor in mice that became deaf in middle age due to genetic reasons (C57Bl/6, Black 6, [C57]; Erway et al., 1993; Davis et al., 2003).

**A New Mouse Model for Studying the Neural and Molecular Genetic Bases of Presbycusis**

A few older human listeners, mostly women, have remarkably good peripheral hearing. This fortunate minority have audiometric thresholds within the normal range of young adults, although they often still have some problems understanding speech in background noise (Frisina and Frisina, 1997). These aged listeners with good cochlear sensitivity are often referred to as “Golden Ears”. Until recently, there was not an optimal animal model for studying these gifted listeners. Frisina et al. (2009) genetically crossed the CBA and C57 mouse strains and discovered a new mouse model for aged human listeners. In old age, this new mouse has an audiogram within the normal murine hearing range: mice with “Golden Ears”. The CBA mouse loses its hearing slowly, similar to most humans, and has a moderate-to-severe hearing loss in the oldest ages. The C57 has a very rapid, accelerated high-frequency age-related hearing loss, where a severe-to-profound cochlear hearing impairment is developed by middle age. The C57 etiology results from a mutation in the mouse ahl gene, causing progressive disruption of the cadherin 23 protein of the cochlear hair cell stereocilia (DiPalma et al., 2001; Xu et al., 2008; Sengupta et al., 2009). It was not surprising that the offspring of the CBA x C57 cross had better hearing than the C57 parental strain, since the offspring had only one copy of the ahl recessive allele. However, the discovery that the offspring had significantly better hearing in old age than the CBA parental strain (lower ABR thresholds, higher otoacoustic emission levels) was quite unexpected, and potentially very useful.

**Summary and Conclusions**

Advances in our understanding of the biological underpinnings of age-related hearing loss in mammals are now occurring at cellular and molecular genetic levels. These discoveries provide critical information about possible therapeutic targets for future biomedical interventions using stem cells or gene therapy, to prevent, slow down or reverse key aspects of presbycusis. Clinical trials utilizing these translational medicine approaches are years off, and will follow extensive experimentation and proof-of-concept in mammalian animal models. In the meantime, as new aspects of how biological changes can affect mammalian complex sound processing become apparent, including coding of speech in background noise, improvements in the design of hearing aids and cochlear implants targeted for our older listeners can be made.

**Acknowledgements**

We thank Dr. Robert Frisina, Sr. for valuable critiques and discussion, John Housel for technical assistance, and Enza Daugherty for project support. Supported by NIH grants: P01 AG09524 from the National
Institute on Aging; P30 DC05409 from the National Institute on Deafness and Communication Disorders.

References


Frisina, R.D., Singh, A., Bak, M., Bozorg, S., Seth, R., Zhu, X. 2009-In Press. F1 (CBA x C57) mice show superior hearing in old age relative to their parental strains: Hybrid vigor or a new animal model for “Golden Ears”? *Neurobiology of Aging.*


