Springer Handbook of Auditory Research

Karen S. Helfer
Edward L. Bartlett
Arthur N. Popper
Richard R. Fay *Editors*

Aging and Hearing

Causes and Consequences

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The Acoustical Society of America

On 27 December 1928 a group of scientists and engineers met at Bell Telephone Laboratories in New York City to discuss organizing a society dedicated to the field of acoustics. Plans developed rapidly, and the Acoustical Society of America (ASA) held its first meeting on 10–11 May 1929 with a charter membership of about 450. Today, ASA has a worldwide membership of about 7000.

The scope of this new society incorporated a broad range of technical areas that continues to be reflected in ASA's present-day endeavors. Today, ASA serves the interests of its members and the acoustics community in all branches of acoustics, both theoretical and applied. To achieve this goal, ASA has established Technical Committees charged with keeping abreast of the developments and needs of membership in specialized fields, as well as identifying new ones as they develop.

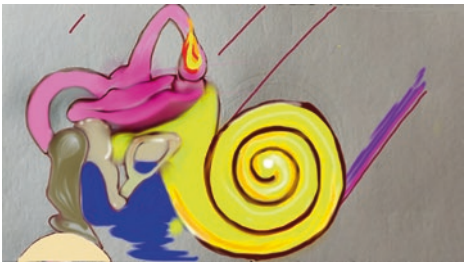
The Technical Committees include acoustical oceanography, animal bioacoustics, architectural acoustics, biomedical acoustics, engineering acoustics, musical acoustics, noise, physical acoustics, psychological and physiological acoustics, signal processing in acoustics, speech communication, structural acoustics and vibration, and underwater acoustics. This diversity is one of the Society's unique and strongest assets since it so strongly fosters and encourages cross-disciplinary learning, collaboration, and interactions.

ASA publications and meetings incorporate the diversity of these Technical Committees. In particular, publications play a major role in the Society. *The Journal of the Acoustical Society of America* (JASA) includes contributed papers and patent reviews. *JASA Express Letters* (JASA-EL) and *Proceedings of Meetings on Acoustics* (POMA) are online, open-access publications, offering rapid publication. *Acoustics Today*, published quarterly, is a popular open-access magazine. Other key features of ASA's publishing program include books, reprints of classic acoustics texts, and videos. ASA's biannual meetings offer opportunities for attendees to share information, with strong support throughout the career continuum, from students to retirees. Meetings incorporate many opportunities for professional and social interactions, and attendees find the personal contacts a rewarding experience. These experiences result in building a robust network of fellow scientists and engineers, many of whom become lifelong friends and colleagues.

From the Society's inception, members recognized the importance of developing acoustical standards with a focus on terminology, measurement procedures, and criteria for determining the effects of noise and vibration. The ASA Standards Program serves as the Secretariat for four American National Standards Institute Committees and provides administrative support for several international standards committees.

Throughout its history to present day, ASA's strength resides in attracting the interest and commitment of scholars devoted to promoting the knowledge and practical applications of acoustics. The unselfish activity of these individuals in the development of the Society is largely responsible for ASA's growth and present stature.

Series Preface



Springer Handbook of Auditory Research

The following preface is the one that we published in volume 1 of the Springer Handbook of Auditory Research back in 1992. As anyone reading the original preface, or the many users of the series, will note, we have far exceeded our original expectation of eight volumes. Indeed, with books published to date and those in the pipeline, we are now set for over 77 volumes in SHAR, and we are still open to new and exciting ideas for additional books.

We are very proud that there seems to be consensus, at least among our friends and colleagues, that SHAR has become an important and influential part of the auditory literature. While we have worked hard to develop and maintain the quality and value of SHAR, the real value of the books is very much because of the numerous authors who have given their time to write outstanding chapters and to our many co-editors who have provided the intellectual leadership to the individual volumes. We have worked with a remarkable and wonderful group of people, many of whom have become great personal friends of both of us. We also continue to work with a spectacular group of editors at Springer. Indeed, several of our past editors have moved on in the publishing world to become senior executives. To our delight, this includes the current president of Springer US, Dr. William Curtis.

But the truth is that the series would and could not be possible without the support of our families, and we want to take this opportunity to dedicate all of the SHAR books, past and future, to them. Our wives, Catherine Fay and Helen Popper, and our children, Michelle Popper Levit, Melissa Popper Levinsohn, Christian Fay, and Amanda Fay Sierra, have been immensely patient as we developed and worked on this series. We thank them and state, without doubt, that this series could not have happened without them. We also dedicate the future of SHAR to our next generation of (potential) auditory researchers – our grandchildren – Ethan and Sophie Levinsohn; Emma Levit; Nathaniel, Evan, and Stella Fay; and Sebastian Sierra-Fay.

Preface 1992

The Springer Handbook of Auditory Research presents a series of comprehensive and synthetic reviews of the fundamental topics in modern auditory research. The volumes are aimed at all individuals with interests in hearing research including advanced graduate students, post-doctoral researchers, and clinical investigators. The volumes are intended to introduce new investigators to important aspects of hearing science and to help established investigators to better understand the fundamental theories and data in fields of hearing that they may not normally follow closely.

Each volume presents a particular topic comprehensively, and each serves as a synthetic overview and guide to the literature. As such, the chapters present neither exhaustive data reviews nor original research that has not yet appeared in peer-reviewed journals. The volumes focus on topics that have developed a solid data and conceptual foundation rather than on those for which a literature is only beginning to develop. New research areas will be covered on a timely basis in the series as they begin to mature.

Each volume in the series consists of a few substantial chapters on a particular topic. In some cases, the topics will be ones of traditional interest for which there is a substantial body of data and theory, such as auditory neuroanatomy (Vol. 1) and neurophysiology (Vol. 2). Other volumes in the series deal with topics that have begun to mature more recently, such as development, plasticity, and computational models of neural processing. In many cases, the series editors are joined by a co-editor having special expertise in the topic of the volume.

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Volume Preface

Age-related hearing loss (ARHL) remains one of the most common chronic maladies of aging. There is increasing realization that this seemingly benign condition may have a host of negative consequences, including social isolation, depression, increased risk of falls, and cognitive decline. And as more people are living longer lives, there is heightened interest in the biological mechanisms of aging and senescence, which includes ARHL. Hence, there is interest in studying ARHL across a broad range of disciplines, including neuroscience, audiology, cognitive psychology, and epidemiology.

Since the earlier volume in the Springer Handbook of Auditory Research (SHAR) series entitled *The Aging Auditory System* (in 2009), there has been a tremendous upsurge in research in basic, translational, and clinical sciences pertinent to age-related changes in auditory system structure and function. The goal of this new volume is to provide an overview of this topic in a format that is accessible and comprehensible to a wide range of researchers interested in ARHL. The hope is that this volume will allow researchers in one area to gain a greater appreciation for research done in other areas, to facilitate the understanding of how others' research might relate to yours, and to inspire future researchers to tackle the complex questions remaining about ARHL.

The volume considers ARHL in 12 chapters that, together, provide a broad view of the topic. Chapter 1, by Karen Helfer and Edward Bartlett, provides an overview of the volume. In Chap. 2, Shinichi Someya and Mi-Jung Kim describe a range of genetic mutations that affect hearing thresholds and function.

Central nervous system changes with aging are then considered in several chapters. In Chap. 3 Kevin Ohlemiller and Christopher Spankovich review the cell types, cell components, and structural and synaptic changes in the cochlea and auditory nerve that lead to presbycusis. Josef Syka then reviews age-related changes in the auditory brainstem and midbrain in Chap. 4 while in Chap. 5 Gregg Recanzone reviews anatomical and physiological changes in the auditory cortex, centered on changes in primate rather than rodent models. Then, in Chap. 6, Kelly Harris reviews human electrophysiological measures and how they are influenced by aging.

More cognitive functions are then considered in several chapters. The focus of Chap. 7, by Frederick Gallun and Virginia Best, is on how aging influences the segregation of sound sources. The epidemiology of age-related hearing loss is covered in Chap. 8 by Jennifer Deal, Nicholas Reed, Emily Pedersen, and Frank Lin. Chapter 9, by Sandra Gordon-Salant, Maureen Shader, and Arthur Wingfield, deals with the complex issue of why older adults have difficulty understanding spoken messages.

In Chap. 10, Stefanie Kuchinsky and Kenneth Vaden cover the complex topic of neuroimaging. The focus of Chap. 11 by Larry Humes, Kathleen Pichora-Fuller, and Louise Hickson is the rehabilitation of older adults with hearing loss. Finally, while earlier chapters mostly focused on the mechanisms, diagnostics, and risk factors associated with ARHL, Chap. 12, by Robert Frisina, Carlos Cruz, Tanika Williamson, Xiaoxia Zhu, and Bo Ding, discusses the latest avenues for treatment of ARHL and technologies that may improve treatments or preclinical research into ARHL.

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Chapter 1
Listening to All Voices: Interdisciplinary Approaches to Understanding Hearing in Aging

Karen S. Helfer and Edward L. Bartlett

Abstract This chapter will introduce the reader to the purpose, content, and structure of *Age-Related Hearing Loss: Causes and Consequences*. In this book, recent advances in the study of age-related hearing loss are reviewed, including basic research with animal and human studies along with translational research. The book is broadly organized into several sections. Chapters 1, 2, 3, 4, and 5 examine age-related changes in the subcortical and cortical anatomy and physiology, drawing primarily on animal studies. Chapters 6 and 7 transition to understanding human age-related changes using electrophysiological measures. Chapter 8 places age-related hearing impairment in the context of overall health, both as an indicator and a major correlative factor in predicting health outcomes. Chapters 9 and 10 focus on behavioral and imaging changes with aging, in order to identify difficult listening situations and the cortical regions and connectivity that are most affected. Finally, Chapters 11 and 12 discuss remediation efforts using a variety of methods. Chapter 1 provides more detailed summaries of each chapter and concludes with thoughts about future directions for research on age-related hearing loss.

Keywords Aging · Auditory processing · Presbycusis

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1.1 Purpose of This Book

Age-related hearing loss (ARHL) remains one of the most common chronic maladies of aging. There is increasing realization that this seemingly benign condition may have a host of negative consequences, including social isolation, depression, increased risk of falls, and cognitive decline. As more people are living longer, there is heightened interest in the biological mechanisms of aging and senescence, including ARHL. Hence, there is interest in studying ARHL across a broad range of disciplines, encompassing neuroscience, audiology, cognitive psychology, and epidemiology.

Increased attention has focused on these areas not only in isolation but also on the intersections and interactions between them. One example of this is the nascent area of cognitive hearing science (e.g., Arlinger et al. 2009), which has contributed much to our understanding that hearing loss associated with aging has both upstream and downstream consequences.

In tandem with interdisciplinary advances at the cognitive level, there have been advances in genetics, neuroanatomy, and electrophysiology that have enabled understanding of ARHL at the cellular and subcellular levels, such as next-generation sequencing (Rehman et al. 2010; Goodwin et al. 2016), CRISPR-Cas genomic editing (Mianne et al. 2016), large-volume neuroanatomy using optical clearing (Urata et al. 2019), multichannel calcium imaging (Issa et al. 2014), multichannel electrophysiology (Panzeri et al. 2010), and optogenetic control of auditory neurons (Natan et al. 2015). These advances include not only mechanisms of neurodegeneration in the auditory system, but also the single-cell and population-level neural responses that are correlated with age-related behavioral changes, primarily in nonhuman mammalian models.

Since publication of the earlier volume in the Springer Handbook of Auditory Research (SHAR) series entitled *The Aging Auditory System* (in 2009), there has been a tremendous upsurge in research in basic, translational, and clinical sciences pertinent to age-related changes in auditory system structure and function. A PubMed search using *Aging* and *Hearing* as key words lists 2335 articles published between 2009 and 2019. The goal of this volume is to provide an overview of this topic in a format that is accessible and comprehensible to a wide range of researchers interested in ARHL. The hope is that this volume will allow researchers in one area to gain a greater appreciation for investigations in other areas, to facilitate the understanding of how others' research might relate to one's own, and to inspire future researchers to tackle the complex questions remaining about ARHL.

1.2 Chapter-by-Chapter Overview

In Chapter 2, Shinichi Someya and Mi-Jung Kim describe a range of genetic mutations that affect hearing thresholds and function. They categorize the mutations and the affected proteins in terms of different mechanisms that are critical for proper

maintenance of hearing and that decline with aging or that accelerate ARHL. These include both nuclear and mitochondrial DNA mutations. The chapter is sectioned topically by the most critical mechanisms for hearing maintenance. Control of oxidative stress and antioxidant defense systems are important for regulating adult and aging hearing. Although cell loss is not rampant with aging, there are multiple factors such as tumor necrosis factor (TNF)-α and BCL genes that control programmed cell death, or apoptosis. More typically, there is significant neurodegeneration that can be affected by β-amyloid, purinergic (ATP) receptors and estrogen receptors. Inflammatory and immune responses and regulation that are critical for healthy aging are also described. Finally, specific screens for ARHL and the candidate genes for further study are discussed.

Next, in Chapter 3 Kevin Ohlemiller and Christopher Spankovich review the cell types, cell components, structural and synaptic changes in the cochlea and auditory nerve that lead to presbycusis. They take a comparative approach, including multiple mammalian species and data from human temporal bones. In addition, different forms of presbycusis, including sensory, metabolic, and neural presbycusis, are identified, and the evidence for them in humans and animal models is presented and evaluated. Not only are the inner and outer hair cells and their cochlear afferents discussed, but the supporting cells that enable the endocochlear potential (stria vascularis) and neural cell health are discussed as well. The age-related changes are evaluated in the classical framing of Schuknecht in terms of different patterns of degeneration (Schuknecht and Gacek 1993). In addition to an analysis of the changes in cells and components that lead to presbycusis, this chapter also reviews risk factors for presbycusis.

In Chapter 4 Josef Syka reviews age-related changes in the auditory brainstem and midbrain. For aging individuals, these brain regions typically receive impoverished auditory nerve responses due to shifts in hearing thresholds and cochlear synaptopathy. In some cases, there may be compensatory activities in these circuits to restore sensitivity to changes in sounds. The chapter is laid out to describe changes in structure and function in the cochlear nucleus, superior olivary complex, olivocochlear efferents, and inferior colliculus with aging. Given the deep internal locations and small sizes of these brain structures, the findings are based primarily on rodent studies. Overall, there are only small changes in cell number, but there are more consistent changes in calcium regulation and buffers and a reduction in synaptic markers and function, especially for inhibitory synapses. Changes in age-altered circuits can be observed mainly for temporal processing rather than spectral processing, and these changes can be observed via unit electrophysiology, auditory evoked potentials, and behavior. Declines in temporal processing with age are most evident when modulation rate is fast and/or modulation depth is small.

In Chapter 5 Gregg Recanzone reviews anatomical and physiological changes in the auditory cortex, centered on changes in primate rather than rodent models. The primate cortical studies were set up in the chapter by a discussion of subcortical anatomical changes in primates with age, including changes in calcium binding proteins, similar to those described in Chapter 4. The subcortical changes could then inform interpretation of the findings for auditory cortex single unit responses,

including increases in spontaneous activity and increases in driven rates in old auditory cortex neurons. Declines in spatial processing were observed. For temporal processing, a shift in firing patterns and processing strategies is described between young and old primates. These data lay the groundwork to understand human auditory cortex studies in later chapters.

In Chapter 6 Kelly Harris provides an overview of human electrophysiological measures and how they are influenced by aging. The chapter begins with the most peripheral measures and works up through higher levels of processing. The CAP (compound action potential)/ABR (auditory brainstem response) wave I is presented as a window into how aging affects the auditory nerve, which appears to be vulnerable to degradation even after the influence of hearing loss is taken into account. Next, research on the ABR for simple click stimuli is summarized, with the idea that age-related changes in later waves of this response are less substantial than those noted for wave I, perhaps suggesting a central gain process where the neural response at central levels is still robust despite reduced input from the periphery. More significant age-related changes can be seen in the frequency following response (FFR) and when cortical auditory evoked potentials are used to assess temporal processing. One theme throughout this chapter is that age-related differences are more apparent in evoked response measures when they are elicited by complex (rather than simple) stimuli. The chapter concludes with an overview of research supporting two intriguing ideas: that training and/or musicianship may influence age-related alterations in brainstem-level and cortical-level responses (and perhaps speech recognition); and that evoked potential measures may be an early marker for cognitive decline.

The focus of Chapter 7, by Frederick Gallun and Virginia Best, is how aging influences the segregation of sound sources. The authors begin by presenting a coherent model of the processes used to segregate sounds. They make a case that deficits in source segregation could be an indicator of "central presbycusis," as segregation relies on suprathreshold processes that fall between peripheral processing and cognitive mediation. The chapter includes a thorough but concise discussion of how aging influences the processing of cues in the temporal, spectral, and spatial domains, which likely underlies problems that older adults experience with sound segregation. Next the authors summarize research on segregation ability in older listeners. The chapter concludes with the important consideration of how older adults function in listening environments more realistic than those typically used in laboratory-based experiments.

The epidemiology of age-related hearing loss is covered in Chapter 8 by Jennifer Deal, Nicholas Reed, Emily Pedersen, and Frank Lin. The chapter begins with an overview of the prevalence and incidence of hearing loss, then proceeds to describe risk factors (including genetics, exposure to noise and toxins, and cardiovascular health and lifestyle) associated with this condition. A brief tutorial on epidemiology is presented as a background for the remaining sections of the chapter, which cover the impact of hearing loss on older adults. One focus of this chapter is how hearing loss is related to cognition in older adults, with evidence provided that supports a link between cognitive decline/dementia and age-related hearing loss. Also discussed is the association between hearing loss and physical function

(mobility, activities of daily living), as well as connections between hearing loss and social isolation. The chapter concludes with information about the novel exploration of how hearing loss is related to health care utilization, providing research evidence showing that this metric is greater in older individuals with (vs. without) hearing loss.

Chapter 9 by Sandra Gordon-Salant, Maureen Shader, and Arthur Wingfield deals with the complex issue of why older adults have difficulty understanding spoken messages. The authors present the framework of a limited resource model, where age-related decline within the peripheral and central auditory systems leads to an increased need for cognitive and linguistic processing (which also are deleteriously affected by aging). The chapter begins with a summary of age-related changes in the peripheral and central auditory systems and how they impact speech perception, with a focus on alterations in temporal processing. Next is a comprehensive discussion of how aging influences the underpinnings of the processing of spoken messages (i.e., phonological and lexical analysis, working memory/attention, inhibitory ability). The chapter also covers the relevant topic of how age-related changes in working memory and hearing sensitivity interact, with the consequence that speech understanding is more effortful in older (than in younger) individuals. It concludes with two clinically relevant areas: an overview of cochlear implants in older adults and the timely issue of how individuals' language experience (specifically, in nonnative English speakers) influences speech understanding.

In Chapter 10 Stefanie Kuchinsky and Kenneth Vaden cover the complex topic of neuroimaging. The overarching premise in this chapter is that these tools can help document listening effort exerted by older adults in challenging listening environments by identifying neural regions used for various tasks. The chapter begins with a review of the benefits and limitations of various imaging methods. It then presents a thorough discussion of listening effort, including the concept of a U-shaped curve relating effort to performance, where effort is at a maximum when the task is moderately difficult and is less when the task is so hard that the individual "gives up." Data from studies using imaging in older adults to identify underlying abilities that contribute to speech understanding are summarized, including the topic areas of functional reorganization/compensation, working memory, and the various forms of attention. The authors conclude with an overview of the limitations of applying current imaging techniques to clinical practice and provide suggestions for future directions in this area, including potential uses of new techniques to identify candidates for and effects of interventions.

The focus of Chapter 11 by Larry Humes, Kathy Pichora-Fuller, and Louise Hickson is the rehabilitation of older adults with hearing loss. The authors use the new World Health Organization (WHO) International Classification of Functioning (ICF) to consider the effects of hearing loss on speech understanding, activities, and self-reported disability. This chapter provides a concise summary of subjective ways of measuring the impact of hearing problems (i.e., questionnaires) as applied both to individuals with hearing loss and to their communication partners. The authors raise the question of whether the current classification system misses individuals with milder forms of hearing loss who might be significantly impacted. Connections between hearing loss and other health conditions, including cognitive

decline, are discussed, as is how the environments in which older adults reside affect communication. Also covered is research on the efficacy of hearing aids in older adults, with attention paid to the critical issue of poor access to affordable hearing health care, and how this might be addressed going forward. The chapter ends with a summary of other types of intervention (communication education, psychological intervention to increase motivation, cognitive training, environmental modification) used to help older adults cope with hearing loss.

Previous chapters focused mostly on the mechanisms, diagnostics, and risk factors associated with ARHL. However, in Chapter 12 Robert Frisina, Carlos Cruz, Tanika Williamson, Xiaoxia Zhu, and Bo Ding discuss the latest avenues for treatment of ARHL and technologies that may improve treatments or preclinical research into ARHL. These avenues include pharmacological approaches to affect estrogen levels, potassium channels, and antioxidants, among others. The authors discuss the challenges of clinical trials and translating basic research into clinical research, as well as the difficulties in identifying appropriate patient populations where treatments may be most effective. Finally, they discuss the obstacles for developing preclinical tools, such as a drug pump for mice that can be used to test longer-term drug treatments, which would enable assessment of new drugs and combination therapies for aging hearing.

1.3 Future Directions

A theme that runs throughout this volume is that future research should focus on multidisciplinary solutions to address the many questions that remain unanswered about age-related hearing loss. The interdependency of the underlying causes and consequences of this condition (e.g., how changes in the auditory periphery lead to changes in central structures; how age-related limitations in working memory affect speech perception) suggests that our understanding of the nature of age-related hearing problems, and how those problems can be remediated, also needs to look beyond individual disciplines or sub-disciplines. Many of the complex issues regarding aging and hearing have been (and will continue to be) best addressed by studies that bring together the points of view and expertise of researchers from two or more areas. Examples of these could include how older adults compensate for age-related changes on both physiological and behavioral levels; the implications of ARHL on a societal level (including economics); and use of the same techniques for diagnostics and treatments in animal models and humans (e.g., auditory evoked potentials, functional magnetic resonance imaging [fMRI], pharmacology).

Just as there is a need to look across disciplines to understand the aging auditory system, it is also critical to look across levels of system organization. For any brain system, subcellular components are organized into specialized cell types, which interact to form circuits for signaling across multiple time scales, which interact with other circuits to form the overall nervous system that interacts with the rest of the organism to generate decisions and behaviors. Each of these levels of organization

changes dramatically over the developmental trajectory from early development to adulthood to aging and senescence. It is important to discover how the different components change and what causes those changes.

Many of these levels of organization are not amenable to study in humans, so researchers rely on animal models, typically using rodents and primates, to understand the cellular mechanisms that underlie behavioral changes. For auditory aging, animal studies allow a level of control for a known history of sound exposure, lifestyle (though it may be impoverished), and genetic background that is not possible in human studies. Furthermore, the subjects in the main rodent models are much shorter-lived than humans, such that it is possible to observe neurodegeneration that has similar characteristics to humans within a much shorter time span. This similarity means that causes of ARHL and hearing deficits associated with ARHL can be identified in animals, and treatments can be tested in humans. However, the translational route from animals to humans is not, and should not be, unidirectional. Researchers, clinicians, and patients all benefit from using human testing and observation of age-related hearing deficits to direct animal studies, as well as using findings from animal studies to drive different means of diagnostics and remediation in humans. The chapters in this book are organized to illustrate this bidirectional communication between researchers working with animal and human subjects. The hope is that this volume convinces present and future researchers of the importance of collaborative research that considers multiple points of reference.

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Chapter 2
Genetic and Molecular Aspects of the Aging Auditory System

Shinichi Someya and Mi-Jung Kim

Abstract Age-related hearing loss (AHL), or presbycusis, is the gradual loss of hearing and is characterized by poor speech comprehension in noisy conditions, impaired temporal resolution, and central auditory processing deficits. The major sites of age-related cochlear pathology typically include inner hair cells (IHCs) and outer hair cells (OHCs), spiral ganglion neurons (SGNs), and stria vascularis. The IHCs are the sensory receptors that relay their electrical response to the central auditory system through the SGNs. Postmitotic hair cells and SGNs are particularly susceptible to injury from a combination of noise exposure and oxidative damage. The blood vessels in the stria vascularis are essential for transporting oxygen into the cochlea. Therefore, age-related degeneration of one or more of these cochlear cells plays a major role in the development of AHL in both humans and animals. This chapter reviews the current literature on genetic and molecular aspects of the aging auditory system, particularly focusing on mouse genetic research from major auditory neuroscience and genetic journals.

Keywords Aging · Apoptosis · Development · Immune response · Neurodegeneration · Oxidative stress

2.1 Introduction

The overall objective of this chapter is to review the current literature on genetic and molecular aspects of the aging auditory system, particularly focusing on mouse studies. This chapter focuses on genes whose functions are involved in oxidative stress, apoptosis, neurodegeneration, development, and immune response, the conditions or cellular events that are known to be directly involved in aging and age-related diseases. Those genes and pathways that have been uncovered have significantly advanced the field of age-related hearing loss (AHL; a full list of

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Table 2.1 Abbreviations used in this chapter

ABR	Auditory brainstem response
AHL	age-related hearing loss
APP	amyloid precursor protein
Aβ	amyloid-β
CARD	caspase recruitment domain
CNS	central nervous system
CR	calorie restriction
CS	citrate synthase
ENU	*N*-ethyl-*N*-nitrosourea
ER	estrogen receptor
ESR1	estrogen receptor alpha
ESRRG	estrogen-related receptor gamma
FADD	FAS-associated death domain
γ-GCS	γ-glutamylcysteine synthetase
GDNF	glial cell-line-derived neurotrophic factor
GH	growth hormone
GST	glutathione *S*-transferase
GWAS	genome-wide association study
IGF-1	insulin-like growth factor-1
IHC	inner hair cell
OHC	outer hair cell
PCR	polymerase chain reaction
PGR	progesterone receptor
PIP3	phosphatidylinositol 3,4,5-trisphosphate
PTEN	phosphatase and tensin homolog
ROS	reactive oxygen species
SGN	spiral ganglion neurons
TCA	tricarboxylic acid
TNF	tumor necrosis factor
XIAP	X-linked inhibitor of apoptosis

abbreviations used in this chapter is provided in Table 2.1) and contributed to the understanding of the molecular mechanisms underlying cochlear aging and both early-onset and late-onset AHL in humans.

2.2 Oxidative Stress

2.2.1 Generation of Reactive Oxygen Species by the Mitochondrial Electron Transport Chain

It is estimated that approximately 90% of intracellular reactive oxygen species (ROS) are continuously generated as a by-product of mitochondrial respiration metabolism during the generation of ATP (Beckman and Ames 1998;

Evans and Halliwell 1999). The production of superoxide (O^{2-}), one of the major ROS, is thought to occur at two electron transport chain sites in mitochondrial respiration: Complex I (NADH dehydrogenase) and Complex III (ubiquinone-cytochrome *c* reductase), but under normal metabolic conditions, Complex III is thought to be the main site of superoxide production. An early study has shown that overexpressing the antioxidant gene superoxide dismutase 2 (*Sod2*) significantly increases longevity in *Drosophila* (Sun et al. 2002). In mice, overexpressing a mitochondrially targeted catalase gene moderately increases lifespan and delays the development of AHL (Schriner et al. 2005; Someya et al. 2009).

2.2.2 *Influence of Aging on Antioxidant Defense*

Cellular components such as DNA, proteins, and lipids are protected against ROS by an interacting network of antioxidant enzymes (Evans and Halliwell 1999; Balaban et al. 2005). For example, mitochondrial SOD2 converts superoxide into hydrogen peroxide, which in turn is decomposed to water and oxygen by peroxiredoxin or glutathione peroxidase. These antioxidant defense enzymes and proteins work with each other to protect key cellular components such as DNA from ROS-induced damage over the course of the lifetime. However, the antioxidant defense system does not keep pace with the age-related increase in ROS production, and hence the balance between the antioxidant defenses and ROS production shifts progressively toward a more pro-oxidant state during aging (Balaban et al. 2005).

2.2.3 *Influence of Aging on* Gpx6, Txnrd1, Idh1, *and* Hspb1 *Expression in Cochlea*

To investigate the association between cochlear antioxidant defense and AHL, Frisina and colleagues examined the expression of antioxidant-related genes in the cochlea of CBA/CaJ mice, a model of late-onset AHL, at various ages by DNA microarray (Tadros et al. 2014). The authors found that middle-aged mice with normal hearing, old mice with mild AHL, and old mice with severe AHL showed large-fold changes in gene expression of four antioxidant-related genes: glutathione peroxidase 6 (*Gpx6*), thioredoxin reductase 1 (*Txnrd1*), isocitrate dehydrogenase 1 (*Idh1*), and heat shock protein beta-1 (*Hspb1*) when compared to young mice with normal hearing: GPX6 catalyzes the reduction of hydrogen peroxide (Evans and Halliwell 1999); cytosolic IDH1 catalyzes the conversion of isocitrate to α-ketoglutarate and $NADP^+$ to NADPH (Reitman and Yan 2010); cytosolic TXNRD1 is a member of the thioredoxin antioxidant defense system that reduces oxidized thioredoxin 1 to reduced thioredoxin 1 and NADPH to $NADP^+$ (Evans and Halliwell 1999); HSPB1 (HSP27) is a member of the heat shock protein family that, in response to stress, acts as a molecular chaperon to inhibit apoptosis

by inhibiting cytochrome-*c*-mediated activation of caspases in the cytosol (Bruey et al. 2000). The results showed that the expression of *Gpx6* was upregulated, while the expression of *Txnrd1* was downregulated in the cochlea of middle-aged mice with normal hearing, old mice with mild AHL, and old mice with severe hearing loss, suggesting a decline in the activities of TXNRD1 is involved in cochlear aging.

2.2.4 Role of NRF2 in Reducing Oxidative Stress in Cochlea

The transcription factor NRF2 regulates transcription of genes encoding phase II detoxification enzymes such as glutathione *S*-transferase (GST) and antioxidant enzymes such as γ-glutamylcysteine synthetase (γ-GCS), the rate-limiting enzyme in glutathione biosynthesis (Kobayashi and Yamamoto 2005; Leiser and Miller 2010). Interestingly, primary skin-derived fibroblasts from long-lived Snell dwarf mutant mice exhibited elevated levels of *Nrf2*, higher levels of glutathione, and resistance to plasma membrane lipid peroxidation, while treatment of the dwarf-derived fibroblasts with arsenite, an inducer of NRF2 activity, and increased resistance to paraquat and hydrogen peroxide compared to untreated cells (Leiser and Miller 2010). Hoshino and colleagues (Hoshino et al. 2011) examined the roles of *Nrf2* in AHL using *Nrf2* knockout mice. There were no differences in auditory brainstem response (ABR) thresholds between young BDF1 control and $Nrf2^{-/-}$ mice. However, middle-aged $Nrf2^{-/-}$ mice displayed significantly higher ABR thresholds compared to age-matched control mice. This was associated with reduced numbers of hair cells and spiral ganglion neurons (SGNs) in the cochlea of middle-aged $Nrf2^{-/-}$ mice compared to age-matched control mice. These results suggest that NRF2 or NRF2-induced antioxidant defense plays a neuroprotective role against ROS in cochlea during aging.

2.2.5 Role of IDH2 in the Mitochondrial Antioxidant Defense in Cochlea

Mitochondrial IDH2 participates in the tricarboxylic acid (TCA) cycle and catalyzes the conversion of isocitrate to α-ketoglutarate and $NADP^+$ to NADPH in the mitochondrial matrix (Reitman and Yan 2010). IDH2 also plays a role in protecting mitochondrial components from oxidative stress by supplying NADPH to both glutathione reductase and thioredoxin reductase 2 within the mitochondrial matrix. White and colleagues (White et al. 2018) investigated the effects of *Idh2* deficiency on age-related cochlear pathology and AHL using $Idh2^{+/+}$ (WT) and $Idh2^{-/-}$ mice. The authors found that old male $Idh2^{-/-}$ mice displayed increased ABR thresholds, increased wave I latency, and decreased wave I amplitude compared to age-matched

WT mice. This was accompanied by increased oxidative DNA damage, increased apoptotic cell death, and profound loss of SGN and HCs in the cochlea of old *Idh2*$^{-/-}$ mice. Loss of *Idh2* also resulted in a decreased NADPH redox state and decreased activity of TXNRD2 in the mitochondria of the inner ear of young mice. These results suggest IDH2 functions as the principal source of NADPH for the mitochondrial thioredoxin antioxidant defense and plays an essential role in protecting cochlear hair cells and neurons against oxidative stress during aging.

2.2.6 Role of SIRT3 in Enhancing the Mitochondrial Antioxidant Defense in Cochlea under Calorie Restriction

Sirtuins are a family of NAD^+-dependent protein deacetylases that extend life span in worms and flies (Finkel et al. 2009). SIRT3, a member of the mammalian sirtuin family, is localized to mitochondria and regulates levels of ATP and the activity of Complex I of the electron transport chain (Ahn et al. 2008). Calorie restriction (CR) or reducing food consumption by 25%–60% without malnutrition, consistently extends life span and delays the onset of age-related diseases in a variety of species, including rodents and monkeys (Kaeberlein 2010; Burnett et al. 2011). Previous studies have shown that CR increases protein levels of SIRT3 in primary mouse cardiomyocytes, while overexpression of *Sirt3* protects these cells from oxidative stress–induced cell death (Sundaresan et al. 2008), suggesting a role of SIRT3 in aging retardation under CR conditions. A subsequent study investigated the effects of *Sirt3* deficiency on cochlear pathology and AHL under control diet and calorie restricted conditions using *Sirt3*$^{+/+}$ (WT) and *Sirt3*$^{-/-}$ mice (Someya et al. 2010). Aging resulted in elevated ABR hearing thresholds in middle-aged WT mice under control diet, while CR delayed the development of AHL in WT mice. However, CR did not delay AHL in *Sirt3*$^{-/-}$ mice. In agreement with the ABR test results, CR reduced oxidative DNA damage and SGN degeneration in the cochlea of middle-aged WT mice, but not *Sirt3*$^{-/-}$ mice. Under CR conditions, SIRT3 also activated IDH2, leading to increased NADPH levels and glutathione redox state in mitochondria. These results suggest that SIRT3 acts as a key player in enhancing the mitochondrial glutathione antioxidant defense system in cochlea and slowing the development of AHL under CR conditions.

2.2.7 Role of SIRT1 in the Antioxidant Defense in Cochlea

Although earlier studies showed that sirtuins extend life span in lower organisms (Finkel et al. 2009), subsequent studies revealed that overexpression of *Sir2* does not increase life span when compared with a genetically standardized control strain

in worms and flies, and that *Sir2* is not required for life span extension by CR (Kaeberlein 2010; Burnett et al. 2011). Li and colleagues have also shown that inhibition of *Sirt1*, a member of the mammalian sirtuin family, protected rat cortical neurons against oxidative stress (Li et al. 2008), suggesting that sirtuins can also accelerate aging. Han and colleagues (Han et al. 2016) examined the effects of *Sirt1* deficiency on cochlear pathology and hearing using $Sirt1^{+/+}$ (WT) and $Sirt1^{-/-}$ mice. The authors found that aging resulted in elevated ABR hearing thresholds in middle-aged WT mice. However, middle-aged $Sirt1^{-/-}$ male mice displayed significantly lower ABR thresholds compared to age-matched WT mice. This was associated with reduced oxidative damage and reduced degeneration of cochlear hair cells (HCs) and SGNs in middle-age $Sirt1^{-/-}$ mice. In mouse inner ear cell lines, *Sirt1* knockdown increased cell viability, increased acetylation status of Foxo3a, and increased activity of catalase under oxidative stress conditions. These results suggest that SIRT1 may promote AHL through suppressing FOXO3a-mediated oxidative stress resistance or catalase activity in mouse cochlea.

2.2.8 Role of Citrate Synthase in the Maintenance of Mitochondrial Function in Cochlea

Citrate synthase (CS) participates in the TCA cycle and catalyzes the synthesis of citrate from oxaloacetate and acetyl coenzyme and generates NADH and FADH2 for oxidative phosphorylation in the mitochondrial matrix (Suissa et al. 1984). C57BL/6 and A/J mice carry the same *ahl* variant of the *Cdh23* gene and exhibit early onset AHL. However, these two inbred strains exhibit dramatically different time courses of AHL: the hearing loss of A/J mice occurs much earlier than that of C57BL/6 and exhibits a rapid loss of hair cells, beginning at the basal turn and progressing toward the apex (Zheng et al. 2009), indicating that additional genetic factors must contribute to the accelerated rate of hearing loss in A/J mice. Two such factors have been identified by Johnson and colleagues: a DNA variant of the mitochondrial tRNA arginine (*mt-Tr*) gene (Johnson et al. 2001) and a missense mutation of the citrate synthase gene (*Cs*) (*ahl4*) (Zheng et al. 2009; Johnson et al. 2012). Johnson et al. (2012) also mapped *ahl4* by analysis of a new linkage backcross and determined a nucleotide variant (H55N) in exon 3 of *Cs* as the underlying cause of *ahl4*-related hearing loss. A subsequent study showed that siRNA knockdown of *Cs* reduced oxygen consumption rates, ATP production level, and increased superoxide formation, resulting in apoptotic cell death in human kidney cell lines (Cai et al. 2017), implicating that a decline in citrate synthase activity can lead to increased oxidative stress and mitochondrial dysfunction.

2.2.9 Role of Mitochondrial DNA Mutations in Cochlear Aging

As discussed in Sect. 2.2.8, A/J mice also carry a variant of the mitochondrial arginine tRNA gene (*mt-Tr*) that contributes to early-onset AHL. An earlier study showed mouse NIH3T3 cell lines carrying mitochondrial DNA (mtDNA) with the A/J strain variant of *mt-Tr* exhibited reduced mitochondrion respiration capacity and increased ROS production compared to control cell lines carrying wild-type mtDNA (Moreno-Loshuertos et al. 2006). In agreement with these reports, mice carrying a mutation (D257A) that disrupts the exonuclease domain of the mtDNA polymerase γ exhibit a variety of premature aging phenotypes, including hair loss and graying, reduced life span, and early-onset AHL compared to age-matched wild-type mice (Kujoth et al. 2005; Someya et al. 2008). Moreover, young mtDNA mutator ($Polg^{D257A/D257A}$) mice displayed a 200- to 500-fold increase in mtDNA point mutations in the brain, heart, and inner ear (Vermulst et al. 2007; Kim et al. 2019), while mtDNA deletions accumulated at an accelerated rate (a 7- to 11-fold increase) in the brain, heart, and inner ear of mtDNA mutator mice with age. These results suggest that mtDNA mutations contribute to premature aging phenotypes in mtDNA mutator mice.

2.3 Apoptosis

2.3.1 Two Major Pathways of Apoptosis

An apoptosis program is thought to play a key role in aging and age-related diseases (Mattson 2000; Someya and Prolla 2010). Neuronal death also contributes to the symptoms of many neurological disorders, including Alzheimer's disease, Parkinson's disease, Huntington's diseases, stroke, and amyotrophic lateral sclerosis (Mattson 2000). Apoptosis can occur through two major pathways: the extrinsic pathway is a sensor for extracellular signals and is initiated through the ligation of tumor necrosis factors (TNFs). The intrinsic pathway or the mitochondrial pathway senses intracellular damage and is initiated when the outer mitochondrial membrane loses its integrity (Lindsten et al. 2000; Youle and Strasser 2008). The mitochondria apoptosis pathway is regulated by BCL-2 family members (Youle and Strasser 2008). Of the BCL-2 family members, the pro-apoptotic proteins BAX and BAK play a central role in promoting mitochondria-mediated apoptosis (Lindsten et al. 2000). Lindsten and colleagues (Lindsten et al. 2000) have shown that $Bak^{-/-}$ mice do not exhibit any gross abnormalities or developmental defects. However, the majority of mice lacking both *Bak* and *Bax* died prenatally with fewer than 10% of the mutants surviving into adulthood. Those $Bax^{-/-}$–$Bak^{-/-}$ mice displayed multiple developmental defects such as accumulation of excess cells within both the central nervous and hematopoietic systems, indicating that BAX and BAK play central roles in the regulation of apoptosis during development and tissue homeostasis. In

the extrinsic pathway, TNF is a cell signaling protein involved in immune and inflammatory responses and a major mediator of extrinsic apoptosis (Chen and Goeddel 2002; Chau et al. 2004). Activation of TNF signaling is involved in the pathogenesis of a wide spectrum of diseases, including diabetes, cancer, osteoporosis, and autoimmune diseases such as multiple sclerosis and inflammatory bowel disease (Chen and Goeddel 2002). TNF signals through two distinct cell surface receptors, TNF-R1 and TNF-R2. Of these receptors, TNF-R1 initiates the majority of TNF's apoptotic activities.

2.3.2 Influence of Aging on Apoptotic Genes in Cochlea

To investigate the association between apoptosis, cochlear aging and AHL, Frisina and colleagues (Tadros et al. 2008) examined by DNA microarray and real-time polymerase chain reaction (PCR) the expression of 318 apoptotic genes in the cochlea of CBA/CaJ mice at various ages: young mice with normal hearing, middle-aged mice with normal hearing, old mice with mild hearing loss, and old mice with severe hearing loss. Of the 318 apoptotic genes, 8 showed significant differences in mRNA expression that were validated by real-time PCR: activating transcription factor3 (*Atf3*), B cell leukemia/lymphoma 2 (*Bcl2*), Bcl2-like 1 (*Bcl2l1*), caspase4 (*Casp4*), Calpain 2 (*Capn2*), dual specificity phosphatase 9 (*Dusp9*), tumor necrosis factor receptor superfamily member 12a (*Tnfrsf12a*), and TNF superfamily member 13b (*Tnfsf13b*). Comparing the gene expressions of middle-aged mice with normal hearing, old mice with mild hearing loss, and old mice with severe hearing loss, seven genes (*Atf3*, *Bcl2*, *Bcl2l1*, *Casp4*, *Dusp9*, *Tnfrsf12a*, *Tnfsf13b*) showed up-regulation with age and hearing loss, while six genes (*Atf3*, *Bcl2*, *Bcl2l1*, *Casp4*, *Capn2*, and *Tnfrsf12a*) showed down-regulation in the middle-aged group compared to young mice with normal hearing. In mammals, there are at least 12 BCL-2 family proteins that have either three-dimensional structural similarity or a secondary structure that is similar to BCL-2 (Youle and Strasser 2008). These apoptosis proteins exhibit a range of bioactivities, from inhibition to promotion of apoptosis. For example, while BAX and BAK promote cell death, BCL-2 and BCL-CL inhibit apoptosis. Caspases are a family of protease enzymes that play an essential role in apoptosis (Cohen 1997; Hakem et al. 1998). The caspases are present in cells as inactive proenzymes that are activated in response to apoptotic stimulation. Activation of caspases during apoptosis leads to the cleavage of a variety of cellular proteins, leading to cell death. In mitochondrial apoptosis, cytochrome *c* initiates apoptosis by inducing the formation of the CASP9/APAF1 complex that is mediated by the interaction of their respective caspase recruitment domain (CARD) (Hakem et al. 1998; Youle and Strasser 2008). CASP4 and CASP8 can associate with APAF1 and CARD (Hu et al. 1998). In the extrinsic pathway, members of the TNF receptor family recruit and activate caspase-8 through the adaptor protein FAS-associated death domain (FADD) at the cell surface, which in turn causes subsequent activation