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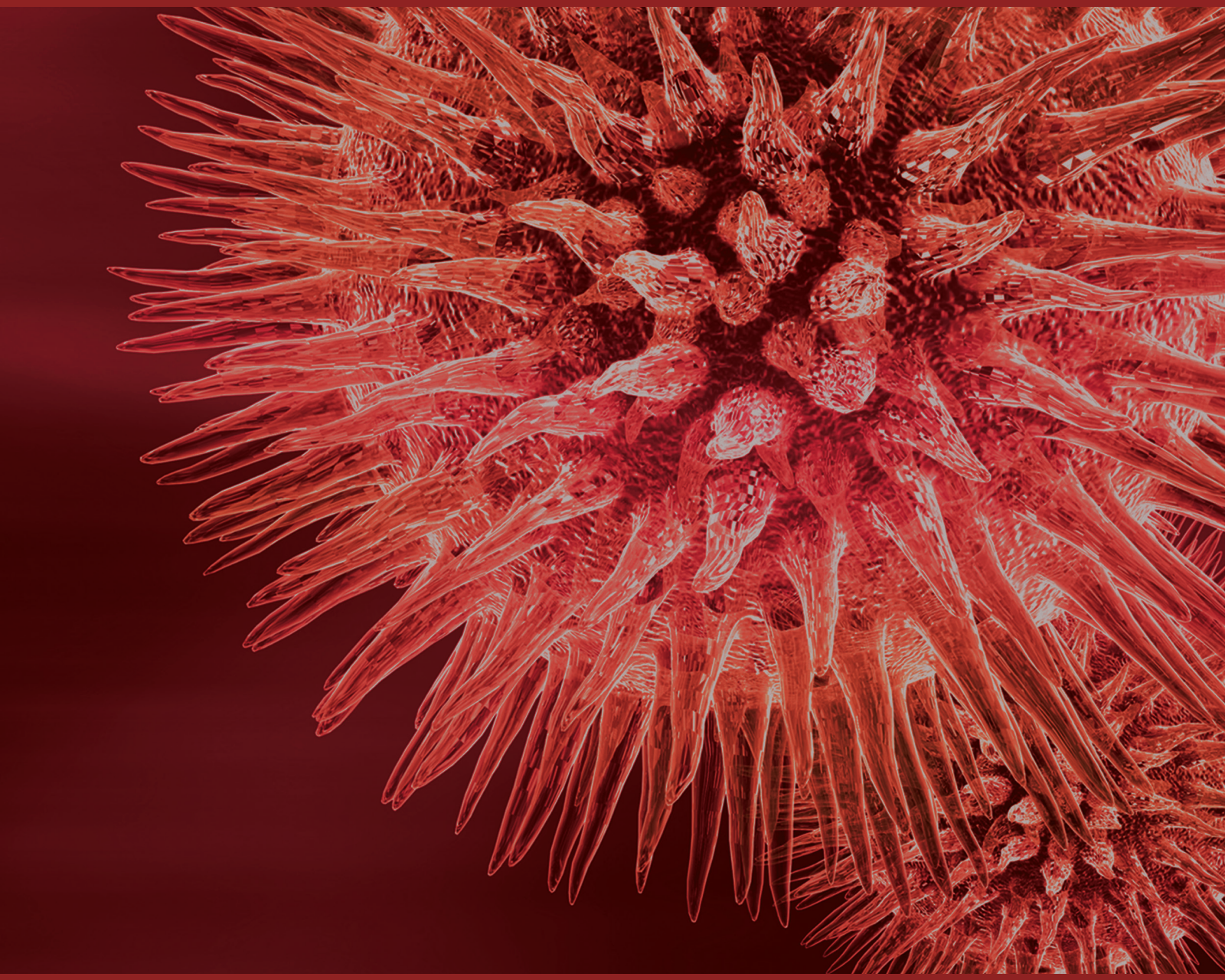
Preventing Hearing Loss and Restoring Hearing: A New Outlook

Guest Editors: Srdjan M. Vlajkovic, Peter R. Thorne, Ramesh Rajan, and Jonathan E. Gale



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Editorial

Preventing Hearing Loss and Restoring Hearing: A New Outlook

Srdjan M. Vlajkovic,[1] Peter R. Thorne,[1] Ramesh Rajan,[2] and Jonathan E. Gale[3]

[1]*Department of Physiology and Centre for Brain Research, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand*
[2]*Department of Physiology, Faculty of Medicine, Nursing and Health Sciences, Monash University, Clayton, Melbourne, VIC 3800, Australia*
[3]*The Ear Institute, University College London, London WCIX BEE, UK*

Correspondence should be addressed to Srdjan M. Vlajkovic; s.vlajkovic@auckland.ac.nz

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People with hearing loss represent one of the largest disability groups worldwide, and the prevalence of hearing loss is predicted to rise with an ageing population. Substantial progress has been made towards understanding some of the biological processes involved in the development of hearing impairment as well as therapeutic ways to prevent or mitigate the hearing loss. For example, we now have a better understanding of the maintenance and regeneration of the sensorineural tissues in the cochlea. The sensory hair cells in the cochlea can potentially be regenerated by reactivating genes that control their development, and various types of stem cells can be transformed into sensory hair cells or auditory neurones. In addition, drugs that reduce oxidative stress or prevent apoptosis have been shown to protect hearing from excessive noise and ototoxic drugs. Furthermore, our knowledge of central auditory changes associated with the peripheral injury or auditory processing problems is rapidly increasing.

In this special issue, we collected review and original research articles presenting current concepts in development, screening, prevention, rehabilitation, and therapeutic management of hearing loss. It is our wish to increase interest in hearing loss research with this special issue and further accelerate the development of novel therapies for hearing disorders.

Several papers are concerned with the therapeutic management of sensorineural hearing loss, including pharmacological and cell replacement therapies. Other papers deal with the mechanisms, biomarkers, early detection, and auditory plasticity in hearing loss. Finally, one paper analyses the risk factors for noise-induced tinnitus.

The review paper by S. Irving and colleagues (Bionics Institute, University of Melbourne) presents the benefits of electroacoustic stimulation to cochlear implant recipients with residual hearing. This review also outlines the experimental and clinical strategies aimed at promoting the survival of sensory hair cells and spiral ganglion neurones in cochlear implant recipients.

Auditory nerve regeneration by human neural precursor cells (HNPC) was demonstrated by Y. Jiao et al. The study shows that BDNF supplementation improves the survival of HNPC and their migration into the brainstem in the vicinity of the cochlear nucleus. However, functional improvement after implantation of HNPC is yet to be confirmed.

Pharmacological treatment of acute noise-induced hearing loss (NIHL) is a relatively novel research field. S. M. Vlajkovic et al. report that adenosine amine congener (ADAC), a selective A_1 adenosine receptor agonist, mitigates NIHL in a dose- and time-dependent manner. ADAC thus holds potential as a clinical treatment for noise-induced cochlear injury.

Ototoxicity is a significant impediment to anticancer treatment by cisplatin; hence different experimental and clinical strategies for prevention of cisplatin ototoxicity are reviewed by F. Chirtes and S. Albu. Gene therapies with

antioxidant enzymes, neurotrophic factors, and antiapoptotic molecules combined with the intratympanic route of administration are proposed as the most promising future therapeutic options.

Understanding speech in background noise poses significant demands on cognitive resources. M. Rudner and T. Lunner in their review paper analyse cognitive spare capacity (CSC) in hearing impaired individuals and suggest ways to improve CSC with cognitive training and optimal balance of visual, phonological, and semantic information.

There is a strong need for fast and effective programs of hearing screening and surveillance. While tools exist in English, there is often a paucity of validated instruments in other languages. N. Vaez and colleagues describe the development of the Speech Understanding in Noise Test in Portuguese language, which can be used to identify individuals with hearing impairment and refer them for further audiological assessment. This provides a template for development of such tests in other languages as necessary.

According to D. Bakhos et al., children with bilateral sensorineural hearing loss and language impairment exhibit atypical temporal cortical auditory evoked potentials (CAEP), not present in hearing impaired children with normal language development. The authors propose that CAEP temporal responses can be used as a biomarker of auditory cortex maturation in children with congenital hearing loss.

The development of spontaneous activity in the auditory system prior to the onset of hearing depends on a transient cochlear structure known as Kölliker's organ. The review article by M. W. N. Dayaratne et al. describes the role of purinergic signalling in this process and how the developmental abnormalities of Kölliker's organ may lead to congenital hearing loss.

Oxidative stress is one of the principal mechanisms of sensorineural hearing loss caused by noise, ototoxic drugs, and ageing process. Excessive production of reactive oxygen species (ROS) in mitochondria reduces the antioxidant defence mechanisms and activates cell death pathways in cochlear tissues. Oxidative stress pathways leading to cell death and hearing loss are succinctly reviewed by T. Kamogashira et al. in this special issue.

Y. Zheng et al. demonstrate that acute sleep disruption does not exacerbate the perception of noise-induced tinnitus in rats, suggesting that insomnia may not be a significant risk factor/predictor for tinnitus severity as previously assumed.

We hope that this special issue will serve as a useful resource for auditory neuroscientists and clinicians and all those who have a keen interest in this research field.

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Finally, as guest editors, we greatly appreciate the contributions of all authors and anonymous reviewers.

Srdjan M. Vlajkovic
Peter R. Thorne
Ramesh Rajan
Jonathan E. Gale

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Review Article

Reactive Oxygen Species, Apoptosis, and Mitochondrial Dysfunction in Hearing Loss

Teru Kamogashira, Chisato Fujimoto, and Tatsuya Yamasoba

Department of Otolaryngology and Head and Neck Surgery, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8665, Japan

Correspondence should be addressed to Tatsuya Yamasoba; tyamasoba-tky@umin.ac.jp

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Reactive oxygen species (ROS) production is involved in several apoptotic and necrotic cell death pathways in auditory tissues. These pathways are the major causes of most types of sensorineural hearing loss, including age-related hearing loss, hereditary hearing loss, ototoxic drug-induced hearing loss, and noise-induced hearing loss. ROS production can be triggered by dysfunctional mitochondrial oxidative phosphorylation and increases or decreases in ROS-related enzymes. Although apoptotic cell death pathways are mostly activated by ROS production, there are other pathways involved in hearing loss that do not depend on ROS production. Further studies of other pathways, such as endoplasmic reticulum stress and necrotic cell death, are required.

1. Introduction

Reactive oxygen species (ROS), such as hydroxyl radicals, superoxide anions, hydrogen peroxide, and singlet oxygen are mainly generated by the mitochondria in most mammalian cells [1, 2]. ROS, which are regarded as toxic products of cellular metabolism, can function as signaling molecules that regulate many physiological processes [3]. ROS play an important role in apoptosis induction under both physiological and pathological conditions; previous studies have shown that oxidative stress can cause cellular apoptosis via both the extrinsic cell death receptor pathway and the intrinsic mitochondrial cell death pathway [4]. The accumulation of ROS and subsequent apoptosis induction is an important contributor to several diseases and aging [5].

Elevated ROS formation and subsequent apoptosis induction have been implicated in the development of several hearing loss pathologies [6]. Furthermore, mitochondrial dysfunction plays an important role in some types of hearing loss [7]. In this review, we will focus on the involvement of ROS, mitochondrial dysfunction, and apoptosis induction in hearing loss pathology.

2. Cochlea and Hearing Loss

The cochlea is the auditory end organ of the inner ear. The organ of Corti is a core component of the cochlea and contains two types of sensory hair cells: inner and outer hair cells. When a sound pressure wave travels from the base to the apex of the cochlea, the basilar membrane of the cochlea vibrates [8]. The displacement of stereocilia—the mechanosensing organelles of the hair cells—results from basilar membrane vibration and opens the transduction ion channels. This causes potassium and calcium ions to enter, generating a transduction current that activates the voltage-dependent calcium channels along the lateral wall and base of the hair cell [9]. The inner hair cells release the neurotransmitter glutamate to encode acoustic signals for the postsynaptic afferent neuron [10]. The outer hair cells are much more sensitive to damage than the inner hair cells.

There are other components and supporting cells in the cochlea which maintain the structure and the environment of the cochlea. The stria vascularis and the spiral ligament are located at the lateral wall of the cochlea and generate the endocochlear potential (EP) [11]. EP is the positive voltage

in the endolymphatic space of the cochlea and is essential for driving the potassium current through the transduction channels and stimulation of the hair cells [12]. The spiral ganglion is located at the modiolus and transfers the neuronal sound information from the hair cells to the brain. Several types of supporting cells are located in the cochlea and maintain homeostasis and the vibration of the cochlea; however, some of the mechanical functions are still under investigation [13].

Hearing loss is a reduction in sound sensitivity and is roughly divided into two types: acquired hearing loss and inherited hearing loss. The well-known types of acquired hearing loss are ototoxic drug-induced hearing loss (ODIHL), age-related hearing loss (ARHL), and noise-induced hearing loss (NIHL). The pathological features of each type of hearing loss are different. ODIHL is the commonly irreversible hearing loss caused by the intake of ototoxic drugs and the main mechanism is the loss of the hair cell [14]. NIHL is the partially irreversible hearing loss caused by exposure to loud sounds and the main mechanisms are mechanical damage to the organ of Corti and the loss of hair cells and the spiral ganglion [15]. ARHL, also known as presbycusis, is the progressive hearing loss associated with aging and the main mechanisms are the loss of hair cells, spiral ganglion cells, and stria vascularis cells [16]. The neural injury without hearing loss in noise exposure or aging is suggested in a mouse model [17]. Inherited hearing loss is caused by the dysfunction of some components of the cochlea, some of which are well researched, such as the hair cell [18], the tectorial membrane [19], and EP [20, 21].

3. Mitochondrial DNA Mutation Diseases Related to Hearing Loss

Many chromosomal genes encoding proteins have been associated with hereditary hearing loss, such as myosin [18], extracellular matrix [19], cadherin [22], ion channels [23], and transfer RNA (tRNA) or ribosomal RNA (rRNA) coding mitochondrial genes [24]. The mechanisms of mitochondrial dysfunction in certain gene mutations that encode mitochondrial rRNA or tRNA have recently been described. Hereditary hearing loss with aminoglycoside hypersusceptibility will be discussed in the next section.

Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome [25] are all associated with hearing loss [26, 27]. MELAS mutations occur in mitochondrial genes [24, 26] and cause mitochondrial dysfunction. The mechanisms of mitochondrial translation are independent of chromosomal translation and the genes encoding mitochondrial tRNA are encoded in the mitochondrial DNA. The most common MELAS mutation is a 3243A>G mutation, which changes the structure of the mitochondrial leucine tRNA. Moreover, the third nucleotide of the anticodon loop of mitochondrial leucine tRNA is uracil and is modified by taurine modifying enzymes. The enzymes are hypothesized to be GTP-binding protein 3 (GTPBP3) and mitochondrial translation optimization 1 (MTO1) [28, 29] encoded on chromosomes. The structural change in tRNA inhibits the taurine modification of the uracil [30]. The modified anticodon loop of tRNA can pair adenine and guanine, the unmodified anticodon loop can pair adenine, and the unmodified tRNA inhibits the translation of UUG to leucine. The ND6 subunit of the mitochondrial oxidative phosphorylation (OXPHOS) enzyme complex I is encoded in mitochondrial DNA and its triplets hold the UUG codon; therefore, enzyme activity decreases in mitochondria with the 3243A>G mutation [31]. The decreased activity of the OXPHOS electron transport chain leads to an increased ROS production. This then induces the opening of nonspecific high conductance permeability transition pores in the mitochondrial inner membrane, decreased mitochondrial membrane potential, increased mitophagy, and apoptotic cell death [32]. MTO1 mutations show similar symptoms to MELAS [33], although their impact on the cochlear function is poorly understood.

The mutation rate of mitochondrial DNA differs between tissues. Rates are higher in the spiral ganglion cells and saccular macula than in the hair cells of the organ of Corti, the stria vascularis, and the facial nerve [34]. There is a good correlation between the mutation rate and histological findings [35]. These results indicate that the differences in OXPHOS activity between tissues affect both the organ activity and specific clinical symptoms.

4. Hearing Loss Induced by Ototoxic Drugs

Two types of ototoxic drug classes are widely known in clinical practice [36]: aminoglycoside antibiotics and platinum-based anticancer drugs. Both drug classes mainly damage the hair cells in the organ of Corti through ROS production via apoptotic pathways.

Aminoglycosides are broad-spectrum antibiotics that require a close monitoring of their potential ototoxicity and nephrotoxicity [37]. The nephrotoxicity is generally reversible because the cells of the proximal convoluted tubules of the kidney can proliferate and recover [38], but the ototoxicity is irreversible because the hair cells of the cochlea cannot proliferate and recover. Aminoglycosides probably damage the outer hair cells relative to the inner hair cells by triggering differential apoptotic signals [39]. In addition, the basal turn hair cells, which process high frequency sounds, are preferentially damaged compared with the hair cells in the apical turn, which process low frequency sound [40]. Thus, aminoglycoside use needs a careful clinical evaluation of the indication.

ROS are now established as the main initiators of aminoglycoside-induced hearing loss [41]. Aminoglycosides tend to accumulate in the mitochondria of the hair cells [42]; gentamicin directly inhibits protein synthesis in mitochondrial ribosomes [43] and triggers mitochondrial permeability transition pore opening [44].

The 1555A>G mitochondrial DNA mutation causes hereditary hearing loss with known aminoglycoside hypersusceptibility [45]. The 12S rRNA gene is encoded at the mutation and changes the rRNA conformation to bind with

aminoglycosides more tightly than when normally configured [43]. However, the precise mechanism of aminoglycoside interaction with rRNA is still under investigation [46]. Other mutations of mitochondrial DNA associated with aminoglycoside hypersusceptible hearing loss have been found in a recent study [47].

Platinum-based anticancer drugs are frequently used in the treatment of many types of cancer including squamous cell carcinoma; adenocarcinoma; and undifferentiated carcinoma of the head, neck, lung, and bladder [48]. However, they have toxic effects on the cochlea, kidney, and neurons. The ototoxicity of cisplatin is widely known [49] and drugs that offer protection against ototoxicity have been studied [50]. Cisplatin acts as a DNA crosslinker in tumor cells, where its platinum atom binds to purine bases and inhibits cell proliferation, which inactivates the cell cycle and causes tumor cell apoptosis [51].

Cisplatin has both acute and chronic toxic effects on cochlear tissue. The acute effect is a reversible inhibition of transduction currents and voltage-dependent calcium currents in the hair cells [52] and the reaction of currents in the stria vascularis. The long lasting toxic reaction makes cochlear tissue to trigger ROS production and potassium conductance change [53], which cause apoptotic cell death [54]. These chronic effects are irreversible and the outer hair cells [55], the marginal cells of the stria vascularis [56], and the spiral ganglion cells [57] tend to degenerate when compared with the inner hair cells. The most common type of cisplatin ototoxicity results in a bilateral, high frequency, and sensorineural hearing loss [58].

ROS formations in the outer hair cells in response to cisplatin represent the binding of cisplatin to the sulfhydryl group of enzymes and the depletion of nicotinamide adenine dinucleotide phosphate (NADPH), copper, or selenium [59]. These processes are essential for glutathione peroxidase and glutathione reductase activity and NADPH oxidase activations [60]. NADPH oxidase 3 (NOX3), one of the six NADPH oxidases, is highly expressed in the organ of Corti [61]. Moreover, superoxide production of NOX3 increases under cisplatin treatment [61]. The other NADPH oxidases are also important in ROS production associated with cisplatin ototoxicity [60].

The increased ROS generation reduces the antioxidant defense mechanisms of the outer hair cells, causing the release of cytochrome c from mitochondria, activating the caspase pathways, and triggering apoptotic cell death [51]. The cytochrome c increase also activates caspases-3 and caspases-9, which trigger deoxyribonuclease activity [62].

Other potential apoptotic pathways in the stria vascularis of the lateral wall or in the spiral ganglion include the activation of nuclear factor kappa B (NF-κB) and the formation of nitric oxide (NO) [56] and the activation of high-mobility group protein 1 (HMG1), NO production, and 4-hydroxynonenal (4-HNE) production [63]. Increased NO levels have been shown in a rat model [64] and increased NF-κB and inductable nitric oxide synthase (iNOS) immunolabeling [65, 66] have been shown. These results indicate that NO and iNOS trigger apoptosis in the stria vascularis [66]. The higher level of HMG1 expression in modiolar tissue than kidney tissue [67] and the increase of iNOS in the spiral ganglion cells one day after cisplatin treatment [67] indicate that different apoptotic cell death pathways exist in the spiral ganglion.

5. Age-Related Hearing Loss

The prevalence of ARHL is expected to increase with aging population [7, 68–70]. Although many factors have been researched including environmental, hereditary, and medical factors [71, 72], the precise mechanism of ARHL is not yet understood.

The accumulation of mitochondrial DNA mutations is hypothesized to cause age-related degenerative diseases such as ARHL [73]. Increases of mitochondrial DNA mutations in cochlear tissue have been shown in humans [74]. Mitochondrial DNA replicates frequently and independently of the cell cycle and mitochondrial DNA mutations tend to accumulate more than chromosomal DNA mutations because mitochondrial DNA lacks protective histones. The same mechanism is suggested in mouse models of ARHL [75, 76]. The major mitochondrial DNA mutations occur in the genes encoding mitochondrial OXPHOS complexes and lead to dysfunctional OXPHOS activity. ROS formation in the dysfunctional mitochondria, decreased mitochondrial membrane potentials, and the activation of apoptotic pathways most likely causes hair cell death; however, other pathways are also hypothesized.

Because ROS play an important role in ARHL [77], the effects of supplementation of antioxidants against ARHL have been studied. In Fischer 344 rats, vitamin C, vitamin E, melatonin, and lazaroid had better effects in preserving auditory sensitivities and reducing mtDNA deletions than a placebo [78]. In C57BL/6 mice, vitamin C did not affect ARHL [79], but a combination of multiple antioxidant agents (L-cysteine-glutathione mixed disulfide, ribose-cysteine, NW-nitro-L-arginine methyl ester, vitamin B12, folate, and ascorbic acid) had significantly better effects on preserving auditory sensitivity than the control agents [80]. In CBA/J mice, supplementation with vitamin A, vitamin C, vitamin E, L-carnitine, and a-lipoic acid significantly increased the antioxidant capacity of inner ear tissues but did not improve the loss of hair cells and spiral ganglion cells and the progression of ARHL [81]. The prevention of ARHL by antioxidant supplementation is influenced by many factors, such as the type and dosage of antioxidant, the duration and timing of the treatment, and the species.

Intrinsic and extrinsic pathways are involved in apoptotic cell death in ARHL. The intrinsic pathway is mitochondrial dependent and is triggered by a loss of the mitochondrial membrane potential. The extrinsic pathway is triggered by ligands that bind to cell surface receptors [82, 83]. Furthermore, the prevention of ARHL following the deletion of the mitochondrial proapoptotic gene, brassinosteroid insensitive-1-associated receptor kinase (*Bak*) [84], indicates that the intrinsic apoptotic pathway is necessary for ARHL.

6. Noise-Induced Hearing Loss

Noise is also a major cause of hearing loss [85]. It is often associated with the military, clubs, discos, and portable audio players; the prevalence of noise-induced hearing loss is predicted to increase over the coming years [15].

Two main pathways result in cochlear damage following noise exposure: mechanical damage [86] and biochemical pathways triggering apoptosis or necrosis. The outer hair cells are much more sensitive to noise exposure than the inner hair cells. Morphological nuclear changes [87] and increases in apoptotic markers, such as caspase [88], tumor necrosis factor receptor [89], and associated promoters [90] occur in mouse or rat models. These indicate the importance of apoptotic pathways in noise-induced hearing loss.

Several pathways that trigger apoptotic cell death in noise-induced hearing loss have been studied in animal models. Studies have revealed common increases in ROS or similar reactive species [91] but ROS formation tends to decrease over time [92]. The mitochondrial release of apoptosis-inducing factor (AIF) and mitochondrial endonuclease G (EndoG) into the cytosol cochlear cells after noise exposure has been shown in guinea pig models [93]. The c-Jun N-terminal kinase (JNK) signaling mitogen-activated protein kinase (MAPK) pathways that mediate cells entering programmed apoptosis are also increased after sound trauma in guinea pig models [94]. In addition, JNK signaling pathways are activated by ROS formation [95] and other apoptotic pathways are hypothesized in noise-induced hearing loss.

Other pathways which do not depend on ROS production have been predicted. The increase of free Ca^{2+} in the outer hair cells [96] or the activation of Ca^{2+} and calmodulin-controlled calcineurin [97] may trigger apoptotic or necrotic cell death pathway without ROS production. The decrease in blood flow [98] caused by vasoactive products [99] leads to ischemia and may contribute to the damage of cochlear tissue. The excessive release of the neurotransmitter glutamate from the inner hair cells can trigger defects in the synaptic connections in the auditory nerve and cause spiral ganglion cell death [100].

7. Conclusion

ROS production and mitochondrial apoptotic pathways play important roles in many types of hearing loss. Major ROS production pathways include OXPHOS dysfunction, increased pro-ROS enzyme activity, and decreased anti-ROS activity. Hearing loss pathways vary and some remain under investigation. Other pathways, such as ER stress and necrotic cell death, are also involved in hearing loss. Further studies of each type of hearing loss are required including the investigation of ROS, apoptosis, and other types of cell death.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Teru Kamogashira and Chisato Fujimoto equally contributed to the study.

References

[1] R. S. Balaban, S. Nemoto, and T. Finkel, "Mitochondria, oxidants, and aging," *Cell*, vol. 120, no. 4, pp. 483–495, 2005.

[2] J. F. Turrens, "Mitochondrial formation of reactive oxygen species," *Journal of Physiology*, vol. 552, no. 2, pp. 335–344, 2003.

[3] L. A. Sena and N. S. Chandel, "Physiological roles of mitochondrial reactive oxygen species," *Molecular Cell*, vol. 48, no. 2, pp. 158–167, 2012.

[4] K. Sinha, J. Das, P. B. Pal, and P. C. Sil, "Oxidative stress: the mitochondria-dependent and mitochondria-independent pathways of apoptosis," *Archives of Toxicology*, vol. 87, no. 7, pp. 1157–1180, 2013.

[5] W. C. Orr and R. S. Sohal, "Extension of life-span by overexpression of superoxide dismutase and catalase in Drosophila melanogaster," *Science*, vol. 263, no. 5150, pp. 1128–1130, 1994.

[6] K. Op de Beeck, J. Schacht, and G. Van Camp, "Apoptosis in acquired and genetic hearing impairment: the programmed death of the hair cell," *Hearing Research*, vol. 281, no. 1-2, pp. 18–27, 2011.

[7] T. Yamasoba, S. Someya, C. Yamada, R. Weindruch, T. A. Prolla, and M. Tanokura, "Role of mitochondrial dysfunction and mitochondrial DNA mutations in age-related hearing loss," *Hearing Research*, vol. 226, no. 1-2, pp. 185–193, 2007.

[8] T. Ren, "Longitudinal pattern of basilar membrane vibration in the sensitive cochlea," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 26, pp. 17101–17106, 2002.

[9] M. LeMasurier and P. G. Gillespie, "Hair-cell mechanotransduction and cochlear amplification," *Neuron*, vol. 48, no. 3, pp. 403–415, 2005.

[10] P. A. Fuchs, E. Glowatzki, and T. Moser, "The afferent synapse of cochlear hair cells," *Current Opinion in Neurobiology*, vol. 13, no. 4, pp. 452–458, 2003.

[11] A. N. Salt, I. Melichar, and R. Thalmann, "Mechanisms of endocochlear potential generation by stria vascularis," *Laryngoscope*, vol. 97, no. 8, part 1, pp. 984–991, 1987.

[12] P. Wangemann, "Supporting sensory transduction: cochlear fluid homeostasis and the endocochlear potential," *Journal of Physiology*, vol. 576, no. 1, pp. 11–21, 2006.

[13] S. S. Gao, A. Xia, T. Yuan et al., "Quantitative imaging of cochlear soft tissues in wild-type and hearing-impaired transgenic mice by spectral domain optical coherence tomography," *Optics Express*, vol. 19, no. 16, pp. 15415–15428, 2011.

[14] M. E. Huth, A. J. Ricci, and A. G. Cheng, "Mechanisms of aminoglycoside ototoxicity and targets of hair cell protection," *International Journal of Otolaryngology*, vol. 2011, Article ID 937861, 19 pages, 2011.

[15] O. Hong, M. J. Kerr, G. L. Poling, and S. Dhar, "Understanding and preventing noise-induced hearing loss," *Disease-A-Month*, vol. 59, no. 4, pp. 110–118, 2013.

[16] H. Chen and J. Tang, "The role of mitochondria in age-related hearing loss," *Biogerontology*, vol. 15, no. 1, pp. 13–19, 2014.

[17] S. G. Kujawa and M. C. Liberman, "Adding insult to injury: Cochlear nerve degeneration after "temporary" noise-induced

hearing loss," *Journal of Neuroscience*, vol. 29, no. 45, pp. 14077–14085, 2009.

[18] X.-Z. Liu, C. Hope, J. Walsh et al., "Mutations in the myosin VIAA gene cause a wide phenotypic spectrum, including atypical usher syndrome," *The American Journal of Human Genetics*, vol. 63, no. 3, pp. 909–912, 1998.

[19] K. Verhoeven, L. Van Laer, K. Kirschhofer et al., "Mutations in the human α-tectorin gene cause autosomal dominant non-syndromic hearing impairment," *Nature Genetics*, vol. 19, no. 1, pp. 60–62, 1998.

[20] Q. Gopen, G. Zhou, K. Whittemore, and M. Kenna, "Enlarged vestibular aqueduct: review of controversial aspects," *The Laryngoscope*, vol. 121, no. 9, pp. 1971–1978, 2011.

[21] X. Li, F. Zhou, D. C. Marcus, and P. Wangemann, "Endolymphatic Na^+ and K^+ concentrations during cochlear growth and enlargement in mice lacking *Slc26a4*/pendrin," *PLoS ONE*, vol. 8, no. 5, Article ID e65977, 2013.

[22] Z. M. Ahmed, S. Riazuddin, J. Ahmad et al., "PCDH15 is expressed in the neurosensory epithelium of the eye and ear and mutant alleles are responsible for both USH1F and DFNB23," *Human Molecular Genetics*, vol. 12, no. 24, pp. 3215–3223, 2003.

[23] X. C. Li, L. A. Everett, A. K. Lalwani et al., "A mutation in PDS causes non-syndromic recessive deafness," *Nature Genetics*, vol. 18, no. 3, pp. 215–217, 1998.

[24] Y.-I. Goto, I. Nonaka, and S. Horai, "A mutation in the $tRNA^{Leu(UUR)}$ gene associated with the MELAS subgroup of mitochondrial encephalomyopathies," *Nature*, vol. 348, no. 6302, pp. 651–653, 1990.

[25] Y. Oka, H. Katagiri, H. Ishihara, T. Asano, T. Kobayashi, and M. Kikuchi, "β-Cell loss and glucose induced signalling defects in diabetes mellitus caused by mitochondrial tRNA(Leu(UUR)) gene mutation," *Diabetic Medicine*, vol. 13, no. 9, supplement 6, pp. S98–S102, 1996.

[26] J. M. W. Van Den Ouweland, H. H. P. J. Lemkes, W. Ruitenbeek et al., "Mutation in mitochondrial tRMALeu(UUR) gene in a large pedigree with maternally transmitted type II diabetes mellitus and deafness," *Nature Genetics*, vol. 1, no. 5, pp. 368–371, 1992.

[27] C. T. Moraes, F. Ciacci, G. Silvestri et al., "Atypical clinical presentations associated with the MELAS mutation at position 3243 of human mitochondrial DNA," *Neuromuscular Disorders*, vol. 3, no. 1, pp. 43–50, 1993.

[28] X. Li and M.-X. Guan, "A human mitochondrial GTP binding protein related to tRNA modification may modulate phenotypic expression of the deafness-associated mitochondrial 12S rRNA mutation," *Molecular and Cellular Biology*, vol. 22, no. 21, pp. 7701–7711, 2002.

[29] M. Villarroya, S. Prado, J. M. Esteve et al., "Characterization of human GTPBP3, a GTP-binding protein involved in mitochondrial tRNA modification," *Molecular and Cellular Biology*, vol. 28, no. 24, pp. 7514–7531, 2008.

[30] T. Suzuki, K. Miyauchi, T. Suzuki et al., "Taurine-containing uridine modifications in tRNA anticodons are required to decipher non-universal genetic codes in ascidian mitochondria," *Journal of Biological Chemistry*, vol. 286, no. 41, pp. 35494–35498, 2011.

[31] T. Yasukawa, Y. Kirino, N. Ishii et al., "Wobble modification deficiency in mutant tRNAs in patients with mitochondrial diseases," *FEBS Letters*, vol. 579, no. 13, pp. 2948–2952, 2005.

[32] S. C. Zapico and D. H. Ubelaker, "mtDNA mutations and their role in aging, diseases and forensic sciences," *Aging and Disease*, vol. 4, no. 6, pp. 364–380, 2013.

[33] D. Ghezzi, E. Baruffini, T. B. Haack et al., "Mutations of the mitochondrial-tRNA modifier MTO1 cause hypertrophic cardiomyopathy and lactic acidosis," *The American Journal of Human Genetics*, vol. 90, no. 6, pp. 1079–1087, 2012.

[34] H. Koda, Y. Kimura, I. Ishige, Y. Eishi, Y. Iino, and K. Kitamura, "Quantitative cellular level analysis of mitochondrial DNA 3243A > G mutations in individual tissues from the archival temporal bones of a MELAS patient," *Acta Oto-Laryngologica*, vol. 130, no. 3, pp. 344–350, 2010.

[35] K. Takahashi, S. N. Merchant, T. Miyazawa et al., "Temporal bone histopathological and quantitative analysis of mitochondrial DNA in MELAS," *The Laryngoscope*, vol. 113, no. 8, pp. 1362–1368, 2003.

[36] J. Schacht, A. E. Talaska, and L. P. Rybak, "Cisplatin and aminoglycoside antibiotics: hearing loss and its prevention," *Anatomical Record*, vol. 295, no. 11, pp. 1837–1850, 2012.

[37] J. M. Lopez-Novoa, Y. Quiros, L. Vicente, A. I. Morales, and F. J. Lopez-Hernandez, "New insights into the mechanism of aminoglycoside nephrotoxicity: an integrative point of view," *Kidney International*, vol. 79, no. 1, pp. 33–45, 2011.

[38] J. S. Duffield and J. V. Bonventre, "Kidney tubular epithelium is restored without replacement with bone marrow-derived cells during repair after ischemic injury," *Kidney International*, vol. 68, no. 5, pp. 1956–1961, 2005.

[39] A. G. Cheng, L. L. Cunningham, and E. W. Rubel, "Hair cell death in the avian basilar papilla: characterization of the in vitro model and caspase activation," *Journal of the Association for Research in Otolaryngology*, vol. 4, no. 1, pp. 91–105, 2003.

[40] H. C. Jensen-Smith, R. Hallworth, and M. G. Nichols, "Gentamicin rapidly inhibits mitochondrial metabolism in high-frequency cochlear outer hair cells," *PLoS ONE*, vol. 7, no. 6, Article ID e38471, 2012.

[41] T. R. van de Water, R. N. Abi-Hachem, and A. Zine, "The injured cochlea as a target for inflammatory processes, initiation of cell death pathways and application of related otoprotective strategies," *Recent Patents on CNS Drug Discovery*, vol. 5, no. 2, pp. 147–163, 2010.

[42] W. Marcotti, S. M. van Netten, and C. J. Kros, "The aminoglycoside antibiotic dihydrostreptomycin rapidly enters mouse outer hair cells through the mechano-electrical transducer channels," *Journal of Physiology*, vol. 567, no. 2, pp. 505–521, 2005.

[43] S. N. Hobbie, S. Akshay, S. K. Kalapala, C. M. Bruell, D. Shcherbakov, and E. C. Böttger, "Genetic analysis of interactions with eukaryotic rRNA identify the mitoribosome as target in aminoglycoside ototoxicity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 52, pp. 20888–20893, 2008.

[44] N. Dehne, U. Rauen, H. de Groot, and J. Lautermann, "Involvement of the mitochondrial permeability transition in gentamicin ototoxicity," *Hearing Research*, vol. 169, no. 1-2, pp. 47–55, 2002.

[45] T. Tono, K. Kiyomizu, K. Matsuda et al., "Different clinical characteristics of aminoglycoside-induced profound deafness with and without the 1555 A → G mitochondrial mutation," *ORL*, vol. 63, no. 1, pp. 25–30, 2001.

[46] E. C. Böttger, "Mutant A1555G mitochondrial 12S rRNA and aminoglycoside susceptibility," *Antimicrobial Agents and Chemotherapy*, vol. 54, no. 7, pp. 3073–3074, 2010.

[47] M. A. Dowlati, P. Derakhshandeh-Peykar, M. Houshmand et al., "Novel nucleotide changes in mutational analysis of mitochondrial 12SrRNA gene in patients with nonsyndromic

and aminoglycoside-induced hearing loss," *Molecular Biology Reports*, vol. 40, no. 3, pp. 2689–2695, 2013.

[48] E. E. Vokes, "Induction chemotherapy for head and neck cancer: recent data," *The Oncologist*, vol. 15, supplement 3, pp. 3–7, 2010.

[49] L. P. Rybak and V. Ramkumar, "Ototoxicity," *Kidney International*, vol. 72, no. 8, pp. 931–935, 2007.

[50] G. W. Hill, D. K. Morest, and K. Parham, "Cisplatin-induced ototoxicity: effect of intratympanic dexamethasone injections," *Otology and Neurotology*, vol. 29, no. 7, pp. 1005–1011, 2008.

[51] L. P. Rybak, C. A. Whitworth, D. Mukherjea, and V. Ramkumar, "Mechanisms of cisplatin-induced ototoxicity and prevention," *Hearing Research*, vol. 226, no. 1-2, pp. 157–167, 2007.

[52] T. Kimitsuki, T. Nakagawa, K. Hisashi, S. Komune, and S. Komiyama, "Cisplatin blocks mechano-electric transducer current in chick cochlear hair cells," *Hearing Research*, vol. 71, no. 1-2, pp. 64–68, 1993.

[53] W. J. Clerici, K. Hensley, D. L. DiMartino, and D. A. Butterfield, "Direct detection of ototoxicant-induced reactive oxygen species generation in cochlear explants," *Hearing Research*, vol. 98, no. 1-2, pp. 116–124, 1996.

[54] J. R. García-Berrocal, J. Nevado, R. Ramírez-Camacho et al., "The anticancer drug cisplatin induces an intrinsic apoptotic pathway inside the inner ear," *British Journal of Pharmacology*, vol. 152, no. 7, pp. 1012–1020, 2007.

[55] S. A. Alam, K. Ikeda, T. Oshima et al., "Cisplatin-induced apoptotic cell death in Mongolian gerbil cochlea," *Hearing Research*, vol. 141, no. 1-2, pp. 28–38, 2000.

[56] J. E. Lee, T. Nakagawa, T. Kita et al., "Mechanisms of apoptosis induced by cisplatin in marginal cells in mouse stria vascularis," *ORL*, vol. 66, no. 3, pp. 111–118, 2004.

[57] J. E. Lee, T. Nakagawa, T. S. Kim et al., "A novel model for rapid induction of apoptosis in spiral ganglions of mice," *The Laryngoscope*, vol. 113, no. 6, pp. 994–999, 2003.

[58] M. Anniko and A. Sobin, "Cisplatin: evaluation of its ototoxic potential," *American Journal of Otolaryngology*, vol. 7, no. 4, pp. 276–293, 1986.

[59] R. S. DeWoskin and J. E. Riviere, "Cisplatin-induced loss of kidney copper and nephrotoxicity is ameliorated by single dose diethyldithiocarbamate, but not mesna," *Toxicology and Applied Pharmacology*, vol. 112, no. 2, pp. 182–189, 1992.

[60] H.-J. Kim, J.-H. Lee, S.-J. Kim et al., "Roles of NADPH oxidases in cisplatin-induced reactive oxygen species generation and ototoxicity," *Journal of Neuroscience*, vol. 30, no. 11, pp. 3933–3946, 2010.

[61] B. Bánfi, B. Malgrange, J. Knisz, K. Steger, M. Dubois-Dauphin, and K.-H. Krause, "NOX_3, a superoxide-generating NADPH oxidase of the inner ear," *The Journal of Biological Chemistry*, vol. 279, no. 44, pp. 46065–46072, 2004.

[62] K.-I. Watanabe, S. Inai, K. Jinnouchi, S. Baba, and T. Yagi, "Expression of caspase-activated deoxyribonuclease (CAD) and caspase 3 (CPP32) in the cochlea of cisplatin (CDDP)-treated guinea pigs," *Auris Nasus Larynx*, vol. 30, no. 3, pp. 219–225, 2003.

[63] J. E. Lee, T. Nakagawa, T. S. Kim et al., "Role of reactive radicals in degeneration of the auditory system of mice following cisplatin treatment," *Acta Oto-Laryngologica*, vol. 124, no. 10, pp. 1131–1135, 2004.

[64] T. C. Kelly, C. A. Whitworth, K. Husain, and L. P. Rybak, "Aminoguanidine reduces cisplatin ototoxicity," *Hearing Research*, vol. 186, no. 1-2, pp. 10–16, 2003.

[65] I. Nagy, A. Monge, A. Albinger-Hegyi, S. Schmid, and D. Bodmer, "NF-κB is required for survival of immature auditory hair cells in vitro," *Journal of the Association for Research in Otolaryngology*, vol. 6, no. 3, pp. 260–268, 2005.

[66] K. Watanabe, S. Inai, K. Jinnouchi et al., "Nuclear-factor kappa B (NF-kappa B)-inducible nitric oxide synthase (iNOS/NOS II) pathway damages the stria vascularis in cisplatin-treated mice," *Anticancer Research*, vol. 22, no. 6, pp. 4081–4085, 2002.

[67] G. Li, W. Liu, and D. Frenz, "Cisplatin ototoxicity to the rat inner ear: a role for HMG1 and iNOS," *NeuroToxicology*, vol. 27, no. 1, pp. 22–30, 2006.

[68] M. A. Gratton and A. E. Vázquez, "Age-related hearing loss: current research," *Current Opinion in Otolaryngology & Head and Neck Surgery*, vol. 11, no. 5, pp. 367–371, 2003.

[69] G. A. Gates and J. H. Mills, "Presbycusis," *The Lancet*, vol. 366, no. 9491, pp. 1111–1120, 2005.

[70] T. N. Roth, D. Hanebuth, and R. Probst, "Prevalence of age-related hearing loss in Europe: a review," *European Archives of Oto-Rhino-Laryngology*, vol. 268, no. 8, pp. 1101–1107, 2011.

[71] B. Gopinath, E. Rochtchina, J. J. Wang, J. Schneider, S. R. Leeder, and P. Mitchell, "Prevalence of age-related hearing loss in older adults: blue mountains study," *Archives of Internal Medicine*, vol. 169, no. 4, pp. 415–416, 2009.

[72] B. Gopinath, J. Schneider, E. Rochtchina, S. R. Leeder, and P. Mitchell, "Association between age-related hearing loss and stroke in an older population," *Stroke*, vol. 40, no. 4, pp. 1496–1498, 2009.

[73] A. W. Linnane, S. Marzuki, T. Ozawa, and M. Tanaka, "Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases," *The Lancet*, vol. 1, no. 8639, pp. 642–645, 1989.

[74] U. Bai, M. D. Seidman, R. Hinojosa, and W. S. Quirk, "Mitochondrial DNA deletions associated with aging and possibly presbycusis: a human archival temporal bone study," *The American Journal of Otology*, vol. 18, no. 4, pp. 449–453, 1997.

[75] C. C. Kujoth, A. Hiona, T. D. Pugh et al., "Medicine: mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging," *Science*, vol. 309, no. 5733, pp. 481–484, 2005.

[76] B. K. Crawley and E. M. Keithley, "Effects of mitochondrial mutations on hearing and cochlear pathology with age," *Hearing Research*, vol. 280, no. 1-2, pp. 201–208, 2011.

[77] I. Darrat, N. Ahmad, K. Seidman, and M. D. Seidman, "Auditory research involving antioxidants," *Current Opinion in Otolaryngology & Head and Neck Surgery*, vol. 15, no. 5, pp. 358–363, 2007.

[78] M. D. Seidman, "Effects of dietary restriction and antioxidants on presbyacusis," *Laryngoscope*, vol. 110, no. 5, part 1, pp. 727–738, 2000.

[79] A. Kashio, A. Amano, Y. Kondo et al., "Effect of vitamin C depletion on age-related hearing loss in SMP30/GNL knockout mice," *Biochemical and Biophysical Research Communications*, vol. 390, no. 3, pp. 394–398, 2009.

[80] S. E. Heman-Ackah, S. K. Juhn, T. C. Huang, and T. S. Wiedmann, "A combination antioxidant therapy prevents age-related hearing loss in C57BL/6 mice," *Otolaryngology—Head and Neck Surgery*, vol. 143, no. 3, pp. 429–434, 2010.

[81] S.-H. Sha, A. Kanicki, K. Halsey, K. A. Wearne, and J. Schacht, "Antioxidant-enriched diet does not delay the progression of age-related hearing loss," *Neurobiology of Aging*, vol. 33, no. 5, pp. e15–e16, 2012.

[82] T. Lindsten, A. J. Ross, A. King et al., "The combined functions of proapoptotic Bcl-2 family members Bak and Bax are essential for normal development of multiple tissues," *Molecular Cell*, vol. 6, no. 6, pp. 1389–1399, 2000.

[83] R. J. Youle and A. Strasser, "The BCL-2 protein family: opposing activities that mediate cell death," *Nature Reviews Molecular Cell Biology*, vol. 9, no. 1, pp. 47–59, 2008.

[84] S. Someya, J. Xu, K. Kondo et al., "Age-related hearing loss in C57BL/6J mice is mediated by Bak-dependent mitochondrial apoptosis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 46, pp. 19432–19437, 2009.

[85] D. I. Nelson, R. Y. Nelson, M. Concha-Barrientos, and M. Fingerhut, "The global burden of occupational noise-induced hearing loss," *The American Journal of Industrial Medicine*, vol. 48, no. 6, pp. 446–458, 2005.

[86] N. Slepecky, "Overview of mechanical damage to the inner ear: noise as a tool to probe cochlear function," *Hearing Research*, vol. 22, no. 1–3, pp. 307–321, 1986.

[87] B. H. Hu, D. Henderson, and T. M. Nicotera, "F-actin cleavage in apoptotic outer hair cells in chinchilla cochleas exposed to intense noise," *Hearing Research*, vol. 172, no. 1-2, pp. 1–9, 2002.

[88] T. M. Nicotera, B. H. Hu, and D. Henderson, "The caspase pathway in noise-induced apoptosis of the chinchilla cochlea," *Journal of the Association for Research in Otolaryngology*, vol. 4, no. 4, pp. 466–477, 2003.

[89] B. H. Hu, Q. Cai, S. Manohar et al., "Differential expression of apoptosis-related genes in the cochlea of noise-exposed rats," *Neuroscience*, vol. 161, no. 3, pp. 915–925, 2009.

[90] M. A. Vicente-Torres and J. Schacht, "A BAD link to mitochondrial cell death in the cochlea of mice with noise-induced hearing loss," *Journal of Neuroscience Research*, vol. 83, no. 8, pp. 1564–1572, 2006.

[91] K. K. Ohlemiller, J. S. Wright, and L. L. Dugan, "Early elevation of cochlear oxygen species following reactive noise exposure," *Audiology and Neuro-Otology*, vol. 4, no. 5, pp. 229–236, 1999.

[92] D. Yamashita, H.-Y. Jiang, J. Schacht, and J. M. Miller, "Delayed production of free radicals following noise exposure," *Brain Research*, vol. 1019, no. 1-2, pp. 201–209, 2004.

[93] D. Yamashita, J. M. Miller, H.-Y. Jiang, S. B. Minami, and J. Schacht, "AIF and EndoG in noise-induced hearing loss," *NeuroReport*, vol. 15, no. 18, pp. 2719–2722, 2004.

[94] J. Wang, J. Ruel, S. Ladrech, C. Bonny, T. R. van de Water, and J.-L. Puel, "Inhibition of the c-Jun N-terminal kinase-mediated mitochondrial cell death pathway restores auditory function in sound-exposed animals," *Molecular Pharmacology*, vol. 71, no. 3, pp. 654–666, 2007.

[95] Y. Y. C. Lo, J. M. S. Wong, and T. F. Cruz, "Reactive oxygen species mediate cytokine activation of c-Jun NH2- terminal kinases," *Journal of Biological Chemistry*, vol. 271, no. 26, pp. 15703–15707, 1996.

[96] A. Fridberger, A. Flock, M. Ulfendahl, and B. Flock, "Acoustic overstimulation increases outer hair cell Ca^{2+} concentrations and causes dynamic contractions of the hearing organ," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 12, pp. 7127–7132, 1998.

[97] S. B. Minami, D. Yamashita, J. Schacht, and J. M. Miller, "Calcineurin activation contributes to noise-induced hearing loss," *Journal of Neuroscience Research*, vol. 78, no. 3, pp. 383–392, 2004.

[98] P. R. Thorne and A. L. Nuttall, "Laser doppler measurements of cochlear blood flow during loud sound exposure in the guinea pig," *Hearing Research*, vol. 27, no. 1, pp. 1–10, 1987.

[99] Y. Ohinata, J. M. Miller, R. A. Altschuler, and J. Schacht, "Intense noise induces formation of vasoactive lipid peroxidation products in the cochlea," *Brain Research*, vol. 878, no. 1-2, pp. 163–173, 2000.

[100] J.-L. Puel, J. Ruel, C. Gervais D'Aldin, and R. Pujol, "Excitotoxicity and repair of cochlear synapses after noise- trauma induced hearing loss," *NeuroReport*, vol. 9, no. 9, pp. 2109–2114, 1998.

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Review Article

Electroacoustic Stimulation: Now and into the Future

S. Irving,[1,2] L. Gillespie,[1,3] R. Richardson,[1,3,4] D. Rowe,[3] J. B. Fallon,[1,4] and A. K. Wise[1,4]

[1] *Bionics Institute, Melbourne, VIC 3002, Australia*
[2] *Department of Psychology, University of Melbourne, Melbourne, VIC 3010, Australia*
[3] *Department of Otolaryngology, University of Melbourne, Melbourne, VIC 3010, Australia*
[4] *Department of Medical Bionics, University of Melbourne, Melbourne, VIC 3010, Australia*

Correspondence should be addressed to S. Irving; sirving@bionicsinstitute.org

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Cochlear implants have provided hearing to hundreds of thousands of profoundly deaf people around the world. Recently, the eligibility criteria for cochlear implantation have been relaxed to include individuals who have some useful residual hearing. These recipients receive inputs from both electric and acoustic stimulation (EAS). Implant recipients who can combine these hearing modalities demonstrate pronounced benefit in speech perception, listening in background noise, and music appreciation over implant recipients that rely on electrical stimulation alone. The mechanisms bestowing this benefit are unknown, but it is likely that interaction of the electric and acoustic signals in the auditory pathway plays a role. Protection of residual hearing both during and following cochlear implantation is critical for EAS. A number of surgical refinements have been implemented to protect residual hearing, and the development of hearing-protective drug and gene therapies is promising for EAS recipients. This review outlines the current field of EAS, with a focus on interactions that are observed between these modalities in animal models. It also outlines current trends in EAS surgery and gives an overview of the drug and gene therapies that are clinically translatable and may one day provide protection of residual hearing for cochlear implant recipients.

1. Introduction

Cochlear implants have successfully provided hearing to over three hundred thousand hearing impaired people worldwide [1]. Traditionally, implantation was carried out only in recipients with profound hearing loss, but improvements in technology and sound processing techniques, coupled with the recent relaxation of the eligibility criteria, has led to more implantees with some degree of low-frequency residual hearing [2, 3]. These recipients receive both electrical stimulation from their cochlear implant and acoustic stimulation via their residual hearing (electroacoustic stimulation; EAS).

The typical EAS recipient is an adult who has lost high frequency hearing postlingually, whilst maintaining usable hearing in the low frequencies, creating a so-called 'ski-slope' hearing loss (Figure 1, [4]). It is likely that the number of undiagnosed partial hearing children is larger than typically accepted [5] and, as such, the number of children using EAS is also likely to rise. Furthermore, the prevalence of high frequency hearing loss is increasing worldwide due to growing environmental and recreational noise and an ageing population. As a result, it is likely that in the future, more cochlear implant recipients will maintain some degree of usable hearing.

EAS recipients display substantial benefits in hearing performance compared to profoundly deaf recipients who rely on electrical stimulation alone in pitch perception [6], speech perception [2, 4, 7–10], listening in background noise [11–14], and music appreciation [8, 15]. Recent reviews have discussed the clinical benefits of EAS over the use of electrical stimulation alone [6, 16], as well as the fitting ranges, outcomes, and clinical practice in EAS [10], and the reader is directed there for more information about these aspects of EAS. Despite the clear clinical benefits, little is known of the mechanisms that contribute towards them, although it is thought that the interactions between the electrical and acoustic stimuli may play a role therein. In order to explore the neural mechanisms of EAS integration, as well as optimising clinical applications, the use of animal models is essential. To date, surprisingly little research has been carried

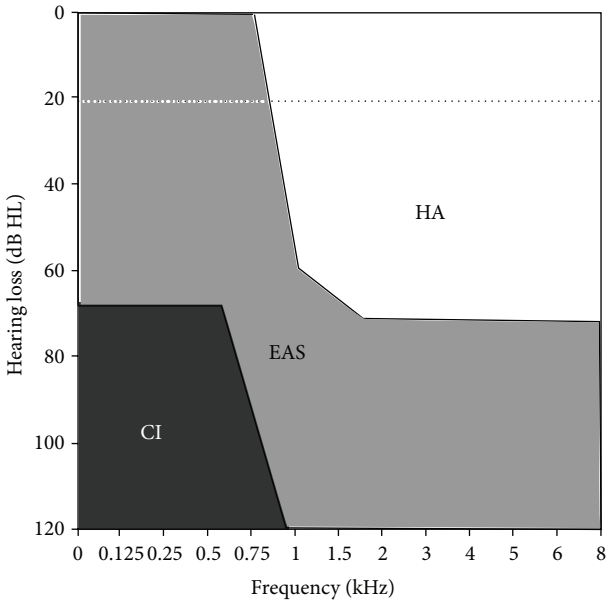

Figure 1: Typical hearing ranges (in dB HL) showing candidature for hearing aids (HA), electroacoustic stimulation (EAS), and cochlear implant use alone (CI).

out into EAS using such models, and fewer still have used animal models with hearing thresholds that reflect those seen in the clinic (with the exception of [17]).

Reports of recipients suffering immediate or delayed loss of low frequency residual hearing following cochlear implantation [4, 6, 18] are concerning: the use of short, atraumatic electrode arrays to minimise cochlear damage, such as the Cochlear Hybrid-S or the Med-EL Flex arrays [2, 18], potentially leaves the more apical regions of the cochlea unstimulated. If cochlear implantation causes residual hearing to deteriorate, these recipients could lose the benefits bestowed by EAS but also do not have the optimum electrical stimulation provided by longer electrode arrays and may therefore require reimplantation [19]. Further investigation into the mechanisms of improved hearing is crucial for optimisation of EAS processing strategies, but also to find ways to minimise the negative effects of cochlear implantation on residual hearing to protect hair cell and spiral ganglion neuron (SGN) function.

The maintenance of existing hearing is critical for EAS, and research has focused on numerous factors that could protect hair cells and SGNs after hearing loss and during cochlear implantation including neurotrophic factors, anti-inflammatory steroidal drugs, antiapoptotic agents, or a combination of these. The means of locally delivering these agents have also been well researched, in particular the challenge of protecting SGNs after hearing loss due to the need for continuous exposure to neurotrophins for long-term SGN survival [20, 21], as survival has not been reported to last beyond 2 weeks after the cessation of neurotrophin delivery [22, 23]. Hence, single-intervention approaches with long-term outcomes such as gene or cell-based therapies are of particular interest.

This review focuses on the current preclinical EAS research, as well as discussing potential therapies that may be combined with electrical stimulation to maintain optimal cochlear and neural health in cochlear implant users with residual hearing. In particular, we will focus upon the interactions and integration between the two stimulation modalities at a neural level, from the cochlea to the auditory cortex, as well as discussing the current practices to reduce loss of residual hearing. Furthermore, we will discuss the potential of gene therapy to provide a long-term or constant supply of neurotrophins from a single intervention to promote SGN survival (and therefore residual hearing) after partial hearing loss, with particular emphasis on the use of viral vectors for cell specific gene expression and discussion of clinical safety.

2. Electroacoustic Stimulation

One of the main areas of research into EAS focuses on the interactions between the responses to the electric and acoustic stimuli. While the clinical evidence indicates improved performance with EAS, it is possible that the stimuli can also effectively mask one another, reducing the quality of the incoming signal. If this were the case, additional clinical benefit may be achieved by segregating the signals either temporally or spatially (with regard to the intracochlear regions that each stimulus type activates) so that masking is minimised. This section presents an overview of the known interactions between electric and acoustic hearing, as well as discussing plastic changes that occur in the brain due to combined stimulation.

2.1. Physiological Interactions between Acoustic and Electric Stimulation. It is important to note that the majority of studies investigating EAS interactions to date have been carried out in normal-hearing animals fitted with intra- or extracochlear stimulating electrodes [24–26]. Although these models give an indication of the interactions in healthy cochlear conditions, they do not necessarily reflect the listening conditions seen in EAS recipients, which will have degraded cochlear processing. Nevertheless, the increasing trend to implant recipients with more and more residual hearing is likely to cause an increase in the number of recipients in which a near normal cochlear region receives stimulation from both electric and acoustic stimulation "overlap," and these results are therefore of considerable interest.

2.1.1. Interactions in the Normal Hearing Cochlea. The first report of the effects of EAS is from the level of the cochlea in the doctoral dissertation of Moxon [27]. Using auditory nerve recordings, Moxon demonstrated that electric stimulation at low current levels within the normal hearing cochlea generated hair cell-mediated response (known as "electrophonic" responses; the β-component of the auditory nerve response). Higher current levels produced a short latency α-component which results from direct stimulation of the auditory neurons (Figure 2). This suggests that, in a cochlea with residual hair cells, electric stimulation activates the auditory nerve through a dual pathway: via the SGNs directly and indirectly

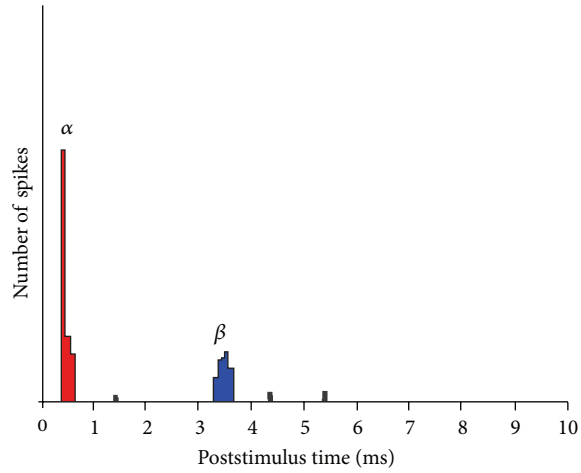

Figure 2: Stylised poststimulus time histogram showing examples of α- and β-responses recorded at the auditory nerve in response to electrical stimulation. The α-response is from direct activation of the auditory nerve and the β-responses are caused by electrophonic activation.

through "normal" transduction of the electrically-generated displacement of the basilar membrane. Electrophonic effects on auditory function are discussed below.

Recordings of compound action potentials (CAPs) under different stimulation combinations have made up the majority of the electrophysiological studies investigating EAS. This work has notably used masking paradigms, including forward masking of an acoustic signal by electric stimulation, forward masking of an electric signal by an acoustic signal and simultaneous EAS.

Experiments in normal hearing animals show that acoustically-evoked CAPs are suppressed by a preceding electric pulse train presented at the base of the cochlea, with the strongest suppression occurring for responses to low intensity, high frequency acoustic stimuli that were masked by high current levels [24]. Suppression of low frequency acoustic stimuli only occurred for higher current levels, likely due to current spread to the apical region of the cochlea, and did not occur in all animals. This suggests that EAS interactions require a physical overlap between the hair cells and the stimulating current. Hence, it is likely that interactions seen in normal hearing experimental animals implanted with cochlear implants would be larger than those seen in partial hearing situations. The observed suppression is not solely due to the refractoriness of the nerve after the masking stimulus, as the latency of the suppressive effect is longer than is seen in spontaneous firing and may be due to suppressive effects from the hair cell (see discussion on electrophonics, below).

Further studies have investigated the effects of an acoustic masker on the electrically-evoked CAP (ECAP) and have shown that broadband noise can decrease both the ECAP amplitude and firing synchrony [28–30], resulting in increased electrical thresholds. This effect was seen both during and after the masking noise was presented (forward- and simultaneous masking), although it was largest for simultaneous masking. Electrical thresholds returned to premasking levels between 100–200 ms post masker offset. Masking was particularly prominent at low electrical pulse rates (>3 ms interpulse interval) but absent at higher rates [29].

The role of background activity in EAS interactions was demonstrated by Miller et al. [30], who showed that adding an acoustic noise to an electric pulse stimulus increased temporal variability of spikes in the auditory neurons ("jitter"), but that in the 20 ms period following the offset of an acoustic masker, electrical responses showed a decrease in jitter. This finding was limited to nerve fibres that exhibited high levels of spontaneous activity, suggesting that the acoustic signal was able to vary firing synchrony across the different auditory nerve fibres. Miller and colleagues [30] have further shown that simultaneous EAS caused an increase in spike rate in auditory nerve fibres compared to electric stimulation alone, corroborating findings by Von Ilberg et al. [25]. Spike rate increased with current level of the electric stimulus, but an increase in spike rate seen in EAS was not equal to the sum of the respective electric and acoustic firing rates. Conversely, the temporal jitter did not vary between EAS and electric stimulation alone conditions.

The tuning of auditory nerve fibres under EAS stimulation has only been investigated in one study [25], which found that EAS did not alter the characteristic frequency (the frequency with the lowest threshold) of the auditory nerve fibres, either acutely or after chronic stimulation. Tuning curves in sharply tuned fibres were broader under combined EAS, but no change was seen in fibres that were already broadly tuned. All characteristics returned to normal after cessation of electrical stimulation.

The dependence of the electric and acoustic masking upon the relative temporal position of the stimuli is of clinical relevance because the latter depends upon a number of factors: (a) the frequency of the stimulus, (b) the relative delay introduced by the speech processor, and (c) the power spectrum of the stimulus.

Stimulus frequency will affect relative timing between the two stimulus modalities due to the travelling wave [31], where apical cochlear regions stimulated by lower frequencies are reached later than the more basal, high-frequency regions, a delay that can vary between 1 and 10 ms from base to apex [32]. A further degree of temporal variability is derived from the processing carried out by the speech processor and the "round robin" stimulation paradigm used in most stimulation strategies, where electrodes are stimulated sequentially (Figure 3. [33]) and therefore depends upon the stimulus, the processing strategy used and the length of the electrode array. It is entirely possible in a region of overlapping electrical stimulation and residual hearing that the same acoustic stimulus could lead to sequential forward masking of both the acoustic response and the electric response by the other. It is currently unknown how such a stimulus is encoded at the level of the auditory nerve and higher up the auditory pathway.

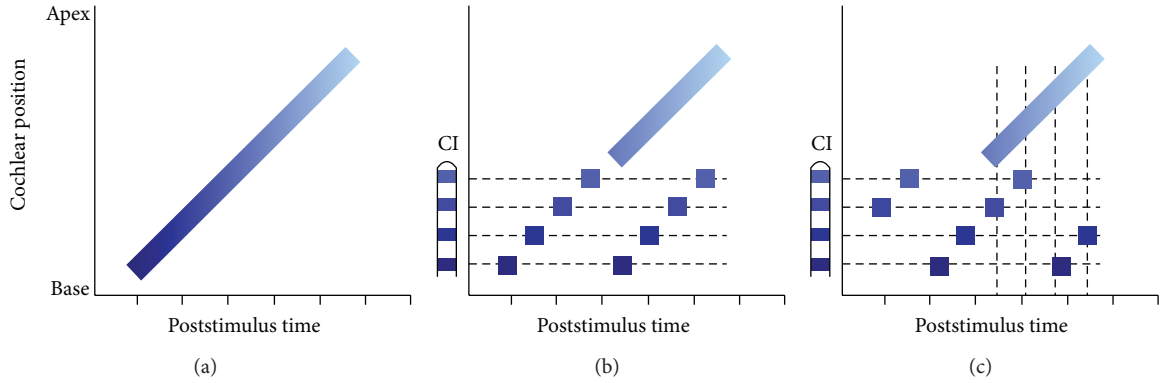

Figure 3: Illustration of potential interference between electric and acoustic stimulation to the same stimulus. (a) represents the normal hearing case, where the travelling wave causes the base of the cochlea to be activated before the apex in a systematic manner. Colour indicates stimulation at a particular cochlear location. (b) and (c) show an EAS cochlea (cochlear implant represented on the left), where the round robin processing strategy causes simultaneous activation of two distinct regions of the cochlea for electric and acoustic stimuli. In (b), the round robin sequence begins at the most basal electrode, whereas (c) shows the stimulation occurring first to the second most apical electrode. These panels represent the response to the same stimulus but depict how the location of the stimulating electrode within the round robin sequence can cause different temporal electrode/cochlear position combinations for the same external stimulus.

2.1.2. Electrophonic Suppression of Auditory Nerve Responses. The electrically-induced basilar membrane motion that gives rise to electrophonic responses was thought to be generated by the electromotile properties of the outer hair cells (OHCs; [16, 34, 35]). However, more recent research investigating the role of the OHCs in electrophonic generation has shown that destruction of OHCs does not abolish the electrophonic component of the CAP [36]. Therefore, as long as some IHCs remain in EAS recipients, there is the opportunity for electrophonics to occur (and at lower current levels, they may be amplified by OHC activation [36]). Stronks et al. [36] also showed that longer electrical pulse widths cause greater electrophonics and suggested that in order to reduce their inhibitory effects, short electrical pulses should be used clinically. Nevertheless, it is currently unclear as to whether these interactions are undesirable from a perceptual perspective and further investigation should aim to answer this question.

2.1.3. Interactions in the Partially Deaf Cochlea. Although most studies investigating EAS in animal models to date have used normal-hearing animals, there has been a recent increase in the number of published studies that aim to emulate a clinically relevant partial hearing loss [28, 37], and we have recently described a model for chronic cochlear implant use in a partial hearing animal model that is directly relevant to EAS [17]. The use of such partial hearing models in EAS research is essential to enable more clinically-relevant questions to be addressed. For example, Stronks et al. [38] observed a significant decrease in CAP suppression by electrical forward masking in guinea pigs that had been partially deafened with a combination of furosemide and kanamycin compared to normal hearing controls. This suggests that partial hearing cochleae implanted with intracochlear electrodesmay notdisplay interactions at the level of the auditory nerve, even when using high current levels to stimulate the apical regions with intact hearing. This finding is important as it suggests that the interactions described in normal hearing animals above may not occur in clinical populations, who typically have a high frequency hearing loss that does not overlap with the cochlear region that is stimulated by the cochlear implant and may therefore have little bearing on "real life" EAS responses. Further research is required to investigate these interactions in partial hearing EAS situations.

A further confound between clinical populations and animal models of partial hearing loss comes in the form of the fitted device. For recipients with residual hearing, it is common for combination devices that couple acoustic amplification with electrical stimulation to be used in the implanted ear [39]. Hearing aids are unavailable for chronic use in animal models, and additional experimental benefits would be seen in mimicking hearing loss that matches that seen in recipients using amplified hearing in the lower frequency region.

2.1.4. Evidence of Central EAS Interactions. EAS interactions at the level of the inferior colliculus (IC) in normal hearing animals have been investigated by Vollmer et al. [40]. As described for the auditory nerve (see above), forward masking of an acoustic stimulus by single biphasic electrical pulses caused a current-dependent decrease in IC responses to acoustic stimuli and resulted in elevated acoustic thresholds. Simultaneous electrical and acoustic presentation with a tone at the neuron's CF resulted in interactions that depended upon the relative levels of each of the two stimuli, with increasing suppression with electric masker level and decreased suppression with acoustic probe level (i.e. if the masker was at a higher level than the probe, then there was a greater suppressive interaction between the two). Simultaneous presentation of acoustic tones with electrical stimulation led to suppression of the electrically evoked

response, which increased with increasing acoustic level. Overall, electrical stimulation at higher levels dominated the acoustic response and combined EAS resulted in increased spike rates, in agreement with findings in the auditory nerve [30].

2.2. Neural Plasticity Seen with Combined EAS. There is evidence that the speech recognition benefits that recipients experience with EAS develop over time [2], suggesting that the combined stimuli are causing plastic changes in the brain that enable the improved performance. Reiss et al. [41] demonstrated that the pitch percept provided by electrical stimulation at a particular cochlear location could change over time in EAS recipients compared to an acoustic reference. This shift could be as large as three octaves in some participants and generally caused the electric pitch to align with the frequency that was allocated to the electrode by the speech processor. The mechanisms of these plastic changes are, to date, unknown. Few studies have looked at the plastic effects of chronic EAS use at the physiological level, primarily due to a lack of suitable animal model (although see [17, 42]).

We have previously reported that chronic intracochlear electrical stimulation in cats with a high frequency hearing loss caused a decrease in the extent of primary auditory cortex that could be activated by acoustic stimulation [42]. Although characteristic frequency of the cortical neurons did not change with EAS, there were fewer neurons responsive to acoustic only stimulation, compared to electrical only and combined stimuli. As this study did not obtain recordings at the beginning of the stimulation period, it is impossible to determine whether these changes correspond to the pitch shifts reported by Reiss et al. [41], although it is apparent that plastic changes do occur in cases of EAS, warranting further examination to enable optimisation of clinical outcomes.

This section has outlined the physiological interactions between electric and acoustic stimulation, which are typically inhibitive for forward and simultaneous masking paradigms. However, there is currently little evidence of these interactions in clinically-relevant partial-hearing animals, although the plastic changes seen in chronically stimulated EAS animal models [41] could contribute to the success of EAS recipients. The use of partial-hearing models is of critical importance for the development and refining of EAS strategies and developing procedures to maintain residual hearing after chronic cochlear implantation. Regardless of the mechanisms involved in improved listening performance with EAS over electrical stimulation alone, the protection of any residual hearing is, by definition, vital to EAS. The following sections describe the potential causes of hearing loss associated with cochlear implantation, as well as discussing the procedures that can be undertaken to minimise loss of hearing during and after cochlear implantation.

3. Potential Causes of Hearing Loss following Cochlear Implantation

Critical to the success of EAS is the health of the cochlea and, in particular, the residual hearing available to the listener. It is well reported in the literature that the insertion and use of a cochlear implant can cause a loss of residual hearing in the stimulated ear. This section outlines potential causes for this loss, which can be surgical or histopathological.

Surgical factors are controlled by the implanting surgeon and include electrode selection, insertion route (cochleostomy or through the round window), insertion depth, and the use of atraumatic surgical techniques. Early traumas such as damage to the osseous spiral lamina, basilar membrane rupture, and lateral wall disruption have all been shown to have occurred during human cochlear implantation [43–45] and animal models of implantation [46, 47] and are likely to manifest as immediate hearing loss. The literature also suggests that implant surgeons have a standardised insertion technique appropriate to the electrode which is unrealistic, given the variations in cochlear anatomy, and could be a potential source of disparity. In addition, it is often assumed that hearing in the implant recipient is stable preoperatively, which is not always the case. As such, it is possible that implantation trauma accelerates the underlying cause of hearing loss and could further cloud outcome measures.

The cochlea's histopathological response to electrode insertion may also contribute to delayed or progressive hearing loss following cochlear implantation. Delayed effects include fibrotic changes around the electrode, new bone formation and foreign body reaction. There are a number of cochlear structures that can be affected by these changes after implantation, including the organ of Corti, the SGNs and their dendrites, and the stria vascularis along the lateral wall [43–45]. Altered electrode performance and hearing/speech outcomes do not always have an obvious causal relationship to damage of these delicate intracochlear structures and interpreting the role and impact of observed histopathological changes in hearing loss can be problematic. In addition, the role of cochlear mechanics and endolymphatic hydrops in affecting outcomes is unclear, and they remain possible contributing factors to the loss of residual hearing.

3.1. Surgical Factors in Loss of Residual Hearing

3.1.1. Electrode Insertion Depth. Successful preservation of residual hearing and prevention of delayed hearing loss have generally been advocated by either surgical technique alone or use of a particular electrode design [8, 9, 48–51]. Gantz and Turner [48] reported that residual low frequency hearing was preserved in a group of 24 volunteers using either a 6 or 10 mm Iowa/Nucleus Hybrid Cochlear Implant, suggesting that a short electrode prevented any damage to the low frequency regions of the cochlea [8]. The authors reported good preservation of low frequency hearing in the long term (up to 20 years) with almost all subjects retaining their residual hearing. The perceived advantage behind the shortened electrode array is that it would cause less damage on insertion to the lateral wall of the cochlea at the basal turn and would not reach the low frequency areas at the

apex of the cochlea. A more recent report by Woodson et al. [52] suggested that delayed hearing loss has occurred in some recipients receiving the Iowa/Cochlear Hybrid cochlear implant. They reported preservation of residual hearing (loss less than 30 dB of preoperative thresholds) in 91% of their subjects. This amount decreased to 75% by the end of the trial period suggesting a progression of hearing loss in some recipients.

Concerns over the potential loss of residual hearing are an important consideration for prospective recipients with significant residual hearing prior to implantation with a "hybrid" or shortened electrode. Given the potential for loss of residual hearing that is either due to the surgery, the biological response to the presence of the electrode array, or to progression of the underlying pathology, there is a potential benefit in having a longer electrode array with more electrodes within the cochlea that would allow for programming flexibility and pitch-matching similar to that used with current electrode arrays [53]. Were recipients to lose their residual hearing in the future, the cochlea would then have adequate coverage with a standard length array in the electrical stimulation only situation and obviate the need for reimplantation with a full length electrode at a later time as seen in some situations [54, 55]. The implantation of longer arrays in patients with residual hearing should be undertaken with caution, however, as a review by Boyd [56] suggests that deeper electrode insertion typically leads to greater cochlear injury. The use of longer electrodes may therefore increase the likelihood of loss of residual hearing compared to shorter electrode arrays, and this risk should be discussed with prospective patients.

3.1.2. Soft Surgery. "Soft surgery," a collection of techniques that would aid in the preservation of hearing following cochlear implantation, was first proposed as a concept by Lehnhardt in 1993 [57]. This protocol aims to minimise cochlear trauma by minimising the size of the cochleostomy, locating it at the level of the promontory to facilitate insertion into the scala tympani, maintaining an intact ossicular chain and not aspirating the perilymph [58]. Furthermore, histological studies have demonstrated less insertion trauma with round window insertions (i.e. without cochleostomy) [59–61] and reported excellent residual hearing preservation using round window insertions of partially inserted MED-EL electrodes. The idea that superior surgical technique (i.e. soft surgery) alone is enough to preserve residual hearing was challenged by Cohen [62] asserting that residual hearing is universally lost following implantation, irrespective of the implanted electrode and the technique of surgical insertion. Despite this assertion, many surgeons still strive to prevent hearing loss following implantation by adopting soft surgical techniques.

3.1.3. Hypothermia. Safe hypothermic induction can be non-invasive by using cooling blankets and ice packs, or a more invasive route can involve safe infusion of large volumes of cold fluids [63]. Hypothermia is used clinically to promote neuronal survival in cardiac surgery and after cardiac arrest [63] by decreasing metabolic rate, reducing tissue oxygen consumption, depressing metabolic acidosis [64], suppressing calcium influx into neurons [65], and diminishing nitric oxide production [66]. Similar protective effects have been described during cochlear implantation, noise trauma and ischemic cochlear injury [67–71]. Further protective effects may arise from decreased glutamate release upon neuronal inflammation after trauma [72]. Despite these findings, the use of hypothermia during cochlear implant surgery has yet to become mainstream practice.

3.2. Histopathology in the Cochlea

3.2.1. Cochlear Reaction to Implantation. Following implantation, the cochlea shows several pathological responses resulting from foreign body response to the electrode array. Apoptosis of auditory hair cells and SGNs [73, 74], inflammation resulting in fibrosis and osteogenesis [45, 75] and foreign body reaction [76] have all been reported. Rizer et al. [77] suggested that, following cochlear implantation, an inflammatory reaction occurred that ultimately resulted in residual hearing loss. A further suggestion was that the presence of the electrode and/or fibrosis within the scala tympani following implantation could act as a space-occupying foreign body that interferes with the natural mechanics of the cochlea [78]. These issues remain current. It should be acknowledged that some delayed hearing loss may reflect progression of the underlying cause of deafness and as such, cochlear implantation may cause an accelerated loss of residual hearing as a result of electrode insertion [79]. Even with meticulous surgical technique, insertion of the electrode through the cochleostomy is essentially a blind technique and some loss of residual hearing is almost a ubiquitous finding [62]. Whether cochlear implantation-related hearing loss is determined at the time of surgery, as a natural consequence of subsequent inflammation, apoptosis and tissue response to a foreign body, or whether the latter may be modulated by pharmacological intervention is under investigation in a number of laboratories.

Fibrosis within the cochlea and around the electrode array is an almost ubiquitous finding following implantation. This is not unique to cochlear implantation as fibrosis can occur as a reaction to other inflammatory processes to disrupt inner ear anatomy without electrode placement [80, 81]. It is common at the cochleostomy site, along the array and also extends beyond the tip of the electrode [45, 82]. Fibrosis appears to be worse along the point on the lateral wall where the electrode turns around the basal turn [45]. The presence of fibrosis along the basal turn is predicted to alter the vibration of the apical basilar membrane and thus interfere with residual low frequency acoustic hearing [83]. Surgical and pharmacological modifications that aim to reduce post-operative fibrosis within the cochlea are therefore important for hearing preservation during cochlear implantation, and their use may enable better understanding of the mechanisms behind this loss.

New bone formation within the cochlea is another commonly observed consequence of cochlear implantation. Osteogenesis typically occurs in similar locations to fibrosis: at the cochleostomy site, along the path of implantation, and even in the nonimplanted apex of the cochlea [79, 82].

Nonspecific inflammatory reactions within the cochlea have been known also to cause loss of residual hearing post-implantation [77]. Most reactions, however, are likely to be in response to the implant itself or to damage within the cochlea upon insertion. Following implantation, histological examination of the fibrosis at the basal turn suggests noninfected inflammatory cells, possibly indicating a foreign body reaction [84]. Closer characterisation of these cells types has shown a wide variety of inflammatory cells within the fibrotic tissue reaction, including mononuclear leukocytes, histiocytes, and foreign body giant cells [79].

Histological evidence suggests that there are many pathological processes associated with electrode insertion. It is unclear whether these pathological processes are associated with any observed hearing loss or cause the observed hearing loss. Direct electrode insertion trauma, apoptosis, acute inflammation, chronic inflammation and hypersensitivity/foreign body reaction can all potentially occur in the cochlea after implantation. With the common finding of inflammatory cells, fibrosis and giant cells, it could be postulated that potent anti-inflammatory drugs like corticosteroids have a role to play in hearing preservation. What remains unclear is what effect these pathological processes have on the hearing mechanisms of the cochlea, although it seems certain that the preservation of residual hearing through maintenance of neuronal survival and cochlear health can only be beneficial to the implant recipient.

4. Interventions to Promote Hair Cell and SGN Survival for EAS

As discussed above, hearing preservation and the survival of both residual hair cells and SGNs in cochlear implant surgery have become an increasingly important goal in order to facilitate EAS outcomes. Along with surgical refinement, the delivery of therapeutic agents to the cochlea has the potential to provide protective effects within the cochlea and is likely to have significant clinical benefits.

Various techniques, treatment regimes, and therapeutic agents are currently being used or are under investigatation for future use in this regard. Localised delivery of therapeutic agents into the cochlea is likely to be the most effective means to promote neuronal health, as it places the therapeutic agents in direct proximity to the target cells they are intended to protect. In addition, this approach minimises the high dose rates and other complications that can arise from systemic delivery.

Direct infusion into the fluid spaces of the cochlea can be achieved via a cochleostomy to insert, for example, a cannula attached to a reservoir containing the drugs of interest, a drug-coated electrode, or to implant cells expressing therapeutic agents. Each of these techniques has been demonstrated to successfully deliver the drug in question to the cochlea and be very efficacious for SGN protection [22, 85–89]; however, the surgical procedures involved can be traumatic within themselves and are likely to cause damage to any existing hearing. As such, in cases where hearing is intact and hair cell preservation is a primary aim, such intracochlear techniques are not suitable. For EAS patients, combining the drug delivery and implantation surgeries is the most efficient way of reducing cochlear trauma and is likely to become the protocol of choice in the future. Drug delivery to the inner ear can also be achieved via diffusion across the round window membrane. The round window membrane is known to be permeable to large molecules including neurotrophic factors, and indeed, the effects of neurotrophic factors have been detected in the perilymphatic fluids of the cochlea following application onto the round window via loading into alginate beads [90], hydrogel [91] or Gelfoam [92], and therapeutic effects on hair cells and SGNs were observed [91, 92]. Importantly, the surgical approach required for this method of application is minimally invasive and has a much lower risk of potential trauma to the hair cells and the patient's existing hearing. This delivery method could be used, for example, when an electrode array is already in place and drug delivery is required. While the delivery methods are important and significant clinical consideration is required for application in human recipients, this section will focus on the therapeutic agents themselves.

Several classes of compounds are available as therapeutic agents to protect the sensory hair cells and SGNs from trauma and degeneration, including neurotrophic factors, antioxidants, antiapoptotic agents, and anti-inflammatory steroids, and a number of companies are currently involved in the commercial development of novel otoprotective drugs. Treatment with these agents, in combination with surgical refinement such as hypothermia, may enhance the outcomes from EAS.

4.1. Neurotrophic Factors. Brain derived neurotrophic factor (BDNF) and neurotrophin-3 (NT3) are neurotrophins that are produced by the hair cells [93, 94] and supporting cells [95, 96] of the organ of Corti, and provide support to the SGNs during both development and adulthood. Loss of these endogenous neurotrophins, as occurs following a sensorineural hearing loss (SNHL), leads to SGN degeneration. However, it is now well known that exogenous application of neurotrophins can rescue SGNs from deafness-induced degeneration [85, 87, 97–101]. Importantly, we have recently reported long-term survival of SGNs following cell-based neurotrophin treatment in both the cat [88] and the guinea pig [86]. In addition, neurotrophic factors including glial cell-derived neurotrophic factor (GDNF), basic fibroblast growth factor (bFGF), and NT3 have been reported to protect hair cells from ototoxic drugs and noise damage [102–106]. Furthermore, auditory thresholds have also been decreased in normal hearing guinea pigs using BDNF diffused across the round window, a procedure that therefore has the potential

to be used to protect residual hearing following cochlear implantation [107] and, thus, optimise EAS outcomes.

While the specific effects of neurotrophins have not been investigated in studies using EAS, enhanced SGN survival, and decreased electrically-evoked auditory brainstem response EABR thresholds were observed when neurotrophins such as BDNF were delivered in conjunction with chronic electrical stimulation from a cochlear implant [99, 108]. In EAS recipients, the remaining apical hair cells may also provide a source of neurotrophins for the SGNs in the damaged but electrically-stimulated regions, a prospect that warrants further investigation. Neurotrophins therefore hold great promise for use as protective therapeutic agents for both SGNs and hair cells, to facilitate and enhance the outcomes of EAS.

4.2. Anti-Inflammatory Steroidal Drugs. Corticosteroid treatments are now a common therapy for many forms of hearing loss, such as autoimmune inner ear disease, sudden hearing loss, and Menière's Disease [109]. More recently, numerous studies have demonstrated that the targeted delivery of steroids can protect hearing during cochlear implant surgery [49, 110–113]. The benefits of corticosteroids seen in these situations are mediated through both anti-inflammatory and antiapoptotic pathways [114]. The most common agent used in these studies is dexamethasone, and protection can be achieved by either pretreatment of the cochlea or postoperative infusion. Local delivery of corticosteroids is superior to systemic delivery, with the additional benefit of reducing any potentially harmful side effects that come with systemic administration [49, 110–113]. Pretreatment of the cochlea with dexamethasone applied onto the round window membrane can prevent the elevation of auditory thresholds that are typically associated with cochlear implantation and preserve the SGNs in the region of implantation [115–117]. A further study in cochlear implant recipients demonstrated that the combination of pre- and intraoperative glucocorticoids improved hearing preservation in adults and children with residual hearing [118]. In addition to the identification of dexamethasone as a potential therapeutic agent for protection of the inner ear against trauma, clinical trials using a novel sustained release delivery system for this drug, known as OTO-104, have reported positive phase 1 trial results [119], and phase IIb clinical trials have recently commenced.

4.3. Antioxidants. Numerous aetiologies of hearing loss occur as a result of the generation of reactive oxygen species and subsequent oxidative stress. For example, cisplatin, a potent antineoplastic agent used for the treatment of a variety of tumours and aminoglycoside antibiotics, which are commonly used to treat aerobic, gram-negative bacterial infections, has ototoxic side effects due to the generation of reactive oxygen species and oxidative stress [73, 120]. In addition, the formation of oxygen free radicals is hypothesised as a major cause of noise-induced hearing loss [104, 121]. Importantly, numerous studies have demonstrated that the application of various antioxidants, including n-acetyl cysteine, glutathione, resveratrol, and superoxide dismutase, can protect the auditory system from the degenerative effects of ototoxin- or noise-induced hearing loss [122–127]. Interestingly, therapeutic agents used for other clinical conditions, such as rasagiline, a monoamine oxidase type B inhibitor that is FDA-approved for use in Parkinson's disease, and metformin, an anti-diabetic drug, have also been demonstrated to attenuate hearing loss following ototoxin exposure [128, 129]. Clinical application of antioxidants for protection in the inner ear is also being developed: a molecule known as as SPI-1005, which induces glutathione peroxidase and reduces reactive oxygen species, is currently undergoing Phase II clinical trials [110].

4.4. Antiapoptotic Agents. Hearing loss has also been associated with the initiation of apoptotic pathways such as those mediated by c-Jun N-terminal kinase (JNK) and the caspases. The inhibition of each of these apoptotic proteins inhibits aminoglycoside-induced hair cell death [130, 131]. Furthermore, the induction of heat shock proteins (HSPs) in response to cell stress can significantly inhibit apoptosis in many systems. In particular, HSP70 has been shown to have protective effects against aminoglycoside-induced hearing loss and associated hair cell degeneration [132]. Clinical developments for the delivery of anti-apoptotic agents are also underway, with phase IIb clinical trials using the JNK inhibitor AM-111 reporting positive results including substantial improvements in hearing thresholds and speech discrimination scores.

4.5. Combinatorial Therapies. An effective treatment strategy to promote residual hearing via preservation of hair cells and SGNs may require a combined application of a number of these therapeutic agents, or agents with multiaction properties which can elicit neuroprotective, antiapoptotic, and antioxidant effects. Indeed, the combined application of neurotrophic factors and antioxidants has previously been shown to protect both SGNs and hair cells against ototoxin-induced damage [133, 134], with the effects significantly enhanced over that observed with the neurotrophic factor alone [134].

An alternative promising combined treatment option in the cochlea is the use of cell-based therapies, in particular, NTCELL, which are alginate encapsulated porcine choroid plexus cells (Living Cell Technologies Pty Ltd) has potential application in the cochlea. Clinical trials for the treatment of Parkinson's Disease by NTCELL have recently begun. NTCELL secretes many neurotrophic and growth factors which can increase neuronal survival in response to traumatic injury, hypoxia or chemical challenges [135]. Specifically, NTCELL has been reported to secrete GDNF, BDNF and vascular endothelial growth factor (VEGF) in quantities sufficient for biological activity [135]. Furthermore, NTCELL also produces high levels of enzymes and proteins with antioxidant activities [135]. We recently reported that long-term implantation of NTCELL into the profoundly deaf cat cochlea promotes the survival of SGNs and their peripheral processes when combined with chronic electrical stimulation from a cochlear implant [88]. The stability and

biocompatibility of NTCELL demonstrates the potential as a long-term technique for the delivery of therapeutic proteins to protect both hair cells and SGNs.

Future therapies may also incorporate the initial application of a steroid such as dexamethasone to protect against implant-related trauma, as well as an ongoing delivery method for neurotrophic factors and/or free radical scavengers for more long-term protective effects. Alternatively, gene therapy may facilitate long-term neurotrophic support to promote preservation of hearing.

5. Long-Term Protective Gene Expression via Gene Therapy

The introduction of neurotrophins into the cochlea has proven to play a key role in the survival of SGNs after SNHL [20, 21, 101] and may play a role in the protection of hearing following cochlear implantation (see above). The finite source of neurotrophins in experimental (pump-based) delivery systems that eventually require replacement has led to increased interest in the use of gene therapy to maintain functional levels of acoustic hearing. Gene therapy has the potential to introduce neurotrophins into the cochlea with long-term gene expression arising from a single surgical intervention to the cochlea, potentially coinciding with cochlear implantation. Gene therapy with neurotrophic factor genes such as NT3, BDNF or GDNF resulted in long-term protection of SGNs after noise-induced, ototoxic, or hereditary hearing loss [100, 136–141]. From a single injection of adenoviral vectors containing neurotrophin genes into the scala tympani or scala media, there were at least 3 months of SGN protection after hearing loss [140, 142], with recent data indicating that adenovirus expression can extend to 6 months post injection [143]. However, a complicating factor in the long-term expression of transgenes in the cochlea is the degeneration of cells that are the potential targets. These include hair cells, supporting cells, and SGNs which can all degenerate after hearing loss (Figure 4).

Previous studies of neurotrophin gene expression, in supporting cells in particular, have demonstrated beneficial effects on SGNs such as survival and neuronal resprouting [100, 138] but did not prevent the supporting cells from degenerating at the same rate as those that were not expressing neurotrophins [140]. Eventually, the supporting cells and the neurotrophic transgene would be lost [143]. This study suggests that neurotrophin gene therapy needs to target cochlear cells that do not degenerate after hearing loss. Further studies have shown that injection of neurotrophin genes into the scala tympani resulted in expression in cells lining the perilymphatic space and protected SGNs after hearing loss [137, 142, 144]. Significantly, this is likely to be the situation for the preservation of residual hearing following cochlear implantation where the sensory and supporting cells are likely to be preserved at the time of implantation. Whether gene therapy can prevent loss of residual hearing after cochlear implantation is yet to be determined.

Similar to studies using other neurotrophin delivery systems, expression of BDNF by adenoviral vectors in combination with electrical stimulation from a cochlear implant promoted SGN survival and improved thresholds for SGN stimulation [142]. It is possible that concurrent electrical stimulation from the cochlear implant will affect the efficiency of the expression of the transgene and the efficacy of the neurotrophins produced, as previous studies have shown improved SGN survival when pump-delivered neurotrophins were combined with electrical stimulation [99].

5.1. Viral Vectors and Cell Specificity. Viral vectors are currently the most efficient way to introduce transgenes into cochlear cells. There are a number of viral vectors that have been tested in the cochlea including adenovirus, adeno-associated virus (AAV) and herpes simplex virus [145–148]. Each vector type has its unique cell specificity expression pattern in the cochlea (tropism), which can be exploited to improve the protective effects of gene therapy. For example, adenovirus type 5 has high tropism for supporting cells of the organ of Corti [149], hence, injection of adenovirus type 5 vectors carrying neurotrophin genes into the scala media was found to result in efficient transduction of supporting cells of the organ of Corti, in turn resulting in protection of SGNs after hearing loss [100]. AAV serotype 5 also has tropism for supporting cells [150], while AAV3 has specificity for inner hair cells [151] and herpes simplex virus targets neuronal cells [152]. There are currently over 100 serotypes of AAV and over 50 serotypes of adenovirus, each with different cell specificities, providing great potential to express transgenes in particular cell types. Cell specific promoters can also be used to achieve cell-specific gene expression, with the myosin VIIa promoter driving exclusive expression in inner hair cells as a striking example [153].

Cell-specific gene expression can have a big impact on the protective effects observed after hearing loss. Gene expression that was localised mainly to supporting cells of the organ of Corti not only had greater SGN survival compared to expression in cells of the scala tympani [100], but also may have a nerve guidance effect on the resprouting nerve fibres [100, 138]. Regenerating nerve fibres were highly disorganised when neurotrophins were introduced via a mini-osmotic pump to the scala tympani [101], but when adenoviral gene therapy was used to introduce neurotrophins into the scala media, nerve fibres were observed in greater density near cells expressing the neurotrophin genes, compared to the control GFP gene alone [100].

Expression of reporter genes in SGNs has been demonstrated after injection into the scala tympani using vectors such as AAV [151, 154] and adenovirus [155]. However, no studies have reported neurotrophic factor transgene expression within SGNs as a means to preserve SGNs, and this is an area for future research as a means to improve EAS outcomes through hearing protection.

5.2. Cochlear Injection Sites for Gene Therapy. The anatomy of the cochlea makes it suitable for localised gene delivery in many ways: It is surgically accessible; the fluid chambers

are partitioned allowing certain cells to be targeted; and the blood-cochlear barrier ensures there is minimal spread of the virus beyond the injection site. The scala tympani is easily accessible via the round window membrane or a cochleostomy, similarly to the insertion route used during cochlear implantation. Injection of viral vectors into the scala tympani results in gene expression predominantly in cells lining the perilymphatic space, but also in hair cells and supporting cells [100, 156]. The scala media is much smaller, surrounded by tight junctions and is more difficult to access either by injection through the basilar membrane or through the lateral wall of the cochlea [100, 157]. Injection into this compartment results in more localised expression in hair cells, supporting cells and interdental cells [100, 156, 157]. Despite the complexity of the surgical approach to the scala media, expression of protective genes such as neurotrophins has a big impact on SGNs due to the proximity of supporting cells to the SGN nerve endings (see Figure 4) [100, 138]. Given that the target for gene therapy is the protection of residual hearing in the apical cochlear regions, delivery of the transgene to the scala tympani would be the best route to enable expression in the apical regions [100]. This also facilitates the administration of viral vectors at the time of cochlear implantation, meaning that only a single surgery is required for the two interventions, and the gene therapy can function from the moment that the electrode array is inserted.

In most cases, injection of viral vectors into the cochlea results in gene expression that is localised to the cochlea. However, there have been reports of viral vector spread to the contralateral ear as well as the cerebellum [158, 159]. Furthermore, the utricle, saccule, or semicircular canals of the vestibular system contain fluids that are continuous with cochlear perilymph and endolymph. Hence, injection into the cochlea also results in gene expression in the vestibular system in many cases [160, 161]. This connection can be exploited as an additional surgical injection site for gene expression in the cochlea, often avoiding the loss of hearing that accompany direct injection into the cochlea [161]. Injection into the cochlea or the vestibular system all require surgical intervention, but studies have shown that viral vectors carrying reporter genes or protective genes can cross the tympanic membrane of the cochlea and exert protective effects, at least on hair cells, without the need for surgery [162, 163].

5.3. Clinical Translation of Gene Therapy. It is important to demonstrate the clinical safety of gene therapy in the cochlea. Gene therapy is finding increased clinical acceptance with multiple clinical trials demonstrating that viral gene therapy in the human nervous system is both beneficial and safe. AAV in particular has been used in numerous clinical trials. For example, AAV2-GAD (glutamic acid decarboxylase) is being trialled for advanced Parkinson's disease and AAV2-RPE65 (retinal pigment epithelium-specific 65 kDa protein) is under investigation for severe retinal dystrophy. No serious adverse effects attributed to the vector were observed and each showed apparent biological effects of the transgene [164–166]. Of particular note is the long-term transgene expression observed from AAV vectors in clinical and preclinical trials, up to eight years in one case in nonhuman primates [167, 168]. In the cochlea, the main issues of viral vector gene delivery are the potential for immunological responses (inflammation) and toxicity. Modern AAV vectors have shown little evidence of toxicity in the cochlea and in particular bovine AAV was shown to be expressed in supporting cells and SGNs with no effect on hearing showing that it does not harm the delicate sensory cells of the cochlea [149]. For adenovirus, strong immune responses were reported for first generation adenoviral vectors [169, 170]. Unfortunately, when an advanced generation virus that lacks all viral coding sequences was introduced into the supporting cells of the cochlea, loss of hearing was still reported indicating enduring ototoxicity [149].

Although more controlled trials will be needed to prove the overall safety and effectiveness of gene therapy, it is encouraging that gene therapy has been conducted in the human nervous system. There is increasing evidence that gene therapy is providing clinical benefits to a range of diseases with ever improving vector design eliminating the adverse events that used to be associated with gene therapy [171]. A recent study by Pinyon et al. [172] used stimulation through a cochlear implant to cause localised electroporation of the mesenchymal cells in the perilynphatic canals in an animal model. This enabled focalised delivery of BDNF genes, and regrowth of SGN neurites close to the cochlear implant electrodes was observed. The combination of cochlear implantation with administration of gene therapies in the same surgery in recipients with low-frequency residual hearing is a compelling prospect. In this way, hearing would be restored (via the implant) and residual hearing would be both maintained and perhaps enhanced, from a single intervention. In order for this to become a reality, a method for long-term expression needs to be developed [173], as this is currently the missing piece to the EAS puzzle.

6. Conclusions

Despite being an emerging field, EAS promises to improve the lives of partially deaf people to whom a hearing aid does not provide a satisfactory listening experience.

There are two clear areas of research required to improve EAS outcomes: improving our understanding of how EAS is processed by the brain, and prevention of the hearing loss that occurs in a significant proportion of EAS listeners. This paper has focused on these two key areas and has shown that the interactions between acoustic and electrical stimulation at the level of the cochlea and along the auditory pathway require further exploration in order to understand their perceptual effects. This research, in turn, will lead to the refinement of processing strategies that will enable the benefits of combined EAS to be optimised to full effect, and minimise any interactions that may hinder ideal results.

The protection of residual hearing, both at the time of implantation and postimplantation, is of critical importance to successful EAS. A number of innovations have recently been made in terms of electrode array design and surgical

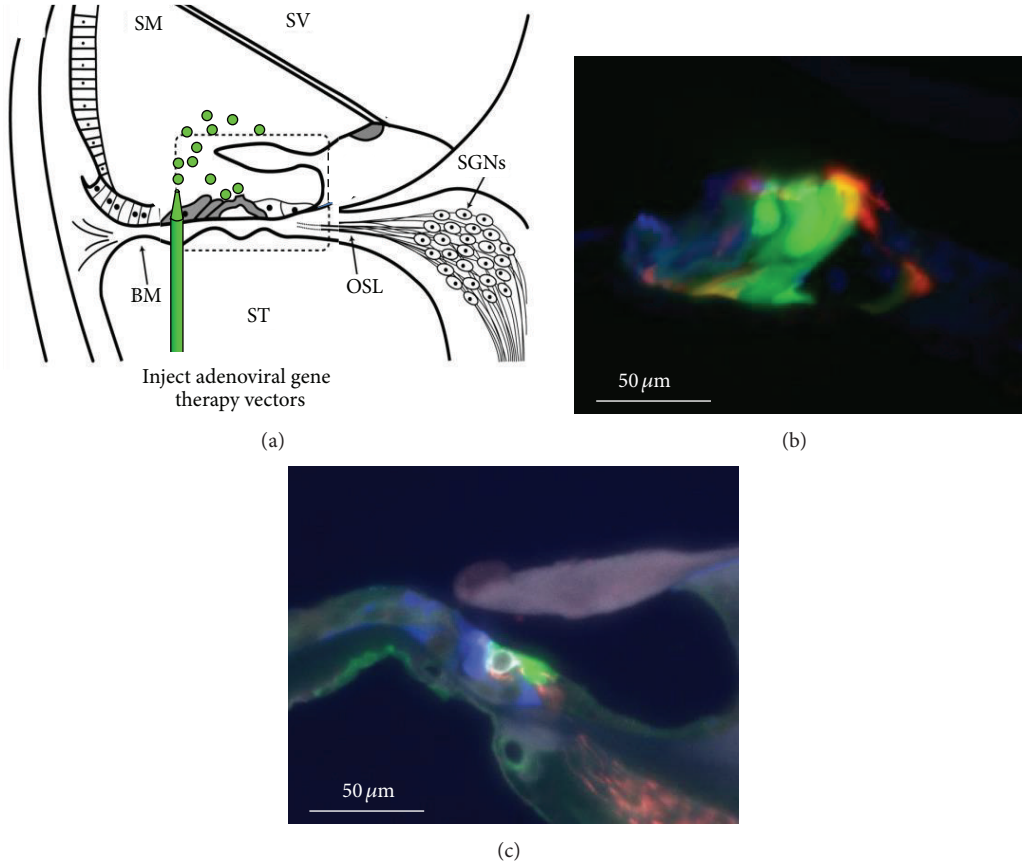

Figure 4: Gene therapy in the cochlea. (a) Injection of adenoviral vectors (green) carrying protective genes such as neurotrophic factors into the scala media of the cochlea results in gene expression in the organ of Corti (shaded cells) and enables protection of hair cells and SGNs. Dashed rectangle shows area of the cochlea shown in (b) and (c). (b) In the normal hearing cochlea, gene expression (green) can be observed in hair cells and supporting cells of the organ of Corti. Pillar cells are shown in red (phalloidin); other supporting cells are shown in blue (calretinin). (c) In a deafened guinea pig, the organ of Corti has degenerated at the time of gene therapy resulting in reduced gene expression (green). A degenerating hair cell is shown in white (myosin VIIa), supporting cells are shown in blue (calretinin) and nerve fibres are red (neurofilament heavy chain). SM: scala media; ST: scala tympani; SV: scala vestibuli; BM: basilar membrane; OSL: osseous spiral lamina; SGNs: spiral ganglion neurons.

technique that aim to promote residual hearing, but there are still reports of implantation-related hearing loss. As such, administration of protective agents prior to, during or after cochlear implantation could provide the required promotion of cochlear health to maintain residual hearing. There are a variety of therapeutic agents that have the potential to protect residual hearing and support SGN survival to enhance the benefits of EAS. Future studies in this field need to elucidate (i) the most suitable therapeutic agents, or combination thereof, (ii) the most effective treatment regime, which may be made up of various regimes and different drugs, and (iii) appropriate delivery methods, including acute administration and longer-lasting gene therapy treatments, in order to preserve residual hearing and SGN survival in cochlear implantation and maximise the benefits and outcomes of EAS.

We have shown that the combination of these agents with electrical stimulation can further promote neuronal growth [88] and it seems that this compelling combination deserves to be explored for the benefit of EAS recipients worldwide.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

[1] P. S. Roland and E. Tobey, "A tribute to a remarkably sound solution," *Cell*, vol. 154, no. 6, pp. 1175–1177, 2013.

[2] B. J. Gantz and C. W. Turner, "Combining acoustic and electrical hearing," *The Laryngoscope*, vol. 113, no. 10, pp. 1726–1730, 2003.

[3] A. L. L. Sampaio, M. F. S. Araujo, and C. A. C. P. Oliveira, "New criteria of indication and selection of patients to cochlear implant," *International Journal of Otolaryngology*, vol. 2011, Article ID 573968, 13 pages, 2011.

[4] W. K. Gstoettner, P. van de Heyning, A. Fitzgerald O'Connor et al., "Electric acoustic stimulation of the auditory system: results of a multi-centre investigation," *Acta Oto-Laryngologica*, vol. 128, no. 9, pp. 968–975, 2008.

[5] A. M. Tharpe and D. P. Sladen, "Causation of permanent unilateral and mild bilateral hearing loss in children," *Trends in Amplification*, vol. 12, no. 1, pp. 17–25, 2008.

[6] K. N. Talbot and D. E. Hartley, "Combined electro-acoustic stimulation: a beneficial union?" *Clinical Otolaryngology*, vol. 33, no. 6, pp. 536–545, 2008.

[7] M. F. Dorman and R. H. Gifford, "Combining acoustic and electric stimulation in the service of speech recognition," *International Journal of Audiology*, vol. 49, no. 12, pp. 912–919, 2010.

[8] B. J. Gantz, C. Turner, K. E. Gfeller, and M. W. Lowder, "Preservation of hearing in cochlear implant surgery: advantages of combined electrical and acoustical speech processing," *The Laryngoscope*, vol. 115, no. 5, pp. 796–802, 2005.

[9] W. Gstoettner, J. Kiefer, W.-D. Baumgartner, S. Pok, S. Peters, and O. Adunka, "Hearing preservation in cochlear implantation for electric acoustic stimulation," *Acta Oto-Laryngologica*, vol. 124, no. 4, pp. 348–352, 2004.

[10] P. V. Incerti, T. Y. C. Ching, and R. Cowan, "A systematic review of electric-acoustic stimulation: device fitting ranges, outcomes, and clinical fitting practices," *Trends in Amplification*, vol. 17, no. 1, pp. 3–26, 2013.

[11] C. C. Dunn, A. Perreau, B. Gantz, and R. S. Tyler, "Benefits of localization and speech perception with multiple noise sources in listeners with a short-electrode cochlear implant," *Journal of the American Academy of Audiology*, vol. 21, no. 1, pp. 44–51, 2010.

[12] Y.-Y. Kong, G. S. Stickney, and F.-G. Zeng, "Speech and melody recognition in binaurally combined acoustic and electric hearing," *Journal of the Acoustical Society of America*, vol. 117, no. 3, pp. 1351–1361, 2005.

[13] H. Skarzynski, A. Lorens, M. Matusiak, M. Porowski, P. H. Skarzynski, and C. J. James, "Partial deafness treatment with the nucleus straight research array cochlear implant," *Audiology and Neurotology*, vol. 17, no. 2, pp. 82–91, 2012.

[14] C. W. Turner, B. J. Gantz, C. Vidal, A. Behrens, and B. A. Henry, "Speech recognition in noise for cochlear implant listeners: benefits of residual acoustic hearing," *Journal of the Acoustical Society of America*, vol. 115, no. 4, pp. 1729–1735, 2004.

[15] K. E. Gfeller, C. Olszewski, C. Turner, B. Gantz, and J. Oleson, "Music perception with cochlear implants and residual hearing," *Audiology & Neurotology*, vol. 11, supplement 1, pp. 12–15, 2006.

[16] C. W. Turner and B. J. Gantz, "Combining acoustic and electric hearing," in *Auditory Prostheses: New Insights*, F. G. Zeng, A. N. Popper, and R. R. Fay, Eds., pp. 59–84, Springer, New York, NY, USA, 2012.

[17] S. Irving, A. K. Wise, R. E. Millard, R. K. Shepherd, and J. B. Fallon, "A partial hearing animal model for chronic electro-acoustic stimulation," *Journal of Neural Engineering*, vol. 11, no. 4, Article ID 046008, 2014.

[18] W. K. Gstoettner, S. Heibig, N. Maier, J. Kiefer, A. Radeloff, and O. F. Adunka, "Ipsilateral electric acoustic stimulation of the auditory system: results of long-term hearing preservation," *Audiology and Neurotology*, vol. 11, no. 1, pp. 49–56, 2006.

[19] C. W. Turner, L. A. J. Reiss, and B. J. Gantz, "Combined acoustic and electric hearing: preserving residual acoustic hearing," *Hearing Research*, vol. 242, no. 1-2, pp. 164–171, 2008.

[20] P. Ernfors, M. L. I. Duan, W. M. Elshamy, and B. Canlon, "Protection of auditory neurons from aminoglycoside toxicity by neurotrophin-3," *Nature Medicine*, vol. 2, no. 4, pp. 463–467, 1996.

[21] H. Staecker, R. Kopke, B. Malgrange, P. Lefebvre, and T. R. Van De Water, "NT-3 and/or BDNF therapy prevents loss of auditory neurons following loss of hair cells," *NeuroReport*, vol. 7, no. 4, pp. 889–894, 1996.

[22] L. N. Gillespie, G. M. Clark, P. F. Bartlett, and P. L. Marzella, "BDNF-induced survival of auditory neurons *in vivo*: cessation of treatment leads to accelerated loss of survival effects," *Journal of Neuroscience Research*, vol. 71, no. 6, pp. 785–790, 2003.

[23] M. J. H. Agterberg, H. Versnel, L. M. van Dijk, J. C. M. J. de Groot, and S. F. L. Klis, "Enhanced survival of spiral ganglion cells after cessation of treatment with brain-derived neurotrophic factor in deafened guinea pigs," *Journal of the Association for Research in Otolaryngology*, vol. 10, no. 3, pp. 355–367, 2009.

[24] H. C. Stronks, H. Versnel, V. F. Prijs, and S. F. L. Klis, "Suppression of the acoustically evoked auditory-nerve response by electrical stimulation in the cochlea of the guinea pig," *Hearing Research*, vol. 259, no. 1-2, pp. 64–74, 2010.

[25] C. Von Ilberg, J. Kiefer, J. Tillein et al., "Electric-acoustic stimulation of the auditory system," *ORL*, vol. 61, no. 6, pp. 334–340, 1999.

[26] C. A. Von Ilberg, U. Baumann, J. Kiefer, J. Tillein, and O. F. Adunka, "Electric-acoustic stimulation of the auditory system: a review of the first decade," *Audiology and Neurotology*, vol. 16, no. 2, pp. 1–30, 2011.

[27] E. C. Moxon, *Neural and Mechanical Responses to Electric Stimulation of the Cat's Inner Ear*, Department of Electrical Engineering, Massachusetts Institute of Technology, Boston, Mass, USA, 1971.

[28] K. V. Nourski, P. J. Abbas, C. A. Miller, B. K. Robinson, and F.-C. Jeng, "Acoustic-electric interactions in the guinea pig auditory nerve: simultaneous and forward masking of the electrically evoked compound action potential," *Hearing Research*, vol. 232, no. 1-2, pp. 87–103, 2007.

[29] K. V. Nourski, P. J. Abbas, C. A. Miller, B. K. Robinson, and F.-C. Jeng, "Effects of acoustic noise on the auditory nerve compound action potentials evoked by electric pulse trains," *Hearing Research*, vol. 202, no. 1-2, pp. 141–153, 2005.

[30] C. A. Miller, P. J. Abbas, B. K. Robinson, K. V. Nourski, F. Zhang, and F.-C. Jeng, "Auditory nerve fiber responses to combined acoustic and electric stimulation," *Journal of the Association for Research in Otolaryngology*, vol. 10, no. 3, pp. 425–445, 2009.

[31] G. V. Békésy, *Experiments in Hearing*, E. G. Wever, Editor, McGraw-Hill, New York, NY, USA, 1960.

[32] M. A. Ruggero and N. C. Rich, "Timing of spike initiation in cochlear afferents: dependence on site of innervation," *Journal of Neurophysiology*, vol. 58, no. 2, pp. 379–403, 1987.

[33] M. W. Skinner, G. M. Clark, L. A. Whitford et al., "Evaluation of a new spectral peak coding strategy for the nucleus 22 channel cochlear implant system," *American Journal of Otology*, vol. 15, supplement 2, pp. 15–27, 1994.

[34] K. I. McAnally and G. M. Clark, "Stimulation of residual hearing in the cat by pulsatile electrical stimulation of the cochlea," *Acta Oto-Laryngologica*, vol. 114, no. 4, pp. 366–372, 1994.

[35] C. A. Miller, P. J. Abbas, B. K. Robinson, K. V. Nourski, F. Zhang, and F.-C. Jeng, "Electrical excitation of the acoustically sensitive auditory nerve: single-fiber responses to electric pulse trains," *Journal of the Association for Research in Otolaryngology*, vol. 7, no. 3, pp. 195–210, 2006.

[36] H. C. Stronks, H. Versnel, V. F. Prijs, J. C. M. J. de Groot, W. Grolman, and S. F. L. Klis, "The role of electrophonics in electroacoustic stimulation of the guinea pig cochlea," *Otology and Neurotology*, vol. 34, no. 3, pp. 579–587, 2013.

[37] N. Hu, P. J. Abbas, C. A. Miller et al., "Auditory response to intracochlear electric stimuli following furosemide treatment," *Hearing Research*, vol. 185, no. 1-2, pp. 77–89, 2003.

[38] H. C. Stronks, H. Versnel, V. F. Prijs, W. Grolman, and S. F. L. Klis, "Effects of electrical stimulation on the acoustically evoked auditory-nerve response in guinea pigs with a high-frequency hearing loss," *Hearing Research*, vol. 272, no. 1-2, pp. 95–107, 2011.

[39] B. U. Seeber, U. Baumann, and H. Fastl, "Localization ability with bimodal hearing aids and bilateral cochlear implants," *Journal of the Acoustical Society of America*, vol. 116, no. 3, pp. 1698–1709, 2004.

[40] M. Vollmer, R. Hartmann, and J. Tillein, "Neuronal responses in cat inferior colliculus to combined acoustic and electric stimulation," *Advances in Oto-Rhino-Laryngology*, vol. 67, pp. 61–69, 2010.

[41] L. A. Reiss, C. W. Turner, S. A. Karsten, and B. J. Gantz, "Plasticity in human pitch perception induced by tonotopically mismatched electro-acoustic stimulation," *Neuroscience*, vol. 256, pp. 43–52, 2014.

[42] J. B. Fallon, R. K. Shepherd, M. Brown, and D. R. F. Irvine, "Effects of neonatal partial deafness and chronic intracochlear electrical stimulation on auditory and electrical response characteristics in primary auditory cortex," *Hearing Research*, vol. 257, no. 1-2, pp. 93–105, 2009.

[43] O. Handzel, B. J. Burgess, and J. B. Nadol Jr., "Histopathology of the peripheral vestibular system after cochlear implantation in the human," *Otology and Neurotology*, vol. 27, no. 1, pp. 57–64, 2006.

[44] J. Lee, J. B. Nadol Jr., and D. K. Eddington, "Depth of electrode insertion and postoperative performance in humans with cochlear implants: a histopathologic study," *Audiology & Neurotology*, vol. 15, no. 5, pp. 323–331, 2010.

[45] J. B. Nadol Jr., B. J. Burgess, B. J. Gantz et al., "Histopathology of cochlear implants in humans," *Annals of Otology, Rhinology and Laryngology*, vol. 110, no. 9, pp. 883–891, 2001.

[46] R. K. Shepherd, G. M. Clark, R. C. Black, and J. F. Patrick, "The histopathological effects of chronic electrical stimulation of the cat cochlea," *The Journal of Laryngology and Otology*, vol. 97, no. 4, pp. 333–341, 1983.

[47] R. L. Webb, G. M. Clark, R. K. Shepherd, B. K.-H. Franz, and B. C. Pyman, "The biologic safety of the cochlear corporation multiple-electrode intracochlear implant," *The American Journal of Otology*, vol. 9, no. 1, pp. 8–13, 1988.

[48] B. J. Gantz and C. Turner, "Combining acoustic and electrical speech processing: iowa/nucleus hybrid implant," *Acta Oto-Laryngologica*, vol. 124, no. 4, pp. 344–347, 2004.

[49] J. Kiefer, W. Gstoettner, W. Baumgartner et al., "Conservation of low-frequency hearing in cochlear implantation," *Acta Oto-Laryngologica*, vol. 124, no. 3, pp. 272–280, 2004.

[50] A. V. Hodges, J. Schloffman, and T. Balkany, "Conservation of residual hearing with cochlear implantation," *The American Journal of Otology*, vol. 18, no. 2, pp. 179–183, 1997.

[51] C. James, K. Albegger, R. Battmer et al., "Preservation of residual hearing with cochlear implantation: how and why," *Acta Oto-Laryngologica*, vol. 125, no. 5, pp. 481–491, 2005.

[52] E. A. Woodson, L. A. J. Reiss, C. W. Turner, K. Gfeller, and B. J. Gantz, "The hybrid cochlear implant: a review," *Advances in Oto-Rhino-Laryngology*, vol. 67, pp. 125–134, 2010.

[53] R. J. S. Briggs, M. Tykocinski, J. Xu et al., "Comparison of round window and cochleostomy approaches with a prototype hearing preservation electrode," *Audiology and Neurotology*, vol. 11, supplement 1, pp. 42–48, 2006.

[54] M. B. Fitzgerald, E. Sagi, M. Jackson et al., "Reimplantation of hybrid cochlear implant users with a full-length electrode after loss of residual hearing," *Otology & Neurotology*, vol. 29, no. 2, pp. 168–173, 2008.

[55] M. L. Carlson, C. L. Driscoll, R. H. Gifford et al., "Implications of minimizing trauma during conventional cochlear implantation," *Otology & Neurotology*, vol. 32, no. 6, pp. 962–968, 2011.

[56] P. J. Boyd, "Potential benefits from deeply inserted cochlear implant electrodes," *Ear and Hearing*, vol. 32, no. 4, pp. 411–427, 2011.

[57] E. Lehnhardt, "Intracochlear placement of cochlear implant electrodes in soft surgery technique," *HNO*, vol. 41, no. 7, pp. 356–359, 1993.

[58] B. Fraysse, Á. R. Macías, O. Sterkers et al., "Residual hearing conservation and electroacoustic stimulation with the nucleus 24 contour advance cochlear implant," *Otology & Neurotology*, vol. 27, no. 5, pp. 624–633, 2006.

[59] O. Adunka, M. H. Unkelbach, M. Mack, M. Hambek, W. Gstoettner, and J. Kiefer, "Cochlear implantation via the round window membrane minimizes trauma to cochlear structures: a histologically controlled insertion study," *Acta Oto-Laryngologica*, vol. 124, no. 7, pp. 807–812, 2004.

[60] P. S. Roland and C. G. Wright, "Surgical aspects of cochlear implantation: mechanisms of insertional trauma," *Advances in Oto-Rhino-Laryngology*, vol. 64, pp. 11–30, 2006.

[61] H. Skarzynski, A. Lorens, A. Piotrowska, and I. Anderson, "Preservation of low frequency hearing in partial deafness cochlear implantation (PDCI) using the round window surgical approach," *Acta Oto-Laryngologica*, vol. 127, no. 1, pp. 41–48, 2007.

[62] N. L. Cohen, "Cochlear implant soft surgery: fact or fantasy?" *Otolaryngology—Head and Neck Surgery*, vol. 117, no. 3, pp. 214–216, 1997.

[63] R. Lee and K. Asare, "Therapeutic hypothermia for out-of-hospital cardiac arrest," *The American Journal of Health-System Pharmacy*, vol. 67, no. 15, pp. 1229–1237, 2010.

[64] M. Hägerdal, J. Harp, L. Nilsson, and B. K. Siesjö, "The effect of induced hypothermia upon oxygen consumption in the rat brain," *Journal of Neurochemistry*, vol. 24, no. 2, pp. 311–316, 1975.

[65] A. Mitani, F. Kadoya, and K. Kataoka, "Temperature dependence of hypoxia-induced calcium accumulation in gerbil hippocampal slices," *Brain Research*, vol. 562, no. 1, pp. 159–163, 1991.

[66] A. Kader, V. I. Frazzini, C. J. Baker, R. A. Solomon, R. R. Trifiletti, and W. Young, "Effect of mild hypothermia on nitric oxide synthesis during focal cerebral ischemia," *Neurosurgery*, vol. 35, no. 2, pp. 272–277, 1994.

[67] S. Takeda, N. Hakuba, T. Yoshida et al., "Postischemic mild hypothermia alleviates hearing loss because of transient ischemia," *NeuroReport*, vol. 19, no. 13, pp. 1325–1328, 2008.

[68] K. R. Henry, "Hyperthermia exacerbates and hypothermia protects from noise-induced threshold elevation of the cochlear nerve envelope response in the C57BL/6J mouse," *Hearing Research*, vol. 179, no. 1-2, pp. 88–96, 2003.

[69] T. J. Balkany, A. A. Eshraghi, H. Jiao et al., "Mild hypothermia protects auditory function during cochlear implant surgery," *The Laryngoscope*, vol. 115, no. 9, pp. 1543–1547, 2005.

[70] F. Watanabe, K. Koga, N. Hakuba, and K. Gyo, "Hypothermia prevents hearing loss and progressive hair cell loss after transient cochlear ischemia in gerbils," *Neuroscience*, vol. 102, no. 3, pp. 639–645, 2001.

[71] K. R. Henry and R. A. Chole, "Hypothermia protects the cochlea from noise damage," *Hearing Research*, vol. 16, no. 3, pp. 225–230, 1984.

[72] C.-J. Chen, Y.-C. Ou, C.-Y. Chang et al., "Glutamate released by Japanese encephalitis virus-infected microglia involves TNF-α signaling and contributes to neuronal death," *GLIA*, vol. 60, no. 3, pp. 487–501, 2012.

[73] P. P. Lefebvre, B. Malgrange, F. Lallemend, H. Staecker, G. Moonen, and T. R. van de Water, "Mechanisms of cell death in the injured auditory system: otoprotective strategies," *Audiology and Neuro-Otology*, vol. 7, no. 3, pp. 165–170, 2002.

[74] T. Huang, A. G. Cheng, H. Stupak et al., "Oxidative stress-induced apoptosis of cochlear sensory cells: otoprotective strategies," *International Journal of Developmental Neuroscience*, vol. 18, no. 2-3, pp. 259–270, 2000.

[75] J. N. Fayad, A. O. Makarem, and F. H. Linthicum Jr., "Histopathologic assessment of fibrosis and new bone formation in implanted human temporal bones using 3D reconstruction," *Otolaryngology—Head and Neck Surgery*, vol. 141, no. 2, pp. 247–252, 2009.

[76] J. B. Nadol Jr., D. K. Eddington, and B. J. Burgess, "Foreign body or hypersensitivity granuloma of the inner ear after cochlear implantation: one possible cause of a soft failure?" *Otology & Neurotology*, vol. 29, no. 8, pp. 1076–1084, 2008.

[77] F. M. Rizer, P. N. Arkis, W. H. Lippy, and A. G. Schuring, "A postoperative audiometric evaluation of cochlear implant patients," *Otolaryngology—Head and Neck Surgery*, vol. 98, no. 3, pp. 203–206, 1988.

[78] W. J. Boggess, J. E. Baker, and T. J. Balkany, "Loss of residual hearing after cochlear implantation," *The Laryngoscope*, vol. 99, no. 10, part 1, pp. 1002–1005, 1989.

[79] J. B. Nadol Jr. and D. K. Eddington, "Histopathology of the inner ear relevant to cochlear implantation," *Advances in Oto-Rhino-Laryngology*, vol. 64, pp. 31–49, 2006.

[80] R. F. Canalis, R. Gussen, E. Abemayor, and J. Andrews, "Surgical trauma to the lateral semicircular canal with preservation of hearing," *Laryngoscope*, vol. 97, no. 5, pp. 575–581, 1987.

[81] E. E. Smouha, "Surgery of the inner ear with hearing preservation: serial histological changes," *The Laryngoscope*, vol. 113, no. 9, pp. 1439–1449, 2003.

[82] M. A. Somdas, P. M. M. C. Li, D. M. Whiten, D. K. Eddington, and J. B. Nadol Jr., "Quantitative evaluation of new bone and fibrous tissue in the cochlea following cochlear implantation in the human," *Audiology and Neurotology*, vol. 12, no. 5, pp. 277–284, 2007.

[83] C.-H. Choi and J. S. Oghalai, "Predicting the effect of post-implant cochlear fibrosis on residual hearing," *Hearing Research*, vol. 205, no. 1-2, pp. 193–200, 2005.

[84] F. J. Cervera-Paz and F. H. Linthicum Jr., "Cochlear wall erosion after cochlear implantation," *Annals of Otology, Rhinology and Laryngology*, vol. 114, no. 7, pp. 543–546, 2005.

[85] L. N. Gillespie, G. M. Clark, and P. L. Marzella, "Delayed neurotrophin treatment supports auditory neuron survival in deaf guinea pigs," *NeuroReport*, vol. 15, no. 7, pp. 1121–1125, 2004.

[86] L. N. Gillespie, M. P. Zanin, and R. K. Shepherd, *Cell-Based Neurotrophin Delivery for Auditory Neuron Survival in Deafness*, Australian Neuroscience Society, Melbourne, Australia, 2013.

[87] L. N. Pettingill, A. K. Wise, M. S. Geaney, and R. K. Shepherd, "Enhanced auditory neuron survival following cell-based bdnf treatment in the deaf guinea pig," *PLoS ONE*, vol. 6, no. 4, Article ID e18733, 2011.

[88] A. K. Wise, J. B. Fallon, A. J. Neil et al., "Combining cell-based therapies and neural prostheses to promote neural survival," *Neurotherapeutics*, vol. 8, no. 4, pp. 774–787, 2011.

[89] A. K. Wise, R. Richardson, J. Hardman, G. Clark, and S. O'Leary, "Resprouting and survival of guinea pig cochlear neurons in response to the administration of the neurotrophins brain-derived neurotrophic factor and neurotrophin-3," *The Journal of Comparative Neurology*, vol. 487, no. 2, pp. 147–165, 2005.

[90] F. Noushi, R. T. Richardson, J. Hardman, G. Clark, and S. O'Leary, "Delivery of neurotrophin-3 to the cochlea using alginate beads," *Otology & Neurotology*, vol. 26, no. 3, pp. 528–533, 2005.

[91] J. Ito, T. Endo, T. Nakagawa, T. Kita, T.-S. Kim, and F. Iguchi, "A new method for drug application to the inner ear," *ORL*, vol. 67, no. 5, pp. 272–275, 2005.

[92] S. Havenith, H. Versnel, M. J. H. Agterberg et al., "Spiral ganglion cell survival after round window membrane application of brain-derived neurotrophic factor using gelfoam as carrier," *Hearing Research*, vol. 272, no. 1-2, pp. 168–177, 2011.

[93] L. C. Schecterson and M. Bothwell, "Neurotrophin and neurotrophin receptor mRNA expression in developing inner ear," *Hearing Research*, vol. 73, no. 1, pp. 92–100, 1994.

[94] J. Ylikoski, U. Pirvola, M. Moshnyakov, J. Palgi, U. Arumae, and M. Saarma, "Expression patterns of neurotrophin and their receptor mRNAs in the rat inner ear," *Hearing Research*, vol. 65, no. 1-2, pp. 69–78, 1993.

[95] K. Stankovic, C. Rio, A. Xia et al., "Survival of adult spiral ganglion neurons requires erbB receptor signaling in the inner ear," *Journal of Neuroscience*, vol. 24, no. 40, pp. 8651–8661, 2004.

[96] Y. Zilberstein, M. C. Liberman, and G. Corfas, "Inner hair cells are not required for survival of spiral ganglion neurons in the adult cochlea," *Journal of Neuroscience*, vol. 32, no. 2, pp. 405–410, 2012.

[97] L. N. Gillespie, G. M. Clark, P. F. Bartlett, and P. L. Marzella, "BDNF-induced survival of auditory neurons in vivo: cessation of treatment leads to accelerated loss of survival effects," *Journal of Neuroscience Research*, vol. 71, no. 6, pp. 785–790, 2003.

[98] R. K. Shepherd, A. Coco, and S. B. Epp, "Neurotrophins and electrical stimulation for protection and repair of spiral ganglion neurons following sensorineural hearing loss," *Hearing Research*, vol. 242, no. 1-2, pp. 100–109, 2008.

[99] R. K. Shepherd, A. Coco, S. B. Epp, and J. M. Crook, "Chronic depolarization enhances the trophic effects of brain-derived neurotrophic factor in rescuing auditory neurons following a sensorineural hearing loss," *Journal of Comparative Neurology*, vol. 486, no. 2, pp. 145–158, 2005.

[100] A. K. Wise, C. R. Hume, B. O. Flynn et al., "Effects of localized neurotrophin gene expression on spiral ganglion neuron

resprouting in the deafened cochlea," *Molecular Therapy*, vol. 18, no. 6, pp. 1111–1122, 2010.

[101] A. K. Wise, R. Richardson, J. Hardman, G. Clark, and S. O'Leary, "Resprouting and survival of guinea pig cochlear neurons in response to the administration of the neurotrophins brain-derived neurotrophic factor and neurotrophin-3," *Journal of Comparative Neurology*, vol. 487, no. 2, pp. 147–165, 2005.

[102] E. M. Keithley, C. L. Ma, A. F. Ryan, J.-C. Louis, and E. Magal, "GDNF protects the cochlea against noise damage," *NeuroReport*, vol. 9, no. 10, pp. 2183–2187, 1998.

[103] R. Kuang, G. Hever, G. Zajic et al., "Glial cell line-derived neurotrophic factor. Potential for otoprotection," *Annals of the New York Academy of Sciences*, vol. 884, pp. 270–291, 1999.

[104] F. Shoji, A. L. Miller, A. Mitchell, T. Yamasoba, R. A. Altschuler, and J. M. Miller, "Differential protective effects of neurotrophins in the attenuation of noise-induced hair cell loss," *Hearing Research*, vol. 146, no. 1-2, pp. 134–142, 2000.

[105] S.-Q. Zhai, J.-C. Cheng, J.-L. Wang, W.-Y. Yang, R. Gu, and S.-C. Jiang, "Protective effect of basic fibroblast growth factor on auditory hair cells after noise exposure," *Acta Oto-Laryngologica*, vol. 122, no. 4, pp. 370–373, 2002.

[106] S.-Q. Zhai, D.-J. Wang, J.-L. Wang, D.-Y. Han, and W.-Y. Yang, "Basic fibroblast growth factor protects auditory neurons and hair cells from glutamate neurotoxicity and noise exposure," *Acta Oto-Laryngologica*, vol. 124, no. 2, pp. 124–129, 2004.

[107] D. J. Sly, A. J. Hampson, R. L. Minter et al., "Brain-derived neurotrophic factor modulates auditory function in the hearing cochlea," *Journal of the Association for Research in Otolaryngology*, vol. 13, no. 1, pp. 1–16, 2012.

[108] S. Kanzaki, T. Stöver, K. Kawamoto et al., "Glial cell line-derived neurotrophic factor and chronic electrical stimulation prevent VIII cranial nerve degeneration following denervation," *Journal of Comparative Neurology*, vol. 454, no. 3, pp. 350–360, 2002.

[109] D. R. Trune and B. Canlon, "Corticosteroid therapy for hearing and balance disorders," *Anatomical Record*, vol. 295, no. 11, pp. 1928–1943, 2012.

[110] P. A. Bird, E. J. Begg, M. Zhang, A. T. Keast, D. P. Murray, and T. J. Balkany, "Intratympanic versus intravenous delivery of methylprednisolone to cochlear perilymph," *Otology and Neurotology*, vol. 28, no. 8, pp. 1124–1130, 2007.

[111] P. A. Bird, D. P. Murray, M. Zhang, and E. J. Begg, "Intratympanic versus intravenous delivery of dexamethasone and dexamethasone sodium phosphate to cochlear perilymph," *Otology & Neurotology*, vol. 32, no. 6, pp. 933–936, 2011.

[112] S. S. Chandrasekhar, R. Y. Rubinstein, J. A. Kwartler et al., "Dexamethasone pharmacokinetics in the inner ear: comparison of route of administration and use of facilitating agents," *Otolaryngology: Head and Neck Surgery*, vol. 122, no. 4, pp. 521–528, 2000.

[113] R. J. Vivero, D. E. Joseph, S. Angeli et al., "Dexamethasone base conserves hearing from electrode trauma-induced hearing loss," *Laryngoscope*, vol. 118, no. 11, pp. 2028–2035, 2008.

[114] T. Rhen and J. A. Cidlowski, "Antiinflammatory action of glucocorticoids—new mechanisms for old drugs," *New England Journal of Medicine*, vol. 353, no. 16, pp. 1658–1723, 2005.

[115] A. Chang, H. Eastwood, D. Sly, D. James, R. Richardson, and S. O'Leary, "Factors influencing the efficacy of round window dexamethasone protection of residual hearing post-cochlear implant surgery," *Hearing Research*, vol. 255, no. 1-2, pp. 67–72, 2009.

[116] D. P. James, H. Eastwood, R. T. Richardson, and S. J. O'Leary, "Effects of round window dexamethasone on residual hearing in a guinea pig model of cochlear implantation," *Audiology and Neurotology*, vol. 13, no. 2, pp. 86–96, 2008.

[117] S. Maini, H. Lisnichuk, H. Eastwood et al., "Targeted therapy of the inner ear," *Audiology and Neurotology*, vol. 14, no. 6, pp. 402–410, 2009.

[118] G. P. Rajan, J. Kuthubutheen, N. Hedne, and J. Krishnaswamy, "The role of preoperative, intratympanic glucocorticoids for hearing preservation in cochlear implantation: a prospective clinical study," *Laryngoscope*, vol. 122, no. 1, pp. 190–195, 2012.

[119] E. Dolgin, "Sound medicine," *Nature Medicine*, vol. 18, no. 5, pp. 642–645, 2012.

[120] J. Schacht, A. E. Talaska, and L. P. Rybak, "Cisplatin and aminoglycoside antibiotics: hearing loss and its prevention," *Anatomical Record*, vol. 295, no. 11, pp. 1837–1850, 2012.

[121] D. Henderson, E. C. Bielefeld, K. C. Harris, and B. H. Hu, "The role of oxidative stress in noise-induced hearing loss," *Ear and Hearing*, vol. 27, no. 1, pp. 1–19, 2006.

[122] H. Eastwood, D. Pinder, D. James et al., "Permanent and transient effects of locally delivered n-acetyl cysteine in a guinea pig model of cochlear implantation," *Hearing Research*, vol. 259, no. 1-2, pp. 24–30, 2010.

[123] T. Erdem, T. Bayindir, A. Filiz, M. Iraz, and E. Selimoglu, "The effect of resveratrol on the prevention of cisplatin ototoxicity," *European Archives of Oto-Rhino-Laryngology*, vol. 269, no. 10, pp. 2185–2188, 2012.

[124] K. Kawamoto, S.-H. Sha, R. Minoda et al., "Antioxidant gene therapy can protect hearing and hair cells from ototoxicity," *Molecular Therapy*, vol. 9, no. 2, pp. 173–181, 2004.

[125] T. Yamasoba, A. L. Nuttall, C. Harris, Y. Raphael, and J. M. Miller, "Role of glutathione in protection against noise-induced hearing loss," *Brain Research*, vol. 784, no. 1-2, pp. 82–90, 1998.

[126] A. C. Yumusakhuylu, M. Yazici, M. Sari et al., "Protective role of resveratrol against cisplatin induced ototoxicity in guinea pigs," *International Journal of Pediatric Otorhinolaryngology*, vol. 76, no. 3, pp. 404–408, 2012.

[127] S.-H. Sha, G. Zajic, C. J. Epstein, and J. Schacht, "Overexpression of copper/zinc-superoxide dismutase protects from kanamycin-induced hearing loss," *Audiology and Neuro-Otology*, vol. 6, no. 3, pp. 117–123, 2001.

[128] J. Chang, H. H. Jung, J. Y. Yang et al., "Protective effect of metformin against cisplatin-induced ototoxicity in an auditory cell line," *Journal of the Association for Research in Otolaryngology*, vol. 15, no. 2, pp. 149–158, 2014.

[129] G. Polony, V. Humli, R. Andó et al., "Protective effect of rasagiline in aminoglycoside ototoxicity," *Neuroscience*, vol. 265, pp. 263–273, 2014.

[130] A. A. Eshraghi, J. Wang, E. Adil et al., "Blocking c-Jun-N-terminal kinase signaling can prevent hearing loss induced by both electrode insertion trauma and neomycin ototoxicity," *Hearing Research*, vol. 226, no. 1-2, pp. 168–177, 2007.

[131] J. I. Matsui, J. M. Ogilvie, and M. E. Warchol, "Inhibition of caspases prevents ototoxic and ongoing hair cell death," *Journal of Neuroscience*, vol. 22, no. 4, pp. 1218–1227, 2002.

[132] L. A. May, I. I. Kramarenko, C. S. Brandon et al., "Inner ear supporting cells protect hair cells by secreting HSP70," *Journal of Clinical Investigation*, vol. 123, no. 8, pp. 3577–3587, 2013.

[133] R. Gabaizadeh, H. Staecker, W. Liu et al., "Protection of both auditory hair cells and auditory neurons from cisplatin induced damage," *Acta Oto-Laryngologica*, vol. 117, no. 2, pp. 232–238, 1997.

[134] J. Maruyama, J. M. Miller, and M. Ulfendahl, "Glial cell line-derived neurotrophic factor and antioxidants preserve the electrical responsiveness of the spiral ganglion neurons after experimentally induced deafness," *Neurobiology of Disease*, vol. 29, no. 1, pp. 14–21, 2008.

[135] S. J. M. Skinner, M. S. Geaney, H. Lin et al., "Encapsulated living choroid plexus cells: potential long-term treatments for central nervous system disease and trauma," *Journal of Neural Engineering*, vol. 6, no. 6, Article ID 065001, 2009.

[136] H. Staecker, R. Gabaizadeh, H. Federoff, and T. R. Van de Water, "Brain-derived neurotrophic factor gene therapy prevents spiral ganglion degeneration after hair ceil loss," *Otolaryngology—Head and Neck Surgery*, vol. 119, no. 1, pp. 7–13, 1998.

[137] T. Nakaizumi, K. Kawamoto, R. Minoda, and Y. Raphael, "Adenovirus-mediated expression of brain-derived neurotrophic factor protects spiral ganglion neurons from ototoxic damage," *Audiology and Neuro-Otology*, vol. 9, no. 3, pp. 135–143, 2004.

[138] S. B. Shibata, S. R. Cortez, L. A. Beyer et al., "Transgenic BDNF induces nerve fiber regrowth into the auditory epithelium in deaf cochleae," *Experimental Neurology*, vol. 223, no. 2, pp. 464–472, 2010.

[139] M. Yagi, S. Kanzaki, K. Kawamoto et al., "Spiral ganglion neurons are protected from degeneration by GDNF gene therapy," *Journal of the Association for Research in Otolaryngology*, vol. 1, no. 4, pp. 315–325, 2000.

[140] P. J. Atkinson, A. K. Wise, B. O. Flynn et al., "Neurotrophin gene therapy for sustained neural preservation after deafness," *PLoS ONE*, vol. 7, no. 12, Article ID e52338, 2012.

[141] H. Fukui, H. T. Wong, L. A. Beyer et al., "BDNF gene therapy induces auditory nerve survival and fiber sprouting in deaf Pou4f3 mutant mice," *Scientific Reports*, vol. 2, article 838, 2012.

[142] J. A. Chikar, D. J. Colesa, D. L. Swiderski, A. D. Polo, Y. Raphael, and B. E. Pfingst, "Over-expression of BDNF by adenovirus with concurrent electrical stimulation improves cochlear implant thresholds and survival of auditory neurons," *Hearing Research*, vol. 245, no. 1-2, pp. 24–34, 2008.

[143] P. J. Atkinson, A. K. Wise, B. O. Flynn, B. A. Nayagam, and R. T. Richardson, "Viability of long-term gene therapy in the cochlea," *Scientific Reports*, vol. 4, article 4733, 2014.

[144] S. Q. Zhai, W. Guo, Y. Y. Hu et al., "Protective effects of brain-derived neurotrophic factor on the noise-damaged cochlear spiral ganglion," *The Journal of Laryngology & Otology*, vol. 125, no. 5, pp. 449–454, 2010.

[145] H. Staecker, D. Li, B. W. O'Malley, and T. R. van de Water, "Gene expression in the mammalian cochlea: a study of multiple vector systems," *Acta Oto-Laryngologica*, vol. 121, no. 2, pp. 157–163, 2001.

[146] M. L. Derby, M. Sena-Esteves, X. O. Breakefield, and D. P. Corey, "Gene transfer into the mammalian inner ear using HSV-1 and vaccinia virus vectors," *Hearing Research*, vol. 134, no. 1-2, pp. 1–8, 1999.

[147] A. K. Lalwani, B. J. Walsh, P. G. Reilly, N. Muzyczka, and A. N. Mhatre, "Development of in vivo gene therapy for hearing disorders: introduction of adeno-associated virus into the cochlea of the guinea pig," *Gene Therapy*, vol. 3, no. 7, pp. 588–592, 1996.

[148] M. A. Weiss, J. C. Frisancho, B. J. Roessler, and Y. Raphael, "Viral-mediated gene transfer in the cochlea," *International Journal of Developmental Neuroscience*, vol. 15, no. 4-5, pp. 577–583, 1997.

[149] A. M. Sheffield, S. P. Gubbels, M. S. Hildebrand et al., "Viral vector tropism for supporting cells in the developing murine cochlea," *Hearing Research*, vol. 277, no. 1-2, pp. 28–36, 2011.

[150] E. Ballana, J. Wang, F. Venail et al., "Efficient and specific transduction of cochlear supporting cells by adeno-associated virus serotype 5," *Neuroscience Letters*, vol. 442, no. 2, pp. 134–139, 2008.

[151] Y. Liu, T. Okada, K. Sheykholeslami et al., "Specific and efficient transduction of cochlear inner hair cells with recombinant adeno-associated virus type 3 vector," *Molecular Therapy*, vol. 12, no. 4, pp. 725–733, 2005.

[152] R. H. Lachmann and S. Efstathiou, "Use of herpes simplex virus type 1 for transgene expression within the nervous system," *Clinical Science*, vol. 96, no. 6, pp. 533–541, 1999.

[153] B. Boëda, D. Weil, and C. Petit, "A specific promoter of the sensory cells of the inner ear defined by transgenesis," *Human Molecular Genetics*, vol. 10, no. 15, pp. 1581–1589, 2001.

[154] M. Li Duan, T. Bordet, M. Mezzina, A. Kahn, and M. Ulfendahl, "Adenoviral and adeno-associated viral vector mediated gene transfer in the guinea pig cochlea," *NeuroReport*, vol. 13, no. 10, pp. 1295–1299, 2002.

[155] Y. Raphael, J. C. Frisancho, and B. J. Roessler, "Adenoviral-mediated gene transfer into guinea pig cochlear cells in vivo," *Neuroscience Letters*, vol. 207, no. 2, pp. 137–141, 1996.

[156] S. B. Shibata, G. Di Pasquale, S. R. Cortez, J. A. Chiorini, and Y. Raphael, "Gene transfer using bovine adeno-associated virus in the guinea pig cochlea," *Gene Therapy*, vol. 16, no. 8, pp. 990–997, 2009.

[157] S.-I. Ishimoto, K. Kawamoto, S. Kanzaki, and Y. Raphael, "Gene transfer into supporting cells of the organ of Corti," *Hearing Research*, vol. 173, no. 1-2, pp. 187–197, 2002.

[158] S. T. Kho, R. M. Pettis, A. N. Mhatre, and A. K. Lalwani, "Safety of adeno-associated virus as cochlear gene transfer vector: analysis of distant spread beyond injected cochleae," *Molecular Therapy*, vol. 2, no. 4, pp. 368–373, 2000.

[159] T. Stöver, M. Yagi, and Y. Raphael, "Transduction of the contralateral ear after adenovirus-mediated cochlear gene transfer," *Gene Therapy*, vol. 7, no. 5, pp. 377–383, 2000.

[160] A. K. Lalwani, B. J. Walsh, G. J. Carvalho, N. Muzyczka, and A. N. Mhatre, "Expression of adeno-associated virus integrated transgene within the mammalian vestibular organs," *The American Journal of Otology*, vol. 19, no. 3, pp. 390–395, 1998.

[161] K. Kawamoto, S.-H. Oh, S. Kanzaki, N. Brown, and Y. Raphael, "The functional and structural outcome of inner ear gene transfer via the vestibular and cochlear fluids in mice," *Molecular Therapy*, vol. 4, no. 6, pp. 575–585, 2001.

[162] D. Mukherjea, S. Jajoo, T. Kaur, K. E. Sheehan, V. Ramkumar, and L. P. Rybak, "Transtympanic administration of short interfering (si)rna for the nox3 isoform of NADPH oxidase protects against cisplatin-induced hearing loss in the rat," *Antioxidants & Redox Signaling*, vol. 13, no. 5, pp. 589–598, 2010.

[163] S. B. Shibata, S. R. Cortez, J. A. Wiler, D. L. Swiderski, and Y. Raphael, "Hyaluronic acid enhances gene delivery into the cochlea," *Human Gene Therapy*, vol. 23, no. 3, pp. 302–310, 2012.

[164] P. A. LeWitt, A. R. Rezai, M. A. Leehey et al., "AAV2-GAD gene therapy for advanced Parkinson's disease: a double-blind, sham-surgery controlled, randomised trial," *The Lancet Neurology*, vol. 10, no. 4, pp. 309–319, 2011.

[165] M. G. Kaplitt, A. Feigin, C. Tang et al., "Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: an open label, phase I trial," *The Lancet*, vol. 369, no. 9579, pp. 2097–2105, 2007.

[166] J. W. B. Bainbridge, A. J. Smith, S. S. Barker et al., "Effect of gene therapy on visual function in Leber's congenital amaurosis," *The New England Journal of Medicine*, vol. 358, no. 21, pp. 2231–2239, 2008.

[167] P. Hadaczek, J. L. Eberling, P. Pivirotto, J. Bringas, J. Forsayeth, and K. S. Bankiewicz, "Eight years of clinical improvement in MPTP-lesioned primates after gene therapy with AAV2-hAADC," *Molecular Therapy*, vol. 18, no. 8, pp. 1458–1461, 2010.

[168] A. C. Nathwani, E. G. D. Tuddenham, S. Rangarajan et al., "Adenovirus-associated virus vector-mediated gene transfer in hemophilia B," *The New England Journal of Medicine*, vol. 365, no. 25, pp. 2357–2365, 2011.

[169] J. R. Holt, D. C. Johns, S. Wang et al., "Functional expression of exogenous proteins in mammalian sensory hair cells infected with adenoviral vectors," *Journal of Neurophysiology*, vol. 81, no. 4, pp. 1881–1888, 1999.

[170] A. E. Luebke, J. D. Steiger, B. L. Hodges, and A. Amalfitano, "A modified adenovirus can transfect cochlear hair cells in vivo without compromising cochlear function," *Gene Therapy*, vol. 8, no. 10, pp. 789–794, 2001.

[171] S. L. Ginn, I. E. Alexander, M. L. Edelstein, M. R. Abedi, and J. Wixon, "Gene therapy clinical trials worldwide to 2012—an update," *Journal of Gene Medicine*, vol. 15, no. 2, pp. 65–77, 2013.

[172] J. L. Pinyon, S. F. Tadros, K. E. Froud et al., "Close-field electroporation gene delivery using the cochlear implant electrode array enhances the bionic ear," *Science Translational Medicine*, vol. 6, no. 233, p. 233ra54, 2014.

[173] R. K. Shepherd and A. K. Wise, "Gene therapy boosts the bionic ear," *Science Translational Medicine*, vol. 6, no. 233, p. 233fs17, 2014.

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Research Article

Development of a Test of Suprathreshold Acuity in Noise in Brazilian Portuguese: A New Method for Hearing Screening and Surveillance

Nara Vaez,[1] Liliane Desgualdo-Pereira,[1] and Alessia Paglialonga[2]

[1] *São Paulo School of Medicine, Federal University of São Paulo (EPM-UNIFESP), Rua Botucatu 802, 04023-900 São Paulo, SP, Brazil*
[2] *National Research Council of Italy (CNR), Institute of Electronics, Computer and Telecommunication Engineering (IEIIT), Piazza Leonardo da Vinci 32, I-20133 Milano, Italy*

Correspondence should be addressed to Alessia Paglialonga; alessia.paglialonga@ieiit.cnr.it

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This paper describes the development of a speech-in-noise test for hearing screening and surveillance in Brazilian Portuguese based on the evaluation of suprathreshold acuity performances. The SUN test (Speech Understanding in Noise) consists of a list of intervocalic consonants in noise presented in a multiple-choice paradigm by means of a touch screen. The test provides one out of three possible results: "a hearing check is recommended" (*red* light), "a hearing check would be advisable" (*yellow* light), and "no hearing difficulties" (*green* light) (Paglialonga et al., Comput. Biol. Med. 2014). This novel test was developed in a population of 30 normal hearing young adults and 101 adults with varying degrees of hearing impairment and handicap, including normal hearing. The test had 84% sensitivity and 76% specificity compared to conventional pure-tone screening and 83% sensitivity and 86% specificity to detect disabling hearing impairment. The test outcomes were in line with the degree of self-perceived hearing handicap. The results found here paralleled those reported in the literature for the SUN test and for conventional speech-in-noise measures. This study showed that the proposed test might be a viable method to identify individuals with hearing problems to be referred to further audiological assessment and intervention.

1. Introduction

Age-related hearing loss and noise-induced hearing loss are common health problems in adults and older adults. The World Health Organization [1, 2] recently indicated hearing loss as the first among the twenty leading causes of moderate-to-severe disability in the adult population. It is estimated that 46% of persons over the age of 60 years, and as many as 83% over 70 years, experience some degree of hearing loss [3]. Epidemiological data suggest that nearly 1 in 25 older adults develops a hearing impairment each year [4] and that almost one in two persons with hearing impairment shows a decline in their hearing over a 5-year period [5]. Yet, despite the high prevalence and incidence, and notwithstanding the significant effects on speech communication, social participation, personal well-being, quality of life, and cognitive decline [6–8], hearing loss in adults is still largely underdetected and undertreated [9, 10]. The impact of hearing problems in adults can be substantial because many people may get used to the slow progression of their impairment and typically do not seek help or seek help very late, on average from eight to ten years after the onset of first complaints and symptoms, once the associated handicap and disability have a severe impact on their lives [10–12]. This brings along considerable burden on the health care system and society as the consequences of untreated hearing loss are huge not only in older adults but also, and increasingly, in middle-aged adults who are still fully engaged in their working life as well as in their social and personal domains [6, 11–13].

Recently, some initiatives and a number of studies have promoted the definition and implementation of effective programs of hearing screening, prevention, surveillance, and care for adults and older adults, particularly in Europe and in the United States [10, 14–16], although the value and

need for community hearing screening in adults are still controversial. As reported by the US Preventive Services Task Force reports, current evidence is insufficient to assess the balance of benefits and harms of screening in adults without symptoms of hearing loss, and additional research is needed to understand the effects of screening for hearing loss compared with no screening on health outcomes [17, 18]. Nevertheless, the research reported by Yueh et al. in 2010 [19] did indeed suggest that hearing screening can be a catalyst in moving individuals from just a hearing loss to actually doing something about it. Moreover, if the outcome of the screening is normal, then it still might provide the individual with an increased awareness of the potential problem and could allow the audiologist to provide people who underestimate their hearing problems with information about the condition, stimulating their motivation and attitude to seek help [20]. Awareness could lead to employment of preventative measures or earlier detection of a hearing problem, before the onset of hearing handicap and disability [16, 21, 22] so that health care can be delivered in a timely manner. Early intervention is particularly important, also in view of recent evidence showing that age-related hearing loss may be independently associated with poorer cognitive functioning [8, 23] and incident dementia [24, 25] and, also, in view of the hypothesis that rehabilitative therapies and devices may, plausibly, help to limit the cascade of negative effects of hearing loss and reduce the risk of cognitive decline [25]. However, the potentially significant benefits of rehabilitation on the cognitive domain were investigated and demonstrated by only one randomized controlled trial up to now [26].

Unfortunately, there is still lack of large scale hearing screening and surveillance programs in many developed and developing countries, including Brazil, even though some recent studies have demonstrated that the implementation of adult hearing screening might be a cost-effective way to reduce unmet need for hearing aids and improve quality of life among older adults [16, 27]. The availability of reliable methods to identify hearing problems is a crucial prerequisite for the implementation of effective screening, prevention, and surveillance programs. In the past ten years, some novel techniques have been specifically developed for hearing screening and surveillance, particularly in adults and older adults. Relevant examples are digits-based tests for remote testing (at-home self-screening) by telephone or Internet [28, 29], adaptive tests based on multiple-choice discrimination of logatomes [30, 31], and tests of suprathreshold acuity in noise such as the SUN (Speech Understanding in Noise) test [32–35]. The SUN test is an automated, user-operated speech-in-noise test which is made up of short list of intervocalic consonants (VCVs, vowel-consonant-vowel, e.g., *afa, aja*, ...) that are presented in a three-alternative forced choice (3AFC) paradigm by means of a touch sensitive screen. Stimuli are delivered monaurally with unmodulated background speech-shaped noise, which is adequately adapted in level for each stimulus. The test score is computed as the number of VCVs correctly identified and, based on this score, the test outcome is given in a self-explanatory way: either "no hearing difficulties" (*green* light), "a hearing check would be advisable" (*yellow* light), or "a hearing check is recommended" (*red* light). Previous studies have shown that this test is a fast, reliable, easy to use tool that proved to be viable to identify early hearing difficulties in clinical as well as nonclinical settings, including unchecked ambient noise (up to 65 dBA) [32, 33].

The aim of this study was to develop a test of suprathreshold acuity in noise, the SUN test, in the Brazilian Portuguese language. This was done through three main steps, that is, (1) the development of the "building blocks" of the test (i.e., speech stimuli and background noise); (2) the definition of the test sequence (i.e., the list of stimuli and the associated levels of background noise); and (3) the optimization of the test outcomes so that the agreement between the test outcomes and hearing impairment was the highest. The development procedure and the most relevant results, as well as the overall findings in a population of 101 subjects, will be described in the following sections.

2. Materials and Methods

2.1. Development of the "Building Blocks": Speech Stimuli and Background Noise. Sixteen VCVs were recorded for the Brazilian Portuguese language (*aba, aca, aça, acha, ada, afa, aga, aja, ala, alha, apa, ara, arra, ata, ava,* and *aza*). Of the 19 consonants in the Brazilian alphabet, three (i.e., /m/, /n/, and/*ɳ*/) were not used because the phonemes *ama*, *ana,* and *aɳa* have a nasal sound which is reflected in the nasalization of the first vowel [36] and would give a cue for discrimination, introducing a bias in the test results. Following the same procedure as in the other language versions of the SUN test [32], VCV utterances were recorded as single exemplars in a sound-treated room by a professional, native-Brazilian, male speaker who was instructed to pronounce the VCVs with no prosodic accent, with the stress on the first vowel and with constant pitch across the list. Stimuli were recorded in a professional recording studio using a Neumann TLM 103 microphone, a SSL S4000 64-channels mixer, Motu HD 192 A/D converters (44.1 kHz, 16 bit), and GENELEC 1025A control room monitor. The level of VCV recordings was digitally equalized across the set to meet the "equal speech level" requirement as set in the ISO 8253-3:1996(E) Standard for Speech Audiometry [37]. The background noise in the test was a steady speech-shaped noise, that is, a white noise filtered with the long-term average speech spectrum of the Brazilian Portuguese language by a male speaker. To deliver the test in the 3AFC multiple-choice task, each VCV was combined with two wrong alternatives. This was done according to the "maximal opposition" criterion: the two wrong alternatives differed from the spoken stimulus in all the relevant feature dimensions: voicing, manner, and place of articulation (e.g., *ata*, *alha*, and *ava*) [32].

2.2. Definition of the Test Sequence. The definition of the test sequence entailed the optimization of the list of stimuli and the associated levels of background noise. The rationale was to set the level of noise for each VCV so that the intelligibility of stimuli was approximately the same across the test list (for details, see [32]). To equalize the intelligibility of stimuli, the presentation SNR (signal-to-noise ratio) for each VCV was

Table 1: Maxima, mean values, and standard deviation (s.d.) of pure-tone thresholds measured in the group of 30 normal hearing young adults (NHY) and in the group of 101 adults with varying degrees of hearing impairment and handicap (ADU).

	Right ear					Left ear				
Frequency (kHz)	0.5	1	2	4	8	0.5	1	2	4	8
NHY										
Max (dB HL)	15	15	10	15	15	20	15	10	15	10
Mean (dB HL)	6.7	4.3	2.3	2.8	2.0	7.5	4.3	2.2	2.3	1.3
S.d.	6.61	5.53	3.88	4.29	3.62	6.79	4.87	3.39	4.30	2.60
ADU										
Max (dB HL)	50	60	75	100	85	65	60	70	85	95
Mean (dB HL)	20.0	18.0	19.6	27.3	32.7	19.4	17.2	18.7	27.4	32.8
S.d.	10.38	13.84	17.91	23.71	26.66	10.68	13.46	17.63	22.80	27.98

set above the reception threshold (RT), which is theoretically defined as the SNR where the psychometric curve reaches 79.4% correct responses in a 3AFC task [38].

To estimate the RTs, the psychometric curves of the 16 VCVs were measured for a wide range of SNRs, from chance performance to 100% recognition, that is, from −10 dB to +6 dB SNR, in 2 dB steps. In this experiment, the output level of VCVs was fixed at 60 dB HL (i.e., above 100% correct responses in normal hearing subjects [39]) and the output level of noise was varied, from 70 dB HL to 52 dB HL, to set the desired SNR. Noise onset and offset were 500 ms before and 100 ms after the VCV onset and offset, respectively. To familiarize participants with the test stimuli and procedure, VCVs were also presented at the beginning of the test sequence at a comfortable presentation SNR of +8 dB (above 100% recognition [40, 41]). Stimuli were presented monaurally through headphones in the right ear and in the left ear, in two separate sessions. At each SNR, stimuli were presented twice, resulting in a total of 320 stimuli presented per ear (16 VCVs × 10 SNRs × 2 repeated presentations). The resulting test sequence was completely randomized across SNRs and across VCVs to limit adaptation to noise levels and learning.

Participants were 30 young adults (15 males, 15 females; age range: 20–25 years; mean: 22.5 years; s.d. 1.89), otologically normal as in the ISO 7029:2000 Standard [42]. Table 1 (upper part) summarizes the pure-tone detection thresholds measured in these subjects.

2.3. Optimization of the Test Outcomes. The optimization of the test outcomes was performed by setting two cut-off scores (T_1 and T_2) between the three categories of test results ("no hearing difficulties": score $\geq T_1$; "a hearing check would be advisable": $T_2 <$ score $< T_1$; and "a hearing check is recommended": score $\leq T_2$) so that the agreement with the reference pure-tone benchmarks for pure-tone screening was the highest (for details, see [32]). On a monaural basis, the outcomes of pure-tone screening in each tested ear were defined by three different classes and the percentage of correct classifications, as well as the sensitivity and specificity of the test, were measured as a function of the different values of T_1 and T_2 to find the cut-off scores that maximised them. The three classes were:

Class I (pure-tone hearing thresholds ≤ 40 dB HL at 1, 2, and 4 kHz), that is, ears that would pass a pure-tone screening at a level of 40 dB HL (a cut-off threshold of 40 dB HL was used as this reference value is recommended for pure-tone screening in nonclinical settings and, in general, where the ambient noise is not controlled [43], as well as for screening adults and older adults [9, 44, 45]).

Class II (pure-tone hearing thresholds > 40 dB HL at 4 kHz and ≤ 40 dB HL at 1 and 2 kHz), that is, ears that might either fail or pass a pure-tone screening at 40 dB HL, depending on whether the frequency 4 kHz is included or not in the pass/refer criterion, respectively [43, 44].

Class III (pure-tone hearing thresholds > 40 dB HL at 1 or 2 kHz), that is, ears that would fail a pure-tone screening, irrespective of the criterion for high-frequency hearing thresholds [43–45].

On a binaural basis, the World Health Organization (WHO) [46] criterion for disabling hearing impairment was considered the reference benchmark (i.e., hearing threshold level, averaged over 0.5, 1, 2, and 4 kHz, >40 dB HL in the better ear; hearing aids are usually recommended). The SUN test results in the two ears were combined in a way that a binaural "fail" was given when the subject received "a hearing check is recommended" (*red* light) in at least one ear, as defined in [32]. The sensitivity and specificity of the test to identify disabling hearing impairment were measured.

Moreover, to evaluate the agreement between the test outcomes and self-perceived hearing handicap, subjects were also asked to fill in the HHIE-S (hearing handicap for the elderly screening) questionnaire [44], in the Brazilian Portuguese version [47]. The HHIE-S is a 10-item questionnaire that scores the emotional and social consequences of hearing impairment on a scale of 0 to 40 and, based on this score, classifies subjects into three categories of hearing handicap: "no handicap" (score ≤ 8), "mild-to-moderate handicap" (score in the range of 10–22), or "significant handicap" (score ≥ 24).

Participants were 101 native-Brazilian adults and older adults (43 males, 58 females; age range: 26–85 years; mean 52.7 years; s.d. 15.15) recruited in order to have a wide range of pure-tone thresholds represented across the study sample,

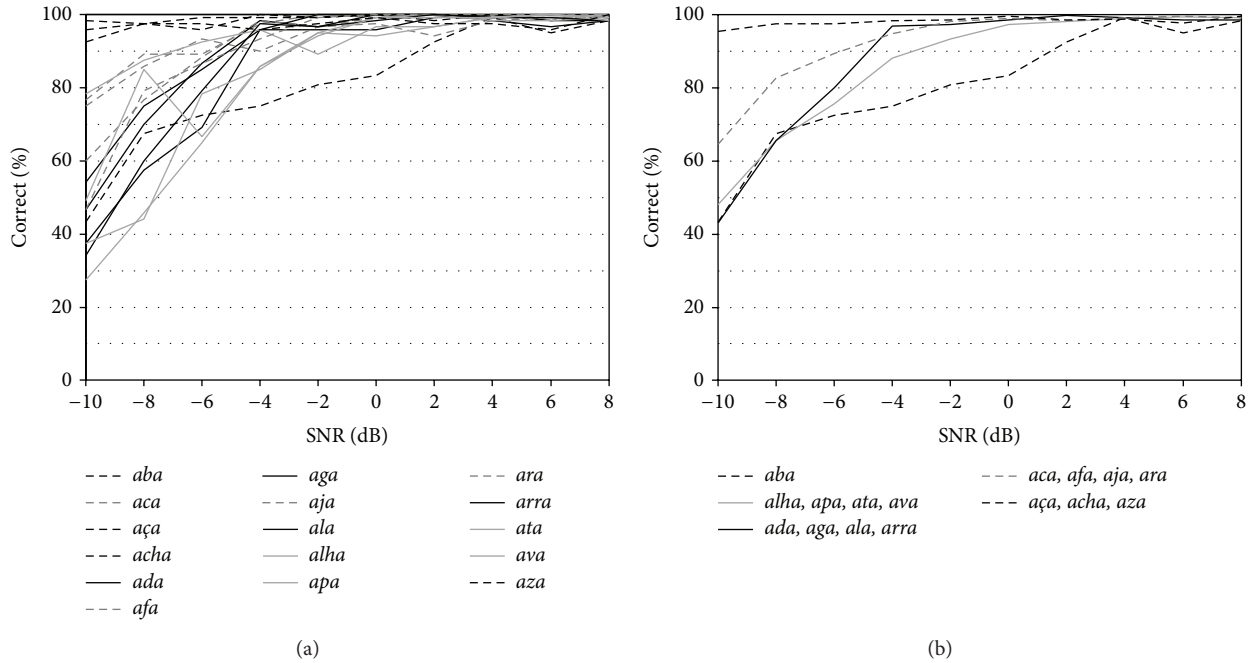

Figure 1: (a) Psychometric curves of the sixteen spoken VCVs as a function of the SNR, from −10 dB to +8 dB, in 2 dB steps. (b) Psychometric curves of the five subsets of VCVs as a function of the SNR.

from normal hearing to severe hearing loss. The maxima, mean values, and standard deviation (s.d.) of pure-tone thresholds measured in this group of subjects are reported in Table 1 (lower part). The SUN test and pure-tone audiometry were administered in both ears, with only one exception, for a total of 201 ears tested.

2.4. Equipment. A PC connected to the RCA analogue input of a clinical audiometer (Auditec PAC-200 with TDH-39 headphones) was used to deliver the stimuli. The speech output was calibrated by using a 1 kHz tone as in the "equal speech level" requirement in the ISO 8253-1:1996 Standard for speech audiometry [48]. A touch sensitive screen (resistive LCD Viper 10.4″; resolution: 800 × 600 pixels; brightness: 350 cd/m2; contrast ratio: 250 : 1) was used to display the written alternatives, record the subjects' responses, and display the test score. The software and user interface for experiments were implemented in MATLAB (R2007b, v. 7.5.0.342, MathWorks). Testing was carried out in low ambient noise (as in the ISO 8253 1:1989 Standard for Pure Tone Audiometry [49]). The experiments were conducted in accordance with the Declaration of Helsinki and were approved by the Ethics Committee for Research at UNIFESP (Comitê de Ética e Pesquisa da Universidade Federal de Sao Paulo, Plataforma Brasil). Participants were informed of the study framework and procedures before testing and agreed to the use of their clinical data for research purposes.

3. Results and Discussions

Figure 1(a) shows the psychometric curves of the 16 VCVs as a function of the SNR, from −10 dB to +8 dB. A wide range of *RTs* was observed across the set, from values lower than 10 dB (e.g., *aza*, *acha*) to about +2 dB (e.g., *aba*), further supporting the need to equalize the intelligibility of test stimuli. Following the analysis of psychometric curves as well as the statistical distribution of results in this group, the presentation SNR was set at +4 dB for the stimulus *aba*, at 0 dB for stimuli *alha*, *apa*, *ata*, and *ava*, at −2 dB for *ada*, *aga*, *ala*, and *arra*, at −4 dB for *aca*, *afa*, *aja*, and *ara*, and at −6 dB for *açа*, *acha*, and *aza*. The average psychometric curves of the five subsets of VCVs are shown in Figure 1(b).

As a result, the SUN test sequence was obtained. It consisted in the set of 16 VCVs, presented in five different subsets at the five presentation SNRs listed above, from the highest (+4 dB) to the lowest SNR (−6 dB). In addition, to help subject familiarize with the test procedure, a short list of VCVs is also presented (but not scored) before the actual test list at a comfortable presentation SNR of +8 dB [32].

Figure 2 shows the percentage of ears in Classes I, II, and III that were classified into the three categories of test outcomes as a function of the two cut-off scores, T_1 (Figure 2(a)) and T_2 (Figure 2(b)). Figure 2(a) shows the percentage of ears in Class I (i.e., ears that would pass a pure-tone screening) with score $\geq T_1$ (black marks) and the percentage of ears in Class II or Class III (i.e., ears that would not pass a pure-tone screening) with score $< T_1$ (white marks) for the different values of T_1. The highest agreement between test results and pure-tone screening results was observed with $T_1 = 11$, which provided a sensitivity of 84% and specificity of 76% to identify ears that might fail a pure-tone screening (Classes II and III). Figure 2(b) shows, the percentage of ears in Class III with score $\leq T_2$ (white marks) and the percentage of ears in Class II with $T_2 <$ score $< T_1$ (black marks) as

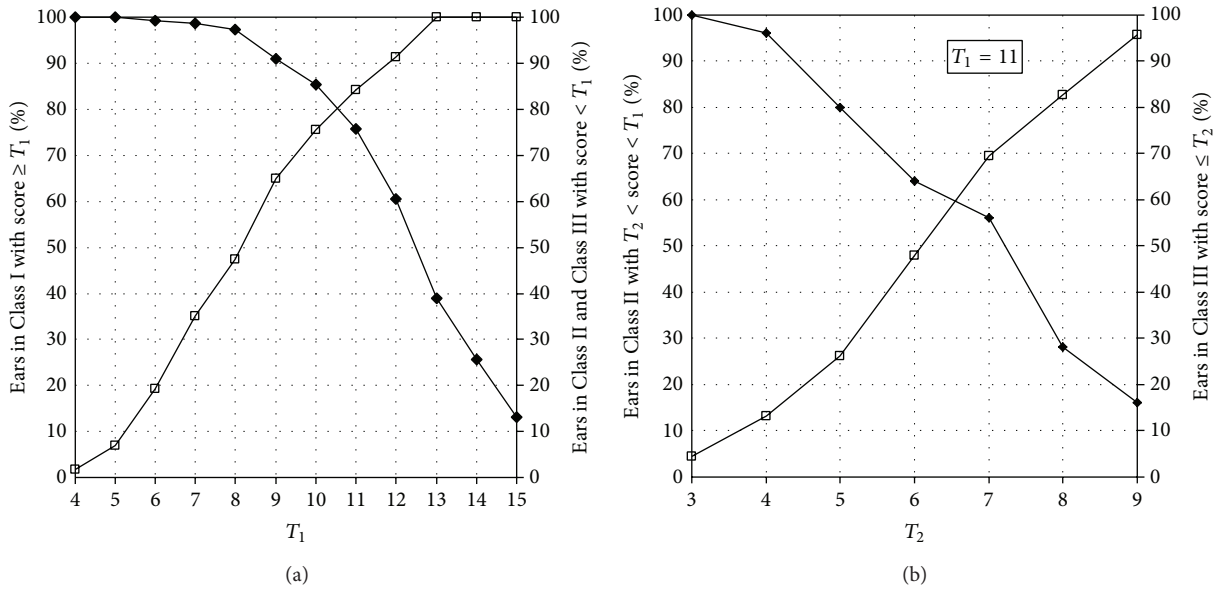

FIGURE 2: (a) Percentage of ears in Class I with score ≥ T_1 (black marks) and percentage of ears in Class II or Class III with score < T_1 (white marks) when T_1 was systematically varied from 4 to 15. (b) Percentage of ears in Class III with score ≤ T_2 (white marks) and percentage of ears in Class II with T_2 < score < T_1 (black marks) when T_2 was systematically varied from 3 to 9, and T_1 was set at 11 (as this value provided the best results, as shown in (a)).

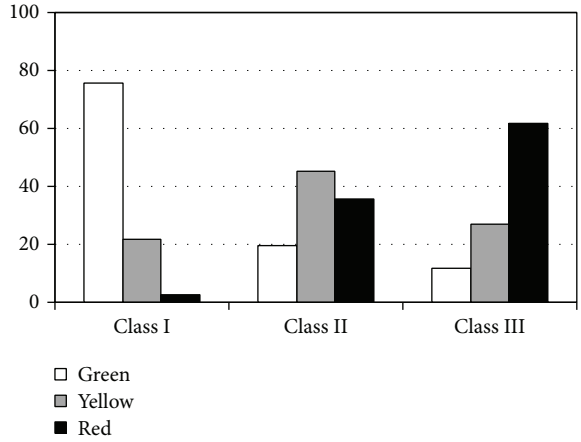

FIGURE 3: Distribution of the three test outcomes (*green*: "no hearing difficulties"; *yellow*: "a hearing check would be advisable"; and *red*: "a hearing check is recommended") in ears classified into the three classes of hearing impairment (Classes I, II, and III).

a function of T_2, with T_1 set at 11. Accordingly, the cut-off score T_2 was set at 7 as this provided about 69.6% and 56% correct subclassification between Class II and Class III and a sensitivity of 62% and specificity of 92% to identify ears in Class III. On a binaural basis, the sensitivity and specificity of the SUN test to identify people with disabling hearing impairment, as defined by the WHO criterion [46], were 83% and 86%, respectively, paralleling results reported by previous investigations (e.g., the sensitivity and specificity were 83.8% and 83.9% for the Italian version of the SUN test [32] and 84% and 75% for the English version [33]).

Figure 3 shows, on a monaural basis, the overall distribution of the three test outcomes in ears classified into the three classes of hearing impairment. Of ears in Class I, 75.7% were classified into the *green* category and only a minor percentage (i.e., 2.8%) were classified as *red*. Vice versa, of ears in Class III, an overall percentage of 88.5% were classified as *yellow* (26.9%) or *red* (61.6%). As to ears in Class II (i.e., ears with pure-tone thresholds higher than 40 dB HL at 4 kHz but *not* at frequencies ≤ 2 kHz), the distribution of results was as follows: 19.3% *green*, 45.2% *yellow*, and 35.5% *red*. This outcome might be explained by the fact that puretone thresholds at 4 kHz are typically not *per se* adequate predictors of speech reception threshold, unless they are combined with thresholds at lower frequencies, at 2 kHz or lower [50]. As a matter of fact, Ventry and Weinstein [44] had reported that relying on a fail with pure-tone thresholds at 4 kHz to screen for hearing handicap produces a high refer rate and fails a high proportion of those who report no handicap. Stated differently, adults with high-frequency hearing loss may—or may not—experience difficulties in speech understanding, as reflected by our results. Conversely, when the hearing loss involves frequencies below 4 kHz (as for ears in Class III), the ability to understand speech, particularly in less than ideal listening conditions, significantly decreases because the mid-to-low frequency regions contribute more to speech understanding than high-frequency regions [51–53]. Accordingly, as shown in Figure 3, the agreement between speech understanding (as measured by the proposed test) and pure-tone thresholds is much higher, as expected, for ears in Class I or Class III (i.e., where pure-tone thresholds at frequencies ≤ 2 kHz were lower or higher than 40 dB, resp.) than for ears in Class II.

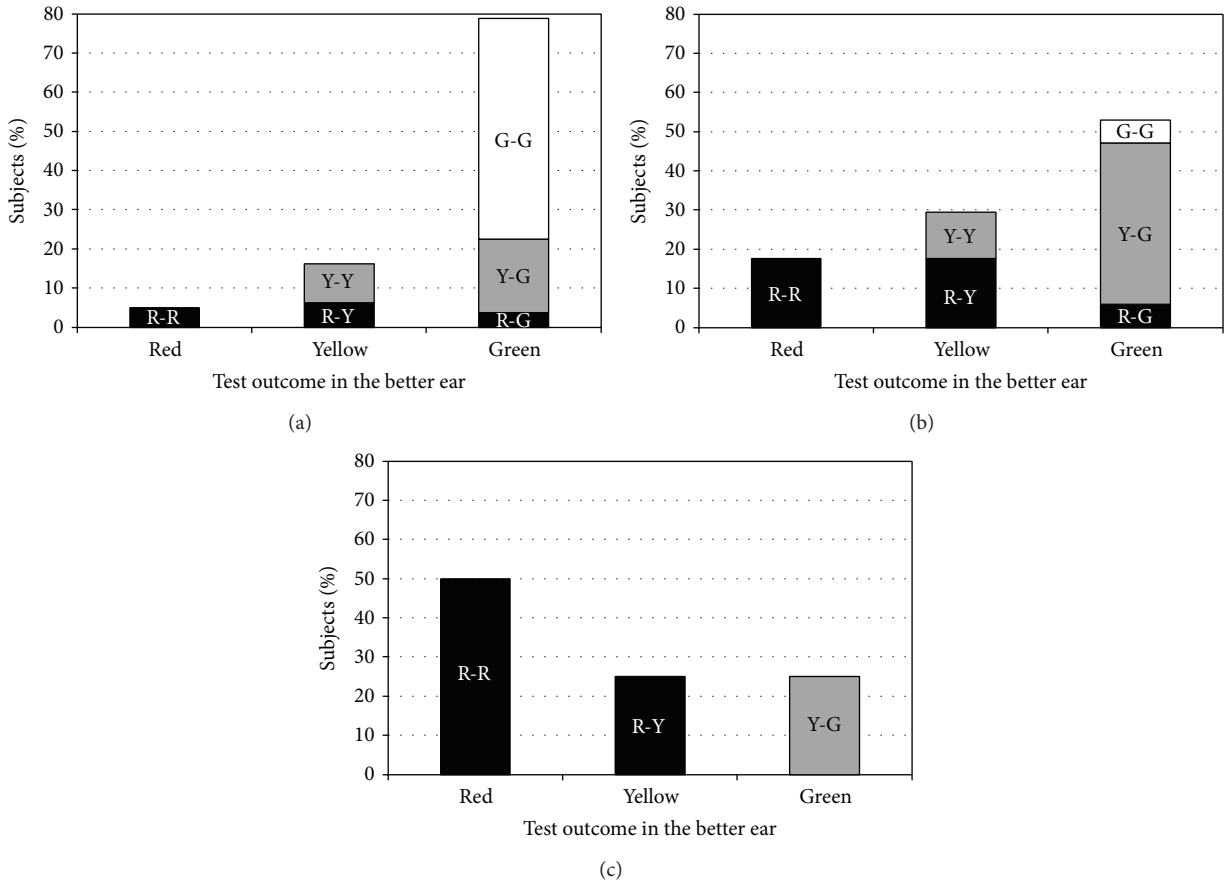

Figure 4: Distribution of the three test outcomes in subjects classified into the three categories of hearing handicap, as measured by the HHIE-S questionnaire: (a) "no handicap" ($N = 80$); (b) "mild-to-moderate handicap" ($N = 17$); (c) "significant handicap" ($N = 4$). For each category of hearing handicap, subjects were divided into three subgroups according to the test results in their better ear, that is, *red* (left-hand bar), *yellow* (center bar), and *green* (right-hand bar). Each of these bars also shows the distribution of scores obtained by those subjects in the other ear: *red* (black bars), *yellow* (grey bars), or *green* (white bars).

It might be observed that a mismatch between results of tests of speech recognition in noise and tests of pure-tone detection in quiet is largely justified, particularly in adults and older adults [51, 54, 55]. For example, in line with this, our results showed that 2.8% of ears in Class I were in the *red* category, which may be explained by possible early hearing difficulties that are not significant yet and are not detected by hearing threshold testing. Vice versa, 11.5% of ears in Class III were in the *green* category, possibly because of gradual adaptation to speech-in-noise recognition in people with hearing difficulties and, also, because of the 33% guess rate in the 3AFC task which may lead to higher-than-expected scores. Noticeably, the results found here are in line with findings reported in the literature for other speech-in-noise tests (sentence, word, or nonsense syllable tests) that are typically used for hearing assessment in the clinic [50, 56–59], overall confirming that the SUN test can estimate speech recognition performance reliably and quickly in adults and older adults. In general, the agreement between speech-in-noise testing and pure-tone testing widely varies as it depends on the speech material, noise, and protocols that are used, but typically the correlation between speech recognition and pure-tone thresholds, particularly for consonants and short phonemes, is below 0.7 [58, 59].

Figure 4 shows the distribution of the three SUN test outcomes in subjects classified into the three categories of hearing handicap, as measured by the HHIE-S questionnaire: "no handicap" (Figure 4(a)), "mild-to-moderate handicap" (Figure 4(b)), and "significant handicap" (Figure 4(c)). In the group of subjects with no handicap ($N = 80$, Figure 4(a)), an overall percentage of 78.8% had *green* in their better ear (right-hand bar); of these, 56.3% had *green* in both ears, whereas 18.7% and 3.8% had *yellow* or *red* in the other ear, respectively. In this group, a minor percentage of subjects (i.e., 5%, left-hand bar) obtained *red* in both ears, whereas about 16% of subjects (center bar) obtained *yellow* either in one ear (6.3%) or in two ears (10%). These data suggest that, in individuals with no self-perceived hearing handicap, the better ear plays a major role as most subjects (78.8%) had very good outcomes (*green* light) in at least one ear. As a matter of fact, good hearing functioning, at least in one ear, is likely to help them to cope with daily (binaural) listening situations, thus lowering the degree of self-perceived hearing handicap. In the group with mild-to-moderate handicap ($N = 17$,

Figure 4(b)), the percentage of subjects who obtained a *red* outcome in both ears increased from 5% to 17.6% (left-hand bar) and the percentage of those who had *green* in at least one ear decreased from 78.8% to 52.9% (right-hand bar), with a parallel decrease in the percentage of those who had *green* in both ears, from 56.3% (45/80) to 5.9% (1/17). The percentage of subjects who obtained *yellow* in their better ear was 29.5% (17.7% had *red* in one ear and 11.8% had *yellow* in the other ear). The distribution of test results in this group indicates that a higher proportion of subjects with mild-to-moderate handicap are likely to have poorer speech recognition in noise, as measured by the SUN test, in both ears (left and center bars: 47.1%). In the small group of subjects who reported significant handicap ($N = 4$, Figure 4(c)), two (50%, left-hand bar) had *red* in both ears, one (25%, center bar) obtained *yellow* in one ear and *red* in the other ear, and one (25%, right-hand bar) had *yellow* in one ear and *green* in the other ear. In summary, the percentage of subjects who obtained *red* outcomes in both ears markedly increased with increasing self-perceived handicap (from 5% to 17.6% and 50%); vice versa, the percentage of subjects who obtained *green* in at least one ear markedly decreased with increasing handicap (from 78.8% to 52.9% and 25%—this last percentage corresponding to one out of four subjects). Despite the very small sample size, results in Figure 4(c) confirm the tendency observed in Figure 4(b): subjects with significant hearing handicap tend to have poor speech recognition in both ears. Additional research in a wider sample of individuals with significant handicap is needed to further support these findings and to fully understand the role of the better (leading) ear in limiting the degree of self-perceived hearing handicap. Overall, these results indicate that the test outcomes were in line with the degree of self-perceived hearing handicap, as measured by the HHIE-S.

4. Conclusions

This paper described the development of a test of suprathreshold acuity in noise in the Brazilian Portuguese language, that is, the SUN (Speech Understanding in Noise) test, a user-operated speech-in-noise test for hearing screening and surveillance, already available in the English, Italian, German, and French languages. This was done by (1) developing its building blocks (speech stimuli and noise); (2) defining the list of stimuli and the associated levels of background noise; and (3) optimizing the test outcomes in a population of adults and older adults with varying degrees of hearing impairment and hearing handicap, including normal hearing. The results obtained with the proposed test fully paralleled those obtained in previous studies in the other language versions [32–35] and were in line with results reported for conventional clinical tests of speech-in-noise recognition [50, 56–59]. Evidence from a group of 101 subjects indicated that the present test can be a viable measure for screening or surveillance programs to identify individuals with hearing impairment and hearing handicap to be referred to further audiological assessment and intervention.

It is recognized that this is a preliminary investigation on a relatively small study sample and that more studies and research need to be conducted on this topic, on a bigger group of subjects. For example, it would be interesting to investigate the association between the different degrees and types of hearing loss (including conductive hearing loss) and the test results on a large representative sample or, also, to measure the correlation with conventional speech-in-noise tests or other clinical assessment measures, including questionnaires different than the HHIE-S. Moreover, additional research is still needed to further evaluate the performance of the proposed test and to fully assess its feasibility as a screening tool, also taking into account the peculiar characteristics of the different populations and countries (either developed or developing). Future studies need to be performed in larger samples of subjects to fully assess the viability of the test for the general population; particularly, it would be important to promote large screening campaigns in Brazil by using the test developed here, also searching for specific solutions to provide accessible and efficient services to help people in remote or underserved areas. The importance of these studies would cross the borders of this specific country as valuable, practical indications would be set that might be useful to the scientific and clinical community at large for the future definition and implementation of successful early detection and intervention services for adults.

Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this paper.

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References

[1] Department of Health Statistics and Informatics in the Information—Evidence and Research Cluster/World Health Organization, *The Global Burden of Disease: 2004 Update. Part 3: Disease Incidence, Prevalence and Disability*, WHO Press, 2004.

[2] World Health Organization and World Bank, *World Report on Disability*, WHO Press, 2011.

[3] K. J. Cruickshanks, T. L. Wiley, T. S. Tweed et al., "Prevalence of hearing loss in older adults in Beaver dam, Wisconsin. The epidemiology of hearing loss study," *The American Journal of Epidemiology*, vol. 148, no. 9, pp. 879–886, 1998.

[4] K. J. Cruickshanks, W. Zhan, and W. Zhong, "Epidemiology of age-related hearing impairment," in *The Aging Auditory System*, R. D. Frisina, A. N. Popper, and R. R. Fay, Eds., pp. 211–257, Springer, New York, NY, USA, 2010.

[5] P. Mitchell, B. Gopinath, J. J. Wang et al., "Five-year incidence and progression of hearing impairment in an older population," *Ear and Hearing*, vol. 32, no. 2, pp. 251–257, 2011.

[6] S. Arlinger, "Negative consequences of uncorrected hearing loss: a review," *International Journal of Audiology*, vol. 42, no. 2, pp. 2S17–2S20, 2003.

[7] J. Nachtegaal, J. H. Smit, C. Smits et al., "The association between hearing status and psychosocial health before the age of 70 years: results from an internet-based national survey on hearing," *Ear and Hearing*, vol. 30, no. 3, pp. 302–312, 2009.

[8] F. R. Lin, K. Yaffe, J. Xia et al., "Hearing loss and cognitive decline in older adults," *JAMA Internal Medicine*, vol. 173, no. 4, pp. 293–299, 2013.

[9] B. Yueh, N. Shapiro, C. H. MacLean, and P. G. Shekelle, "Screening and management of adult hearing loss in primary care: scientific review," *The Journal of the American Medical Association*, vol. 289, no. 15, pp. 1976–1985, 2003.

[10] A. Davis, P. Smith, M. Ferguson, D. Stephens, and I. Gianopoulos, "Acceptability, benefit and costs of early screening for hearing disability: a study of potential screening tests and models.," *Health Technology Assessment*, vol. 11, no. 42, pp. 1–294, 2007.

[11] A. Davis and P. Smith, "Adult hearing screening: health policy issues-what happens next?" *American Journal of Audiology*, vol. 22, no. 1, pp. 167–170, 2013.

[12] C. H. Yamamoto and D. V. Ferrari, "Relation between hearing thresholds, handicap and the time taken to seek treatment for hearing loss [Relação entre limiares audiométricos, handicap e tempo para procura de tratamento da deficiência auditiva]," *Sociedade Brasileira de Fonoaudiologia*, vol. 17, no. 2, pp. 135–141, 2012 (Portuguese).

[13] F. Hjalte, J. Brännström, and U. Gerdtham, "Societal costs of hearing disorders: a systematic and critical review of literature," *International Journal of Audiology*, vol. 51, no. 9, pp. 655–662, 2012.

[14] A. Donahue, J. R. Dubno, and L. Beck, "Guest editorial: accessible and affordable hearing health care for adults with mild to moderate hearing loss," *Ear Hear*, vol. 31, pp. 2–6, 2010.

[15] F. Grandori, M. Parazzini, G. Tognola, and A. Paglialonga, "Hearing screening in adults and elderly is gaining momentum," in *Proceedings of the Phonak Conference Hearing Care for Adults: The Challenge of Aging*, L. Hickson, Ed., pp. 191–202, Chicago, Ill, USA, 2009.

[16] A. E. Morris, M. E. Lutman, A. J. Cook, and D. Turner, "An economic evaluation of screening 60-to 70-year-old adults for hearing loss," *Journal of Public Health*, vol. 35, no. 1, pp. 139–146, 2013.

[17] R. Chou, T. Dana, C. Bougatsos, C. Fleming, and T. Beil, "Screening adults aged 50 years or older for hearing loss: a review of the evidence for the U.S. preventive services task force," *Annals of Internal Medicine*, vol. 154, no. 5, pp. 347–355, 2011.

[18] V. A. Moyer, "Screening for hearing loss in older adults: U.S. preventive services task force recommendation statement," *Annals of Internal Medicine*, vol. 157, no. 9, pp. 655–661, 2012.

[19] B. Yueh, M. P. Collins, P. E. Souza et al., "Long-term effectiveness of screening for hearing loss: the screening for auditory impairment—which hearing assessment test (SAI-WHAT) randomized trial," *Journal of the American Geriatrics Society*, vol. 58, no. 3, pp. 427–434, 2010.

[20] E. P. Whitlock, C. T. Orleans, N. Pender, and J. Allan, "Evaluating primary care behavioral counseling interventions: an evidence-based approach," *The American Journal of Preventive Medicine*, vol. 22, no. 4, pp. 267–284, 2002.

[21] M. Morettin, M. R. Cardoso, M. L. Lebrão, and M. L. Lebrão, "Factors related to self-perception of hearing loss among an elderly population in São Paulo—SABE project [Fatores relacionados à auto-percepção da audição entre idosos do município de São Paulo—Projeto SABE]," *Saúde Coletiva*, vol. 5, no. 4, pp. 168–172, 2008 (Portuguese).

[22] K. M. Paiva, C. L. Cesar, M. C. Alves, M. B. Barros, L. Carandina, and M. Goldbaum, "Aging and self-reported hearing loss: a population-based study," *Cadernos de Saúde Pública*, no. 7, pp. 1292–1300, 2011.

[23] F. R. Lin, L. Ferrucci, E. J. Metter, Y. An, A. B. Zonderman, and S. M. Resnick, "Hearing loss and cognition in the Baltimore Longitudinal study of aging," *Neuropsychology*, vol. 25, no. 6, pp. 763–770, 2011.

[24] F. R. Lin, E. J. Metter, R. J. O'Brien, S. M. Resnick, A. B. Zonderman, and L. Ferrucci, "Hearing loss and incident dementia," *Archives of Neurology*, vol. 68, no. 2, pp. 214–220, 2011.

[25] F. R. Lin and M. Albert, "Hearing loss and dementia—who is listening?" *Aging & Mental Health*, vol. 18, no. 6, pp. 671–673, 2014.

[26] C. D. Mulrow, C. Aguilar, J. E. Endicott et al., "Quality-of-life changes and hearing impairment: a randomized trial," *Annals of Internal Medicine*, vol. 113, no. 3, pp. 188–194, 1990.

[27] C. F. Liu, M. P. Collins, P. E. Souza, and B. Yueh, "Long-term cost-effectiveness of screening strategies for hearing loss," *Journal of Rehabilitation Research and Development*, vol. 48, no. 3, pp. 235–244, 2011.

[28] C. Smits, T. S. Kapteyn, and T. Houtgast, "Development and validation of an automatic speech-in-noise screening test by telephone," *International Journal of Audiology*, vol. 43, no. 1, pp. 15–28, 2004.

[29] C. S. Watson, G. R. Kidd, J. D. Miller, C. Smits, and L. E. Humes, "Telephone screening tests for functionally impaired hearing: current use in seven countries and development of a US version," *Journal of the American Academy of Audiology*, vol. 23, no. 10, pp. 757–767, 2012.

[30] K. Meisenbacher, *Development and evaluation of an adaptive logatom test to determine consonant understandability [Graduate thesis]*, University of Applied Sciences, Oldenburg, Germany, 2008, (German).

[31] V. Spitzlei, R. Herbig, T. Geissler et al., "Online hearing screening using logatomes in fluctuating noise," in *Proceedings of the International Conference on Adult Hearing Screening (AHS '10)*, Cernobbio, Italy, 2010.

[32] A. Paglialonga, G. Tognola, and F. Grandori, "A user-operated test of suprathreshold acuity in noise for adult hearing screening: the SUN (Speech Understanding in Noise) test," *Computers in Biology and Medicine*, vol. 52, pp. 66–72, 2014.

[33] A. Paglialonga, F. Grandori, and G. Tognola, "Using the Speech Understanding in Noise (SUN) test for adult hearing screening," *The American Journal of Audiology*, vol. 22, no. 1, pp. 171–174, 2013.

[34] A. Paglialonga, G. Tognola, and F. Grandori, "SUN-test (Speech Understanding in Noise): a method for hearing disability screening," *Audiology Research*, vol. 1, pp. 49–54, 2011.

[35] A. Paglialonga, G. Tognola, and F. Grandori, "Pilot initiatives of adult hearing screening in Italy," *Audiology Research*, vol. 1, no. 1, pp. 64–68, 2011.

[36] P. S. Beddor, *Phonological and phonetic effects of nasalization on vowel height [Ph. D. Dissertation]*, University of Minnesota, 1983, Reproduced by Indiana University Linguistics Club.

[37] "Acoustics—audiometric test methods—part 3: speech audiometry," ISO Standard 8253-3, 1996.

[38] M. R. Leek, "Adaptive procedures in psychophysical research," *Perception and Psychophysics*, vol. 63, no. 8, pp. 1279–1292, 2001.

[39] P. L. M. Albernaz, "Logoaudiometria," in *Processamento Auditivo Central*, L. D. Pereira and E. Schochat, Eds., pp. 37–47, Lovise, São Paulo, Brazil, 1997.

[40] P. Assmann and Q. Summerfield, "The perception of speech under adverse conditions," in *Speech Processing in the Auditory System*, S. Greenberg, W. Ainsworth, W. Popper, and R. Fay, Eds., vol. 18 of *Springer Handbook of Auditory Research*, pp. 231–308, Springer, New York, NY, USA, 2004.

[41] I. J. Hirsh, E. G. Reynolds, and M. Joseph, "Intelligibility of different speech materials," *Journal of the Acoustical Society of America*, vol. 26, pp. 530–537, 1954.

[42] "Acoustics—Statistical distribution of hearing thresholds as a function of age," ISO Standard 7029, 2000.

[43] T. Frank and D. R. Petersen, "Accuracy of a 40 dB HL audioscope and audiometer screening for adults," *Ear and Hearing*, vol. 8, no. 3, pp. 180–183, 1987.

[44] I. M. Ventry and B. E. Weinstein, "Identification of elderly people with hearing problems," *American Speech-Language-Hearing Association*, vol. 25, no. 7, pp. 37–42, 1983.

[45] W. S. McBride, C. D. Mulrow, C. Aguilar, and M. R. Tuley, "Methods for screening for hearing loss in older adults," *The American Journal of the Medical Sciences*, vol. 307, no. 1, pp. 40–42, 1994.

[46] World Health Organization, Grades of hearing impairment, http://www.who.int/pbd/deafness/hearing_impairment_grades/en/.

[47] M. B. Wieselberg, *Self-assessment of handicap in elderly with hearing impairment: the use of HHIE [Master's degree in communication disorders]*, Pontifícia Universidade Católica, São Paulo, Brazil, 1997.

[48] "Acoustics—audiometric test methods—part 3: speech audiometry," ISO Standard 8253-3, 1996.

[49] "Acoustics—Audiometric test methods—Part 1: Basic pure tone air and bone conduction threshold audiometry," *ISO Standard 8253-1, 1989*.

[50] G. F. Smoorenburg, "Speech reception in quiet and in noisy conditions by individuals with noise-induced hearing loss in relation to their tone audiogram," *Journal of the Acoustical Society of America*, vol. 91, no. 1, pp. 421–437, 1992.

[51] L. E. Humes and J. R. Dubno, "Factors affecting speech understanding in older adults," in *The Aging Auditory System*, S. Gordon-Salant, R. D. Frisina, A. N. Popper, and R. R. Fay, Eds., pp. 211–257, Springer, New York, NY, USA, 2010.

[52] R. A. DePaolis, C. P. Janota, and T. Frank, "Frequency importance functions for words, sentences, and continuous discourse," *Journal of Speech, Language, and Hearing Research*, vol. 39, no. 4, pp. 714–723, 1996.

[53] C. V. Pavlovic, "Band importance functions for audiological applications," *Ear and Hearing*, vol. 15, no. 1, pp. 100–104, 1994.

[54] L. E. Humes, "Understanding the speech-understanding problems of older adults," *The American Journal of Audiology*, vol. 22, no. 2, pp. 303–305, 2013.

[55] M. C. Killion and P. A. Niquette, "What can the pure-tone audiogram tell us about a patient's SNR loss?" *Hearing Journal*, vol. 53, no. 3, pp. 46–53, 2000.

[56] R. H. Wilson, R. A. McArdle, and S. L. Smith, "An evaluation of the BKB-SIN, HINT, QuickSIN, and WIN materials on listeners with normal hearing and listeners with hearing loss," *Journal of Speech, Language, and Hearing Research*, vol. 50, no. 4, pp. 844–856, 2007.

[57] A. J. Bosman and G. F. Smoorenburg, "Intelligibility of Dutch CVC syllables and sentences for listeners with normal heaving and with three types of hearing impairment," *Audiology*, vol. 34, no. 5, pp. 260–284, 1995.

[58] P. M. Zurek and L. A. Delhorne, "Consonant reception in noise by listeners with mild and moderate sensorineural hearing impairment.," *Journal of the Acoustical Society of America*, vol. 82, no. 5, pp. 1548–1559, 1987.

[59] R. H. Wilson and R. McArdle, "Speech signals used to evaluate functional status of the auditory system," *Journal of Rehabilitation Research and Development*, vol. 42, no. 4, pp. 79–94, 2005.

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Research Article

Adenosine Amine Congener as a Cochlear Rescue Agent

Srdjan M. Vlajkovic,[1,2] Hao Chang,[1] Song Yee Paek,[1] Howard H.-T. Chi,[1,3] Sreevalsan Sreebhavan,[4] Ravindra S. Telang,[1,2] Malcolm Tingle,[5] Gary D. Housley,[6] and Peter R. Thorne[1,2,3]

[1] *Department of Physiology, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand*
[2] *Centre for Brain Research, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand*
[3] *Section of Audiology, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand*
[4] *Auckland Cancer Society Research Centre, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand*
[5] *Department of Pharmacology, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand*
[6] *Translational Neuroscience Facility and Department of Physiology, School of Medical Sciences, UNSW Australia, Sydney, NSW 2052, Australia*

Correspondence should be addressed to Srdjan M. Vlajkovic; s.vlajkovic@auckland.ac.nz

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We have previously shown that adenosine amine congener (ADAC), a selective A_1 adenosine receptor agonist, can ameliorate noise- and cisplatin-induced cochlear injury. Here we demonstrate the dose-dependent rescue effects of ADAC on noise-induced cochlear injury in a rat model and establish the time window for treatment. *Methods*. ADAC (25–300 μg/kg) was administered intraperitoneally to Wistar rats (8–10 weeks old) at intervals (6–72 hours) after exposure to traumatic noise (8–16 kHz, 110 dB sound pressure level, 2 hours). Hearing sensitivity was assessed using auditory brainstem responses (ABR) before and 12 days after noise exposure. Pharmacokinetic studies investigated ADAC concentrations in plasma after systemic (intravenous) administration. *Results*. ADAC was most effective in the first 24 hours after noise exposure at doses >50 μg/kg, providing up to 21 dB protection (averaged across 8–28 kHz). Pharmacokinetic studies demonstrated a short (5 min) half-life of ADAC in plasma after intravenous administration without detection of degradation products. *Conclusion*. Our data show that ADAC mitigates noise-induced hearing loss in a dose- and time-dependent manner, but further studies are required to establish its translation as a clinical otological treatment.

1. Introduction

Hearing loss is one of the greatest causes of disability (WHO), affecting up to 1 in 6 of the population. It is estimated that approximately 20% of the burden is generated from excessive noise exposure in occupational and leisure settings [1]. Hearing loss comes at a great economic cost (estimated at 1.6–3.2% GDP in Australia; Access Economics Report, 2006) and reduces the quality of life of the affected individuals. On a personal level, hearing loss results in considerable communication difficulties, social isolation, and depression and appears to be associated with early cognitive decline [2]. The damage from noise exposure is cumulative over time and exacerbates the effects of aging on hearing loss.

Noise-induced hearing loss (NIHL) is particularly common in the military, in industrial settings (construction workers, mining, forestry, and aircraft industry), and in

the music industry. The proportion of nonwork related NIHL is also considered to be on the rise contributing significantly to the overall impact of NIHL. Hearing conservation programs are generally ineffective [3], as there are many instances of unprotected exposure to excessive noise, particularly in the military and heavy industry. Cumulative NIHL associated with recreational activities and loud music from personal listening devices is also contributing to the growth in hearing disability. Sensory hair cells in the cochlea damaged by noise do not regenerate, so their loss is permanent. Prosthetic rehabilitation via hearing aids and cochlear implants is the only current treatment for hearing loss from inner ear injury and these have significant limitations. It is therefore important to develop therapies that can prevent or repair injury to the delicate inner ear structures rather than relying upon medical devices that boost residual hearing functionality. We and others have identified adenosine receptors as one of the most promising targets for the treatment of NIHL.

Adenosine is a cytoprotective substance released from tissues in response to stress. Acting on adenosine receptors (AR), adenosine augments antioxidant defences, increases oxygen supply, improves blood flow, inhibits the release of neurotransmitters, stabilises cells by stimulating K^+ channels and inhibiting Ca^{2+} channels, triggers anti-inflammatory responses, and promotes antiapoptotic pathways [4–6]. Neuroprotective actions of adenosine receptors in CNS disorders such as stroke, epilepsy, migraine, neurodegenerative, and neuropsychiatric disorders have been well documented [7–9]. Four distinct adenosine receptor subtypes have been characterised and designated as A_1, A_{2A}, A_{2B}, and A_3 [10]. Selective AR agonists are now being developed as cardioprotective and neuroprotective (A_1 and A_3), anti-inflammatory (A_{2A} and A_3), and antinociceptive (A_1) agents, whilst AR antagonists show therapeutic potential as neuroprotective (A_{2A}) and antiglaucoma (A_3) agents [10]. Some of AR agonists either are FDA approved or are currently being investigated in clinical trials [11].

All four adenosine receptors are expressed in the mammalian cochlea and they are differentially distributed in cochlear tissues [12]. Immunohistochemistry demonstrates that the sensory hair cells, supporting Deiters' cells and spiral ganglion neurons, express multiple adenosine receptors [12]. The sources of extracellular adenosine in cochlear fluids include active transport from the intracellular compartment by nucleoside transporters, adenosine release from damaged cells, and extracellular ATP hydrolysis [13, 14]. Adenosine activates adenosine receptors on target cells in a paracrine or autocrine fashion, whilst the clearance of adenosine from the extracellular space is provided by nucleoside transporters [14].

Previous studies have shown that prophylactic treatment with adenosine receptor agonists mitigates hearing loss from noise [15, 16] and the anticancer drug cisplatin [17]. We have shown that the local or systemic administration of selective A_1 adenosine receptor (A_1R) agonists, such as 2-chloro-N^6-cyclopentyladenosine (CCPA) and adenosine amine congener (ADAC), can ameliorate cochlear injury and hearing loss following noise exposure [18, 19]. The important aspect of this finding is that the drugs were administered after the cessation of noise exposure, suggesting that A_1R agonists could be useful for the treatment of acute noise-induced cochlear injury within a therapeutic window, and not only as prophylactics. In contrast to other drugs acting on A_1R, ADAC lacks cardiovascular side effects at the dose used to treat NIHL in these experiments [19–21], which suggests its suitability for systemic administration. The lack of systemic side effects within the therapeutic dose range for NIHL is due to a modified chemical structure, and its increased ability to cross the blood-brain barrier [22, 23] likely reflects permeability across the blood/perilymph partition within the cochlea. We previously showed that a five-day treatment of daily ADAC injections, starting six hours after exposure to noise, rescued up to 25 dB of otherwise permanent hearing loss [19]. For reference, 10 dB rescue in hearing thresholds is considered clinically significant [24]. The improvement of hearing thresholds was supported by increased survival of sensory hair cells and reduced expression of oxidative stress markers in the cochlea. We have also shown that ADAC ameliorates cisplatin-induced cochlear injury and hearing loss [25].

In the present study, using a rat model, we demonstrate the time window for ADAC otoprotective treatment after noise exposure and the optimal doses for systemic administration. Pharmacokinetic studies demonstrate changes in ADAC concentrations in plasma after systemic administration. These data provide a background for drug development studies which aim to establish the suitability of ADAC as a treatment for acute NIHL.

2. Materials and Methods

2.1. Animals. The studies were performed on male Wistar rats (8–10 weeks old) sourced from the animal facility at the University of Auckland. All procedures in this study were approved by the University of Auckland Animal Ethics Committee and conformed to international guidelines for the ethical use of animals. After completion of manipulations, animals were euthanised using sodium pentobarbital (100 mg/kg, i.p.) and cochlear tissues collected for histology.

2.2. Noise Exposure. Rats were exposed to 8–16 kHz octave band noise for 2 hours at 110 dB SPL to induce permanent hearing loss in untreated animals. Noise exposures were carried out in a custom-built acoustic chamber (Shelburg Acoustics, Sydney, Australia) with internal speakers and external controls (sound generator and frequency selector). The sound intensity inside the chamber was measured using a calibrated Bruel & Kjaer 2232 sound level meter to ensure minimal deviations of sound intensity. Control animals were housed in the animal facility at ambient sound conditions (45–55 dB SPL, 0.5–20 kHz).

2.3. Auditory Brainstem Responses (ABR). ABR thresholds in response to 8–28 kHz tone pips were measured in a sound attenuating chamber (Shelburg Acoustics, Sydney, Australia)

before and 12 days after noise exposure. Rats were anaesthetised with a mixture of ketamine (90 mg/kg) and xylazine (10 mg/kg) intraperitoneally and then placed onto a heating pad, to maintain body temperature at 37°C. ABRs were obtained by placing fine platinum electrodes subdermally at the mastoid region of the ear of interest (active electrode), scalp vertex (reference), and mastoid region of the opposite ear (ground electrode). The acoustic stimuli were supplied via a TD48 Beyer dynamic transducer connected to a 10 cm plastic tube that was placed into the external auditory canal of the left ear. A Tucker-Davis Technology (TDT) auditory physiology workstation System 3 (Alachua, FL, USA), equipped with a computer-based digital signal processing package and software (BioSig, Alachua), was used to produce the acoustic stimuli and record the ABR responses. Tone pips (5 ms, 0.5 ms rise-fall time) were presented at frequencies between 8 and 28 kHz at varying intensity levels. The threshold of the ABR complex (waves I–V) were determined by progressively attenuating the sound intensity in 5 dB steps until the wave I–V complex of the averaged ABR waveforms (1024 repeats with stimulus polarity alternated) was no longer distinguishable from noise floor in recorded traces. The ABR threshold was defined as the lowest intensity (to the nearest 5 dB) at which a response could be visually detected above the noise floor. Repeat waveforms were analysed at each frequency to determine the consistency of the responses and to identify the recurring peaks.

2.4. ADAC Treatment. ADAC (Sigma-Aldrich) was dissolved in 1 M HCl and then in 0.1 M phosphate buffered saline (PBS; pH 7.4) to prepare a 100 μg/mL stock solution. The stock solution was then aliquoted and stored at −20°C for later use. Light-protected ADAC aliquots were thawed at 37°C for 30 min before administration. In study one, the following single ADAC dosages were used to optimize the rescue dose: 25, 50, 100, 200, and 300 μg/kg/day. An equal volume of vehicle solution was given to control animals. ADAC or control vehicle solution was administered intraperitoneally (i.p.) for five consecutive days at 24 h intervals, beginning six hours after the cessation of noise exposure. In study two, one of the higher ADAC doses (200 μg/kg) was used to determine the time window for treatment after noise exposure. ADAC treatment (five daily injections at 24 h intervals) commenced at 12, 24, 48, or 72 hours after noise exposure.

2.5. Measurement of ADAC Concentrations in Rat Plasma after Intravenous Administration. Male Wistar rats (8 weeks old) were anaesthetised with ketamine (90 mg/kg i.p.) plus xylazine (10 mg/kg i.p.) and the femoral vein was surgically exposed. ADAC (400 μg/kg) was injected (1 mL/min) through the femoral vein, and blood samples (0.5 mL) were taken from the heart. Up to 1.5 mL of blood was taken from each animal, and the first sample was drawn 1 min after drug delivery. Subsequent extraction and purification procedures were modified from Stocchi et al. [26]. Briefly, after deproteination with 0.1 M KOH, plasma was extracted from the blood using an Amicon Ultra-4 50 K centrifugal filtering device (Merck Millipore, Tullagreen, Carrigtwohill, IRL).

Table 1: Chromatographic conditions

Min	%B	Flow rate (mL/min)
0	20	0.4
1	20	0.4
5	40	0.4
5.5	90	0.6
6.5	90	0.6
7	20	0.6
9.5	20	0.6
10	20	0.4

The filtrate was mixed with 50 μL of 1 M KH_2PO_4 and subjected to HPLC analysis using an Agilent 1100 series instrument (Agilent Technologies, Santa Clara, California, USA). Reverse phase HPLC separation of ADAC was achieved on a Phenomenex Gemini 3 μm C18 column (150 × 3.00 mm, 110 Å) protected with a Phenomenex Gemini C18 guard column (4 × 2.0 mm i.d.). The mobile phase used for the separation of ADAC from the matrix peaks consisted of two eluents: 45 mM ammonium formate in water, pH 7 (aqueous mobile phase A), and 100% acetonitrile (organic mobile phase B). The chromatographic conditions are shown in (Table 1).

Peaks were recorded with a diode-array detector at 254 nm detection wavelength and identified by comparison of retention times (RT) with the standard. Integration of peak areas was performed using Chemstation software version B.01.03 (Agilent).

2.6. Data Analysis. Results are presented as the mean ± SEM and the α level was set at $P = 0.05$. Each set of posttreatment threshold shifts compared between the control group and ADAC-treated groups using one-way ANOVA and post hoc Holm-Sidak multiple pairwise comparison test. Pharmacokinetic (PK) data were analysed by PKSolver [27].

3. Results

3.1. Dose-Response Study. ADAC (25–300 μg/kg) was administered to noise-exposed Wistar rats for five consecutive days, commencing six hours after noise exposure (8–16 kHz, 110 dB SPL for 2 hours). Auditory thresholds were assessed using auditory brainstem responses (ABR) before and 12 days after noise exposure. Figure 1 shows the baseline and final ABR thresholds in ADAC- and vehicle-treated (control) rats. Baseline ABR thresholds were comparable in all groups. All ADAC-treated groups showed a broad reduction of final thresholds across the 8–28 kHz frequency range compared with the hearing loss in the control group. ABR threshold shifts for each frequency are shown in Figure 2. In the control noise-exposed and nontreated group, the average threshold shift across the frequencies was 37 dB, with the largest shift of 40 dB at 12 kHz. In this study, all ADAC doses significantly ($P < 0.05$) reduced the extent of the threshold shift at some or all frequencies. The most effective ADAC doses (100 μg/kg and 200 μg/kg) reduced average noise-induced threshold shift across the frequencies by 21 dB and 18 dB,

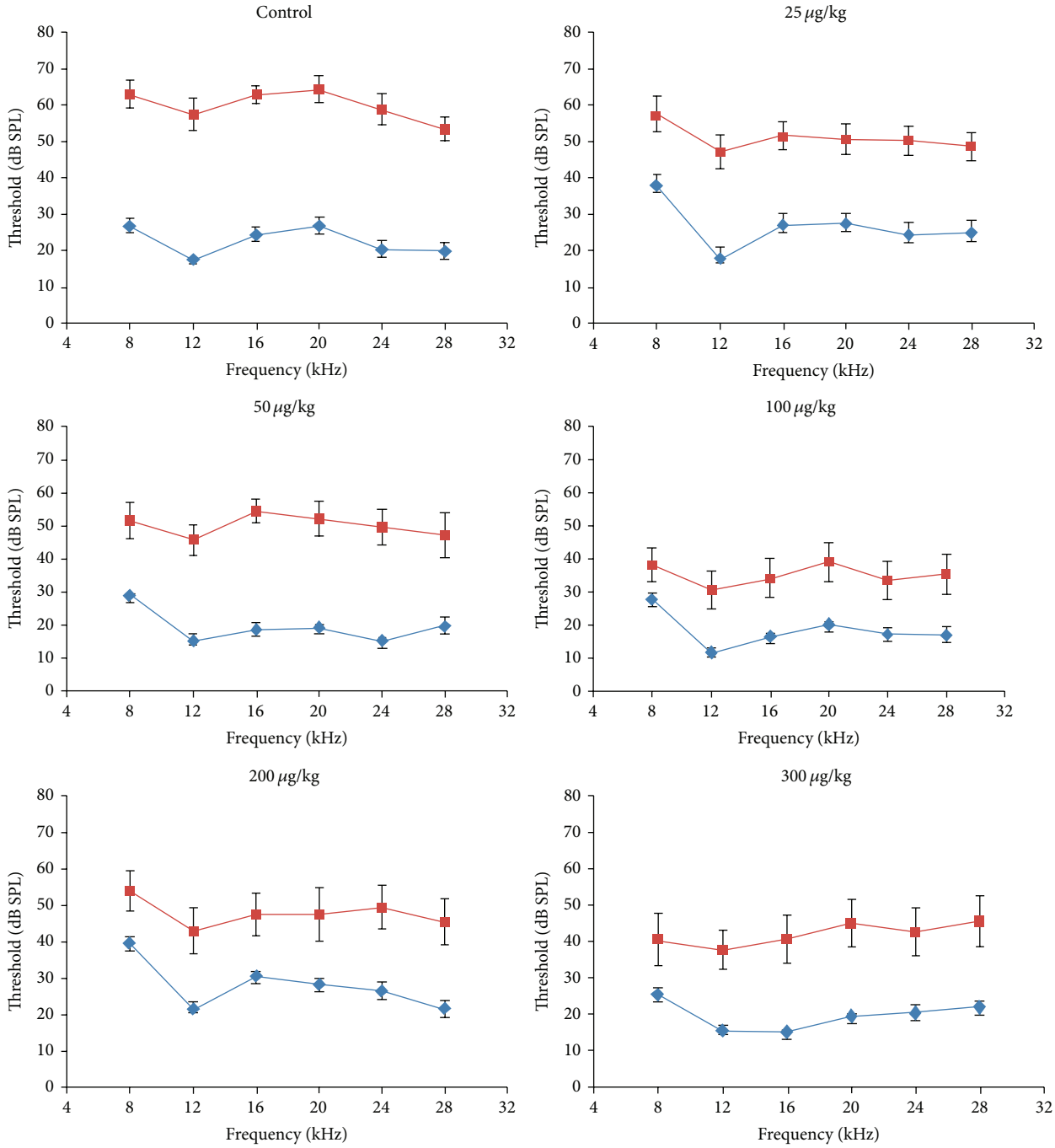


Figure 1: ADAC dose-response study in Wistar rats: the effect on auditory brainstem responses (ABR) before (blue line) and 12 days after (red line) traumatic noise exposure (8–16 kHz, 110 dB SPL, 2 hours). ADAC (25–300 μg/kg) was administered intraperitoneally for five consecutive days at 24 h intervals, commencing six hours after noise exposure. In the control group, injections of the vehicle solution were administered at the same intervals as ADAC. ABR were measured in response to tone pips (8–28 kHz). Data are expressed as mean ± SEM (n = 8–10).

respectively ($P < 0.001$; ANOVA). These two ADAC doses significantly ($P < 0.05$) reduced threshold shifts at 8–24 kHz frequencies, but thresholds at the highest frequency (28 kHz) improved only with a 100 μg/kg ADAC dose. The 300 μg/kg and 25 μg/kg doses were also effective, reducing average threshold shift by 15 dB and 13 dB, respectively (Figure 2). The 50 μg/kg ADAC doses were the least effective, reducing average threshold shifts by 7 dB ($P < 0.05$). The effect of this dose was significantly ($P < 0.01$) lower compared to ADAC doses over 100 μg/kg.

3.2. ADAC Efficacy Study. The optimal time to commencement of treatment was investigated using 5 daily ADAC

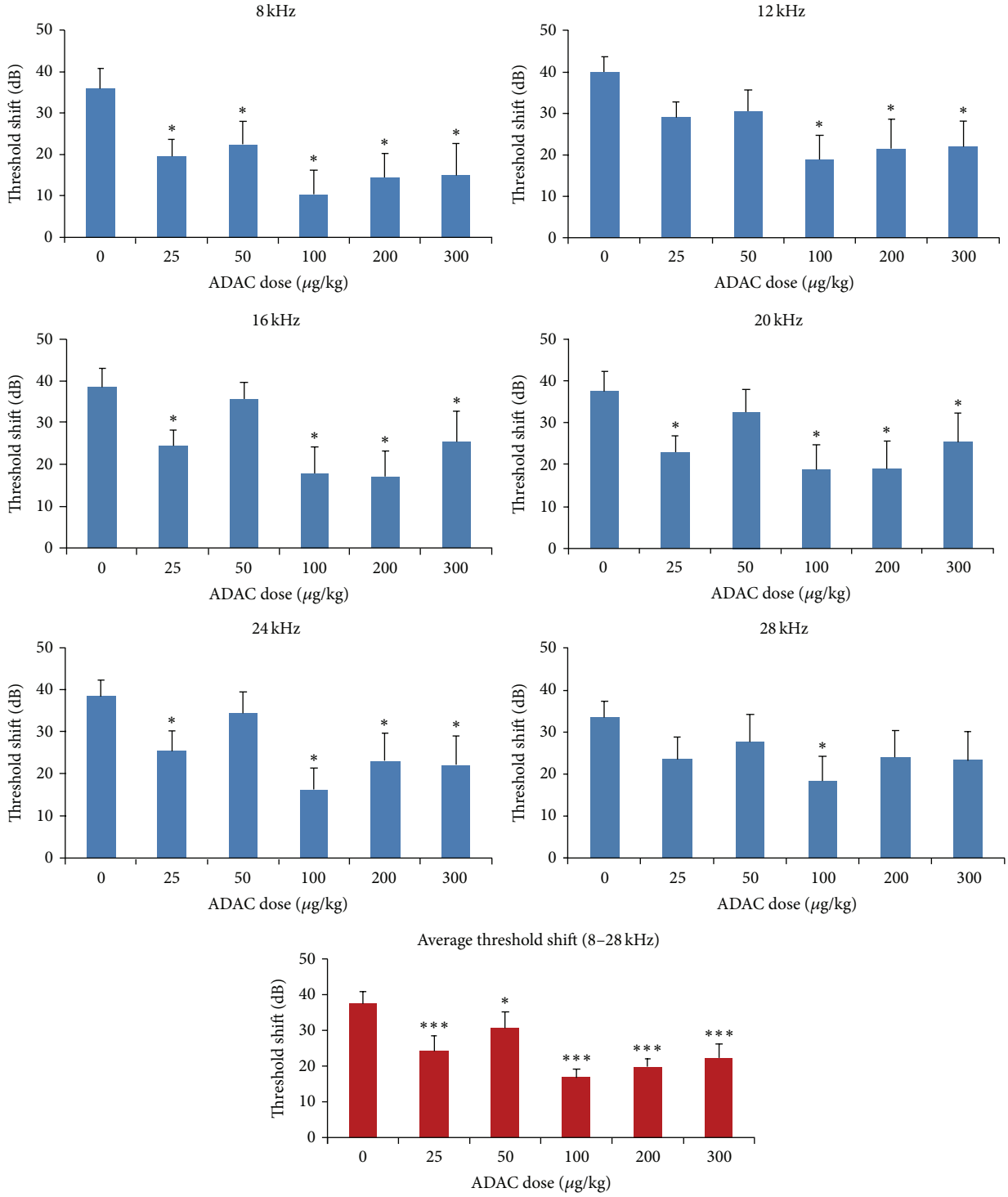


Figure 2: ADAC dose-response study: the effect on noise-induced ABR threshold shifts (defined as the difference between ABR thresholds before and after noise exposure at a single frequency). Threshold shifts averaged across all frequencies (8–28 kHz) are also shown. ADAC (25–300 μg/kg i.p.) was administered for five consecutive days at 24 h intervals, commencing six hours after noise exposure. In the control group (ADAC dose "0"), injections of the vehicle solution were administered at the same intervals as ADAC. Data are expressed as mean ± SEM (n = 8–10). $^{*}P < 0.05$; $^{***}P < 0.001$ versus control, one-way ANOVA with pairwise comparison.

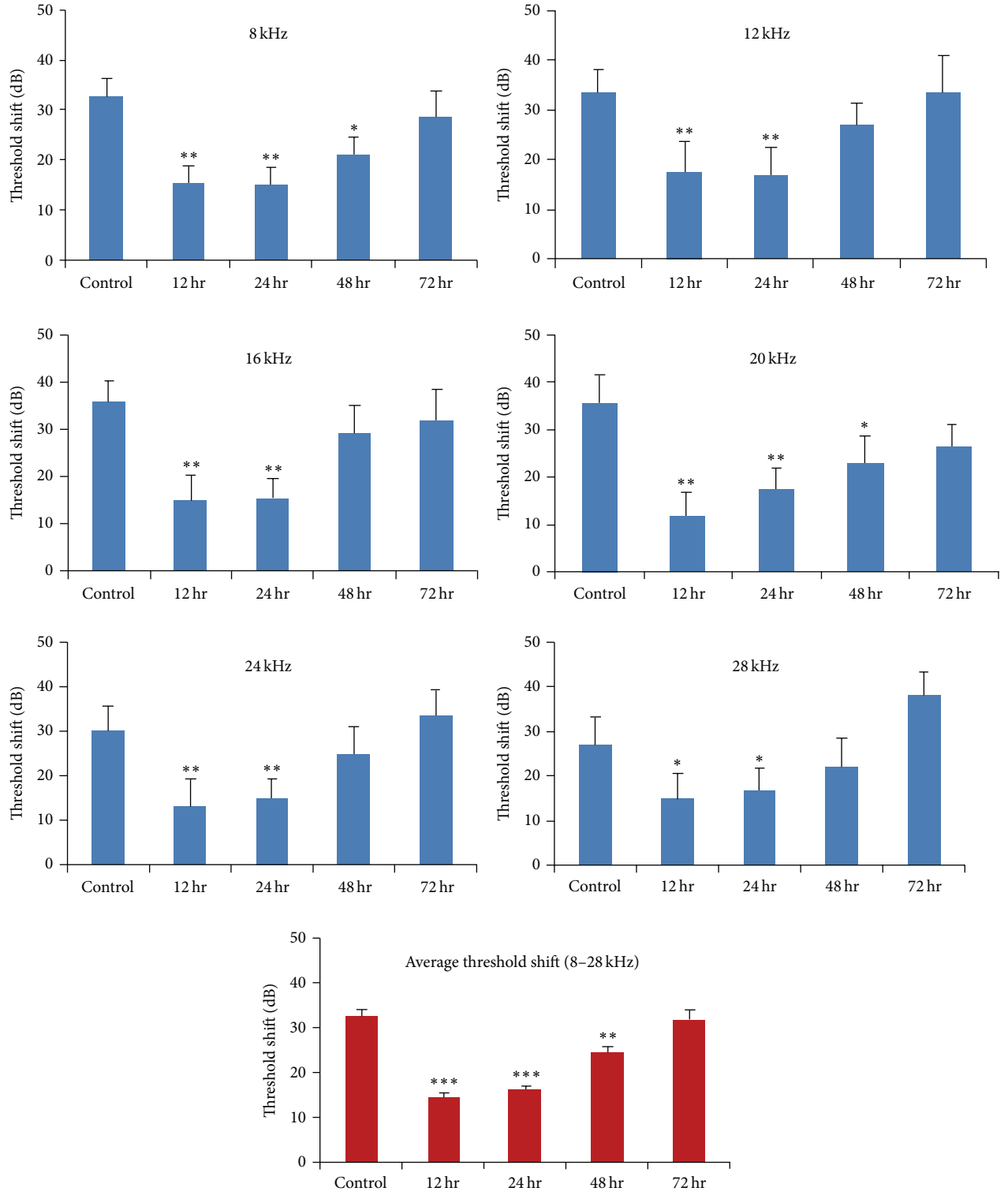
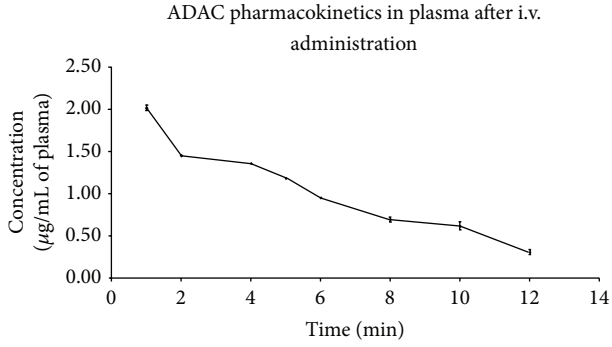


Figure 3: ADAC efficacy study. ADAC (200 μg/kg i.p.) was administered as 5 daily injections commencing 12, 24, 48, or 72 hours after traumatic noise exposure, and the outcomes were measured using auditory brainstem responses (ABR) before and 12 days after noise exposure. In the control group, injections of the vehicle solution were administered at the same intervals as ADAC starting 12 hours after noise exposure. Data are expressed as ABR threshold shifts at different frequencies (mean ± SEM; n = 8–10) and threshold shifts averaged across all frequencies (8–28 kHz). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ versus control, one-way ANOVA with pairwise comparison.



Dose = 100 μg
C_0 = 2.2 μg/mL
$T_{1/2}$ = 5 min
Ke = 0.14 ng/min
AUC_{0-t} = 12.8 ng·min/mL

Figure 4: Pharmacokinetic properties of ADAC in plasma after administration through the femoral vein (400 μg/kg, 1 mL/min). Data are expressed as mean ± SEM (n = 4 per time point). Pharmacokinetic properties were calculated using an Excel plugin PKSolver [24]. C_0, extrapolated maximum concentration; $T_{1/2}$, half-life; Ke, elimination constant; AUC_{0-t}, the integral area under the curve, measuring the overall amount of ADAC in the compartment.

injections (200 μg/kg) commencing 12, 24, 48, or 72 hours after traumatic noise exposure (Figure 3). The outcomes were measured using ABR before and 12 days after noise exposure. ADAC treatment significantly ($P < 0.001$) reduced ABR threshold shifts in the treatment groups that commenced 12 and 24 hours postnoise exposure by 18 dB and 16 dB, respectively, averaged across the measured frequencies. The treatment regime that commenced 48 hours after noise exposure produced an average protection of 8 dB, which was also statistically significant ($P < 0.01$). At 72 hours delay before commencing ADAC treatment, the threshold shifts were similar to nontreated animals. This study suggests that ADAC treatment is most effective in the first 24 hours after noise exposure.

3.3. Pharmacokinetic Properties of ADAC after Systemic Administration. ADAC concentrations in plasma were determined using HPLC analysis. As expected, ADAC concentration was highest shortly after i.v. administration, followed by a rapid distribution and elimination phase (Figure 4), but without detection of degradation products. The graph shows concentration changes of ADAC in rat plasma for the time period of 12 minutes; after that, ADAC concentrations dropped below the limit of UV-Vis detection (~0.1 μg/mL). The pharmacokinetic properties of ADAC in plasma were analysed by PKSolver, an add-in program for pharmacokinetic data analysis in Microsoft Excel [27]. Figure 4 shows that ADAC has a short (5 min) half-life ($T_{1/2}$) in plasma and a fast elimination rate (Ke). The cochlear tissue was also analysed for ADAC in these experiments using UV-Vis detection. ADAC was detected at 5 min after injection in 4 out of 6 cochleae obtained from 3 animals but at 15 min postinjection ADAC was no longer detectable (data not shown). More sensitive assay is required to accurately assess ADAC concentrations in cochlear perilymph.

4. Discussion

This study shows that ADAC mitigates noise-induced hearing loss in a dose- and time-dependent manner. The ADAC was effective across the broad dose range, from 25 μg/kg–300 μg/kg i.p., reducing noise-induced threshold shifts by clinically significant levels [24] across the tested frequency range. At some frequencies, threshold improvement was up to 25 dB (Figure 2). ADAC was most effective at rescuing NIHL when treatment commenced within 24 hours after noise exposure. After 48 hours, ADAC improved ABR thresholds by more than 10 dB at two frequencies, 8 kHz and 20 kHz, suggesting that even delayed treatment could be useful. Pharmacokinetic studies demonstrated a short half-life of ADAC in plasma after intravenous administration without detection of degradation products. This rapid clearance suggests broad uptake into tissue compartments.

The dose-response curve for individual ADAC doses was nonlinear (nonmonotonic). The doses over 100 μg/kg were the most effective in reducing threshold shifts, followed by 25 and 50 μg/kg. Nonmonotonic dose response curves are often U-shaped or inverted U-shape (biphasic) but can also show complex multiphasic shape [28]. Similar nonmonotonic dose-response curves were observed in gerbils, in the study investigating the neuroprotective effect of ADAC in experimentally induced cerebral ischaemia [21]. In that study, acute prophylactic administration of ADAC at doses ranging from 25 to 200 μg/kg was effective in preserving neurons, apart from the 50 μg/kg dose, which failed to improve neuronal survival. In the same study, chronic treatment with ADAC was effective at lower doses: 10–100 μg/kg in reducing mortality, and 25–100 μg/kg in neuronal preservation. Another study showed that an acute treatment with ADAC (100 μg/kg) is strongly neuroprotective in a model of Huntington's disease [29], whilst chronic administration at the same dose was ineffective. Together with our results, these studies provide a good indication of the neuro- and otoprotective dose range for ADAC, with the caveats such as different dosing schedules and disease models, and possible species-related differences in tissue distribution and affinity of A_1R.

The present study also demonstrates pharmacokinetic properties of ADAC in rat plasma. The ADAC concentration curve follows a one-compartment bolus model with first-order output. The short half-life (5 minutes) of ADAC in plasma after intravenous injection is most likely due to rapid distribution in tissues, and it may explain a lack of side effects reported previously [19, 20]. ADAC was detected in the cochlea 5 min after administration, but the low sensitivity of the UV-Vis detection precluded the pharmacokinetic study after systemic administration. The peak cochlear level of ADAC detected following intravenous administration was consistent with pharmacological action, given the high affinity of A_1 receptors for ADAC [5]. More sensitive method of detection, such as LC/MS, will be required to characterise

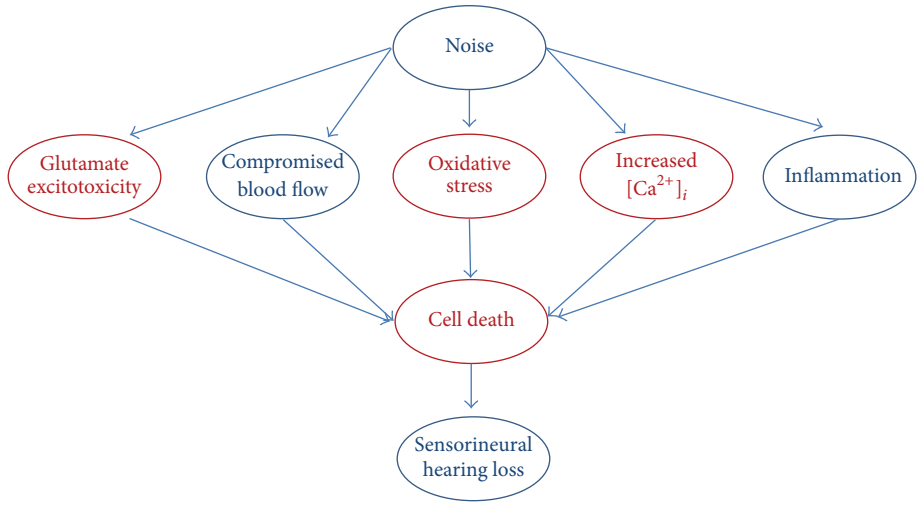


Figure 5: Overview of the basic mechanisms of noise-induced cochlear injury and proposed model of cochlear protection by ADAC. Putative therapeutic targets of ADAC are shown in red.

ADAC concentrations in cochlear perilymph with different delivery routes.

In NIHL, postinsult time to the initiation of the treatment is an important factor which significantly affects treatment outcomes. Oxidative stress and the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the cochlea is one of the major mechanisms of cochlear injury during and after noise exposure [30]. ROS levels increase postexposure, due to heavy cellular energy demands and reperfusion [30]. Production of superoxide and other free radicals reaches a maximum 7 to 10 days after exposure [31], and the production of ROS/RNS correlates with a gradual spread of hair cell loss postexposure. The peroxidation of membrane lipids, along with oxidative damage to DNA and cellular proteins, results in cell death after noise exposure [31]. Oxidative stress and other cellular events, such as inflammation and calcium overload, contribute to the development of cochlear injury after exposure to traumatic noise but also provide a window of opportunity to treat cochlear injury postexposure. We have previously shown that adenosine receptor agonists provide an effective postexposure treatment of acoustic injury and NIHL [18, 19], and the present study defines the window of opportunity for cochlear rescue. ADAC treatment provides partial rescue in the first 24 hours after noise exposure, but after that cochlear injury becomes less responsive to treatment. This is consistent with other studies demonstrating partial recovery of auditory thresholds in the first 24 hours after exposure to traumatic noise after administration of antioxidants [32–34] or antiapoptotic agents [35]. Despite the fact that free radical production appears to continue for up to 10 days after exposure, it appears that oxidative stress rapidly leads to irreversible cochlear injury and the substantial loss of critical tissues such as sensory hair cells beyond the first 24 hours, which limits the window of opportunity for pharmacological treatment of hearing loss from acute noise exposure.

The basic cochlear protection model by ADAC has been summarised in Figure 5. Acoustic trauma can induce excessive generation of free radicals in the cochlea by overdriving the mitochondria; it can reduce cochlear blood flow, cause excitotoxic swelling of afferent nerve terminals, induce intracellular Ca^{2+} overload in sensory hair cells, and cause inflammation in cochlear tissues [24, 36]. Cellular damage results in cell death from a combination of necrosis and apoptosis, which leads to hearing loss (Figure 5). Our experimental evidence suggests that ADAC can reduce oxidative stress in the noise-exposed cochlea, leading to protection of sensory hair cells [19]. We have also demonstrated that ADAC can reduce cisplatin-induced apoptosis in cochlear tissues, particularly in sensory hair cells and strial marginal cells [25]. Other putative mechanisms of otoprotection by ADAC include inhibition of glutamate release via presynaptic A_1 receptors and inhibition of voltage-gated Ca^{2+} channels, which can prevent activation of apoptotic and necrotic cell death pathways [14, 36]. However, further studies are required to fully understand the otoprotective mechanisms of ADAC, particularly with regard to cochlear afferent neurons.

5. Conclusion

Our study suggests that ADAC has a potential to be developed as a clinical otological treatment for acute hearing loss caused by exposure to traumatic noise. We show that systemic administration route is effective in mitigating cochlear injury. Previous studies suggest that the use of ADAC is not contraindicated by cardiovascular side effects at the doses used for otoprotection and neuroprotection; however, clinical trials will be necessary to confirm its safety for human use. Currently, the intratympanic administration to achieve uptake via the round window is a preferred otological drug development pathway, as it obviates possible systemic side effects (unlikely

to be a factor with ADAC, but of significant regulatory body concern), and this also counters metabolism/elimination. On the other hand, intratympanic drug delivery requires expert otological intervention, whereas systemic drug administration would be more practical in a broader range of clinical situations, or circumstances where specialist surgical intervention is untenable. This is particularly relevant given that the 24-hour therapeutic window that this study indicates is therapeutically effective. Future studies are thus required to establish the optimal drug delivery method but also to establish the mechanisms of action and the optimum usage situations for ADAC. The therapeutic efficacy of ADAC for NIHL should also be evaluated in other animal models.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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References

[1] P. R. Thorne, S. N. Ameratunga, J. Stewart et al., "Epidemiology of noise-induced hearing loss in New Zealand," *New Zealand Medical Journal*, vol. 121, no. 1280, pp. 33–44, 2008.

[2] K. Parham, B. J. McKinnon, D. Eibling, and G. A. Gates, "Challenges and opportunities in presbycusis," *Otolaryngology—Head and Neck Surgery*, vol. 144, no. 4, pp. 491–495, 2011.

[3] J. H. Verbeek, E. Kateman, T. C. Morata, W. A. Dreschler, and C. Mischke, "Interventions to prevent occupational noise-induced hearing loss," *Cochrane Database Systematic Reviews*, vol. 10, 2012.

[4] B. B. Fredholm, "Adenosine, an endogenous distress signal, modulates tissue damage and repair," *Cell Death and Differentiation*, vol. 14, no. 7, pp. 1315–1323, 2007.

[5] K. A. Jacobson and Z. Gao, "Adenosine receptors as therapeutic targets," *Nature Reviews Drug Discovery*, vol. 5, no. 3, pp. 247–264, 2006.

[6] J. Linden, "Adenosine in tissue protection and tissue regeneration," *Molecular Pharmacology*, vol. 67, no. 5, pp. 1385–1387, 2005.

[7] R. A. Cunha, "Neuroprotection by adenosine in the brain: from A_1 receptor activation to A_{2A} receptor blockade," *Purinergic Signalling*, vol. 1, no. 2, pp. 111–134, 2005.

[8] D. Boison, "Adenosine as a neuromodulator in neurological diseases," *Current Opinion in Pharmacology*, vol. 8, no. 1, pp. 2–7, 2008.

[9] T. W. Stone, S. Ceruti, and M. P. Abbracchio, "Adenosine receptors and neurological disease: neuroprotection and neurodegeneration," in *Handbook of Experimental Pharmacology*, vol. 193, pp. 535–587, 2009.

[10] K. A. Jacobson, "Introduction to adenosine receptors as therapeutic targets," *Handbook of Experimental Pharmacology*, vol. 193, pp. 1–24, 2009.

[11] B. B. Fredholm, A. P. IJzerman, K. A. Jacobson, J. Linden, and C. E. Müller, "International union of basic and clinical pharmacology. LXXXI. Nomenclature and classification of adenosine receptors—an update," *Pharmacological Reviews*, vol. 63, no. 1, pp. 1–34, 2011.

[12] S. M. Vlajkovic, S. Abi, C. J. H. Wang, G. D. Housley, and P. R. Thorne, "Differential distribution of adenosine receptors in rat cochlea," *Cell and Tissue Research*, vol. 328, no. 3, pp. 461–471, 2007.

[13] A. F. Khan, P. R. Thorne, D. J. B. Muñoz, C. J. H. Wang, G. D. Housley, and S. M. Vlajkovic, "Nucleoside transporter expression and adenosine uptake in the rat cochlea," *NeuroReport*, vol. 18, no. 3, pp. 235–239, 2007.

[14] S. M. Vlajkovic, G. D. Housley, and P. R. Thorne, "Adenosine and the auditory system," *Current Neuropharmacology*, vol. 7, no. 3, pp. 246–256, 2009.

[15] B. H. Hu, X. Y. Zheng, S. L. McFadden, R. D. Kopke, and D. Henderson, "R-phenylisopropyladenosine attenuates noise-induced hearing loss in the chinchilla," *Hearing Research*, vol. 113, no. 1-2, pp. 198–206, 1997.

[16] N. G. Hight, S. L. McFadden, D. Henderson, R. F. Burkard, and T. Nicotera, "Noise-induced hearing loss in chinchillas pretreated with glutathione monoethylester and R-PIA," *Hearing Research*, vol. 179, no. 1-2, pp. 21–32, 2003.

[17] C. A. Whitworth, V. Ramkumar, B. Jones, N. Tsukasaki, and L. P. Rybak, "Protection against cisplatin ototoxicity by adenosine agonists," *Biochemical Pharmacology*, vol. 67, no. 9, pp. 1801–1807, 2004.

[18] A. C. Y. Wong, C. X. Guo, R. Gupta, G. D. Housley, P. R. Thorne, and S. M. Vlajkovic, "Post exposure administration of A1 adenosine receptor agonists attenuates noise-induced hearing loss," *Hearing Research*, vol. 260, no. 1-2, pp. 81–88, 2010.

[19] S. M. Vlajkovic, K. Lee, A. C. Y. Wong et al., "Adenosine amine congener mitigates noise-induced cochlear injury," *Purinergic Signalling*, vol. 6, no. 2, pp. 273–281, 2010.

[20] D. K. J. E. von Lubitz, R. C. Lin, I. A. Paul et al., "Postischemic administration of adenosine amine congener (ADAC): analysis of recovery in gerbils," *European Journal of Pharmacology*, vol. 316, no. 2-3, pp. 171–179, 1996.

[21] D. K. J. E. von Lubitz, R. C. Lin, N. Bischofberger et al., "Protection against ischemic damage by adenosine amine congener, a potent and selective adenosine A_1 receptor agonist," *European Journal of Pharmacology*, vol. 369, no. 3, pp. 313–317, 1999.

[22] K. A. Jacobson, K. L. Kirk, W. L. Padgett, and J. W. Daly, "Functionalized congeners of adenosine: preparation of analogues with high affinity for A1-adenosine receptors," *Journal of Medicinal Chemistry*, vol. 28, no. 9, pp. 1341–1346, 1985.

[23] K. A. Jacobson and J. W. Daly, "Purine functionalized congeners as molecular probes for adenosine receptors," *Nucleosides and Nucleotides*, vol. 10, no. 5, pp. 1029–1038, 1991.

[24] N. Oishi and J. Schacht, "Emerging treatments for noise-induced hearing loss," *Expert Opinion on Emerging Drugs*, vol. 16, no. 2, pp. 235–245, 2011.

[25] N. Gunewardene, C. X. Guo, A. C. Y. Wong, P. R. Thorne, and S. M. Vlajkovic, "Adenosine amine congener ameliorates cisplatin-induced hearing loss," *World Journal of Otorhinolaryngology*, vol. 3, no. 3, pp. 100–107, 2013.

[26] V. Stocchi, L. Cucchiarini, M. Magnani, L. Chiarantini, P. Palma, and G. Crescentini, "Simultaneous extraction and reverse-phase high-performance liquid chromatographic determination of adenine and pyridine nucleotides in human red blood cells," *Analytical Biochemistry*, vol. 146, no. 1, pp. 118–124, 1985.

[27] Y. Zhang, M. Huo, J. Zhou, and S. Xie, "PKSolver: an add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel," *Computer Methods and Programs in Biomedicine*, vol. 99, no. 3, pp. 306–314, 2010.

[28] L. N. Vandenberg, T. Colborn, T. B. Hayes et al., "Hormones and endocrine-disrupting chemicals: low-dose effects and non-monotonic dose responses," *Endocrine Reviews*, vol. 33, no. 3, pp. 378–455, 2012.

[29] D. Blum, D. Gall, M. Galas, P. D'Alcantara, K. Bantubungi, and S. N. Schiffmann, "The adenosine A1 receptor agonist adenosine amine congener exerts a neuroprotective effect against the development of striatal lesions and motor impairments in the 3-nitropropionic acid model of neurotoxicity," *The Journal of Neuroscience*, vol. 22, no. 20, pp. 9122–9133, 2002.

[30] K. K. Ohlemiller, J. S. Wright, and L. L. Dugan, "Early elevation of cochlear oxygen species following reactive noise exposure," *Audiology and Neuro-Otology*, vol. 4, no. 5, pp. 229–236, 1999.

[31] D. Yamashita, H. Jiang, J. Schacht, and J. M. Miller, "Delayed production of free radicals following noise exposure," *Brain Research*, vol. 1019, no. 1-2, pp. 201–209, 2004.

[32] D. Yamashita, H.-Y. Jiang, C. G. Le Prell, J. Schacht, and J. M. Miller, "Post-exposure treatment attenuates noise-induced hearing loss," *Neuroscience*, vol. 134, no. 2, pp. 633–642, 2005.

[33] K. Tanaka, T. Takemoto, K. Sugahara et al., "Post-exposure administration of edaravone attenuates noise-induced hearing loss," *European Journal of Pharmacology*, vol. 522, no. 1–3, pp. 116–121, 2005.

[34] K. Campbell, A. Claussen, R. Meech, S. Verhulst, D. Fox, and L. Hughes, "D-methionine (d-met) significantly rescues noise-induced hearing loss: timing studies," *Hearing Research*, vol. 282, no. 1-2, pp. 138–144, 2011.

[35] M. Suckfuell, M. Canis, S. Strieth, H. Scherer, and A. Haisch, "Intratympanic treatment of acute acoustic trauma with a cell-permeable JNK ligand: a prospective randomized phase I/II study," *Acta Oto-Laryngologica*, vol. 127, no. 9, pp. 938–942, 2007.

[36] A. C. Y. Wong, K. E. Froud, and Y. S.-Y. Hsieh, "Noise-induced hearing loss in the 21st century: a research and translational update," *World Journal of Otorhinolaryngology*, vol. 3, no. 3, pp. 58–70, 2013.

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Research Article

BDNF Increases Survival and Neuronal Differentiation of Human Neural Precursor Cells Cotransplanted with a Nanofiber Gel to the Auditory Nerve in a Rat Model of Neuronal Damage

Yu Jiao,[1,2,3] Björn Palmgren,[1,2] Ekaterina Novozhilova,[1,2] Ulrica Englund Johansson,[4] Anne L. Spieles-Engemann,[1,2] Ajay Kale,[1,2] Samuel I. Stupp,[5] and Petri Olivius[2,6,7]

[1] *Center for Hearing and Communication Research, Karolinska University Hospital, 171 76 Stockholm, Sweden*
[2] *Department of Clinical Sciences, Intervention and Technology (CLINTEC), Section of Otorhinolaryngology, Karolinska Institutet, Karolinska University Hospital, 171 76 Stockholm, Sweden*
[3] *Department of Otolaryngology, Head and Neck Surgery, Beijing Tongren Hospital, Capital Medical University, Beijing 100730, China*
[4] *Department of Ophthalmology, Institution of Clinical Sciences in Lund, Lund University, 221 84 Lund, Sweden*
[5] *Departments of Chemistry, Materials Science and Engineering, and Medicine and Institute for Bionanotechnology in Medicine, Northwestern University, Chicago, IL 60208, USA*
[6] *Division of Otorhinolaryngology, Linköping University Hospital, 581 85 Linköping, Sweden*
[7] *Division of Otorhinolaryngology, Department of Clinical and Experimental Medicine, University of Linköping, 581 85 Linköping, Sweden*

Correspondence should be addressed to Björn Palmgren; bjorn.palmgren@karolinska.se

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Objectives. To study possible nerve regeneration of a damaged auditory nerve by the use of stem cell transplantation. *Methods*. We transplanted HNPCs to the rat AN trunk by the internal auditory meatus (IAM). Furthermore, we studied if addition of BDNF affects survival and phenotypic differentiation of the grafted HNPCs. A bioactive nanofiber gel (PA gel), in selected groups mixed with BDNF, was applied close to the implanted cells. Before transplantation, all rats had been deafened by a round window niche application of β-bungarotoxin. This neurotoxin causes a selective toxic destruction of the AN while keeping the hair cells intact. *Results*. Overall, HNPCs survived well for up to six weeks in all groups. However, transplants receiving the BDNF-containing PA gel demonstrated significantly higher numbers of HNPCs and neuronal differentiation. At six weeks, a majority of the HNPCs had migrated into the brain stem and differentiated. Differentiated human cells as well as neurites were observed in the vicinity of the cochlear nucleus. *Conclusion*. Our results indicate that human neural precursor cells (HNPC) integration with host tissue benefits from additional brain derived neurotrophic factor (BDNF) treatment and that these cells appear to be good candidates for further regenerative studies on the auditory nerve (AN).

1. Introduction

Although stem cell therapy in many ways is still in its infancy for treating neurodegenerative diseases or nerve trauma, cell transplantation provides a promising strategy for treatment of several lesions in the central nervous system (CNS). In humans, degeneration of the auditory nerve (AN) in the inner ear secondary to hair cell loss is an irreversible process, which eventually reduces residual hearing in any hearing impaired patient [1, 2]. Additionally, there are a number of lesions and diseases that primarily affect the AN, inducing profound to total hearing loss. Such lesions include tumors (most commonly caused by neurofibromatosis type 2), surgery, trauma, and auditory neuropathy [3]. Such central hearing impairment cannot be improved by hearing aids or cochlear implants (CI), since these are designed to aid patients with

a cochlear sensorineural hearing loss illustrating that these are dependent on a functioning AN. For example, it has been demonstrated that the success of a CI is dependent on the proper transduction of electrical signals and subsequently on the function of the AN [4, 5]. Auditory brain stem implants (ABI) have been developed as one possible treatment for central hearing loss (i.e., along the AN). Even though several studies have reported that for some patients excellent speech understanding is possible with an ABI [6, 7], other results obtained from such implants have only improved speech recognition as compared with lip reading [8]. Thus, there is a need for an improved treatment strategy for hearing loss caused by permanently malfunctioning or lesioned spiral ganglion neurons (SGNs). The AN is composed of bipolar SGNs with peripheral processes and central axons and is located in Rosenthal's canal within the modiolus in the cochlea. The SGNs constitute the afferent innervation from the hair cells and transduce the nerve impulses to the next relay station in the cochlear nucleus.

A theoretical method of restoring hearing for patients with a permanent neuronal hearing loss may be to regenerate the injured auditory pathway with stem cells. Neural stem cells are precursor cells with the ability to differentiate into neurons and glial cells. These can be not only derived from embryonic stem cells (ESCs) but also found within the adult nervous system [9]. *In vitro*, we have demonstrated the capability of several different cell types to integrate into the cochlear nucleus, being the second order neuron in the auditory neuronal pathway [10–12]. Furthermore, in a previous *in vivo* study we demonstrated good survival and differentiation of mouse tau-green fluorescent protein (GFP) embryonic stem cells transplanted to the AN in deafened rats [13]. However, good survival, differentiation, and, most importantly, functional improvement of hearing are still challenges for any *in vivo* experiment with stem cells transplanted to the AN. To achieve a properly restored neuronal circuitry, the transplanted cells need to differentiate into neurons and display regulated outgrowth of axons and dendrites with accurate target selection [14].

Human neural precursor cells (HNPCs) have in several studies proved an excellent capacity to generate neurons after *in vivo* intracerebral transplantation [15–17]. Such cells have been studied in animal models of neural degenerative diseases such as Huntington's and Parkinson's diseases with a remarkable ability to form site-specific neurons and to some extent have a functional effect [17–20]. In addition, the possibility to culture human spiral ganglion tissue to obtain neurons indicates a presence of HNPCs also in the adult human auditory nervous system [21]. Encouragingly, a recent report describes a protocol to induce differentiation of human ESCs into otic neuroprogenitors. The differentiated cells were subsequently implanted into a gerbil auditory neuropathy model [22]. Significantly improved auditory-evoked response thresholds were detected for up to 10 weeks after implantation indicating functional recovery.

Neurotrophic factors are important for the development and maintenance of SGNs. For example, exogenous brain derived neurotrophic factor (BDNF) has in *in vitro* as well as *in vivo* studies proved to promote SGN survival [23–25]. It has further been shown that ESCs express the tropomyosin-related kinase (TRK) receptors that mediate antiapoptotic signals, including the BDNF receptor trkB [26]. Thus, a more beneficial approach of regeneration or enhancement of the AN may be to use a combination of transplanted cells and neurotrophic factors.

Tissue engineering is an approaching research field, and numerous studies are describing the advantages with using nanomaterials for improving graft-host integration [27]. Our laboratory has studied a bioactive nanofiber gel consisting of self-assembling peptide amphiphile (PA) molecules designed to present the neurite-promoting laminin epitope isoleucine-lysine-valine-alanine-valine (IKVAV) to the transplanted cells [28]. When mouse-derived neural precursor cells are encapsulated *in vitro* in these gels, rapid and selective differentiation of the cells into neurons is observed [28]. Furthermore, the PA gel has also been shown to have an inhibitory effect on astrocytes, thus preventing scar formations [29]. In an earlier study we have shown a beneficial effect of BDNF and PA gel on mouse tau-GFP cells injected to the internal auditory meatus (IAM) or the modiolus [13].

Here, we used a previously established rodent model of selective AN lesion [30] and injected HNPCs either with the PA gel only or with BDNF added in the PA gel. In order to cause minimal damage to the donor site, due to the limited space in the thin AN, the PA gel was applied over the injection site subsequent to the deposition of the HNPCs. HNPCs were carrying the reporter gene GFP. Previously, GFP expression has been found long-term in neurons as well as in glial cells after transplantation of similar HNPCs [15, 31]. The GFP distribution found throughout the entire cytoplasm and fine structures of the HNPCs allowed for detailed morphological analysis.

In the present study we demonstrate the beneficial effect of BDNF contained in the PA gel. This treatment rendered a significant larger number of cells, increased neuronal differentiation, and migration of the GFP+ HNPCs after injection into the AN. Furthermore, histological analysis demonstrated neurite outgrowths and arborisation in the transplanted HNPCs as well as fiber growth into the cochlear nucleus area of the brain stem (BS).

2. Materials and Methods

2.1. Animals. All animal experiments followed the national approved protocol for care and use of animals in Sweden (N3/11; N4/11). Young adult female Sprague-Dawley rats ($n = 13$; 200–250 g) were used in this study. To exclude any visible middle ear infections, preoperative otoscopic examinations were performed.

2.2. Application of β-Bungarotoxin to the Round Window Niche. Three weeks prior to HNPC injection, the animals ($n = 13$) were deafened by application of β-bungarotoxin (β-BuTx) to the round window niche as previously described [30]. In brief, after anesthesia with xylazine (10 mg/kg i.p.) and ketamine (50 mg/kg i.p.), the round window niche was

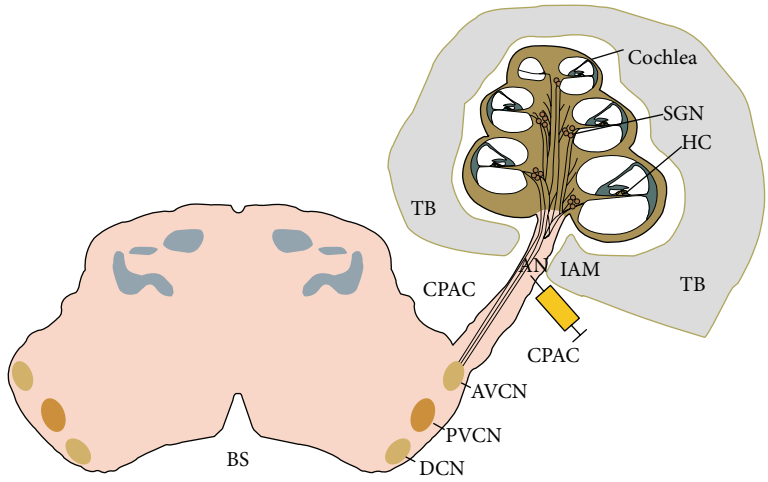

Figure 1: Schematic of the cochlea, the auditory nerve, and the brain stem. The syringe indicates the HNPC injection site in the auditory nerve trunk by the internal auditory meatus. AN: auditory nerve; TB: temporal bone; CPAC: cerbellopontine angle cistern; IAM: internal auditory meatus; HC: hair cell; SGN: spiral ganglion neuron; AVCN: anteroventral cochlear nucleus; PVCN: posteroventral cochlear nucleus; DCN: dorsal cochlear nucleus; BS: brain stem.

exposed by a retroauricular incision. Five microliters of β-BuTx (0.05 μg/mL, Alexis Biochemicals) was absorbed by gel foam and applied to completely fill the round window niche. A piece of fascia was placed to cover the hole in the bulla and the wound was sutured.

2.3. Human Neural Precursors (HNPCs) Cell Line. The human neural precursor cell line used for this study was originally established by L. Wahlberg, Å. Seiger, and colleagues at the Karolinska University Hospital (original work with the cell line was described by Carpenter et al. [32] and was kindly provided by Professor A. Björklund (Department of Experimental Medical Science, Lund University Sweden)). Briefly, forebrain tissue was obtained from a nine-week-old (postconception) human embryo and isolated under compliance with the National Institute of Health guidelines, Swedish Government guidelines, and the local ethical committee. The HNPCs were cultured in DMEM-F12 medium (Invitrogen) supplemented with 2.0 mM L-glutamine (Sigma), 0.6% glucose (Sigma), N2 supplement (Invitrogen), and 2.0 mg/mL heparin (Sigma) and were cultured as free floating clusters (neurospheres). The growth factors: human basic fibroblast growth factor (hbFGF, 20 ng/mL; R&D Systems), human epidermal growth factor (hEGF, 20 ng/mL; Invitrogen), and human leukemia inhibitory factor (hLIF, 20 ng/mL; Sigma), were added every 3–5 days to the culture. The neurospheres were passaged by mechanical dissociation every 7–10 days and reseeded as single cells at a density of 1×10^5 cells/mL. HNPCs expressed the reporter gene GFP which was previously transduced to the cells using a lentiviral infection at MOI = 0, 1 (for details on lentiviral infection see [33]).

2.4. Surgical Approach. The surgical approach for cell injections (C.Inj.) into the AN trunk by the IAM (Figure 1) has been previously described [34]. In brief, all animals were anaesthetized with an intraperitoneal (i.p.) injection of a mixture of Ketalar (50 mg/kg) and Rompun (10 mg/kg) and the skull was put in a fixed position with the aid of a stereotactic frame. Under a surgical microscope, an incision was made through the skin and underlying soft tissue. Using a drill, a hole was made in the suboccipital bone and the underlying dura was opened and reflected towards the edge of the hole. The cerebellar hemisphere was retracted medially to reveal the AN entering through the IAM.

2.5. Cell Injections. A microsyringe mounted in the clamping device of the stereotactic frame was used for all C.Injs. The needle was positioned above the AN trunk with the angle of the tip adjusted towards the IAM. The needle was lowered into the AN trunk by the use of a micromanipulator and 5 μL of HNPCs dissociated into a single cell suspension in culture medium (10.000 cells/μL, i.e., a total of 50.000 cells/implant) was injected over one minute. After injection, the needle was left in place for 10 minutes. The wound cavity was filled with sterile saline and a piece of fascia was applied to cover the hole in the dura and occipital bone. The wound was sutured in layers. Following surgery and removal from the stereotactic frame, the animals were given subcutaneous injections of 3 mL saline and 0.2 mL Temgesic (0.3 mg/mL) and placed in a warm cage to recover before being transferred to their home cage.

To prevent postoperative infection and immune response rejection, all animals received daily doses of tetracycline (1.8 mg/mL, i.p.) and cyclosporine (4.2 mg/mL, i.p.) until sacrificed.

2.6. BDNF and PA Gel Applications. In Groups 1–3, 1 wt% IKVAV peptide amphiphile nanofiber gel (Nanotope, USA) was applied over the C.Inj. site by the IAM in the same surgical session as the C.Inj. In two groups (Groups 2 and 3) an additional 10 μL high concentration BDNF (20 mg/mL) was mixed with the applied PA gel.

Table 1: Animal groups. Group 1 ($n = 3$; survival time 3 weeks) had HNPCs injected to the AN with PA gel applied on the injection site. Group 2 ($n = 4$; survival time 3 weeks) had HNPCs injected to the AN with PA gel including BDNF applied on the injection site. Group 3 ($n = 3$; survival time 6 weeks) had HNPCs injected to the AN with PA gel with BDNF applied on the injection site. Group 4 was the control group ($n = 3$; 3-week survival time) and had medium only without cells injected to the nerve.

Group	Number of animals	β-ButTx	HNPC injections	BDNF	PA gel	Survival time
1	3	+	+		+	3 weeks
2	4	+	+	+	+	3 weeks
3	3	+	+	+	+	6 weeks
4	3	+				3 weeks

β-ButTx, β-bungarotoxin; HNPC, human neural precursor cells; BDNF, brain derived neurotrophic factor; PA, peptide amphiphile.

2.7. Experimental Groups. The animal groups are described in Table 1. In Groups 1–3 the HNPCs were injected into the left AN trunk near the IAM in the central portion of the AN (Figure 1). Group 4 served as the control group with culture medium (vehicle) only injected.

2.8. Tissue Preparation. After three or six weeks of survival, rats were sacrificed by an overdose of pentobarbital (60 mg/mL, i.p.) and transcardially perfused with body warm 0.9% saline followed by ice-cold 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer saline (PBS). The cochlea, the AN, and part of the BS were carefully removed from the temporal bone en bloc. The specimens (cochlea plus AN including BS) were further dissected and a small hole was made in the apex of the cochlea through which the cochlea was perfused with PFA (initially with 4% and then with 0.5%). The cochlea was decalcified for seven days using 1% ethylenediaminetetraacetic acid in 0.1 M PBS. After decalcification, the specimens spent 24 hours in 20% sucrose solution and were embedded and frozen in Optimal Cutting Temperature Compound (Tissue-Tek; Sakura Finetek, Torrance, CA, USA). The specimens were orientated in the compound so that the midmodiolar sections would contain the cochlea, the AN, and the BS as a continuum. The 12 μm midmodiolar cryosections were mounted on glass slides.

2.9. Immunohistochemistry. Sections were fixed in 4% PFA in phosphate buffered saline (PBS, pH 7.4) for 1 h at room temperature (RT). After washing 3 times in PBS, the sections were treated with ice cold 20% methanol in PBS for 5 min at RT. After 3 more rinses in PBS, the tissue was permeabilized using 0.5% Triton-X in PBS overnight at 4°C. The sections were washed 3 times with PBS and incubated with 20% bovine serum albumin (Sigma) blocking solution for 12 h at 4°C prior to incubation with primary antibodies. The sections were incubated at 4°C overnight with fluorescein isothiocyanate conjugated goat polyclonal GFP antibody for transplanted cell detection (1 : 200 dilution; Abcam, Cambridge, UK). For double immunostaining, the sections were incubated at 4°C overnight with a primary rabbit polyclonal β-tubulin (TUJ1) antibody (1 : 200 dilution; Covance Research Products, Berkeley, CA, USA). Following incubation with the primary antibodies, the sections were incubated with secondary goat-anti-rabbit Cy3 antibody (1 : 2000 dilution; Jackson Immuno Research) for four hours at RT. Omission of the primary antibody served as negative control. Cell nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI). After three more rinses with PBS the sections were mounted with Prolong Gold mounting medium (Invitrogen) and examined using a fluorescence microscope (Axio Observer Z1, Zeiss). The brightness and contrast of the presented images were adjusted to aid visualization (Adobe Photoshop CS5 12.0, Adobe Systems Inc., USA).

2.10. Quantification of Transplanted and Differentiated Cells. The specimens were oriented and cryosectioned with the cochlea, the AN, and the BS in the same section. Surviving transplanted cells were defined as cell profiles with coexpression of GFP and DAPI. Differentiated transplanted cells were defined as cell profiles with coexpression of GFP and TUJ1. Quantification and statistical analysis were performed on all groups assessing the number of GFP- and DAPI-stained cells and GFP- and TUJ1 double-labeled cells. We further quantified the number of differentiated HNPCs with nerve fiber outgrowths.

From a total of approximately 30 serial sections from each specimen, the number of HNPCs was in quantified in four regions. These regions include the scala tympani (ST) in the cochlea, the modiolus in the cochlea, the AN, and the BS including the cochlear nucleus. The restrictions of the regions were set in the microscope in accordance with histological characteristics (e.g., the transitional zone between the AN and modiolus). Quantification was performed using stereology, as previously described [13, 35].

Throughout the entire specimen, quantification was performed in every third section. Since the average diameter of the profiles quantified was 15 μm and the sections had a thickness of 12 μm, this avoided double counting of profiles. In every analyzed section, cells were quantified in each of the four different regions individually. Every third section of a region was subdivided into fields where each field was equivalent to the microscopic optic field at 40x. In order to get a mean value of cells per section for each region, the total number of counted cells in each region was divided by the number of evaluated sections. Further, for every region, the mean number of cells in one section was multiplied with the total number of sections. The number of TUJ1 positive cells was calculated using the same technique. The differentiation rate was estimated by dividing the number of differentiated cells by the number of surviving cells per specimen. All data are presented as the mean of each experimental group.

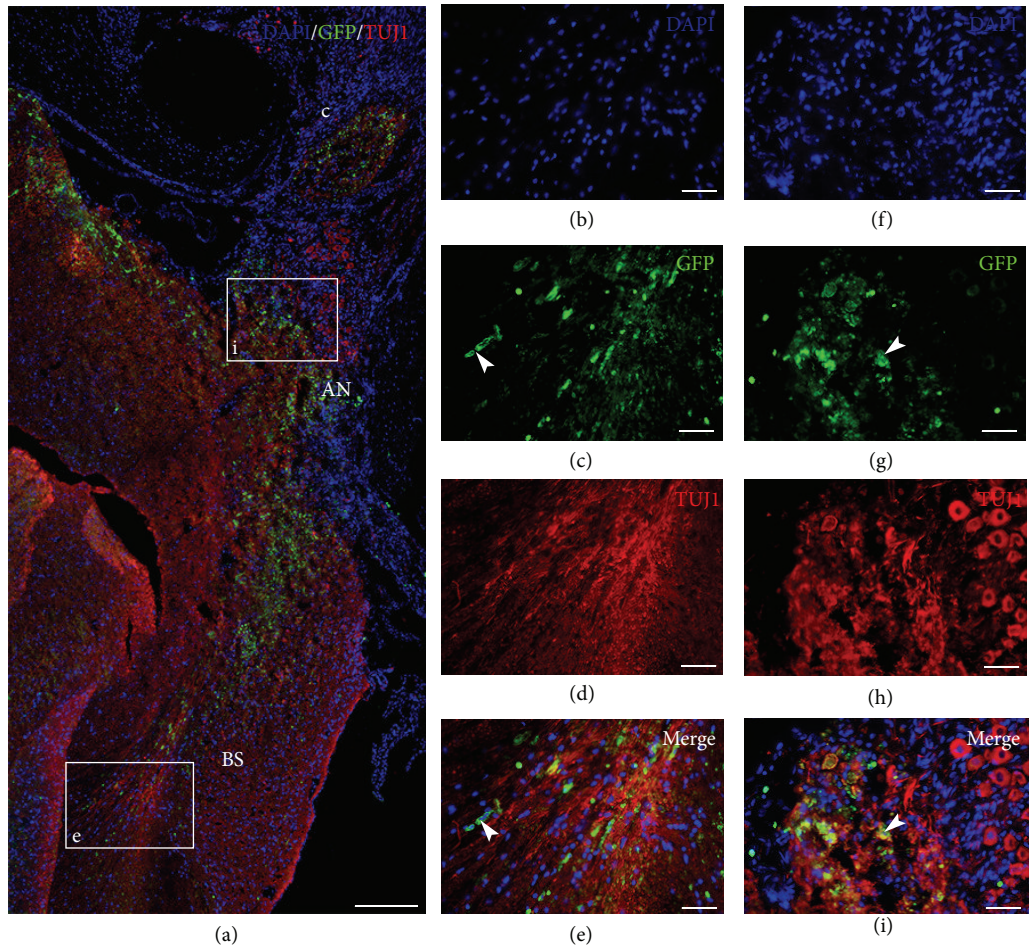

Figure 2: GFP+ HNPCs injected to the internal auditory meatus with application of PA gel only (Group 1). Immunohistochemical staining was performed with DAPI ((b), (f)) for cell nuclei, GFP ((c), (g)) for transplanted cells, and TUJ1 ((d), (h)) to verify differentiation. At three weeks following transplantation, GFP positive cells were identified along the auditory nerve "i" and in the brain stem "e." Only a few cells have differentiated as verified by yellow double staining (GFP and TUJ1; (a), (c), (d), (e), (g), (h), and (i)). White arrowheads indicating surviving GFP+ HNPCs in the auditory nerve "i" and in the BS "e." AN: auditory nerve; BS: brain stem; C: cochlea. Scale bar (a) 400 μm, (b–i) 100 μm.

2.11. Statistics. Two-way ANOVA followed by Tukey's HSD test was used to determine statistically significant differences in cell numbers between the groups of animals. All results are expressed as mean ± standard error of the mean (SEM).

3. Results

The results described include four different animal groups (three or four rats in each group) shown in Table 1. In two groups, the survival time was three weeks and in one group it was six weeks. All groups were subject to the same surgical approach with HNPC cell injections to the AN by the IAM (Figure 1). The cells were harvested and processed from forebrain tissue obtained from a nine-week-old (post-conception) human embryo. In all cell injection groups the BDNF was applied in the PA gel previously described. One control group did not receive any cell injections or BDNF. Immunohistochemistry and staining were used to identify transplanted cells and to detect possible differentiation. Cells double labeled (yellow) with GFP (green) and TUJ1 (red) were quantified as differentiated cells. The results are presented as comparisons between the groups with respect to cell survival and migration patterns as well as cell differentiation. The results of the cell quantification are summarized in Figure 5. As no cells were found in the modiolus or in the ST, these regions are not further mentioned in this section.

3.1. Higher Numbers of GFP+ HNPCs after BDNF-Treatment. Quantification of the number of GFP+ cells includes both the GFP+ HNPCs with immature profiles and GFP+ HNPCs differentiated into TUJ1 positive cells (Figure 5(a)).

Surviving GFP+ HNPCs were found in all grafted animals. Overall, Groups 2 and 3 (both receiving BDNF-treatment) had significantly higher numbers of GFP+ cells (9562 ± 699 and 8489 ± 561, resp.) as compared to Group 1, which did not receive BDNF-treatment (1699 ± 383; $P < 0.001$; Figure 5(a), cf. Figures 2–4). When the regions were quantified separately, Groups 2 and 3 displayed a significantly

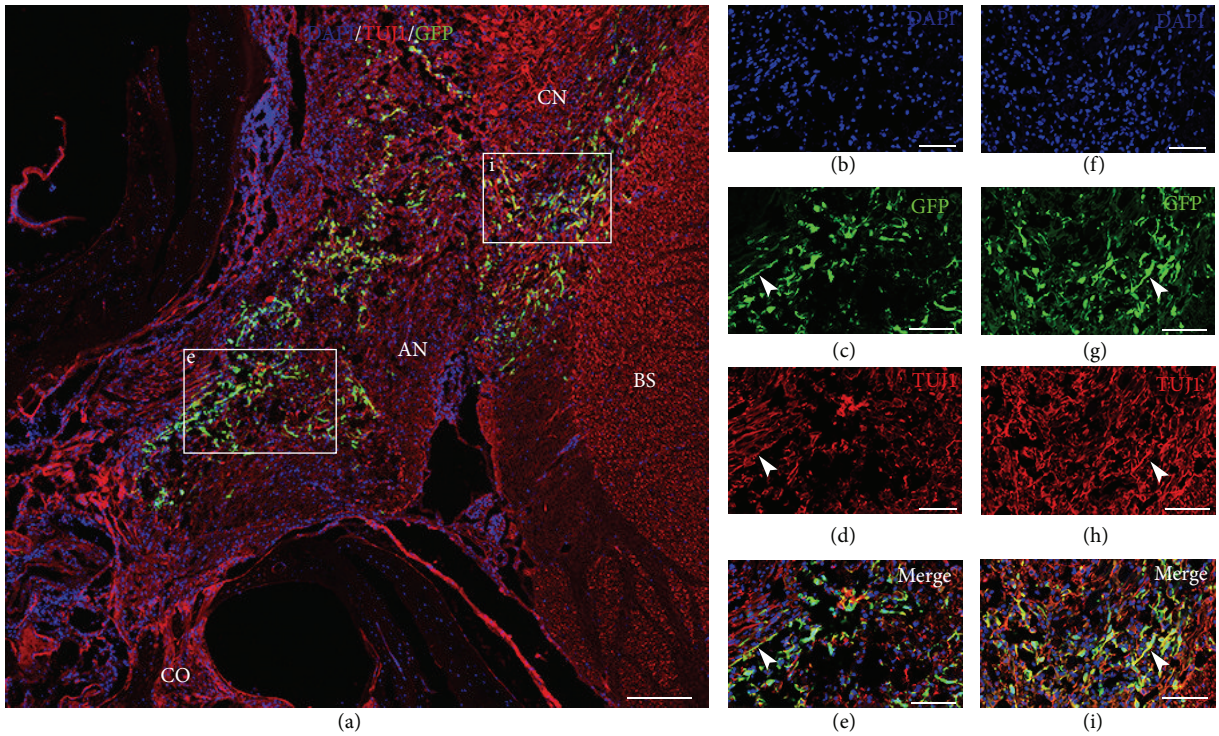

Figure 3: GFP+ HNPCs three weeks after transplantation with BDNF in PA gel (Group 2). Immunohistochemical staining was performed with DAPI ((b), (f)) for cell nuclei, GFP ((c), (g)) for transplanted cells, and TUJ1 ((d), (h)) to verify differentiation. Transplanted HNPCs were identified along the auditory nerve "e" and had also migrated to the brain stem "i." Double-labeled yellow cells (GFP and TUJ1) and neurites (arrowheads) indicate neuronal differentiation ((a), (c), (d), (e), (g), (h), and (i)). CO: cochlea; AN: auditory nerve; BS: brain stem; CN: cochlear nucleus. Scale bar (a) 200 μm, (b–i) 100 μm.

higher number of GFP+ cells in both the AN and the BS (Group 2: 5196 ± 375 (AN); 4366 ± 08 (BS), Group 3: 1989 ± 226 (AN); 6500 ± 362 (BS)) as compared to Group 1 (481 ± 112 (AN), 1217 ± 274 (BS); Figure 5(a), cf. Figures 3 and 4). In Group 3 a majority of GFP+ cells were found in the BS as compared to Group 2, where the majority of GFP+ cells were found in the AN (Figure 5(a), cf. Figures 3 and 4).

3.2. BDNF in the PA Gel Promotes HNPC Differentiation. Transplanted cells were considered to have differentiated into a neuronal lineage if these were double stained with GFP and TUJ1. Overall, Groups 2 and 3 displayed significantly more neuronal differentiated cells (7590 ± 639 and 6088 ± 84, resp.) as compared to Group 1 (147 ± 71; Figure 5(b), cf. Figures 2–4). When the regions were analyzed separately, Groups 2 and 3 had significantly more differentiated cells in both the AN and the BS (Group 2: 4002 ± 359 (AN); 3588 ± 381 (BS), Group 3: 1577 ± 137 (AN); 4511 ± 57 (BS)) as compared to Group 1 (22 ± 22 (AN), 126 ± 50 (BS); $P < 0.001$; Figure 5(b), cf. Figures 3 and 4). Thus, out of the GFP+ HNPCs the most prominent neuronal differentiation was found in Groups 2 (79%) and 3 (72%). There were similar numbers of differentiated cells in the BS in Groups 2 and 3 (Figure 5(b)) but significantly more cells in the AN in Group 2 as compared to Group 3 (Figure 5(b); $P < 0.001$).

3.3. BDNF in the PA Gel Promotes Neuronal Outgrowths/Extensions in Differentiated Cells. Quantification of numbers of GFP+ HNPC with neuronal outgrowths was performed as described above. These were defined as GFP and TUJ1 positive cells that had outgrowths that were estimated to be at least equal in length to the cell soma. Overall, Groups 2 and 3 had significantly more cells with neuronal outgrowth (4240 ± 474 and 5473 ± 87, resp.; Figure 5(c)) as compared to Group 1 (17 ± 4; $P < 0.001$). When we analyzed each region individually we found that there were zero cells with outgrowths in the AN and only 17 ± 4 in the BS in Group 1 (Figure 5(c)). Group 2 had approximately an equal number of cells with outgrowths in the AN (2454 ± 237) as in the BS (1786 ± 446). However, after 6 weeks of survival (Group 3), there were 4030 ± 33 cells with outgrowth in the BS as compared to 1443 ± 104 in the AN. This is illustrated in Figure 5(c).

4. Discussion

Here we present a novel nerve regeneration protocol for HNPC transplantation into a damaged rat AN with coapplication of PA gel and BDNF. In the present study, surviving and differentiated GFP+ HNPCs were detected in all groups in both the AN and the BS for up to six weeks after transplantation into the β-BuTx-damaged rat AN. At six weeks

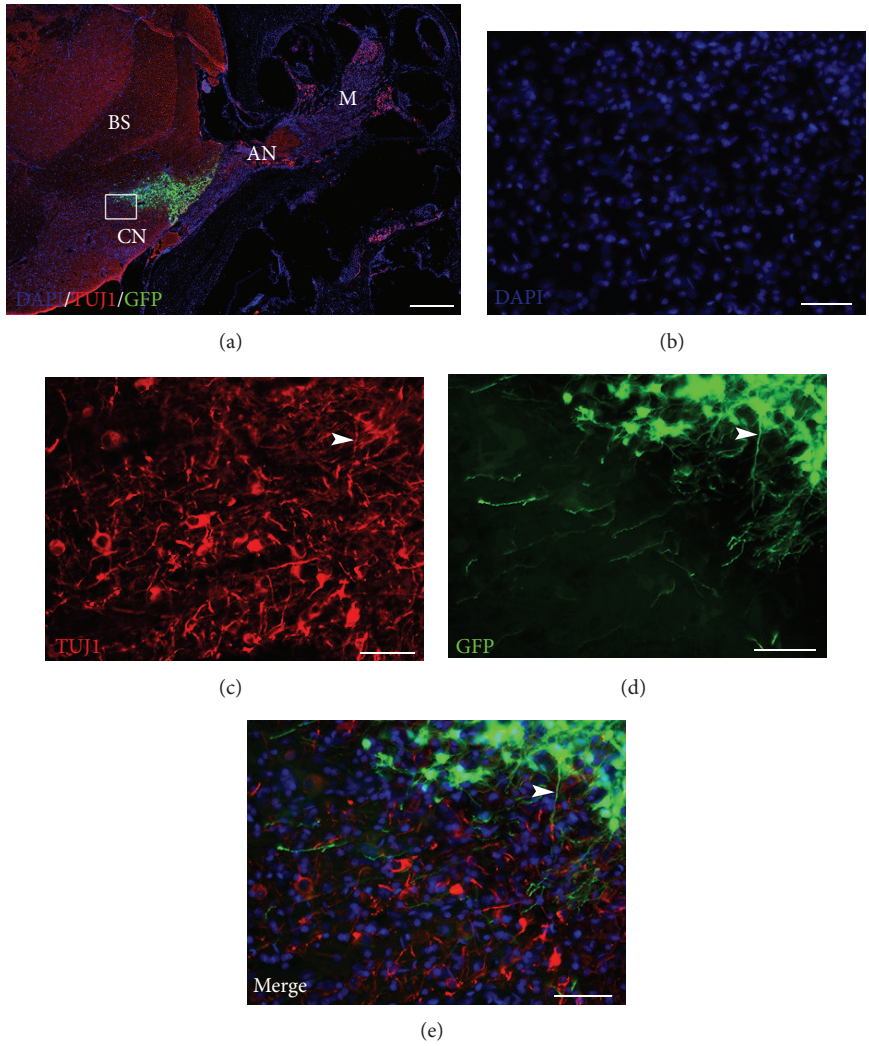

Figure 4: GFP+ HNPCs six weeks after transplantation with BDNF in PA gel (Group 3) (a). Cryostat section of dissected rat cochlea, auditory nerve, and brain stem following transplantation of HNPCs. Inset box in (a) shown at higher magnification in ((b)–(e)). Immunohistochemical staining was performed with DAPI (b) for cell nuclei, GFP (d) for transplanted cells, and TUJ1 (c) to verify differentiation. Double labeling (yellow) of GFP and TUJ1 indicates neuronal differentiation of the transplanted HNPCs. Double-labeled GFP, TUJ1 positive cells, and their arborisation of fibers can be identified in vicinity of the cochlear nucleus ((c)–(e)); arrowhead indicates GFP/TUJ1 double-labeled fiber. M: modiolus; AN: auditory nerve; BS: brain stem; CN: cochlear nucleus. Scale bar (a) 400 μm, ((b)–(e)) 50 μm.

after transplantation, a higher number of GFP+ HNPCs were found in the BS as compared to the number of cells at three weeks, demonstrating central-directed (from the C.Inj. site) cell migration over time. Additionally, significantly more fiber outgrowths were seen at the six-week time point, demonstrating a progressive neuronal maturation over time of the grafted HNPC.

Further, the results indicate that BDNF had a significant effect on numbers of GFP+ cells and differentiation of the transplanted HNPCs. BDNF was mixed and applied in a PA gel over the injection site. This neurite-promoting laminin epitope containing gel has in several studies been shown to promote the regeneration of sensory nerve fibers, to increase survival and differentiation of stem cells and neural progenitors, and to have a suppressing effect on astrogliosis [28, 36]. This gel is intended to be mixed with transplanted cells and applied directly to the nerve lesion. However, due to limited space in the AN trunk in our experimental setup, we were unable to use the gel as intended but instead we applied the gel mixed with BDNF on the C.Inj. site. Although this application approach of the gel may not have been ideal, it is possible that our cell transplantation strategy benefited from the inhibitory effect of the PA gel on the glial scarring that can occur around the injection site, thus allowing for an increased migration of transplanted cells. Further, the cylindrical and hydrated nanofibers in the PA gel may bind and localize neurotrophic factor protecting it from enzymatic degradation. The gel network may also allow for sustained release of BDNF over time. Recently Angeloni and coworkers, using a different PA gel, demonstrated the

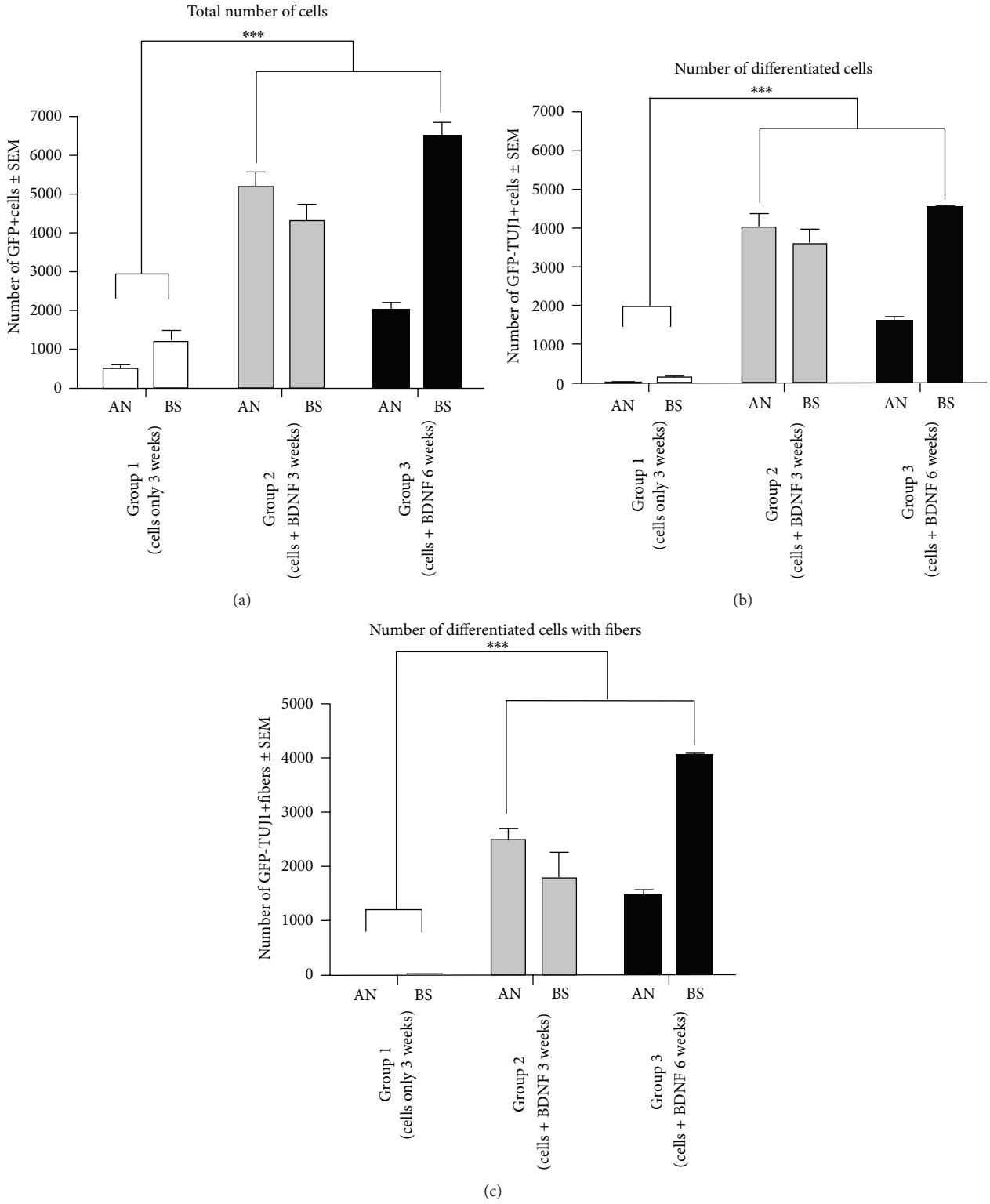

Figure 5: Graphs illustrating the quantification of numbers of GFP+ HNPCs, differentiated cells, and cells with neuronal outgrowths. (a) Total number of GFP+ HNPCs. Groups 2 and 3 had significantly better cell survival compared to Group 1 ($^{***}P \leq 0.001$). After 3 weeks the majority of the surviving cells were observed in the AN whereas after 6 weeks the majority of the cells were found in the BS. No cells were found in Group 4 (data not shown). (b) Number of double-labeled (GFP and TUJ1) differentiated cells. Groups 2 and 3 had significantly more differentiated cells than Group 1 ($^{***}P \leq 0.001$). After six weeks, the majority of the differentiated cells were found in the BS. (c) Number of differentiated cells with outgrowth of fibers. Groups 2 and 3 had significantly more cells with fiber outgrowth as compared to Group 1 ($^{***}P \leq 0.001$).

possibility of regenerating the cavernous nerve by delivering Sonic hedgehog from a PA gel but not when the same morphogen was encapsulated in conventional gel beads [37].

To evaluate the effect of BDNF on the numbers of GFP+ cells, differentiation, and migration of the transplanted HNPCs, we quantified the number of GFP+ cells (Figure 5(a)) as well as the number of differentiated GFP and TUJ1 positive cells in the AN and BS (Figure 5(b)). Further we evaluated the number of differentiated GFP and TUJ1 positive cells with fiber outgrowth (Figure 5(c)). Branching is a necessary feature for axons in the development of complex neuronal circuits [38]. Studies of the retinotectal system have illustrated that BDNF, through activation of the TrkB, promotes branching of retinal axons [38, 39]. Further, the arborisation of neurite processes in the period of neurite outgrowth adds to the complexity of neuronal circuits [40]. Later retraction and pruning of inappropriate branches result in a more mature pattern of connectivity [41].

Regeneration of a sensory cranial nerve such as the AN presents several obstacles. First, cells suitable for the task need to be selected. There are several cell candidates in various stages of differentiation to choose from. Most favorable would be to transplant autogenic cells as this circumvents the issue with immunogenic responses and host versus graft rejection. Since the eventual goal of our experiments is to develop a cell replacement strategy that can be used clinically, we presently used HNPCs as cell candidate. In contrast to rodent stem cells, HNPCs have the potential to be used as a future allogeneic cell replacement therapy in humans. The HNPCs were initially obtained from first-trimester human embryonic forebrain tissue and can be long term expanded into high numbers *in vitro* [42] maintaining the capacity to form neurons, astrocytes, or oligodendrocytes, the three main phenotypes in the CNS [32]. Second, there is the issue of surgical approach. The AN is embedded in the cochlea and the temporal bone. To access the distal part of the nerve, it is therefore necessary to perform a cochleostomy; that is, penetrate the basal turn of the cochlea and access the nerve via the modiolus. This approach may therefore jeopardize the integrity and homeostasis of the cochlea. However, the central portion of the AN near the IAM can be accessed via a craniotomy. Once the craniotomy is performed, the cerebellum is retracted medially to reveal the AN going into the IAM. In a previous experiment, we have demonstrated that this central approach does not significantly affect the hearing of the experimental animals as measured by auditory brain stem response testing [34]. Third, the injected cells need to differentiate into neurons and find the proper connections both peripherally with the hair cells and centrally with cochlear nucleus neurons. BDNF has been shown to promote the neural differentiation of stem cells [43–45]. BDNF has also been shown to promote axonal branching in retinal ganglion cells through activation of the TrkB receptor [38]. Since the transplanted cells may need support by exogenous factors to survive and differentiate, one impediment may be how to give the cells trophic support over time. This may be achieved by prolonged infusions via miniosmotic pumps or containers with slow releasing agents such as a PA gel. Indeed one *in vitro* study has demonstrated that BDNF tethered with a nanofiber scaffold enhanced the proliferation and differentiation of cultured NSCs as compared to soluble BDNF [44]. Here we used a PA gel mixed with BDNF in order to provide prolonged trophic support to the HNPCs.

Overall, we observed very high neuronal differentiation rates of the transplanted GFP+ HNPCs in the BDNF-treated groups. At 3 weeks, 79% of the GFP+ HNPCs were TUJ1 positive. After 6 weeks of survival 72% of the GFP+ HNPCs were TUJ1 positive, which may indicate good survival of the human neurons over time. Additionally, from the total number of differentiated cells we observed a higher rate of cells with fiber outgrowth at 6 weeks 90% as compared to cells with fiber outgrowths at 3 weeks 56% (Figure 5), in the BDNF-treated groups, indicating that these cells appear to be maturing over time. These effects on numbers of GFP+ cells, differentiation, and sprouting appear to be due to BDNF since significantly more GFP+ cells, neuronally differentiated cells, and sprouts were found in Groups 2 and 3 as compared to the untreated Group 1.

In the BDNF-treated groups, even though total GFP+ cell numbers were similar between the three and six weeks of survival groups, the locations of the surviving cells were different. The differentiated GFP+ cells at the 3-week time point were evenly distributed between the AN (54% GFP, 53% GFP/TUJ1) and the BS (46% GFP, 47% GFP/TUJ1). However, at the 6-week time point the majority of surviving and differentiated cells had migrated into the BS (77% GFP, 74% GFP/TUJ1) as compared to the AN (23% GFP, 26% GFP/TUJ1) indicating a central migration of the cells over time. The environment in the BS being more favorable for harboring the transplanted cells could explain this and that site-specific cues in the BS may trigger migration, differentiation, and sprouting of these cells.

Additionally, the number of double-stained cells with fiber outgrowths in the BDNF-treated groups was also significantly higher in the BS than in the AN after 6 weeks as compared to the 3-week group. Therefore, it appears that BDNF promoted differentiation and these differentiated cells developed fibers as the cells matured over time.

5. Conclusion

Regeneration of cranial nerves constitutes a major challenge both in the control of the microcellular environment and in getting the proper surgical access without damaging sensitive structures. The current study comprises a first step in finding novel therapeutic techniques for aiding patients with a severely damaged AN. As CIs and ABIs are getting more advanced, higher demands on the function of the residual AN and spiral ganglions are to be expected. A combination therapy with CI, cell transplantation, and neurotrophic factor stimulation may be a feasible approach. For a future clinical use, the cells need to be derived from human sources. In this study, we observed high survival and neuronal differentiation of HNPCs transplanted to the rat AN. Our results also indicate that HNPC integration with host tissue benefits from additional BDNF. As this study did not include verification of synapses or functional assessment, we cannot draw any conclusions on the integration of the transplanted cells into

the existing neuronal circuitry. However, the fact that we found differentiated HNPCs and newly formed nerve fibers in close proximity to the cochlear nucleus suggests the need for further studies to explore the functional effectiveness of this cell transplantation paradigm.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Yu Jiao and Björn Palmgren contributed equally to this work.

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References

[1] J.B. Nadol Jr., "Patterns of neural degeneration in the human cochlea and auditory nerve: implications for cochlear implantation," *Otolaryngology-Head and Neck Surgery*, vol. 117, no. 3, pp. 220–228, 1997.

[2] C. E. Zimmermann, B. J. Burgess, and J. B. Nadol Jr., "Patterns of degeneration in the human cochlear nerve," *Hearing Research*, vol. 90, no. 1-2, pp. 192–201, 1995.

[3] A. Starr, Y. S. Sininger, and H. Pratt, "The varieties of auditory neuropathy," *Journal of Basic and Clinical Physiology and Pharmacology*, vol. 11, no. 3, pp. 215–230, 2000.

[4] A. M. Khan, O. Handzel, B. J. Burgess, D. Damian, D. K. Eddington, and J. B. Nadol Jr., "Is word recognition correlated with the number of surviving spiral ganglion. Cells and electrode insertion depth in human subjects with cochlear implants?" *Laryngoscope*, vol. 115, no. 4, pp. 672–677, 2005.

[5] A. Incesulu and J. B. Nadol Jr., "Correlation of acoustic threshold measures and spiral ganglion cell survival in severe to profound sensorineural hearing loss: implications for cochlear implantation," *Annals of Otology, Rhinology & Laryngology*, vol. 107, no. 11, part 1, pp. 906–911, 1998.

[6] L. Sennaroglu, I. Ziyal, A. Atas et al., "Preliminary results of auditory brainstem implantation in prelingually deaf children with inner ear malformations including severe stenosis of the cochlear aperture and aplasia of the cochlear nerve," *Otology and Neurotology*, vol. 30, no. 6, pp. 708–715, 2009.

[7] V. Colletti and R. V. Shannon, "Open set speech perception with auditory brainstem implant?" *Laryngoscope*, vol. 115, no. 11, pp. 1974–1978, 2005.

[8] M. S. Schwartz, S. R. Otto, R. V. Shannon, W. E. Hitselberger, and D. E. Brackmann, "Auditory Brainstem Implants," *Neurotherapeutics*, vol. 5, no. 1, pp. 128–136, 2008.

[9] F. H. Gage, "Mammalian neural stem cells," *Science*, vol. 287, no. 5457, pp. 1433–1438, 2000.

[10] C. Thonabulsombat, S. Johansson, C. Spenger, M. Ulfendahl, and P. Olivius, "Implanted embryonic sensory neurons project axons toward adult auditory brainstem neurons in roller drum and Stoppini co-cultures," *Brain Research*, vol. 1170, pp. 48–58, 2007.

[11] A. Glavaski-Joksimovic, C. Thonabulsombat, M. Wendt, M. Eriksson, H. Ma, and P. Olivius, "Morphological differentiation of tau-green fluorescent protein embryonic stem cells into neurons after co-culture with auditory brain stem slices," *Neuroscience*, vol. 162, no. 2, pp. 472–481, 2009.

[12] A. Glavaski-Joksimovic, C. Thonabulsombat, M. Wendt et al., "Survival, migration, and differentiation of Sox1GFP embryonic stem cells in coculture with an auditory brainstem slice preparation," *Cloning and Stem Cells*, vol. 10, no. 1, pp. 75–87, 2008.

[13] B. Palmgren, Y. Jiao, E. Novozhilova, S. I. Stupp, and P. Olivius, "Survival, migration and differentiation of mouse tau-GFP embryonic stem cells transplanted into the rat auditory nerve," *Experimental Neurology*, vol. 235, no. 2, pp. 599–609, 2012.

[14] J. Dodd and T. M. Jessell, "Axon guidance and the patterning of neuronal projections in vertebrates," *Science*, vol. 242, no. 4879, pp. 692–699, 1988.

[15] U. Englund, R. A. Fricker-Gates, C. Lundberg, A. Björklund, and K. Wictorin, "Transplantation of human neural progenitor cells into the neonatal rat brain: Extensive migration and differentiation with long-distance axonal projections," *Experimental Neurology*, vol. 173, no. 1, pp. 1–21, 2002.

[16] R. A. Fricker, M. K. Carpenter, C. Winkler, C. Greco, M. A. Gates, and A. Björklund, "Site-specific migration and neuronal differentiation of human neural progenitor cells after transplantation in the adult rat brain," *Journal of Neuroscience*, vol. 19, no. 14, pp. 5990–6005, 1999.

[17] R. J. E. Armstrong, C. Watts, C. N. Svendsen, S. B. Dunnett, and A. E. Rosser, "Survival, neuronal differentiation, and fiber outgrowth of propagated human neural precursor grafts in an animal model of huntington's disease," *Cell Transplantation*, vol. 9, no. 1, pp. 55–64, 2000.

[18] C. N. Svendsen, M. A. Caldwell, J. Shen et al., "Long-term survival of human central nervous system progenitor cells transplanted into a rat model of Parkinson's disease," *Experimental Neurology*, vol. 148, no. 1, pp. 135–146, 1997.

[19] F. J. Rubio, C. Bueno, A. Villa, B. Navarro, and A. Martínez-Serrano, "Genetically perpetuated human neural stem cells engraft and differentiate into the adult mammalian brain," *Molecular and Cellular Neurosciences*, vol. 16, no. 1, pp. 1–13, 2000.

[20] O. Lindvall and A. Björklund, "Cell Therapeutics in Parkinson's Disease," *Neurotherapeutics*, vol. 8, no. 4, pp. 539–548, 2011.

[21] H. Rask-Andersen, M. Boström, B. Gerdin et al., "Regeneration of human auditory nerve. In vitro/in video demonstration of neural progenitor cells in adult human and guinea pig spiral ganglion," *Hearing Research*, vol. 203, no. 1-2, pp. 180–191, 2005.

[22] W. Chen, N. Jongkamonwiwat, L. Abbas et al., "Restoration of auditory evoked responses by human ES-cell-derived otic progenitors," *Nature*, vol. 490, no. 7419, pp. 278–282, 2012.

[23] T. Shinohara, G. Bredberg, M. Ulfendahl et al., "Neurotrophic factor intervention restores auditory function in deafened animals," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 3, pp. 1657–1660, 2002.

[24] J. M. Miller, C. G. Le Prell, D. M. Prieskorn, N. L. Wys, and R. A. Altschuler, "Delayed neurotrophin treatment following deafness rescues spiral ganglion cells from death and promotes regrowth of auditory nerve peripheral processes: effects of brain-derived neurotrophic factor and fibroblast growth factor," *Journal of Neuroscience Research*, vol. 85, no. 9, pp. 1959–1969, 2007.

[25] A. Warnecke, K. Wissel, A. Hoffmann et al., "The biological effects of cell-delivered brain-derived neurotrophic factor on cultured spiral ganglion cells," *NeuroReport*, vol. 18, no. 16, pp. 1683–1686, 2007.

[26] A. D. Pyle, L. F. Lock, and P. J. Donovan, "Neurotrophins mediate human embryonic stem cell survival," *Nature Biotechnology*, vol. 24, no. 3, pp. 344–350, 2006.

[27] A. C. A. Wan and J. Y. Ying, "Nanomaterials for in situ cell delivery and tissue regeneration," *Advanced Drug Delivery Reviews*, vol. 62, no. 7-8, pp. 731–740, 2010.

[28] G. A. Silva, C. Czeisler, K. L. Niece et al., "Selective differentiation of neural progenitor cells by high-epitope density nanofibers," *Science*, vol. 303, no. 5662, pp. 1352–1355, 2004.

[29] V. M. Tysseling-Mattiace, V. Sahni, K. L. Niece et al., "Self-assembling nanofibers inhibit glial scar formation and promote axon elongation after spinal cord injury," *Journal of Neuroscience*, vol. 28, no. 14, pp. 3814–3823, 2008.

[30] B. Palmgren, Z. Jin, H. Ma, Y. Jiao, and P. Olivius, "β-Bungarotoxin application to the round window: An in vivo deafferentation model of the inner ear," *Hearing Research*, vol. 265, no. 1-2, pp. 70–76, 2010.

[31] U. Englund, A. Björklund, and K. Wictorin, "Migration patterns and phenotypic differentiation of long-term expanded human neural progenitor cells after transplantation into the adult rat brain," *Developmental Brain Research*, vol. 134, no. 1-2, pp. 123–141, 2002.

[32] M. K. Carpenter, X. Cui, Z. Hu et al., "In vitro expansion of a multipotent population of human neural progenitor cells," *Experimental Neurology*, vol. 158, no. 2, pp. 265–278, 1999.

[33] U. Englund, C. Ericson, C. Rosenblad et al., "The use of a recombinant lentiviral vector for ex vivo gene transfer into the rat CNS," *NeuroReport*, vol. 11, no. 18, pp. 3973–3977, 2000.

[34] B. Palmgren, Z. Jin, Y. Jiao, B. Kostyszyn, and P. Olivius, "Horseradish peroxidase dye tracing and embryonic statoacoustic ganglion cell transplantation in the rat auditory nerve trunk," *Brain Research*, vol. 1377, pp. 41–49, 2011.

[35] H. J. G. Gundersen, T. F. Bendtsen, L. Korbo et al., "Some new, simple and efficient stereological methods and their use in pathological research and diagnosis," *Acta Pathologica, Microbiologica, et Immunologica Scandinavica*, vol. 96, no. 5, pp. 379–394, 1988.

[36] V. M. Tysseling, V. Sahni, E. T. Pashuck et al., "Self-assembling peptide amphiphile promotes plasticity of serotonergic fibers following spinal cord injury," *Journal of Neuroscience Research*, vol. 88, no. 14, pp. 3161–3170, 2010.

[37] N. L. Angeloni, C. W. Bond, Y. Tang et al., "Regeneration of the cavernous nerve by Sonic hedgehog using aligned peptide amphiphile nanofibers," *Biomaterials*, vol. 32, no. 4, pp. 1091–1101, 2011.

[38] H. Schmidt and F. G. Rathjen, "Signalling mechanisms regulating axonal branching in vivo," *BioEssays*, vol. 32, no. 11, pp. 977–985, 2010.

[39] K. J. M. Marler, E. Becker-Barroso, A. Martínez et al., "A TrkB/EphrinA interaction controls retinal axon branching and synaptogenesis," *Journal of Neuroscience*, vol. 28, no. 48, pp. 12700–12712, 2008.

[40] M. Tessier-Lavigne and C. S. Goodman, "The molecular biology of axon guidance," *Science*, vol. 274, no. 5290, pp. 1123–1133, 1996.

[41] A. Nikolaev, T. McLaughlin, D. D. M. O'Leary, and M. Tessier-Lavigne, "APP binds DR6 to trigger axon pruning and neuron death via distinct caspases," *Nature*, vol. 457, no. 7232, pp. 981–989, 2009.

[42] U. Englund, A. Björklund, K. Wictorin, O. Lindvall, and M. Kokaia, "Grafted neural stem cells develop into functional pyramidal neurons and integrate into host cortical circuitry," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 26, pp. 17089–17094, 2002.

[43] J. H. Reyes, K. S. O'Shea, N. L. Wys et al., "Glutamatergic neuronal differentiation of mouse embryonic stem cells after transient expression of neurogenin 1 and treatment with BDNF and GDNF: in vitro and in vivo studies," *Journal of Neuroscience*, vol. 28, no. 48, pp. 12622–12631, 2008.

[44] M. K. Horne, D. R. Nisbet, J. S. Forsythe, and C. L. Parish, "Three-dimensional nanofibrous scaffolds incorporating immobilized BDNF promote proliferation and differentiation of cortical neural stem cells," *Stem Cells and Development*, vol. 19, no. 6, pp. 843–852, 2010.

[45] N. Kamei, N. Tanaka, Y. Oishi et al., "BDNF, NT-3, and NGF released from transplanted neural progenitor cells promote corticospinal axon growth in organotypic cocultures," *Spine*, vol. 32, no. 12, pp. 1272–1278, 2007.

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Review Article

Kölliker's Organ and the Development of Spontaneous Activity in the Auditory System: Implications for Hearing Dysfunction

M. W. Nishani Dayaratne,[1,2] Srdjan M. Vlajkovic,[1,2] Janusz Lipski,[1,2] and Peter R. Thorne[1,2,3]

[1] *Department of Physiology, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand*
[2] *Centre for Brain Research, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand*
[3] *Section of Audiology, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand*

Correspondence should be addressed to M. W. Nishani Dayaratne; n.dayaratne@auckland.ac.nz

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Prior to the "onset of hearing," developing cochlear inner hair cells (IHCs) and primary auditory neurons undergo experience-independent activity, which is thought to be important in retaining and refining neural connections in the absence of sound. One of the major hypotheses regarding the origin of such activity involves a group of columnar epithelial supporting cells forming Kölliker's organ, which is only present during this critical period of auditory development. There is strong evidence for a purinergic signalling mechanism underlying such activity. ATP released through connexin hemichannels may activate P2 purinergic receptors in both Kölliker's organ and the adjacent IHCs, leading to generation of electrical activity throughout the auditory system. However, recent work has suggested an alternative origin, by demonstrating the ability of IHCs to generate this spontaneous activity without activation by ATP. Regardless, developmental abnormalities of Kölliker's organ may lead to congenital hearing loss, considering that mutations in ion channels (hemichannels, gap junctions, and calcium channels) involved in Kölliker's organ activity share strong links with such types of deafness.

1. Introduction

As first described in 1863 by a Swiss anatomist and physiologist Albert von Kölliker, Kölliker's organ is an epithelial structure present in the developing auditory sensory organ in a wide variety of mammals, including cattle, rabbits, cats, dogs, and humans [1, 2]. It is one of the earliest visible epithelial structures of the developing cochlea and is the source of the sensory cells. After sensory cell differentiation, the residual Kölliker's organ remains as a large collection of epithelial cells on the medial aspect of the sensory organ, the organ of Corti (named after one of Kölliker's students, Alfonso Corti), while it is still in its developmental stage. As a transient structure, Kölliker's organ undergoes extensive remodelling in the embryonic or early postnatal stages and is eventually transformed into the inner sulcus region of the organ of Corti after the sensory structures become sensitive to external sound. Although Kölliker's organ was described well over a century ago, its function, especially in the period after sensory cell differentiation, is still largely unknown. This review looks at the structure and putative function of Kölliker's organ, with a major focus on purinergic intercellular signalling in the structure after sensory cell differentiation.

2. Morphology and Transformation

The cochlea develops from its base to apex in a time-dependent manner, both structurally and functionally. In the mouse, the putative sensory epithelium becomes visible around day 14 of gestation (embryonic day 14), when the endolymphatic duct is composed of tall columnar epithelial

cells of ectodermal origin surrounded by mesenchymal tissue [3]. This mass of epithelial tissue which gives rise to the sensory IHCs is sometimes referred to as "Kölliker's organ" in the literature [4]. However, the sensory cell development and differentiation are not the topic of the review. Instead, it will focus on the functional role of Kölliker's organ after sensory hair cell differentiation and how genetic mutations may lead to abnormal function of this tissue and deafness.

As the sensory structures mature in the cochlea, the epithelium forms two domains starting from around embryonic day 16 (mouse): the greater epithelial ridge (GER) containing Kölliker's organ lying on its medial aspect and the lesser epithelial ridge (LER) in the lateral portion. The epithelial cells that separate these two regions become the inner and outer pillar cells of the organ of Corti [3]. While IHCs are thought to originate from the GER, the outer hair cells (OHCs) are derived from the LER [4]. The differentiation process between the sensory and nonsensory cells begins without any visibly distinguishable features between the two cell types, as nerve fibres begin to invade specific areas of the GER [5, 6]. Kölliker's organ in hamsters and other rodents initially appears in the basal turn, along with the lateral wall and Reissner's membrane, while the apical turn is still in its undifferentiated state [3, 7].

2.1. Structure of the Differentiated Kölliker's Organ. The differentiated Kölliker's organ is composed of tightly packed columnar epithelial cells, but due to its dense nature, the nuclei of these cells can be present in different regions of the cells (though mostly in the basal half), giving it a stratified appearance when viewed in cross section [8] (Figure 1). The supporting cells are generally separated by an extracellular space of about 200 Å, while some intercellular spaces measure as little as 30 Å [8–10]. In kittens, these supporting cells are approximately 65 μm in height 3-4 μm in width, and have microvilli covering their apical or luminal surface. An occasional kinocilium has been observed on the surface of the epithelial cells [8], although others have suggested that all cells within Kölliker's organ contain kinocilium surrounded by microvilli [10]. In mice, the microvilli are approximately 3-4 μm in length and are present up to 10 days after birth [3]. The cytoplasm at the apical or luminal end of these cells is dense with organelles such as mitochondria, endoplasmic reticulum, and secretory vesicles [11]. Based on their morphological appearance, it was proposed that these cells secrete the tectorial membrane [12], as during early developmental stages (at birth in mice), the tectorial membrane is in contact with Kölliker's organ through a network of fine filaments, which later detach [3, 8, 10]. The tectorial membrane then extends to the OHC region two weeks after birth [3].

Cells of Kölliker's organ as well as other supporting cells in the immature organ of Corti are extensively connected through gap junctions, forming a syncytium [13]. Gap junctions are composed of two connexin hemichannels (connexons) from adjacent cells and are made of six subunits, each containing 4 transmembrane regions. These gap junctions are formed before the functional maturation of the cochlea [13] and comprise mainly connexin 26 (Cx26) and connexin 30 (Cx30) subunits (capable of forming heteromeric channels) which share similar expression patterns in Kölliker's organ epithelial cells in mice and are associated with congenital deafness [6].

2.2. Transformation. As the cochlea matures, columnar cells constituting Kölliker's organ are replaced by cuboidal cells, which are approximately 10 μm in height, forming the mature inner sulcus [8]. The replacement process increases the intercellular space and greatly reduces the number of cells so that the final number of cuboidal cells is approximately 12% of the original columnar cell count [8]. This transformation occurs in a basal to apical and medial to lateral manner. Although the exact process behind this refinement is unclear, it may involve apoptosis of columnar cells, followed by formation of the inner sulcus containing cuboidal cells, while supporting cells lining the inner hair cells (border cells and phalangeal cells) are maintained through to the mature cochlea [8]. This transformation process appears to be very sensitive to thyroid hormone, as its deficiency leads to prolonged survival of Kölliker's organ in rats as old as 30 days and the malformed structure of the organ of Corti, particularly the tectorial membrane [11, 14, 15]. Supplementation of thyroid hormone at this age stimulates the transformation of the tall columnar cells of Kölliker's organ to the cuboidal cells of the inner sulcus [14].

3. Purinergic Signalling in Kölliker's Organ

Purines have been shown to have considerable influence on the cellular activity in Kölliker's organ. The actions of adenine compounds on cells and tissues were first described by Drury and Szent-Györgyi in 1929 [16]. However, the term "purinergic signalling" was not introduced until 1972, when adenosine triphosphate (ATP) was recognised as a neurotransmitter, followed by identification of purinergic receptors in 1976 [17]. Purines such as adenosine, ATP, and guanosine triphosphate (GTP) and pyrimidines such as uridine triphosphate (UTP) act as neurotransmitters, gliotransmitters, and paracrine signalling molecules in a variety of sensory systems including vision, smell, taste, and hearing [18]. In the peripheral auditory system, purines have multiple roles, including regulation of cochlear sensitivity and electrochemical homeostasis, synaptic transmission, and signalling between sensory and supporting cells after sensory cell injury [18]. There are two major classes of purinergic receptors, each with multiple subtypes. The P1 receptors (A_1, A_{2A}, A_{2B}, and A_3) are G protein-coupled and are activated by adenosine. These are further subdivided into receptors which stimulate the production of cyclic AMP (A_{2A} and A_{2B}) and those which inhibit its production (A_1 and A_3). P2 receptors are classified into two major groups, P2X and P2Y (for review, see [19]). The P2X receptors are nonselective ATP-gated ion channels, with high permeability for Na^+, K^+, and Ca^{2+}. P2X subtypes range from P2X1 to P2X7 with various roles throughout the body. On the other hand, P2Y receptors are G protein-coupled metabotropic receptors, which activate phospholipase C (PLC), resulting in activation of the second messengers diacylglycerol and inositol trisphosphate (IP_3).

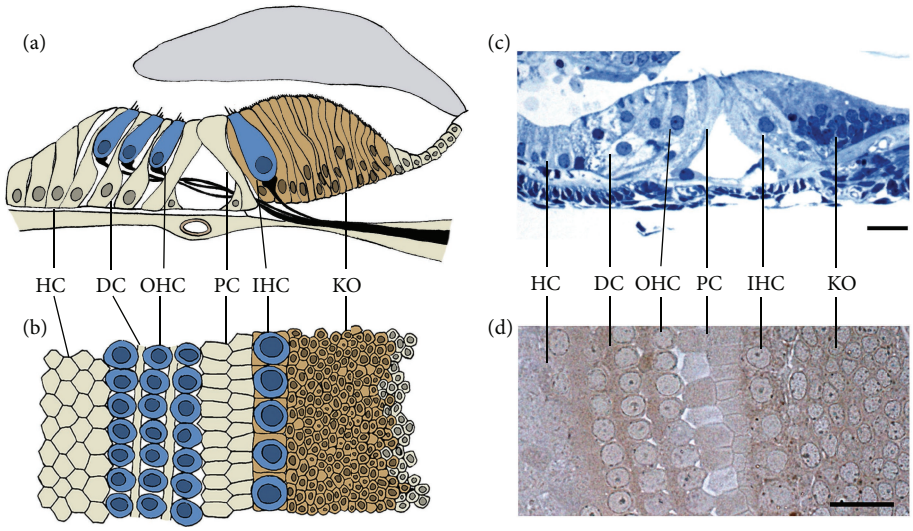

FIGURE 1: The immature organ of Corti and the adjacent Kölliker's organ. (a) A cross sectional diagram of a rat organ of Corti (approximately 10 days old) outlining the sensory hair cells and supporting epithelia. Kölliker's organ is found immediately adjacent to the inner hair cells (IHCs), shaded in darker colour (HC-Hensen's cells; DC-Deiters' cells; OHC-outer hair cells; PC-pillar cells; IHC-inner hair cells; and KO-Kölliker's organ). (b) A diagram outlining a horizontal section of the same developing organ of Corti, detailing the positions of various sensory and nonsensory cells. (c) Resin embedded toluidine-blue stained 1 μm cross section of a 10-day-old Wistar rat organ of Corti. (d) Resin embedded horizontal section of an 11-day-old Wistar rat organ of Corti, postfixed in 1% osmium tetroxide. Scale bar: 20 μm.

IP_3 increases intracellular Ca^{2+} levels by releasing these ions from internal stores [20]. P2Y receptors can be further subdivided into two groups. The first is mainly coupled to G_q/G_{11}, activating the PLC/IP_3 pathway, and includes $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$ [21]. The second group consists of $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$ and is coupled to $G_{i/o}$ and adenylyl cyclase [22].

3.1. P2 Receptors in the Developing Cochlea. In the developing rat cochlea, $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{12}$ receptors are expressed in sensory and nonsensory cells of the organ of Corti and the spiral ganglion neurons [23], although this review focuses on the cells of Kölliker's organ (for review of receptor expression in IHC and OHC, see [18]). $P2Y_2$ and $P2Y_4$ receptors are localised in cells of the GER [23] and are vital for its function. $P2X_{2\text{-}3}$ and the transiently expressed $P2X_3$ contribute to specific innervation of sensory cells by the spiral ganglion neurons (SGNs) [24]. During development, both type I and type II SGNs innervate IHCs and OHCs. This is followed in rodents by programmed withdrawal of the type I SGNs from the outer hair cells and the type II fibres from the inner hair cells few days after birth [24]. At this stage, SGNs are supported by neurotrophins secreted by the hair cells, such as brain-derived neurotrophic factor and neurotrophin 3 [25, 26]. P2X receptor signalling via $P2X_{2/3}$ receptors inhibits this neurotrophic support [27]. $P2X_7$ receptors are also expressed in hair cells and supporting cells within the cochlea from embryonic stages through to adulthood and are thought to be involved in ion homeostasis as well as apoptosis in the cochlea [28]. $P2X_1$ receptors are transiently expressed during development and are downregulated by postnatal day 10 (P10) in rats, suggesting an involvement of this P2X subunit only in early stages of cochlear development [18, 29]. This may occur through regulation of cell death and differentiation [29].

3.2. Activity in Kölliker's Organ. Neural connections develop throughout the auditory system during development to form the necessary circuits. This formation and its refinement occur during a stage when there is no sound-driven activity (prior to the "onset of hearing") in the cochlea. However, there is strong evidence for experience-independent action potentials throughout various regions of the developing auditory circuit, from the cochlear neurons to auditory nuclei in the brain [30, 31]. This is similar to other developing neural circuits, including the spinal cord [32], cerebellum [33], hippocampus [34], and the retina [35–39]. The origin of this neural activity was first found to be in the cochlea as tetrodotoxin applied to the round window membrane of the developing avian cochlea resulted in the elimination of neural activity throughout the developing auditory system [36]. The activity, therefore, appears to first begin spontaneously in the cochlea, generating action potentials in the auditory pathways through to the auditory cortex through the activation of IHC and their primary afferent neurons. The electrical activity in the immature auditory cortex thus appears to result from the auditory neural input in the absence of sound. Maintaining activity throughout the auditory circuit in this manner retains and refines the important synaptic connections made during very early development. Although spontaneous electrical activity was first observed in the developing IHCs over a decade ago [40], the link to refinement of the auditory system is only recently starting to emerge [31].

Tritsch et al. [35] identified Kölliker's organ as the area of the developing organ of Corti that could be responsible for generating the intrinsic spontaneous activity which drives the primary afferent auditory neurons [35]. Clusters (approximately 60 μm wide) of supporting cells of Kölliker's organ show synchronous spontaneous activity, which could lead to a synchronous event involving adjacent IHCs because of their close proximity [35]. Their work on prehearing rats revealed spontaneous inward currents within epithelial supporting cells of Kölliker's organ and ruled out the direct involvement of IHCs or neural activity in initiating these events [30, 35, 41]; a finding that has been disputed by later studies [42–44] and is discussed in the next section. Furthermore, in the experiments by Tritsch and Bergles, spontaneous activity in supporting cells, IHCs, and SGNs was reduced by P2 purinergic receptor antagonists and extracellular ATP-hydrolysing enzymes (ectonucleotidases), suggesting an involvement of ATP released from Kölliker's organ and a subsequent activation of P2 receptors in surrounding cells. Predictably, experience-independent neural activity is transient, just like Kölliker's organ itself (it is only present during the prehearing stages of development). During the earliest stages of development (P0–3), the spontaneously generated currents are smaller, faster, and more frequent when compared to the activity observed later (P7–10) [30].

3.3. Possible Mechanisms of Spontaneous Activity Generation. As mentioned earlier, the possible involvement of purinergic signalling in generation of spontaneous activity in the auditory nerve was first introduced by Tritsch et al., as P2 receptor antagonists such as suramin and pyridoxalphosphate-6-azophenyl-2′,4′-disulphonate (PPADS) inhibited experience-independent activity [35]. In addition, application of ATP to the epithelial nonsensory cells of Kölliker's organ resulted in bursts of excitatory postsynaptic currents in primary auditory afferents [30, 35]. Both P2X and P2Y receptors (in particular $P2X_2$, $P2X_7$, and $P2Y_4$) are expressed in the developing organ of Corti, suggesting their likely involvement in ATP-induced currents [23, 41].

It is proposed that release of ATP from Kölliker's organ may occur through connexin hemichannels expressed extensively in Kölliker's organ [45, 46]. In particular, this could involve Cx26 and Cx30 which are expressed throughout the organ of Corti, excluding sensory cells [6]. This is supported by the inhibitory effects of gap junction blockers (such as octanol and carbenoxolone) on spontaneous activity as well as the observation of an increase of activity in response to hemichannel opening with Ca^{2+}-free external solutions [30, 46, 47]. It is however important to note that pharmacological studies of such channels or hemichannels lack specificity, limiting their effectiveness in isolating the correct proteins [48]. Pannexins are another candidate for ATP release, as Panx1 and Panx2 are also strongly expressed in supporting cells of the adult cochlea [49], although their expression during development is not clear. Interestingly, genetic loci for the Panx1 gene also contain part of the gene sequence responsible for a dominant form of nonsyndromic sensorineural deafness (DFNA11) [50]. A cochlear organotypic culture study [47] provided further support for connexins as the channels responsible for ATP-induced Ca^{2+} waves over Panx1 and $P2X_7$, though their work was limited to the outer sulcus [47]. While specific connexin activity has not yet been confirmed, it appears that ATP release in Kölliker's organ likely occurs through these hemichannels.

Therefore, as first proposed by Tritsch et al., the generation of intrinsic activity in the developing cochlea may include periodic release of ATP from inner supporting cells of Kölliker's organ, leading to P2 receptor activation at the adjacent IHCs, and subsequent glutamate release [35]. This, in turn, initiates bursts of action potentials in SGNs, connecting spontaneous activity of the cochlea to upstream neural activity in the auditory circuit. Studies carried out on epithelial cells of LER origin indicate that a large portion of Ca^{2+} rise following purinergic activation occurs from internal stores, as Ca^{2+} spikes persist in these cells even in Ca^{2+}-free external solution [51–54]. Although developed from different domains, this may give an insight into mechanisms behind Ca^{2+} spikes observed during spontaneous activity generated in Kölliker's organ. While the rise in intracellular Ca^{2+} can lead to glutamate release from IHCs, it is also thought to be involved in inducing spontaneous morphological changes within Kölliker's organ. The ATP-induced activity is likely biphasic, as the P2X receptors respond more rapidly than P2Y receptors [35]. This is evident during the response of supporting cells to the specific P2Y receptor agonist UTP, which is consistently slower than the responses elicited by ATP [35]. Following its release from supporting cells, ATP is likely degraded through the actions of ectonucleotidases (extracellular ATP-hydrolysing enzymes), resulting in generation of adenosine [55]. Although adenosine stimulates P1 (adenosine) receptors in the adult cochlea, it does not have any effect on the spontaneous activity in the developing organ of Corti [35]. The combined action of ATP release through connexins and its continuous degradation in the extracellular space by ectonucleotidases may be responsible for the rhythmic regulation of spontaneous activity. A recent study on adult guinea pigs also suggested the involvement of ATP in mediating gap junctional coupling [56]. This study demonstrated an uncoupling effect on gap junctions by ATP in cochlear supporting cells, an effect mediated by P2X rather than P2Y receptors [56].

In contrast to the purinergic mechanism proposed by Tritsch et al. [35], more recent findings suggest that the initiation of spontaneous activity occurs through IHCs, without ATP-induced depolarisation [42–44]. Johnson et al. [43] recorded sustained spontaneous activity from mouse IHCs which was independent from purinergic signalling by Kölliker's organ. In addition, IHCs still fire spontaneous action potentials in the presence of broadly selective P2 receptor antagonists such as PPADS and suramin and in the absence of the $P2X_4$ receptor subunit [43, 44]. Both studies revealed a modulatory role of the efferent neurotransmitter acetylcholine using $\alpha 9\alpha 10$ nicotinic receptor antagonist strychnine [43, 44]. This again challenges the original work by Tritsch et al. [35], which reported no change of spontaneous activity in response to strychnine [35]. The major differences

between the purinergic and IHC theory of spontaneous activity generation may be due to variation in ionic compositions (such as K^+ concentrations and Ca^{2+} buffering), which may affect the resting potential of IHCs and therefore their ability to generate action potentials. However, if ATP release by Kölliker's organ is not responsible for generating spontaneous activity which drives the auditory system prior to the "onset of hearing," questions remain regarding the role of spontaneous purinergic activity in Kölliker's organ.

3.4. Rhythmic Morphological Changes within Kölliker's Organ. Strongly correlated with the onset of inward currents, supporting cells of Kölliker's organ undergo spontaneous morphological changes where the cytoplasm pulls away from the membrane, resulting in crenation of the cell and increased extracellular space between individual cells. These events are tightly linked as demonstrated by the fact that 93% of optical changes resulting from cell shrinking are correlated with inward currents [35]. This allows real-time visual detection of the spontaneous activity in Kölliker's organ by recording changes in the optical density/refractive indices of supporting cells involved. Such optical changes occur at a frequency of 0.034 ± 0.003 Hz and could be observed at random locations within the length of Kölliker's organ [32]. Morphological changes are relatively specific to Kölliker's organ but are also observed in the processes of phalangeal cells away from the OHCs [41]. Much like the inward currents, these spontaneously generated morphological changes are also inhibited by nonselective P2 receptor antagonists such as suramin and activated by both ATP and UTP, suggesting a similar purinergic control [30, 35, 41]. Although spontaneous currents and Ca^{2+} waves are present in Kölliker's organ from birth in rodents, morphological activity is only observed a few days after birth, indicating that a certain level of development is required to induce changes in cell shape. While low expression of purinergic receptors is a possibility, ATP still induces inward currents and Ca^{2+} waves in Kölliker's organ of young rodents, suggesting that the expression levels of purinergic receptors cannot solely account for the lack of morphological changes at these earlier stages [23, 35]. In order to identify the mechanisms of the morphological changes, supporting cells of Kölliker's organ were depolarised in the presence of high intracellular Ca^{2+} concentrations. Neither large current injections nor rise in extracellular K^+ resulted in the spontaneous morphological changes [41]. In contrast, rises in intracellular Ca^{2+} alone induced changes in cell diameter of the activated cell as well as adjacent cells. These results indicate that intracellular Ca^{2+} rise alone can lead to rhythmic morphological changes, which spread to adjacent cells within Kölliker's organ [41].

A possible mechanism for the changes in cell shape involves the activation of Cl^- channels or nonselective cation channels by Ca^{2+}, leading to the expulsion of water. It has been suggested that cultured cochlea has the ability to secrete water, providing further support to this theory [57]. Other possibilities include the involvement of contractile proteins such as actin, although there is a lack of evidence to support this. If these morphological changes in the supporting cells do in fact result in the secretion of water, this process may be involved in forming the cochlear compartmental fluids (endolymph and perilymph) during development.

3.5. Calcium Signalling in Kölliker's Organ. Calcium is a major intracellular messenger in the cochlea, being involved in a number of signalling pathways. Within Kölliker's organ, a strong correlation was initially observed between spontaneously generated currents, morphological changes, and Ca^{2+} spikes. This likely occurs through the combined rise in intracellular $[Ca^{2+}]$ from internal stores and inward currents through P2X receptors-channel complexes [30, 35]. The Ca^{2+} waves in Kölliker's organ are comparable to those found between groups of astrocytes connected via gap junctions [58]. Each wave is initiated in a small group of cells (one to four) and then spreads radially. Ca^{2+} waves are also observed within the outer sulcus cells following ATP release or mechanical stimulation [51]. These Ca^{2+} waves lead to further ATP release, resulting in a regenerative wave that allows the synchronisation of nearby cells [30, 35]. ATP-induced Ca^{2+} waves can be observed from very early stages of development, with their frequency increasing dramatically (by 5-fold), along with an increase (1.7-fold) of the area of activation [30]. Although extracellular ATP can induce Ca^{2+} waves throughout Kölliker's organ at very early stages (P0-P1), naturally occurring Ca^{2+} waves are rare at that age, possibly due to lower levels of ATP release. The ATP-induced currents are also of smaller amplitudes at that age [30, 41].

Clinically, lack of regular Ca^{2+} action potentials in IHCs during development could lead to hearing impairment, particularly due to defects in the Ca^{2+} channel $Ca_v1.3$, as its dysfunction is detrimental to cochlear functioning [59]. This particular *L*-type channel is the predominant Ca^{2+} channel in IHCs of developing cochlea and is responsible for exocytosis of glutamate and potentially of other neurotrophic factors [60]. It is likely that Ca^{2+} is also involved in rhythmic morphological changes within supporting cells of Kölliker's organ. One intriguing possibility is that the increase in intracellular Ca^{2+} within supporting cells can activate Ca^{2+}-activated Cl^- channels, leading to efflux of Cl^-. A recent study has demonstrated a strong expression of Ca^{2+}-activated Cl^- channels (Anoctamin-1) in Kölliker's organ, particularly in supporting cells immediately adjacent to IHCs [61]. While Anoctamin-1 is thought to be involved in pacemaker activity and fluid secretion in the digestive system [62], its functional significance in Kölliker's organ is yet to be established. The osmotic gradient created by this Cl^- efflux could in turn cause the movement of water out of these cells, resulting in morphological changes within Kölliker's organ [35, 41]. This is supported by the negative effect of Cl^- channel inhibitor 4,4′diisothiocyano-2,2′stilbene disulfonic acid on spontaneous morphological activity in Kölliker's organ [41].

4. Kölliker's Organ and Deafness

The role of Kölliker's organ is quickly evolving from simply providing support for adjacent IHCs, to potentially initiating

activity that is necessary for the development of a fully functional auditory system. While no direct link between Kölliker's organ dysfunction and deafness has yet been established, critical components of its organisation appear to be linked to major forms of deafness. One of the strongest potential links lies within mutations of the *GJB2* gene, which encodes Cx26 and accounts for about 50% of prelingual childhood deafness [63]. Both Cx26 and Cx30 (with strong links to both syndromic and nonsyndromic congenital hearing loss [64, 65]) are highly expressed throughout Kölliker's organ and are likely to play a key role in generating and transmitting synchronised spontaneous activity. In rodents, mutations and blockers of Cx30 reduce Ca^{2+} transients within Kölliker's organ, and the Cx30-null mice also show an elevation of auditory thresholds [66, 67]. Furthermore, $Ca_v1.3$ channels are essential in creating and maintaining synapses during the critical spontaneous activity driven period of development, and adverse effects are seen as early as the first postnatal week in their absence, starting with OHC degeneration [59].

In addition, the function as well as the transformation of Kölliker's organ to the inner sulcus is crucial for the maturation of the cochlear structure and function. Thyroid gland deficiencies have been shown to affect this process by maintaining Kölliker's organ past the onset of hearing, and in humans, impairment of thyroid hormone signalling is associated with hearing loss [68–70]. This may be due to malformed structural changes during development, although its exact cause and incidence are still not well characterised (for review, see [71]).

5. Summary

First discovered over a century ago, Kölliker's organ is present transiently in the developing cochlea, until the cochlea becomes sensitive to external sound. It is an epithelial structure composed of long columnar cells and lies immediately adjacent to IHCs. From this close proximity, epithelial cells of Kölliker's organ have been suggested to initiate spontaneous electrical activity in the IHCs, driving action potentials in the primary auditory neurons, and auditory nuclei in the brainstem. Although the exact mechanism remains unclear, rhythmic ATP release from connexin hemichannels and activation of purinergic P2 receptors may contribute to rhythmic current/morphological oscillations of Kölliker's organ. The rise in intracellular Ca^{2+} or ectonucleotidase activity may act as a feedback for ATP release and thus control the rhythmicity of ATP release in the developing cochlea. Interestingly, both types of ion channels (connexins and P2 receptors) have been linked to hearing loss. Following sound detection by the cochlea (the "onset of hearing"), Kölliker's organ disappears, transforming into the adult inner sulcus region which contains cuboidal epithelial cells. From a physiological perspective, this is an important step in cochlear adaptation to external environment. From a developmental point of view, purinergic signalling in Kölliker's organ may provide a sophisticated mechanism central to the tonotopic organisation of the cochlea which starts to develop in the absence of sound.

Abbreviations

ATP: Adenosine triphosphate
Cx: Connexin
GER: Greater epithelial ridge
IHCs: Inner hair cells
IP_3: Inositol trisphosphate
LER: Lesser epithelial ridge
OHCs: Outer hair cells
PLC: Phospholipase C
PPADS: Pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonate
SGNs: Spiral ganglion neurons
UTP: Uridine triphosphate.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

[1] V. Hensen, "Zur morphologie der schnecke des menschen und der säugethiere," *Zeitschrift für Wissenschaftliche Zoologie*, vol. 13, pp. 481–512, 1863.

[2] A. Kölliker, *Handbuch der Gewebelehre des Menschen*, Engelmann, 1902.

[3] D. J. Lim and M. Anniko, "Developmental morphology of the mouse inner ear. A scanning electron microscopic observation," *Acta Oto-Laryngologica*, vol. 422, pp. 1–69, 1985.

[4] L. Simonneau, M. Gallego, and R. Pujol, "Comparative expression patterns of T-, N-, E-cadherins, β-catenin, and polysialic acid neural cell adhesion molecule in rat cochlea during development: implications for the nature of Kölliker's organ," *The Journal of Comparative Neurology*, vol. 459, no. 2, pp. 113–126, 2003.

[5] R. Pujol, M. Lavigne-Rebillard, and M. Lenoir, "Development of sensory and neural structures in the mammalian cochlea," in *Development of the Auditory System*, E. W. Rubel, A. N. Popper, and R. R. Fay, Eds., vol. 9 of *Springer Handbook of Auditory Research*, pp. 146–192, Springer, New York, NY, USA, 1998.

[6] P. Majumder, G. Crispino, L. Rodriguez et al., "ATP-mediated cell-cell signaling in the organ of Corti: the role of connexin channels," *Purinergic Signalling*, vol. 6, no. 2, pp. 167–187, 2010.

[7] W. Giebel and N. Wei, "The early postnatal development of the hamster cochlea," *Laryngo- Rhino- Otologie*, vol. 80, no. 12, pp. 725–730, 2001.

[8] R. Hinojosa, "A note on development of Corti's organ," *Acta Oto-Laryngologica*, vol. 84, no. 3-4, pp. 238–251, 1977.

[9] D. J. Lim and M. Anniko, "Developmental morphology of the mouse inner ear. A scanning electron microscopic observation," *Acta Oto-Laryngologica*, vol. 99, no. 422, 1985.

[10] A. Zine and R. Romand, "Development of the auditory receptors of the rat: a SEM study," *Brain Research*, vol. 721, no. 1-2, pp. 49–58, 1996.

[11] A. Uziel, J. Gabrion, M. Ohresser, and C. Legrand, "Effects of hypothyroidism on the structural development of the organ of Corti in the rat," *Acta Oto-Laryngologica*, vol. 92, no. 5-6, pp. 469–480, 1981.

[12] M. Anniko, "Embryogenesis of the mammalian middle ear. III. Formation of the tectorial membrane of the CBA/CBA mouse in vivo and in vitro," *Anatomy and Embryology*, vol. 160, no. 3, pp. 301–313, 1980.

[13] M. Cohen-Salmon, F. J. del Castillo, and C. Petit, "Connexins responsible for hereditary deafness—the tale unfolds," in *Gap Junctions in Development and Disease*, P. D. E. Winterhager, Ed., pp. 111–134, Springer, Berlin, Germany, 2005.

[14] A. Uziel, "Periods of sensitivity to thyroid hormone during the development of the organ of Corti," *Acta Oto-Laryngologica*, vol. 102, no. 429, pp. 23–27, 1986.

[15] C. Legrand, A. Bréhier, M. C. Clavel, M. Thomasset, and A. Rabié, "Cholecalcin (28-kDa CaBP) in the rat cochlea. Development in normal and hypothyroid animals. An immunocytochemical study," *Brain Research*, vol. 466, no. 1, pp. 121–129, 1988.

[16] A. N. Drury and A. Szent-Györgyi, "The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart," *The Journal of Physiology*, vol. 68, no. 3, pp. 213–237, 1929.

[17] G. Burnstock, "Purinergic receptors," *Journal of Theoretical Biology*, vol. 62, no. 2, pp. 491–503, 1976.

[18] G. D. Housley, A. Bringmann, and A. Reichenbach, "Purinergic signaling in special senses," *Trends in Neurosciences*, vol. 32, no. 3, pp. 128–141, 2009.

[19] G. Burnstock and C. Kennedy, "Is there a basis for distinguishing two types of P2-purinoceptor?" *General Pharmacology: The Vascular System*, vol. 16, no. 5, pp. 433–440, 1985.

[20] M. P. Abbracchio, G. Burnstock, A. Verkhratsky, and H. Zimmermann, "Purinergic signalling in the nervous system: an overview," *Trends in Neurosciences*, vol. 32, no. 1, pp. 19–29, 2009.

[21] A. Verkhratsky, "Physiology and pathophysiology of the calcium store in the endoplasmic reticulum of neurons," *Physiological Reviews*, vol. 85, no. 1, pp. 201–279, 2005.

[22] M. P. Abbracchio, G. Burnstock, J.-M. Boeynaems et al., "International Union of Pharmacology LVIII: update on the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy," *Pharmacological Reviews*, vol. 58, no. 3, pp. 281–341, 2006.

[23] L.-C. Huang, P. R. Thorne, S. M. Vlajkovic, and G. D. Housley, "Differential expression of P2Y receptors in the rat cochlea during development," *Purinergic Signalling*, vol. 6, no. 2, pp. 231–248, 2010.

[24] L.-C. Huang, D. Greenwood, P. R. Thorne, and G. D. Housley, "Developmental regulation of neuron-specific P2X3 receptor expression in the rat cochlea," *Journal of Comparative Neurology*, vol. 484, no. 2, pp. 133–143, 2005.

[25] K. Mou, C. L. Hunsberger, J. M. Cleary, and R. L. Davis, "Synergistic effects of BDNF and NT-3 on postnatal spiral ganglion neurons," *Journal of Comparative Neurology*, vol. 386, no. 4, pp. 529–539, 1997.

[26] U. Pirvola and J. Ylikoski, "Neurotrophic factors during inner ear development," *Current Topics in Developmental Biology*, vol. 57, pp. 207–223, 2003.

[27] D. Greenwood, D. J. Jagger, L. Huang et al., "P2X receptor signaling inhibits BDNF-mediated spiral ganglion neuron development in the neonatal rat cochlea," *Development*, vol. 134, no. 7, pp. 1407–1417, 2007.

[28] P. Nikolic, G. D. Housley, and P. R. Thorne, "Expression of the P2X7 receptor subunit of the adenosine 5′-triphosphate-gated ion channel in the developing and adult rat cochlea," *Audiology & Neuro-Otology*, vol. 8, no. 1, pp. 28–37, 2003.

[29] P. Nikolic, G. D. Housley, L. Luo, A. F. Ryan, and P. R. Thorne, "Transient expression of P2X1 receptor subunits of ATP-gated ion channels in the developing rat cochlea," *Developmental Brain Research*, vol. 126, no. 2, pp. 173–182, 2001.

[30] N. X. Tritsch and D. E. Bergles, "Developmental regulation of spontaneous activity in the Mammalian cochlea," *Journal of Neuroscience*, vol. 30, no. 4, pp. 1539–1550, 2010.

[31] S. L. Johnson, S. Kuhn, C. Franz et al., "Presynaptic maturation in auditory hair cells requires a critical period of sensory-independent spiking activity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 21, pp. 8720–8725, 2013.

[32] L. T. Landmesser and M. J. O'Donovan, "Activation patterns of embryonic chick hind limb muscles recorded in ovo and in an isolated spinal cord preparation," *The Journal of Physiology*, vol. 347, pp. 189–204, 1984.

[33] A. J. Watt, H. Cuntz, M. Mori, Z. Nusser, P. J. Sjöström, and M. Häusser, "Traveling waves in developing cerebellar cortex mediated by asymmetrical Purkinje cell connectivity," *Nature Neuroscience*, vol. 12, no. 4, pp. 463–473, 2009.

[34] Y. Ben-Ari, E. Cherubini, R. Corradetti, and J.-L. Gaiarsa, "Giant synaptic potentials in immature rat CA3 hippocampal neurones," *The Journal of Physiology*, vol. 416, pp. 303–325, 1989.

[35] N. X. Tritsch, E. Yi, J. E. Gale, E. Glowatzki, and D. E. Bergles, "The origin of spontaneous activity in the developing auditory system," *Nature*, vol. 450, no. 7166, pp. 50–55, 2007.

[36] W. R. Lippe, "Rhythmic spontaneous activity in the developing avian auditory system," *Journal of Neuroscience*, vol. 14, no. 3, pp. 1486–1495, 1994.

[37] T. A. Jones, S. M. Jones, and K. C. Paggett, "Primordial rhythmic bursting in embryonic cochlear ganglion cells," *The Journal of Neuroscience*, vol. 21, no. 20, pp. 8129–8135, 2001.

[38] M. Sonntag, B. Englitz, C. Kopp-Scheinpflug, and R. Rübsamen, "Early postnatal development of spontaneous and acoustically evoked discharge activity of principal cells of the medial nucleus of the trapezoid body: an in vivo study in mice," *The Journal of Neuroscience*, vol. 29, no. 30, pp. 9510–9520, 2009.

[39] N. Dale, "Dynamic ATP signalling and neural development," *The Journal of Physiology*, vol. 586, no. 10, pp. 2429–2436, 2008.

[40] C. J. Kros, J. P. Ruppersberg, and A. Rüsch, "Expression of a potassium current inner hair cells during development of hearing in mice," *Nature*, vol. 394, no. 6690, pp. 281–284, 1998.

[41] N. X. Tritsch, Y. Zhang, G. Ellis-Davies, and D. E. Bergles, "ATP-induced morphological changes in supporting cells of the developing cochlea," *Purinergic Signalling*, vol. 6, no. 2, pp. 155–166, 2010.

[42] S. L. Johnson, H. J. Kennedy, M. C. Holley, R. Fettiplace, and W. Marcotti, "The resting transducer current drives spontaneous activity in prehearing mammalian cochlear inner hair cells," *Journal of Neuroscience*, vol. 32, no. 31, pp. 10479–10483, 2012.

[43] S. L. Johnson, T. Eckrich, S. Kuhn et al., "Position-dependent patterning of spontaneous action potentials in immature cochlear inner hair cells," *Nature Neuroscience*, vol. 14, no. 6, pp. 711–717, 2011.

[44] G. Sendin, J. Bourien, F. Rassendren, J.-L. Puel, and R. Nouvian, "Spatiotemporal pattern of action potential firing in developing inner hair cells of the mouse cochlea," *Proceedings of the*

National Academy of Sciences, vol. 111, no. 5, pp. 1999–2004, 2014.

[45] A. Forge, D. Becker, S. Casalotti, J. Edwards, N. Marziano, and G. Nevill, "Gap junctions in the inner ear: comparison of distribution patterns in different vertebrates and assessement of connexin composition in mammals," *Journal of Comparative Neurology*, vol. 467, no. 2, pp. 207–231, 2003.

[46] H. B. Zhao, "Connexin26 is responsible for anionic molecule permeability in the cochlea for intercellular signalling and metabolic communications," *European Journal of Neuroscience*, vol. 21, no. 7, pp. 1859–1868, 2005.

[47] F. Anselmi, V. H. Hernandez, G. Crispino et al., "ATP release through connexin hemichannels and gap junction transfer of second messengers propagate Ca^{2+} signals across the inner ear," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 48, pp. 18770–18775, 2008.

[48] D. C. Spray, Z. C. Ye, and B. R. Ransom, "Functional connexin "hemichannels": a critical appraisal," *Glia*, vol. 54, no. 7, pp. 758–773, 2006.

[49] X. Wang, M. Streeter, Y. Liu, and H. Zhao, "Identification and characterization of pannexin expression in the mammalian cochlea," *Journal of Comparative Neurology*, vol. 512, no. 3, pp. 336–346, 2009.

[50] Y. Tamagawa, K. Kitamura, T. Ishida et al., "A gene for a dominant form of non-syndromic sensorineural deafness (*DFNA11*) maps within the region containing the *DFNB2* recessive deafness gene," *Human Molecular Genetics*, vol. 5, no. 6, pp. 849–852, 1996.

[51] J. E. Gale, V. Piazza, C. D. Ciubotaru, and F. Mammano, "A mechanism for sensing noise damage in the inner ear," *Current Biology*, vol. 14, no. 6, pp. 526–529, 2004.

[52] V. Piazza, C. D. Ciubotaru, J. E. Gale, and F. Mammano, "Purinergic signalling and intercellular Ca^{2+} wave propagation in the organ of Corti," *Cell Calcium*, vol. 41, no. 1, pp. 77–86, 2007.

[53] J. F. Ashmore and H. Ohmori, "Control of intracellular calcium by ATP in isolated outer hair cells of the guinea-pig cochlea," *The Journal of Physiology*, vol. 428, pp. 109–131, 1990.

[54] L. Lagostena, J. F. Ashmore, B. Kachar, and F. Mammano, "Purinergic control of intracellular communication between Hensen's cells of the guinea-pig cochlea," *Journal of Physiology*, vol. 531, no. 3, pp. 693–706, 2001.

[55] S. M. Vlajkovic, P. R. Thorne, G. D. Housley, D. J. B. Muñoz, and I. S. Kendrick, "Ecto-nucleotidases terminate purinergic signalling in the cochlear endolymphatic compartment," *NeuroReport*, vol. 9, no. 7, pp. 1559–1565, 1998.

[56] Y. Zhu and H. Zhao, "ATP activates P2X receptors to mediate gap junctional coupling in the cochlea," *Biochemical and Biophysical Research Communications*, vol. 426, no. 4, pp. 528–532, 2012.

[57] H. M. Sobkowicz, J. M. Loftus, and S. M. Slapnick, "Tissue culture of the organ of Corti," *Acta Oto-Laryngologica*, vol. 502, pp. 3–36, 1993.

[58] T. A. Fiacco and K. D. McCarthy, "Astrocyte calcium elevations: properties, propagation, and effects on brain signaling," *Glia*, vol. 54, no. 7, pp. 676–690, 2006.

[59] R. Glueckert, G. Wietzorrek, K. Kammen-Jolly et al., "Role of class D L-type Ca^{2+} channels for cochlear morphology," *Hearing Research*, vol. 178, no. 1-2, pp. 95–105, 2003.

[60] A. Brandt, J. Striessnig, and T. Moser, "CaV1.3 channels are essential for development and presynaptic activity of cochlear inner hair cells," *Journal of Neuroscience*, vol. 23, no. 34, pp. 10832–10840, 2003.

[61] E. Yi, J. Lee, and C. Lee, "Developmental role of anoctamin-1/TMEM16A in Ca^{2+}-dependent volume change in supporting cells of the mouse Cochlea," *Experimental Neurobiology*, vol. 22, no. 4, pp. 322–329, 2013.

[62] S. J. Hwang, P. J. A. Blair, F. C. Britton et al., "Expression of anoctamin 1/TMEM16A by interstitial cells of Cajal is fundamental for slow wave activity in gastrointestinal muscles," *Journal of Physiology*, vol. 587, no. 20, pp. 4887–4904, 2009.

[63] J. B. Hochman, T. L. Stockley, D. Shipp, V. Y. W. Lin, J. M. Chen, and J. M. Nedzelski, "Prevalence of Connexin 26 (GJB2) and Pendred (SLC26A4) mutations in a population of adult cochlear implant candidates," *Otology and Neurotology*, vol. 31, no. 6, pp. 919–922, 2010.

[64] Y. Sun, W. Tang, Q. Chang, Y. Wang, W. Kong, and X. Lin, "Connexin30 null and conditional connexin26 null mice display distinct pattern and time course of cellular degeneration in the cochlea," *The Journal of Comparative Neurology*, vol. 516, no. 6, pp. 569–579, 2009.

[65] Y. Takada, L. Beyer, D. Swiderski et al., "Connexin 26 null mice exhibit spiral ganglion degeneration that can be blocked by BDNF gene therapy," *Hearing Research*, vol. 309, pp. 124–135, 2014.

[66] M. Schütz, P. Scimemi, P. Majumder et al., "The human deafness-associated connexin 30 T5M mutation causes mild hearing loss and reduces biochemical coupling among cochlear non-sensory cells in knock-in mice," *Human Molecular Genetics*, vol. 19, no. 24, pp. 4759–4773, 2010.

[67] L. Rodriguez, E. Simeonato, P. Scimemi et al., "Reduced phosphatidylinositol 4,5-bisphosphate synthesis impairs inner ear Ca^{2+} signaling and high-frequency hearing acquisition," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 35, pp. 14013–14018, 2012.

[68] M. Vanderschueren-Lodeweyckx, F. Debruyne, L. Dooms, E. Eggermont, and R. Eeckels, "Sensorineural hearing loss in sporadic congenital hypothyroidism," *Archives of Disease in Childhood*, vol. 58, no. 6, pp. 419–422, 1983.

[69] F. Brucker-Davis, M. C. Skarulis, A. Pikus et al., "Prevalence and mechanisms of hearing loss in patients with resistance to thyroid hormone," *The Journal of Clinical Endocrinology and Metabolism*, vol. 81, no. 8, pp. 2768–2772, 1996.

[70] F. W. Newell and K. R. Diddie, "Typical monochromacy, congenital deafness, and resistance to intracellular action of thyroid hormone (author's transl)," *Klinische Monatsblatter fur Augenheilkunde*, vol. 171, no. 5, pp. 731–734, 1977.

[71] L. Ng, M. W. Kelley, and D. Forrest, "Making sense with thyroid hormone-the role of T 3 in auditory development," *Nature Reviews Endocrinology*, vol. 9, no. 5, pp. 296–307, 2013.

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Research Article

Cortical Electrophysiological Markers of Language Abilities in Children with Hearing Aids: A Pilot Study

David Bakhos,[1,2,3] Hélène Delage,[4] John Galvin,[5] Emmanuel Lescanne,[1,2,3] Sylvie Roux,[2,3] Frédérique Bonnet-Brilhault,[2,3,6] and Nicole Bruneau[2,3]

[1] *CHRU de Tours, Service ORL et Chirurgie Cervico-Faciale, boulevard Tonnellé, 37044 Tours, France*
[2] *INSERM U930, 37044 Tours, France*
[3] *Université François-Rabelais de Tours, CHRU de Tours, UMR-S930, 37044 Tours, France*
[4] *Laboratoire de Psycholinguistique Expérimentale, Faculté de Psychologie et des Sciences de l'Education, Université de Genève, 40 boulevard du pont d'Arve, 1211 Genève 4, Switzerland*
[5] *Department of Head and Neck Surgery, David Geffen School of Medicine, UCLA, Los Angeles, CA 90095, USA*
[6] *CHRU de Tours, Service de Pédopsychiatrie, boulevard Tonnellé, 37044 Tours, France*

Correspondence should be addressed to David Bakhos; david.bakhos@univ-tours.fr

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Objective. To investigate cortical auditory evoked potentials (CAEPs) in pediatric hearing aid (HA) users, with and without language impairment. *Design*. CAEPs were measured in 11 pediatric HA users (age: 8–12 years) with moderate bilateral sensorineural hearing loss (HL); participants were classified according to language ability. CAEPs were also measured for a control group of 11 age-matched, normal-hearing (NH) children. *Results*. HL children without language impairment exhibited normal CAEPs. HL children with language impairment exhibited atypical temporal CAEPs, characterized by the absence of N1c; frontocentral responses displayed normal age-related patterns. *Conclusion*. Results suggest that abnormal temporal brain function may underlie language impairment in pediatric HA users with moderate sensorineural HL.

1. Introduction

The human cochlea is mature at birth; however, axonal, dendritic, and synaptic maturation and myelination continue to develop in the brainstem into early childhood and in the cerebral cortex into late childhood [1]. Auditory development and speech perception are guided by relevant acoustic and linguistic information experienced early in life to assure cortical maturation [2]. Hearing loss (HL) can be deleterious to children's speech and language development due to reduced quality and quantity of auditory input, and these developmental difficulties can have a cascading effect on social, academic, and (later) occupational success [3]. Given the restricted auditory input, abnormal cortical auditory maturation can occur in children with sensorineural HL, suggesting that the root cause may lie in the inner ear [4].

In case of moderate HL in childhood, hearing aids (HAs) can improve speech audibility and facilitate language development, assuming that the auditory cortical areas are functional [5]. However, individual variation in language performance has been observed in children with mild to moderate sensorineural HL [6]. Approximately 50% of children fitted with HAs for moderate HL have language impairment despite normal aided audiometric thresholds [7, 8]; the same finding was observed in adolescents [9]. Other studies have suggested that impairment in basic auditory processing might contribute to language difficulties in children with specific language impairment [10–12]. Language impairment might therefore be related to abnormal cortical auditory processing, which can be investigated using cortical auditory evoked potentials (CAEPs).

The successive peaks of CAEPs correspond to the spatiotemporal involvement of the cortical auditory generators and are therefore influenced by cortical maturation [13–15]. Whereas the morphology of frontocentral CAEPs is strongly influenced by age and provides an important

index of auditory function and plasticity, the morphology of temporal CAEPs remains stable throughout childhood [16], with the successive negative N1a and N1c peaks occurring at approximately 80 and 160 ms, respectively [17]. These temporal responses (T-complex) represent the activity of the secondary auditory cortex [17]. The frontocentral CAEPs of children mainly exhibit two successive positive-negative peaks (P100 and N250) occurring at approximately 100 and 200 ms, respectively. At 8–11 years, adult-like CAEP waveforms progressively emerge, with the successive N1b, P2, and N250 peaks occurring at approximately 100, 180, and 220 ms, respectively. Given that speech information is transmitted at somewhat rapid rates (100 to 200 words per minute) [18], short interstimulus intervals (<750 ms) have sometimes been used to study the effect of the stimulus rate on frontocentral CAEPs in children that were categorized according to age [13]. Indeed, stimulus rate has been recognized as a marker of cortical auditory maturation [13]. Only one study has examined the influence of stimulus rate on temporal responses in children [19]. In that study, the amplitude of the temporal negative peak corresponding to Tb increased with interstimulus interval (350, 700, 1400, and 2000 ms), indicating a long refractory period for the underlying generator. Thus, it appears that long interstimulus intervals may be favorable for identifying successive peaks of the T-complex, and their asymmetry.

The aim of this study was to use CAEPs to investigate cortical auditory processing in regard to pediatric HA users with different levels of language ability. We hypothesized that temporal auditory responses and/or their sensitivity to stimulus rate would reflect different levels of language ability in these patients.

2. Patients and Method

2.1. Subjects. In this cortical electrophysiological study, we included children with symmetrical bilateral sensorineural HL fitted with HAs and aged between 8 and 12 years. Children in this age range were chosen for this pilot study as they are likely to understand and follow instructions during testing (i.e., CAEP, audiometry, and language tests). Participants were recruited from the Pediatric Unit of the Otolaryngology Department during clinical follow-up visits.

We reviewed patient charts of pediatric HA users. Thirty children had symmetrical bilateral sensorineural HL and were between 8 and 12 years old. Eleven children (8 males, 3 females), aged between 8.3 and 12.8 years (mean: 10.9 yrs), fitted with bilateral HAs for a bilateral moderate sensorineural HL were accepted into the study. A control group of 11 age- and gender-matched NH children with normal language development, as evaluated using the battery of oral language (evaluated with BILO battery including receptive and expressive language skills; see below for explanation), was also recruited for the study. For all participants, French was the main language spoken at home. All participants were right-handed.

Aided and unaided pure tone average (PTA) thresholds (averaged across audiometric frequencies 0.5, 1, 2, and 4 kHz) were <20 dB for pediatric HA and NH participants, respectively. Demographic data for pediatric HA users are shown in Table 1. All participants used oral communication and were enrolled in mainstream schools.

The Ethics Committee of the University Hospital of Tours approved the protocol, and written informed consent was obtained from the parents and assent from the children.

Spoken language and literacy skills were assessed using BILO, a set of standardized, computerized French language tests [20]. The BILO battery was standardized over a population of 272 primary and middle school students. In the BILO battery, phonology is assessed by a word repetition task using 42 words of increasing length and/or complexity. Expressive vocabulary is assessed using a classical naming task. Expressive grammar is evaluated using a sentence completion task and assesses the ability to produce a variety of specific grammatical morphemes: nominal, adjectival, and verbal inflexion, irregular plurals, prepositions, passive structure, and pronominal clitics. Reading is assessed using a timed task in which the child has to read, in 60 seconds, as many words as possible from a list of words of increasing difficulty. Spelling is tested using a word identification task, in which the child is presented with words that are correctly spelled, contain homophonic or nonhomophonic misspellings, or merely belong to the same semantic field and must decide whether the word corresponding to the picture is correct. Lexical judgment is tested by asking the child to decide whether there is concordance between a word and picture presented simultaneously. Grammatical judgment is tested by asking the child to decide whether a sentence corresponding to a picture is grammatically correct.

As described in Delage and Tuller [9], BILO scores were converted to z-scores. Language impairment was defined as scores on two or more of the language subtests that were 1.2 standard deviations below the norm. Six of the pediatric HA participants were deemed as having "good" language ability (HL+), with BILO > 1.2, while five were deemed as having "fair" language ability (HL−), with BILO < 1.2 (see Table 1). They were age-matched to control group of 11 NH children categorized according to similar language ability (HL+ controls and HL− controls). Six children (4 males and 2 females) were included in the HL+ controls (mean age: 11.6 years ± 0.7) and 5 children (4 males and one female) were included in the HL− controls (mean age: 9.4 years ± 1.9).

2.2. CAEPs Assessments

2.2.1. Stimuli and Procedure. Participants were tested while sitting on an armchair in a dimly lit, sound-insulated room; pediatric HA participants were tested while wearing their HAs. Participants' mother or father accompanied them in the room during testing. The stimuli were comprised of 50-ms tone bursts (1100 Hz) delivered through two loudspeakers placed symmetrically on each side of the computer screen. The tone stimuli were presented via Neuroscan Stim^2 software. The stimuli were presented at four different interstimulus intervals: 700 (i1), 1100 (i2), 1500 (i3), and 3000 (i4) ms.

Table 1: Demographic information for the pediatric HA users with bilateral moderate sensorineural HL.

Subject	Gender	Age test (yrs; months)	Duration of auditory deprivation (months)	Age at which child received HA (yrs; months)	Experience xith HA (yrs; months)	Aetiology	Right PTA	Left PTA	BILO score	Group
1	M	8; 3	6	3; 5	4; 10	Unknown	63	69	−1.34	HL−
2	M	8; 5	3	2; 1	6; 4	Familial	64	65	−3.53	HL−
3	M	8; 11	4	2	6; 11	Familial	66	66	−1.46	HL−
4	F	9; 7	2	6; 1	3; 6	Familial	56	56	−5.31	HL−
5	M	10; 5	2	5; 11	4; 6	Unknown	45	41	−0.97	HL+
6	M	11; 5	2	5; 11	5; 6	Unknown	41	43	−0.2	HL+
7	M	11; 8	1	3; 5	8; 3	Familial	55	66	−0.46	HL+
8	F	12; 1	18	8; 11	3; 2	Unknown	40	41	−0.16	HL+
9	F	12; 2	6	4; 10	7; 4	Familial	59	45	1.31	HL+
10	M	12; 6	5	8; 5	4; 1	Familial	41	41	−1.25	HL−
11	M	12; 10	6	5; 2	7; 8	Unknown	41	42	−0.5	HL+

Note: M: male; F: female; yrs: years; HA: hearing aid; PTA: aided pure tone audiometric threshold averaged over 0.5, 1, 2, and 4 kHz; BILO: Batterie Informatisée du Langage Oral; HL+: good language ability; HL−: fair language ability.

The sound intensity was 70 dBA measured at the head of the participant.

2.2.2. Electroencephalogram (EEG) Recording. EEG recordings were obtained using 28 Ag-AgCl cup electrodes (Ffz, Fz, Cz, Pz, O1, F3, FC1, FT3, C3, T3, CP1, TP3, P3, T5, and F7) and their counterparts on the right hemiscalp. Electrodes were placed according to the 10-20 system, as well as the left and right mastoids (M1 and M2), and referenced to the nose. In addition, to detect ocular artifacts, vertical electrooculogram (EOG) data were recorded from two electrodes above and below the right eye (vertical bipolar).

The EEG and EOG were digitized (Neuroscan Synamps amplifier, Scan 4.3, Compumedics Corp., El Paso, TX) at a sampling rate of 500 Hz. The EEG was amplified and bandpass-filtered (0.3–100 Hz). Electrode impedances were kept below 10 kΩ. Eye movement artifacts were eliminated using a spatial filter transform developed by Neuroscan, and EEG periods with movement artifacts were rejected manually. A digital zero-phase-shift low-pass filter (30 Hz) was then applied to the EEG.

2.2.3. Data Analysis. CAEPs were analyzed with the ELAN software [21]. Analysis was performed for waveform peaks occurring at frontocentral sites (N1b-P2-N250) and at temporal sites: N1a, N1c, P1t (the positive peak between these N1a and N1c), and P2t (the positive peak following N1c).

The influence of interstimulus interval on each peak of the CAEPs was analyzed using Friedman nonparametric analyses of variance. Amplitudes and latency peaks measured in HL+ and HL− children and the control group were compared using nonparametric Mann-Whitney rank tests.

3. Results

HL+ and HL− children did not differ, using Wilcoxon Mann-Whitney test, on age at testing (HL+: 11.7 years old ± 0.8; HL−: 9.5 years old ± 1.7; $P = 0.08$) or experience with hearing aids (HL+: 6.4 years, HL−: 5.7 years; $P = 0.4$).

3.1. Frontocentral Responses. Frontocentral CAEPs displayed similar successive N1b-P2-N250 peaks across the four groups. No significant difference was observed for the N1b peak amplitudes and latencies between HL+ and HL+ controls, or between HL− and HL− controls (Figure 1). The N1b peak amplitude was smaller for HL− than for HL+ participants and for HL− controls than for HL+ controls. This might be related to age differences, as the HL− and HL− control participants were younger (mean age was, resp., 9.5 and 9.4 years) than the HL+ and HL+ control participants (mean age was, resp., 11.7 and 11.6 years). A significant effect of interstimulus interval was observed on N1b amplitude for all groups except for the HL−. The N1b peak increased with interstimulus interval in HL+ ($P = 0.01$), HL+ controls ($P = 0.03$), and HL− controls ($P = 0.04$). There was a greater P2 peak amplitude in HL+ than in HL+ controls and was significant at i3 ($P = 0.02$) and at i4 ($P = 0.004$). The P2 peak was better individualized in HL− children than in matched controls. The N250 peak amplitude and latency did not vary with interstimulus interval and no significant differences across groups were observed.

3.2. Temporal Responses. Because no significant effect of interstimulus interval was found for the amplitude and latency of the successive peaks recorded at temporal sites N1a, P1t, N1c, and P2t, the CAEPs were averaged across the 4 interstimulus intervals to increase the signal to noise ratio (SNR).

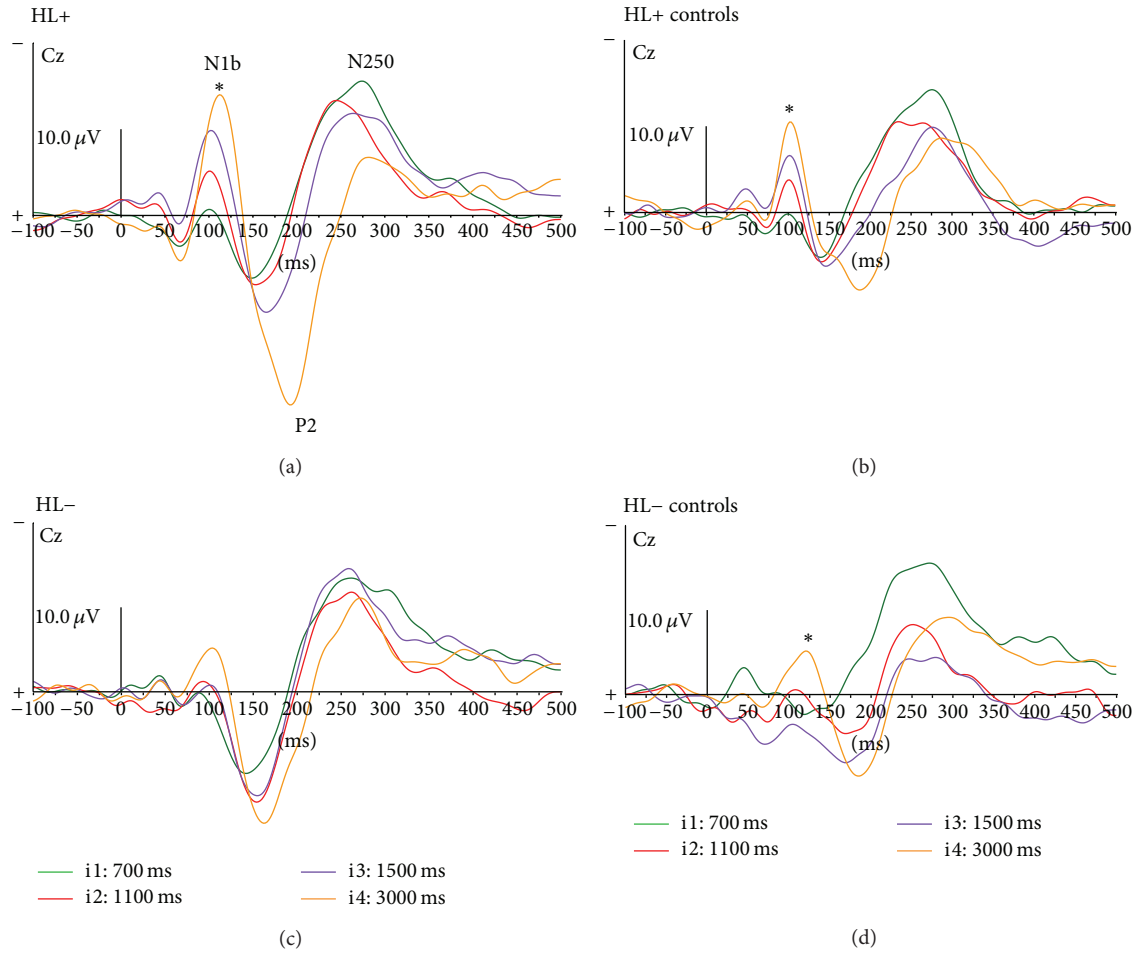

FIGURE 1: Cortical AEPs. Midline responses (Cz) at different interstimulus intervals (i1, i2, i3, and i4) for the HL+ (a), HL+ controls (b), HL− (c), and HL− controls (d). The asterisks indicate significant effect of interstimulus interval ($P < 0.05$).

Although the grand average N1a peak amplitude was greater in the HL+ and HL− groups than in their respective controls (mainly on the left temporal site), the difference was not statistically significant. The subsequent P1t, N1c, and P2t waves also were not significantly different between the HL+ and HL+ control groups. However, the N1c and P2t amplitudes were significantly smaller (only on the right temporal site) in the HL− group than in the HL− controls (N1c: HL− = −0.8 μV ± 1.3, HL− controls = −3.1 μV ± 0.9, U = 2, $P = 0.03$; P2t: HL− = 3.1 μV ± 0.6, HL+ controls = 5.4 μV ± 1.4, U = 2, $P = 0.03$). This difference between the HL− group and the HL− controls was not observed for either latency or amplitude of the preceding P1t (Figure 2).

4. Discussion

The present pilot study provided interesting preliminary findings regarding the relationship between CAEP characteristics and language ability in 8- to 12-year-old pediatric HA users. Atypical CAEPs were observed at temporal recording sites for pediatric HA users with some degree of language impairment. In normal development, temporal CAEPs typically display a stable morphology through childhood and particularly for the age range of the present study, with successive N1a and N1c peaks [16, 17], suggesting that cortical auditory maturation does not change the morphology of temporal responses for children between 8 and 12 years old.

In our study, despite a normal (or greater than normal) amplitude of the early temporal peaks (N1a, P1t) for all pediatric HA patients, the later temporal responses (N1c and P2t) were reduced or absent in the HL− group. This does not appear to be due to an absence of cortical auditory input because waves N1a and P1t were present and normal in all the pediatric HA patients. N1c wave was absent or smaller in the HL− group. This relationship between N1c abnormalities (N1c being reduced or absent) and language impairment has previously been shown in other clinical populations with language impairment such as in children with autism [22], Down's syndrome [23], or specific language impairment [24]. Because generators of N1c are located at the lateral part of the superior temporal gyrus [25–27], the present results emphasize the importance of these cortical areas in language processing.

Relatively few studies have investigated CAEP temporal responses in pediatric HA users. Most of these studies have focused on frontocentral responses (especially the latency of

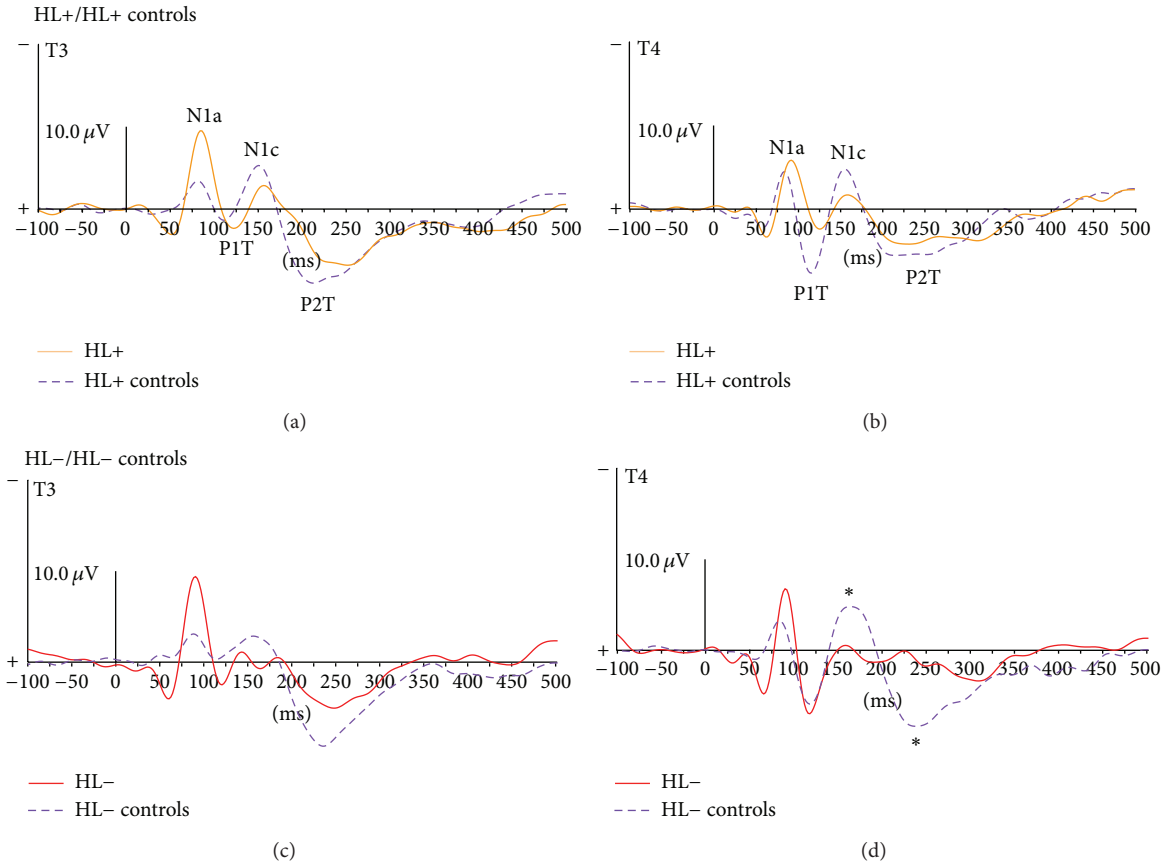

Figure 2: CAEP T3 (a and c) and T4 temporal responses (b and d) at i1, i2, i3, and i4 for the HL+ and HL+ control groups (a and b) and for the HL− and HL− control groups (c and d). The asterisks indicate significant differences ($P < 0.05$) for the peak amplitude wave.

the P1 peak recorded at the vertex) in deaf children who use cochlear implants [28–30]. These studies found that P1 peak latency can be a biomarker of auditory cortex maturation in children with congenital hearing loss and that this P1 peak latency will decrease with auditory rehabilitation. In the present study, the P1 peak latency did not differ between HA patients and NH controls, suggesting that the HA provided enough auditory input to allow maturation of the primary auditory cortex as found in cochlear-implanted children.

Unlike the temporal responses, the frontocentral responses of normally developing children are greatly influenced by age. The smaller N1b peak amplitude in the HL− and HL− control groups, compared to the well-defined peaks and greater amplitude of N1b in the HL+ and HL+ control groups, might be due to age differences between groups. This result is in agreement with the literature indicating the emergence of N1b at approximately 8–10 years of age and greater N1b amplitude at 10–12 years of age. The greater P2 peak amplitude observed in HL+ and HL− groups than in the controls may be related to HA amplification, as described in previous studies [31, 32]. N1b peak amplitude increased with interstimulus interval in the HL+ group, similar to the HL+ controls and previous studies [5]; the effect of interstimulus interval was not observed for the HL− group. This finding might be related to language impairment or the younger age of the HL− group, as a significant effect of interstimulus interval was observed for the HL− controls.

In this study, children with HA and good language ability (HL+) were older than those with language impairment (HL−). Language abilities were evaluated with BILO, which is calibrated in order to allow comparisons in language ability across age groups. Moreover, CAEPs were compared with age- and gender-matched control children.

Although further longitudinal studies are needed with larger sample of children, these preliminary results suggest that abnormal CAEP responses recorded at temporal sites might underlie language impairment in pediatric HA users who have a moderate sensorineural HL.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

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References

[1] J. K. Moore and F. H. Linthicum Jr., "The human auditory system: a timeline of development," *International Journal of Audiology*, vol. 46, pp. 460–478, 2007.

[2] P. K. Kuhl, "A new view of language acquisition," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 22, pp. 11850–11857, 2000.

[3] H. W. Catts, M. E. Fey, J. B. Tomblin, and X. Zhang, "A longitudinal investigation of reading outcomes in children with language impairments," *Journal of Speech, Language, and Hearing Research*, vol. 45, no. 6, pp. 1142–1157, 2002.

[4] J. K. Moore, J. K. Niparko, M. R. Miller, and F. H. Linthicum, "Effect of profound hearing loss on a central auditory nucleus," *The American Journal of Otology*, vol. 15, no. 5, pp. 588–595, 1994.

[5] J. B. Tombin, J. J. Oleson, S. E. Ambrose, E. Walker, and M. P. Moeller, "The influence of hearing aids on the speech and language development of children with hearing loss," *JAMA Otolaryngology—Head & Neck Surgery*, vol. 140, pp. 403–409, 2014.

[6] J. Briscoe, D. V. Bishop, and C. F. Norbury, "Phonological processing, language, and literacy: a comparison of children with mild-to-moderate sensorineural hearing loss and those with specific language impairment," *The Journal of Child Psychology and Psychiatry*, vol. 42, no. 3, pp. 329–340, 2001.

[7] K. Hansson, J. Forsberg, A. Löfqvist, E. Mäki-Torkko, and B. Sahlén, "Working memory and novel word learning in children with hearing impairment and children with specific language impairment," *International Journal of Language and Communication Disorders*, vol. 39, no. 3, pp. 401–422, 2004.

[8] P. G. Stelmachowicz, A. L. Pittman, B. M. Hoover, and D. E. Lewis, "Novel-word learning in children with normal hearing and hearing loss," *Ear and Hearing*, vol. 25, no. 1, pp. 47–56, 2004.

[9] H. Delage and L. Tuller, "Language development and mild-to-moderate hearing loss: does language normalize with age?" *Journal of Speech, Language, and Hearing Research*, vol. 50, no. 5, pp. 1300–1313, 2007.

[10] P. Tallal, "Rapid auditory processing in normal and disordered language development," *Journal of Speech and Hearing Research*, vol. 19, no. 3, pp. 561–571, 1976.

[11] B. A. Wright, L. J. Lombardino, W. M. King, C. S. Puranik, C. M. Leonard, and M. M. Merzenich, "Deficits in auditory temporal and spectral resolution in language- impaired children," *Nature*, vol. 387, no. 6629, pp. 176–178, 1997.

[12] G. M. McArthur and D. V. Bishop, "Which people with specific language impairment have auditory processing deficits?" *Cognitive Neuropsychology*, vol. 21, pp. 79–94, 2004.

[13] E. Sussman, M. Steinschneider, V. Gumenyuk, J. Grushko, and K. Lawson, "The maturation of human evoked brain potentials to sounds presented at different stimulus rates," *Hearing Research*, vol. 236, no. 1-2, pp. 61–79, 2008.

[14] C. W. Ponton, J. J. Eggermont, B. Kwong, and M. Don, "Maturation of human central auditory system activity: evidence from multi-channel evoked potentials," *Clinical Neurophysiology*, vol. 111, no. 2, pp. 220–236, 2000.

[15] J. L. Wunderlich and B. K. Cone-Wesson, "Maturation of CAEP in infants and children: a review," *Hearing Research*, vol. 212, no. 1-2, pp. 212–223, 2006.

[16] I. Tonnquist-Uhlen, C. W. Ponton, J. J. Eggermont, B. Kwong, and M. Don, "Maturation of human central auditory system activity: the T-complex," *Clinical Neurophysiology*, vol. 114, no. 4, pp. 685–701, 2003.

[17] N. Bruneau, S. Roux, P. Guërin, C. Barthélémy, and G. Lelord, "Temporal prominence of auditory evoked potentials (N1 wave) in 4-8- year-old children," *Psychophysiology*, vol. 34, no. 1, pp. 32–38, 1997.

[18] J. C. Krause and L. D. Braida, "Investigating alternative forms of clear speech: the effects of speaking rate and speaking mode on intelligibility," *Journal of the Acoustical Society of America*, vol. 112, pp. 2165–2172, 2002.

[19] R. Ceponiene, M. Cheour, and R. Näätänen, "Interstimulus interval and auditory event-related potentials in children: evidence for multiple generators," *Electroencephalography and Clinical Neurophysiology*, vol. 108, no. 4, pp. 345–354, 1998.

[20] A. Khomsi, J. Khomsi, F. Pasquet, and A. Parbeau-Gueno, *Bilan Informatisé du Langage Oral (BILO)*, Editions du Centre de Psychologie Appliquée, Paris, France, 2007.

[21] P.-E. Aguera, K. Jerbi, A. Caclin, and O. Bertrand, "ELAN: a software package for analysis and visualization of MEG, EEG, and LFP signals," *Computational Intelligence and Neuroscience*, vol. 2011, Article ID 158970, 11 pages, 2011.

[22] N. Bruneau, S. Roux, J. L. Adrien, and C. Barthélémy, "Auditory associative cortex dysfunction in children with autism: evidence from late auditory evoked potentials (N1 wave-T complex)," *Clinical Neurophysiology*, vol. 110, no. 11, pp. 1927–1934, 1999.

[23] M. A. Groen, P. Alku, and D. V. M. Bishop, "Lateralisation of auditory processing in Down syndrome: a study of T-complex peaks Ta and Tb," *Biological Psychology*, vol. 79, no. 2, pp. 148–157, 2008.

[24] V. L. Shafer, R. G. Schwartz, and B. Martin, "Evidence of deficient central speech processing in children with specific language impairment: The T-complex," *Clinical Neurophysiology*, vol. 122, no. 6, pp. 1137–1155, 2011.

[25] G. G. Celesia, "Organization of auditory cortical areas in man," *Brain*, vol. 99, no. 3, pp. 403–414, 1976.

[26] C. C. Wood and J. R. Wolpaw, "Scalp distribution of human auditory evoked potentials. II. Evidence for overlapping sources and involvement of auditory cortex," *Electroencephalography and Clinical Neurophysiology*, vol. 54, no. 1, pp. 25–38, 1982.

[27] M. Scherg and D. von Cramon, "Evoked dipole source potentials of the human auditory cortex," *Electroencephalography and Clinical Neurophysiology*, vol. 65, no. 5, pp. 344–360, 1986.

[28] C. W. Ponton, M. Don, J. J. Eggermont, M. D. Waring, and A. Masuda, "Maturation of human cortical auditory function: differences between normal-hearing children and children with cochlear implants," *Ear and Hearing*, vol. 17, no. 5, pp. 430–437, 1996.

[29] A. Sharma, M. F. Dorman, and A. J. Spahr, "A sensitive period for the development of the central auditory system in children with cochlear implants: implications for age of implantation," *Ear and Hearing*, vol. 23, no. 6, pp. 532–539, 2002.

[30] A. Sharma, K. Martin, P. Roland et al., "P1 latency as a biomarker for central auditory development in children with hearing impairment," *Journal of the American Academy of Audiology*, vol. 16, no. 8, pp. 564–573, 2005.

[31] P. A. Korczak, D. Kurtzberg, and D. R. Stapells, "Effects of sensorineural hearing loss and personal hearing aids on cortical event-related potential and behavioral measures of speech-sound processing," *Ear and Hearing*, vol. 26, no. 2, pp. 165–185, 2005.

[32] C. J. Billings, K. L. Tremblay, P. E. Souza, and M. A. Binns, "Effects of hearing aid amplification and stimulus intensity on cortical auditory evoked potentials," *Audiology and Neurotology*, vol. 12, no. 4, pp. 234–246, 2007.

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Research Article

The Effects of Acute Stress-Induced Sleep Disturbance on Acoustic Trauma-Induced Tinnitus in Rats

Yiwen Zheng,[1,2] Lucy Stiles,[1,2] Yi-Ting Chien,[1,2] Cynthia L. Darlington,[1,2] and Paul F. Smith[1,2]

[1] *Department of Pharmacology and Toxicology, School of Medical Sciences, University of Otago, P.O. Box 913, Dunedin 9016, New Zealand*
[2] *Brain Health Research Centre, University of Otago, Dunedin 9016, New Zealand*

Correspondence should be addressed to Yiwen Zheng; yiwen.zheng@otago.ac.nz

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Chronic tinnitus is a debilitating condition and often accompanied by anxiety, depression, and sleep disturbance. It has been suggested that sleep disturbance, such as insomnia, may be a risk factor/predictor for tinnitus-related distress and the two conditions may share common neurobiological mechanisms. This study investigated whether acute stress-induced sleep disturbance could increase the susceptibility to acoustic trauma-induced tinnitus in rats. The animals were exposed to unilateral acoustic trauma 24 h before sleep disturbance being induced using the cage exchange method. Tinnitus perception was assessed behaviourally using a conditioned lick suppression paradigm 3 weeks after the acoustic trauma. Changes in the orexin system in the hypothalamus, which plays an important role in maintaining long-lasting arousal, were also examined using immunohistochemistry. Cage exchange resulted in a significant reduction in the number of sleep episodes and acoustic trauma-induced tinnitus with acoustic features similar to a 32 kHz tone at 100 dB. However, sleep disturbance did not exacerbate the perception of tinnitus in rats. Neither tinnitus alone nor tinnitus plus sleep disturbance altered the number of orexin-expressing neurons. The results suggest that acute sleep disturbance does not cause long-term changes in the number of orexin neurons and does not change the perception of tinnitus induced by acoustic trauma in rats.

1. Introduction

Chronic tinnitus ("ringing in the ears") is a debilitating condition affecting about 10% of the adult population [1]. As ageing occurs, its prevalence increases, affecting 14.3% of the population between the ages of 60 and 69 [1]. Despite numerous studies investigating the underlying mechanisms and therapeutic options, to date, there is no effective treatment for tinnitus. Tinnitus treatment is complicated not only by a poor understanding of its mechanisms but also by the variations in individuals' reactions to its perception. For example, while some people can tolerate their tinnitus, others feel severely distressed and handicapped [2–5]. It has been shown that patients with tinnitus that causes distress often have high rates of psychopathological conditions (see [6–8] for reviews). Specifically, tinnitus severity has been associated with high levels of anxiety, depression, and sleeping disturbance [9–14].

Among tinnitus-related problems, sleeping disturbance is the second most frequent comorbid condition [12], affecting 50–77% of tinnitus patients [15, 16]. Both the subjective (i.e., self-rated) and objective (i.e., electroencephalography (EEG), electromyography (EMG), and electrooculography (EOG) recordings) sleep measurements in tinnitus patients with disturbed sleep are very similar to those that occur in insomnia [14], a sleep disorder characterised by difficulties in initiating/maintaining sleep. It is tempting to think that insomnia is a consequence of people's reaction to the annoyance of tinnitus; however, studies have suggested that insomnia may, in fact, be a risk factor/predictor for tinnitus-related distress [17, 18] and the two conditions may share common neurobiological mechanisms (see [19] for a review).

Recent research has suggested that hyperarousal might play an important role in the pathophysiology of both insomnia and the conscious perception of tinnitus (see [19–22] for

reviews). It is assumed that insomnia and tinnitus perception are the result of active cognitive appraisal behaviour that are reinforced by a vicious cycle of biased attention and negative behaviour. While stress-reactivity is the most important risk factor in the development of insomnia, stressful changes in one's life, such as divorce, accidents, or sickness in family members, have been linked to the development of tinnitus and stress is especially important for tinnitus to transit from mild to severe (see [23, 24] for reviews). Therefore, there might be a neurobiological system that regulates the normal arousal level under physiological conditions and a malfunction of this system under stressful conditions could serve as a promoter for hyperarousal in pathological conditions, such as tinnitus and insomnia.

One candidate system is the orexin system located in the lateral hypothalamus. Discovered in 1998, the orexin system is well recognised for its role in sleep-wake regulation, appetite control, reward, emotional responses to stress, and learning and memory (see [25] for a review). There are two peptides (orexin-A and -B) and their corresponding receptors are distributed widely in brain areas involved in arousal, sensory processing, and autonomic function, including key structures involved in processing auditory information, such as the inferior colliculus and the ventral cochlear nucleus [26]. There is evidence that orexin neurons are capable of undergoing experience-induced synaptic plasticity, which is thought to be responsible for the maintenance of long-lasting arousal (see [27] for a review). Inappropriate activation of the orexin system has been attributed to the pathophysiology of insomnia based on the beneficial effects of orexin receptor antagonists [28, 29]. However, the possibility that inappropriate activation of the orexin system may also reset the arousal threshold, to promote tinnitus, has never been explored.

Sleep patterns across many different species have been characterised and rodents, such as rats and mice, normally spend most of their time asleep during the light phase and awake during the dark phase. Compared with humans, sleep in rodents is broken into small segments, with the duration of each sleep episode being only 10–14 min in rats (see [30, 31] for reviews). The different rodent models of insomnia, such as the stress-induced model, caffeine-induced model, genetic model, and brain lesion model, have been used to resemble some aspects of the sleep disturbance that insomnia patients experience; however, none of the models can convincingly represent the human insomniac condition (see [31] for a review). Among these models, the stress-induced insomnia model using cage exchange has been shown to result in sleep disturbance similar to that of patients with stress-induced insomnia [32]. Given that stress is one of the risk factors associated with both tinnitus and sleep disorders (see [23, 33, 34] for reviews), this model is ideal for studying the relationship between stress, sleep disturbance, and tinnitus. Therefore, in this study, we used the stress-inducing cage exchange method to induce sleep disturbance in rats and investigated the susceptibility of these rats to acoustic trauma-induced tinnitus. Furthermore, the number of orexin-expressing neurons in the hypothalamus was also assessed using immunohistochemistry.

2. Methods

2.1. Animals. Thirty-two male Wistar rats (300–350 g, 2 months old, at the beginning of the experiment) were obtained from the Hercus-Taieri Resource Unit, Dunedin, New Zealand, and were divided into 3 groups ($n = 12$ per group): (1) control (no cage exchange and no acoustic trauma); (2) acoustic trauma + clean cage; and (3) acoustic trauma + dirty cage. The animals were maintained on a 12 : 12 h light : dark cycle at 22°C and had free access to food but were water deprived throughout the tinnitus behavioural test. All procedures were approved by the University of Otago Committee on Ethics in the Care and Use of Laboratory Animals.

2.2. Acoustic Trauma to Induce Tinnitus. Unilateral acoustic trauma was delivered using a procedure described previously [35, 36]. Briefly, the animals were anaesthetised with fentanyl citrate (0.2 mg/kg, s.c.), medetomidine hydrochloride (Domitor, Novartis; 0.5 mg/kg, s.c.), and atropine sulphate (50 μg/kg, s.c.) and placed inside a sound attenuation chamber. A 16 kHz 110 dB pure tone generated by a NI 4461 Dynamic Signal Acquisition and Generation system (National Instruments New Zealand Ltd.) was delivered to one of the ears for 1 h through a closed field magnetic speaker (Tucker-Davis Technologies) connected to a custom made speculum. Acoustic values were calibrated by connecting the speaker to a 0.25 inch prepolarised free-field microphone (Type 40BE, GRAS Sound & Vibration) via the speculum used to fit into the external auditory canal. The unexposed ear was blocked with cone-shaped foam and taped against the foam surface. The exposure was counterbalanced between the left and the right ears. The control animals were kept under anaesthesia for the same duration as the acoustic trauma animals, but without exposure.

2.3. Auditory Brainstem Responses. Auditory function in the exposed ears before and immediately after the acoustic trauma was measured using auditory brainstem-evoked response (ABR) thresholds described previously [37]. Briefly, the animals were anaesthetised as previously described and subdermal needle electrodes were placed at the vertex and over the bullae with a reference electrode at the occiput. Tone bursts of 5 ms duration (2 ms rise/decay, 1 ms plateau) were presented at a rate of 50/sec and in a series of decreasing intensity, which began at a level that resulted in distinct evoked potentials. Tone intensities progressed in 20-, 10-, and 5-dB steps, at 32, 20, 16, and 8 kHz, and the ABR threshold was determined as the lowest intensity that produced a visually distinct potential.

2.4. Cage Exchange to Induce Sleep Disturbance. Twenty-four hours after acoustic trauma, sleep disturbance was induced using the cage exchange method described by Cano et al. [32]. Briefly, rats in group 3 were placed in dirty cages previously occupied by another male rat for 1 week and left undisturbed for 5.5 h. The control and the acoustic trauma-only group rats were placed in clean cages for the same duration. The 5.5 h duration was chosen because insomnia and neuronal

activation have been shown to be induced at this time point [32]. In order to confirm that cage exchange induced sleep disturbances and stress, the animal's behaviour inside the exchanged cage (dirty or clean cage) was recorded for 5.5 h and the sleep-wake cycle, that is, the latency to fall asleep, duration of sleep, and number of sleep episodes, was analysed [32].

2.5. Tinnitus Assessment. At 3 weeks after acoustic trauma, the animals were tested for the behavioural signs of tinnitus inside an operant conditioning test chamber (ENV-007, Med Associates Inc.) using the conditioned lick suppression method routinely used in our laboratory [37–40]. The animals were water deprived to 95% of their normal body weight and this ensured that the animals reliably produced approximately 2000 to 6000 licks per 15 min testing session. A broadband noise (BBN) was played throughout the 15 min session except at 10 random intervals, at which point 15 sec acoustic stimulus presentations were inserted. Two of the 10 presentations were always speaker- off periods (i.e, silence) and the remaining 8 were one of BBN, 20 kHz tones, or 32 kHz tones at one of 4 different intensity levels (BBN = 30 dB, 40 dB, 50 dB, 70 dB; 20 kHz = 70 dB, 80 dB, 90 dB, 100 dB; 32 kHz = 70 dB, 80 dB, 90 dB, 100 dB) in a random order with each stimulus repeated twice within each session. The type of stimulus varied randomly between sessions but remained constant within a session. The animals were trained to establish a conditioned lick suppression response by pairing the silence (conditioned stimulus) with mild foot shock (0.35 mA, 3 sec; unconditioned stimulus) produced by a constant current shock source (ENV-410B, Med Associates Inc.) through a scrambler (ENV-412, Med Associates Inc.). The magnitude of lick suppression was measured by comparing the number of licks in the period preceding the stimulus presentation (A) and the period during the stimulus presentation (B), that is, the suppression ratio (SR):

$$\mathrm{SR} = \frac{B}{A + B}. \quad (1)$$

Once the lick suppression was established (SR < 0.2), the rats were subjected to the frequency discrimination test, during which the acoustic stimuli were presented in the same way as in the acclimation and the suppression training. However, the foot shock was delivered only if the SR for the speaker off period was >0.2. If a rat did not have tinnitus, the presentation of the stimuli had no effect on its licking activity. However, if a rat had tinnitus, the tinnitus sound would serve as the conditioned stimulus instead of the silence. Therefore, a testing stimulus with similar acoustic features to its tinnitus should produce greater lick suppression in this rat than in control rats.

2.6. Immunohistochemistry. Four rats from the sham group and four rats that exhibited the behavioural signs of tinnitus were selected from each exposed-clean cage and exposed-dirty cage group. At the conclusion of the tinnitus behavioural testing, the rats were overdosed with sodium pentobarbital and perfused transcardially with 4% paraformaldehyde. The brains were dissected out, postfixed, and frozen. Forty μm serial sagittal sections throughout the hypothalamus were collected according to a random, systematic sampling design for free floating immunolabelling. Antigen retrieval was achieved by incubating the sections in citrate buffer (pH 6) at 90°C for 10 min. The sections were then blocked with a blocking buffer (5% normal goat serum, 1% bovine serum albumin, and 5% Triton X-100 in 0.01 M PBS) for 2 h at room temperature before being incubated with a rat anti-orexin-A antibody (1 : 2000; Millipore, AB 3704) for 48 h at 4°C. This was followed by an incubation with a secondary antibody (horseradish peroxidase-conjugated goat anti-rabbit IgG, 1 : 200; Santa Cruz, sc-2004) for 2 h at room temperature. The orexin immunocomplex was visualised using a DAB kit. Every orexin-positive neuron was counted under a 63x oil objective lens throughout the thickness of the section in one set of the serial sections collected and the total number of orexin-positive neurons in the left and right hypothalamus was then estimated using a modified fractionator method [41–43].

2.7. Statistical Analysis. All data were tested for the normal parametric assumptions of normality and homogeneity of variance [44]. Where these assumptions were violated, the data were natural log or square root transformed and retested. Data were analysed using 2-way ANOVAs with repeated measures and multiple comparisons were conducted using Bonferroni tests. The number of animals exhibiting clear signs of tinnitus in each group was analysed using a chi-squared test. $P \leq 0.05$ was considered significant.

3. Results

3.1. The Effect of Acoustic Trauma on Animals' ABR Thresholds. Unilateral acoustic trauma caused an immediate increase in the ABR thresholds across all the frequencies examined (Figure 1). There was a significant exposure effect for both clean and dirty cage animals ($F_{3,175} = 216.3$, $P \leq 0.0001$) and a significant frequency-exposure interaction ($F_{9,175} = 10.76$, $P \leq 0.0001$), which suggests that the ABR threshold elevation was much greater at the higher frequencies compared with the lower frequencies.

3.2. The Effect of Cage Exchange on Animals' Sleeping Patterns. The general behaviour of the rats placed in the dirty cages was not obviously different from those placed in the clean cages. However, after 5.5 h, the cage bedding was noticeably dishevelled, which suggests that the animals in the dirty cages were more unsettled. When the sleeping patterns were analysed, animals placed in the dirty cages had significantly fewer sleep episodes during the 5.5 h period when compared with those in the clean cages ($t = 3.86$, $P \leq 0.0012$; Figure 2). A close inspection of the sleep episodes within each hourly period revealed that the reduced number of sleep episodes occurred mainly during the first 3 h inside the dirty cages, with the significant reduction being observed between 2 and 3 h ($F_{1,17} = 14.86$, $P \leq 0.0013$; Figure 2). There was no difference between the clean and dirty cage groups in the time taken to fall asleep and the duration of sleep, whether it

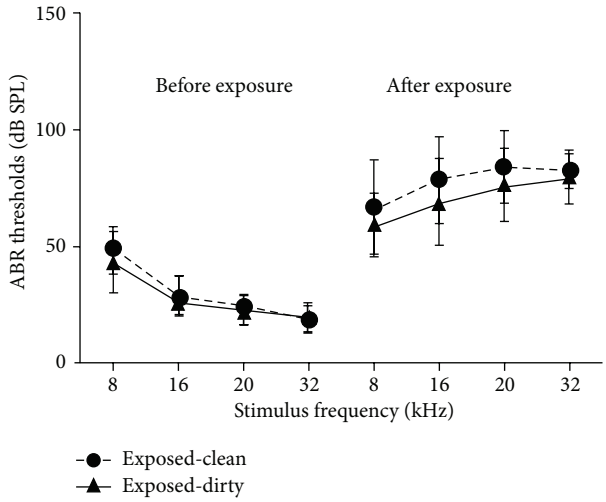

Figure 1: ABR thresholds at different stimulus frequencies in the ipsilateral ear of the animals from the exposed-clean and exposed-dirty cage groups measured pre- and postexposure to acoustic trauma. Symbols represent means ± 1 SEM.

was measured during the 5.5 h or within each hourly period (Figure 2).

3.3. The Effects of Cage Exchange on Animals' Tinnitus Perception. Frequency discrimination curves for all of the groups were plotted using the SRs against the intensity of each frequency of the stimulus (Figure 3). As expected, SR values differed significantly with different stimulus intensities for BBN ($F_{4,108} = 134.7$, $P \leq 0.0001$), 20 kHz ($F_{4,108} = 64.42$, $P \leq 0.0001$), and 32 kHz ($F_{4,108} = 43.08$, $P \leq 0.0001$) stimuli. While there was no significant group difference when tested for any of the 3 stimuli presentations, there was a significant intensity x group interaction specifically for the 32 kHz tones ($F_{8,108} = 2.954$, $P \leq 0.005$). Multiple comparisons revealed that 32 kHz tones elicited a significant downward shift of the curves at 100 dB for both the exposed-clean and exposed-dirty groups compared with the sham group ($t = 3.071$, $P \leq 0.05$, for the exposed-clean group; $t = 2.950$, $P \leq 0.05$, for the exposed-dirty group). The number of animals exhibiting clear behavioural signs of tinnitus was not significantly different between the exposed-clean (4 out 12) and exposed-dirty (5 out of 12) groups ($P = 1.000$, chi-square test).

3.4. The Effects of Cage Exchange and Tinnitus on the Number of Orexin Neurons in the Hypothalamus. Orexin immunohistochemistry was carried out in four nontinnitus rats from the sham group, four tinnitus rats from the exposed-clean group, and four tinnitus rats from the exposed-dirty group and revealed selective and specific staining in the cytoplasm of the cell bodies and the processes of neurons throughout the hypothalamus (Figure 4(a)). Orexin-positive fibres were present in many areas of the brain including the cochlear nucleus; however, no orexin-positive cell bodies were observed in any areas of the brain other than the hypothalamus (data not shown). Stereological cell counting showed that there was no significant difference in the total number of orexin-positive neurons in the hypothalamus either between the ipsilateral and contralateral sides to the acoustic trauma-exposed ear or among the sham, exposed-clean, and exposed-dirty groups (Figure 4(b)), which suggests that neither tinnitus alone nor tinnitus plus sleep disturbance altered the number of orexin-expressing neurons.

4. Discussion

Our results showed that stress-inducing cage exchange caused a sleep disturbance and acoustic trauma-induced tinnitus in rats. However, sleep disturbance did not affect tinnitus perception caused by acoustic trauma. Furthermore, sleep disturbance and tinnitus did not change the number of orexin neurons in the hypothalamus.

The cage exchange method to induce sleep disturbance is based on the notion that stress is the most common cause of sleep disorders. This method, by placing the rat in a dirty cage previously occupied by another male rat for 1 week, has been shown to successfully induce sleep disturbances in rats with similar behavioural and electrophysiological patterns to those observed in stress-induced insomnia in humans [32]. In addition, using c-Fos as an indication of neuronal activation, the study also showed an activation of the brain areas involved in arousal and emotional control. It was hypothesized that "being inescapably surrounded by the territory that has been marked by another male rat" is the psychological stressor rather than the odour of another rat itself, since the animals were housed next to each other [32]. Indeed, compared with the rats being placed in clean cages, the rats in the dirty cages were noticeably unsettled, which indicated an increase in stress. Analysis of the sleeping patterns revealed that animals in dirty cages had significantly fewer sleep episodes compared with the animals in clean cages, albeit with no difference in sleep durations. The decrease in the number of sleep episodes was observed during the third hour after cage exchange, which is similar to what was reported previously [32]. However, the dirty cage animals in our study had similar latencies to fall asleep and slept for a similar duration as the clean cage animals. These findings are in contrast with the previous study where a longer latency to fall asleep and an increased wakefulness were reported [32]. One of the factors that might contribute to the differences between the studies is that our rats received anaesthesia 24 h prior to the cage exchange and the anaesthesia might have affected the level of stress induced by cage exchange. Although it is difficult to determine whether the duration of sleep or the number of sleep episodes is more important for the quality of sleep in rats, the fact that sleep in rats was highly fragmented suggests that the reduced sleep episodes in dirty cage animals may reflect a reduced quality of sleep. However, changes in sleep quality did not exacerbate the behavioural signs of tinnitus in these animals.

Tinnitus was induced by exposing the rats to a 16 kHz pure tone at 110 dB SPL for 1 h. Acoustic stimuli at the intensities between 110 and 115 dB SPL have successfully induced tinnitus in rats in our previous studies [37–40, 45].

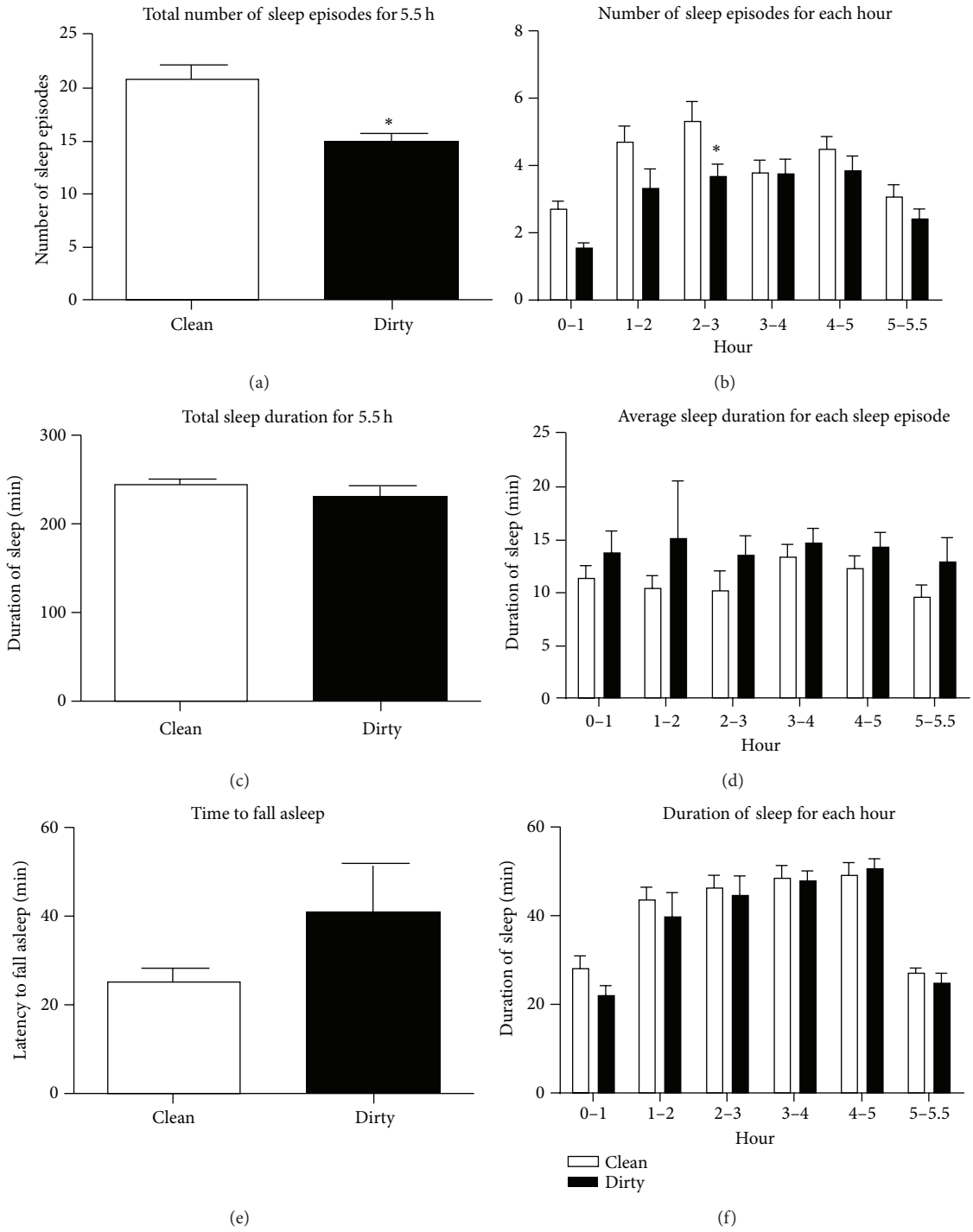

Figure 2: Sleep patterns during the 5.5 h cage exchange period. (a) Total number of sleep episodes during the 5.5 h period. (b) Number of sleep episodes during each hourly period. (c) Total duration of sleep during the 5.5 h period. (d) Average sleep duration for each sleep episode during each hourly period. (e) Time taken to fall asleep. (f) Sleep duration during each hourly period. Bars represent means ± 1 SEM.

We chose to use the intensity at the lower end, that is, 110 dB SPL, for this study with the intention of causing a moderate level of tinnitus in the exposed-clean group and to increase the sensitivity to detect a difference between animals in the clean and dirty cages if the dirty cages made tinnitus worse. As expected, this acoustic trauma parameter induced clear behavioural signs of tinnitus in 33% of the animals (4 out of 12 animals) in the clean cage group, which is lower than our previous tinnitus induction rate (62.5–75%) when a 115 dB tone was used. However, dirty cages did not significantly increase the number of animals exhibiting tinnitus (5 out of 12 animals). We found acoustic trauma-induced tinnitus with

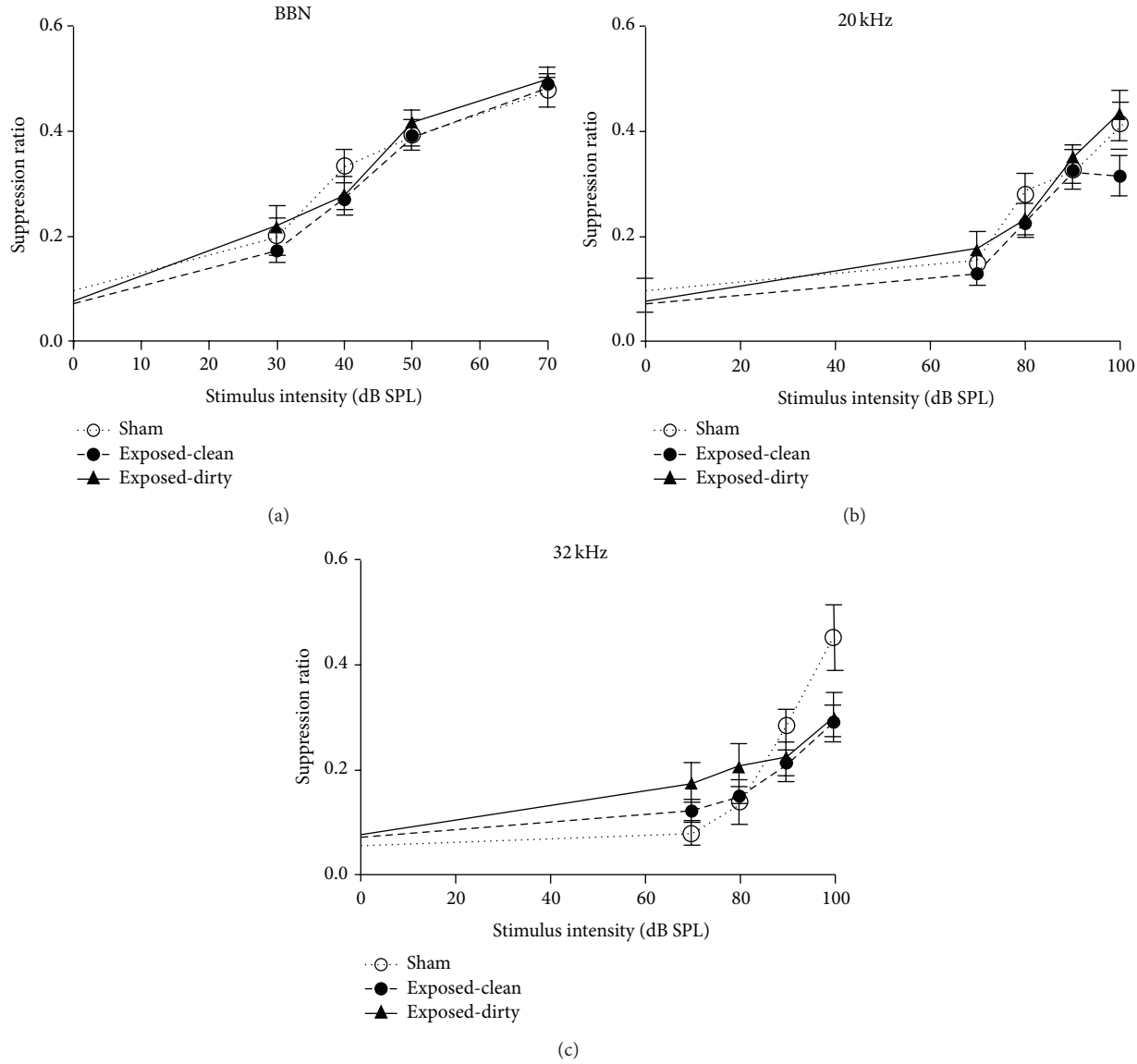

Figure 3: Frequency discrimination curves for suppression ratio in sham, exposed-clean, and exposed-dirty animals in response to BBN, 20 kHz or 32 kHz stimuli at different intensities. Symbols represent means ± 1 SEM.

acoustic features similar to a 32 kHz tone of 100 dB in both the clean and dirty cage animals and there was no significant difference between the clean and dirty cage animals. Given that acoustic trauma caused comparable elevations of the ABR thresholds immediately after the exposure in both groups of animals, the peripheral damage caused by acoustic trauma should also be similar between the two groups. Therefore, additional stress and sleep disturbance 24 h after acoustic trauma did not increase tinnitus severity in this experiment. If a stress factor was introduced during or immediately after the acoustic trauma, it might be possible to increase tinnitus severity. However, under the current animal ethics regulation, that is, the acoustic trauma was delivered under anaesthesia, this possibility could not be explored. Another reason that sleep disturbance failed to intensify tinnitus might be that the stress-inducing cage exchange was delivered only once following acoustic trauma and the sleep disturbance did not last long enough. Methods to induce chronic stress or chronic sleep disturbance may be needed in order to further investigate the relationship between stress and tinnitus perception.

Since hyperarousal has been proposed to underlie both tinnitus and insomnia (see [19] for a review) and the orexin system plays an important role in maintaining long-lasting arousal (see [27] for a review), it remains unknown whether the orexin system could be altered in animals that developed tinnitus and/or sleep disturbance. In this study, the total number of orexin-expressing neurons was estimated using immunohistochemistry and stereology throughout the hypothalamus. No significant difference was found between

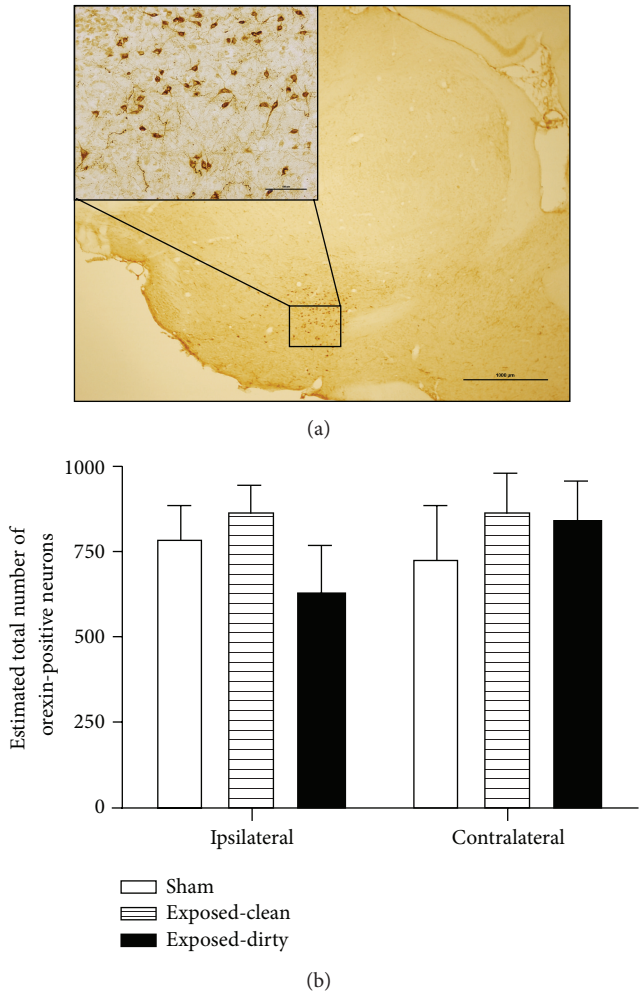

FIGURE 4: Orexin immunoreactivity in the hypothalamus. (a) A photograph showing orexin immunostaining located in the hypothalamus under a low magnification (scale bar: 1000 μm). Inserted picture showing the presence of orexin-positive staining in the cytoplasm and the processes of the neurons (scale bar: 100 μm). (b) Estimated total number of orexin-positive neurons in the ipsilateral and contralateral hypothalamus in sham, exposed-clean, and exposed-dirty animals. Bars represent means ± 1 SEM.

the different groups. Different types of stimulations, such as insulin-induced hypoglycemia, caffeine, and stress, have been shown to activate orexin neurons in the hypothalamus evaluated by measuring c-Fos expression in orexin neurons [46]. It is possible that changes in the orexin system are mediated by activation of the existing orexin neurons rather than the induction of new orexin neurons. Another possibility is that there is an increased orexin projection to the target areas, since orexin neurons project to a wide range of brain areas including the inferior colliculus and the ventral cochlear nucleus [26]. Therefore, an increase in orexin neurotransmission in these auditory brain areas might be related to tinnitus. Further studies are needed to examine these possibilities following acoustic trauma-induced tinnitus.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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References

[1] J. Shargorodsky, G. C. Curhan, and W. R. Farwell, "Prevalence and characteristics of tinnitus among US adults," *The American Journal of Medicine*, vol. 123, no. 8, pp. 711–718, 2010.

[2] A. Axelsson and A. Ringdahl, "Tinnitus—a study of its prevalence and characteristics," *British Journal of Audiology*, vol. 23, no. 1, pp. 53–62, 1989.

[3] S. I. Erlandsson, L. R. M. Hallberg, and A. Axelsson, "Psychological and audiological correlates of perceived tinnitus severity," *Audiology*, vol. 31, no. 3, pp. 168–179, 1992.

[4] K. M. Holgers, S. Zöger, and K. Svedlund, "Predictive factors for development of severe tinnitus suffering-further characterisation," *International Journal of Audiology*, vol. 44, no. 10, pp. 584–592, 2005.

[5] E. Wallhäusser-Franke, J. Brade, T. Balkenhol, R. D'Amelio, A. Seegmüller, and W. Delb, "Tinnitus: distinguishing between subjectively perceived loudness and tinnitus-related distress," *PLoS ONE*, vol. 7, no. 4, Article ID e34583, 2012.

[6] H. Belli, S. Belli, M. F. Oktay, and C. Ural, "Psychopathological dimensions of tinnitus and psychopharmacologic approaches in its treatment," *General Hospital Psychiatry*, vol. 34, no. 3, pp. 282–289, 2012.

[7] B. Langguth, "A review of tinnitus symptoms beyond "ringing in the ears": a call to action," *Current Medical Research and Opinion*, vol. 27, no. 8, pp. 1635–1643, 2011.

[8] J. M. Malouff, N. S. Schutte, and L. A. Zucker, "Tinnitus-related distress: a review of recent findings," *Current Psychiatry Reports*, vol. 13, no. 1, pp. 31–36, 2011.

[9] S. Zöger, J. Svedlund, and K. Holgers, "Relationship between tinnitus severity and psychiatric disorders," *Psychosomatics*, vol. 47, no. 4, pp. 282–288, 2006.

[10] J. Harrop-Griffiths, W. Katon, R. Dobie, C. Sakai, and J. Russo, "Chronic tinnitus: association with psychiatric diagnoses," *Journal of Psychosomatic Research*, vol. 31, no. 5, pp. 613–621, 1987.

[11] M. D. Sullivan, W. Katon, R. Dobie, C. Sakai, J. Russo, and J. Harrop-Griffiths, "Disabling tinnitus. Association with affective disorder," *General Hospital Psychiatry*, vol. 10, no. 4, pp. 285–291, 1988.

[12] R. Asplund, "Sleepiness and sleep in elderly persons with tinnitus," *Archives of Gerontology and Geriatrics*, vol. 37, no. 2, pp. 139–145, 2003.

[13] A. O. Lasisi and O. Gureje, "Prevalence of insomnia and impact on quality of life among community elderly subjects with tinnitus," *Annals of Otology, Rhinology and Laryngology*, vol. 120, no. 4, pp. 226–230, 2011.

[14] T. Crönlein, B. Langguth, P. Geisler, and G. Hajak, "Tinnitus and insomnia," *Progress in Brain Research*, vol. 166, pp. 227–233, 2007.

[15] R. S. Hallam, "Correlates of sleep disturbance in chronic distressing tinnitus," *Scandinavian Audiology*, vol. 25, no. 4, pp. 263–266, 1996.

[16] J. Alster, Z. Shemesh, M. Ornan, and J. Attias, "Sleep disturbance associated with chronic tinnitus," *Biological Psychiatry*, vol. 34, no. 1-2, pp. 84–90, 1993.

[17] M. Langenbach, M. Olderog, O. Michel, C. Albus, and K. Köhle, "Psychosocial and personality predictors of tinnitus-related distress," *General Hospital Psychiatry*, vol. 27, no. 1, pp. 73–77, 2005.

[18] D. M. Nondahl, B. E. K. Klein, R. Klein, K. J. Cruickshanks, T. S. Tweed, and T. L. Wiley, "Factors predicting severity of tinnitus: a population-based assessment.," *Journal of the American Academy of Audiology*, vol. 16, no. 3, pp. 196–197, 2005.

[19] E. Wallhäusser-Franke, M. Schredl, and W. Delb, "Tinnitus and insomnia: Is hyperarousal the common denominator?" *Sleep Medicine Reviews*, vol. 17, no. 1, pp. 65–74, 2013.

[20] D. Riemann, K. Spiegelhalder, B. Feige et al., "The hyperarousal model of insomnia: a review of the concept and its evidence," *Sleep Medicine Reviews*, vol. 14, no. 1, pp. 19–31, 2010.

[21] M. H. Bonnet and D. L. Arand, "Hyperarousal and insomnia: state of the science," *Sleep Medicine Reviews*, vol. 14, no. 1, pp. 9–15, 2010.

[22] H. Zenner and I. M. Zalaman, "Cognitive tinnitus sensitization: behavioral and neurophysiological aspects of tinnitus centralization," *Acta Oto-Laryngologica*, vol. 124, no. 4, pp. 436–439, 2004.

[23] C. J. Harvey, P. Gehrman, and C. A. Espie, "Who is predisposed to insomnia: a review of familial aggregation, stress-reactivity, personality and coping style," *Sleep Medicine Reviews*, vol. 18, no. 3, pp. 237–247, 2014.

[24] M. T. Minen, J. Camprodon, R. Nehme, and Z. Chemali, "The neuropsychiatry of tinnitus: a circuit-based approach to the causes and treatments available," *Journal of Neurology, Neurosurgery & Psychiatry*, 2014.

[25] T. E. Scammel and C. J. Winrow, "Orexin receptors: pharmacology and therapeutic opportunities," *Annual Review of Pharmacology and Toxicology*, vol. 51, pp. 243–266, 2011.

[26] M. A. Greco and P. J. Shiromani, "Hypocretin receptor protein and mRNA expression in the dorsolateral pons of rats," *Molecular Brain Research*, vol. 88, no. 1-2, pp. 176–182, 2001.

[27] X.-B. Gao and A. H. Wang, "Experience-dependent plasticity in hypocretin/orexin neurones: re-setting arousal threshold," *Acta Physiologica*, vol. 198, no. 3, pp. 251–262, 2010.

[28] C. J. Winrow, A. L. Gotter, C. D. Cox et al., "Pharmacological characterization of MK-6096—a dual orexin receptor antagonist for insomnia," *Neuropharmacology*, vol. 62, no. 2, pp. 978–987, 2012.

[29] S. M. Richey and A. D. Krystal, "Pharmacological advances in the treatment of Insomnia," *Current Pharmaceutical Design*, vol. 17, no. 15, pp. 1471–1475, 2011.

[30] S. S. Campbell and I. Tobler, "Animal sleep: a review of sleep duration across phylogeny," *Neuroscience & Biobehavioral Reviews*, vol. 8, no. 3, pp. 269–300, 1984.

[31] L. A. Toth and P. Bhargava, "Animal models of sleep disorders," *Comparative Medicine*, vol. 63, no. 2, pp. 91–104, 2013.

[32] G. Cano, T. Mochizuki, and C. B. Saper, "Neural circuitry of stress-induced insomnia in rats," *Journal of Neuroscience*, vol. 28, no. 40, pp. 10167–10184, 2008.

[33] B. Canlon, T. Theorell, and D. Hasson, "Associations between stress and hearing problems in humans," *Hearing Research*, vol. 295, pp. 9–15, 2013.

[34] B. Mazurek, H. Haupt, H. Olze, and A. J. Szczepek, "Stress and tinnitus—from bedside to bench and back," *Frontiers in Systems Neuroscience*, vol. 6, article 47, 2012.

[35] C. A. Bauer and T. J. Brozoski, "Assessing tinnitus and prospective tinnitus therapeutics using a psychophysical animal model," *Journal of the Association for Research in Otolaryngology*, vol. 2, no. 1, pp. 54–64, 2001.

[36] T. J. Brozoski, T. J. D. Spires, and C. A. Bauer, "Vigabatrin, a GABA transaminase inhibitor, reversibly eliminates tinnitus in an animal model," *Journal of the Association for Research in Otolaryngology*, vol. 8, no. 1, pp. 105–118, 2007.

[37] Y. Zheng, E. Hamilton, L. Stiles et al., "Acoustic trauma that can cause tinnitus impairs impulsive control but not performance accuracy in the 5-choice serial reaction time task in rats," *Neuroscience*, vol. 180, pp. 75–84, 2011.

[38] Y. Zheng, E. McNamara, L. Stiles, C. L. Darlington, and P. F. Smith, "Evidence that memantine reduces chronic tinnitus caused by acoustic trauma in rats," *Frontiers in Neurology*, vol. 3, article 127, 2012.

[39] Y. Zheng, E. Hamilton, S. Begum, P. F. Smith, and C. L. Darlington, "The effects of acoustic trauma that can cause tinnitus on spatial performance in rats," *Neuroscience*, vol. 186, pp. 48–56, 2011.

[40] Y. Zheng, S. Vagal, E. McNamara, C. L. Darlington, and P. F. Smith, "A dose-response analysis of the effects of L-baclofen on chronic tinnitus caused by acoustic trauma in rats," *Neuropharmacology*, vol. 62, no. 2, pp. 940–946, 2012.

[41] F. Yang, J.-C. Wang, J.-L. Han, G. Zhao, and W. Jiang, "Different effects of mild and severe seizures on hippocampal neurogenesis in adult rats," *Hippocampus*, vol. 18, no. 5, pp. 460–468, 2008.

[42] J. E. Malberg, A. J. Eisch, E. J. Nestler, and R. S. Duman, "Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus," *The Journal of Neuroscience*, vol. 20, no. 24, pp. 9104–9110, 2000.

[43] K. Nixon and F. T. Crews, "Temporally specific burst in cell proliferation increases hippocampal neurogenesis in protracted abstinence from alcohol," *Journal of Neuroscience*, vol. 24, no. 43, pp. 9714–9722, 2004.

[44] M. H. Kutner, C. J. Nachtsheim, J. Neter, and W. Li, *Applied Linear Statistical Models*, Applied Linear Statistical Models, McGraw-Hill Irwin, Boston, Mass, USA, 2005.

[45] Y. Zheng, E. Hamilton, E. McNamara, P. F. Smith, and C. L. Darlington, "The effects of chronic tinnitus caused by acoustic trauma on social behaviour and anxiety in rats," *Neuroscience*, vol. 193, pp. 143–153, 2011.

[46] A. Kiss, "Immobilization induced fos expression in the medial and lateral hypothalamic areas: a limited response of hypocretin neurons.," *Ideggyógyászati szemle*, vol. 60, no. 3-4, pp. 192–195, 2007.

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Review Article

Prevention and Restoration of Hearing Loss Associated with the Use of Cisplatin

Felician Chirtes[1] and Silviu Albu[2]

[1] *Otolaryngology Department, Military Hospital Cluj-Napoca, 22 General Traian Mosoiu Street, 3400 Cluj-Napoca, Romania*
[2] *II-nd Department of Otolaryngology, Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca, Strada Republicii 18, 400015 Cluj-Napoca, Romania*

Correspondence should be addressed to Silviu Albu; silviualbu63@gmail.com

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Background. Cisplatin is a well known platinum-based chemotherapeutic agent used for the treatment of various malignant tumours. A frequent side effect of cisplatin therapy is ototoxicity. Unfortunately, currently there are no available treatments. *Material and Methods*. Experimental, clinical studies and reviews published between 2004 and 2014 in the English medical literature concerning ototoxicity were selected using Medline, PubMed, and Google Scholar databases. Inclusion criteria were cisplatin-induced ototoxicity and therapy aimed at preventing or curing this disorder. Molecular mechanisms and clinical, audiological, and histological markers of cisplatin-induced ototoxicity are described. Moreover, experimental and clinical strategies for prevention or treatment of hearing loss were also reviewed. *Results and Discussion*. Experimental studies demonstrate a wide range of otoprotective molecules and strategies efficient against cisplatin-induced hearing loss. However, only dexamethasone proved a slight otoprotective effect in a clinical study. *Conclusion*. Further research must be completed to bring future therapeutic options into clinical setting.

1. Introduction

Cisplatin (cis-diamminedichloroplatinum II) is a well-known chemotherapeutic alkylating agent very effective in the treatment of various malignant tumors, especially squamous cell cancers of the head and neck regions, in both the pediatric and adult age groups [1]. Though synthesized already since 1845 by Peyrone owing to him its name of Peyrone's salt, only in the late 1960s was it used clinically in oncologic therapy for head and neck, lung, bladder, cervical, ovarian, testicular, and gastrointestinal cancers, as well as malignant gliomas and metastatic cancers such as melanoma, mesothelioma, and those of the prostate and breast [2]. One of its major side effects is irreversible neurosensorial hearing loss which affects ears symmetrically: high frequencies in the first place followed by low, speech range frequencies in a dose related and cumulative fashion. Cisplatin-induced hearing loss is favoured either by preexisting afflictions like hypoalbuminemia, anaemia, renal failure, and noise-induced hearing loss or by risk factors like therapy with loop diuretics, aminoglycoside antibiotics, radiotherapy fields which includes the inner ear, extreme ages (very young or very old), duration and dose schedule of cisplatin infusion, and genetic factors. Between 11% and 97% of patients treated with cisplatin develop hearing loss, with an average incidence of 62% [3]. Cisplatin-induced tinnitus is not an infrequent occurrence either. Ototoxicity may occur within hours to days after cisplatin administration. As subsequent multiple cisplatin regimens for the control of cancer may be necessary, side effects should be prevented or treated without reducing the efficacy of the antitumor mechanisms.

2. Material and Methods

A review of the literature from 2004 to 2014 was performed, using the Medline, PubMed, and Google Scholar databases. The search terms included cisplatin-induced hearing loss, protective therapy for inner ear diseases, intratympanic therapy, systemic therapy, and gene therapy. Only original experimental and clinical research papers were included. A total of 43 relevant papers were selected for the present review.

Table 1: Mechanisms of ototoxicity.

Cellular mechanisms of ototoxicity	Molecular mechanisms of ototoxicity
(i) Damage to outer hair cells (ii) Damage to supporting cells (iii) Damage to marginal cells of stria vascularis (iv) Damage to the spiral ligament (v) Damage to spiral ganglion cells	(i) Creation of reactive oxygen species (ii) Depletion of antioxidant glutathione and its regenerating enzymes (iii) Increased rate of lipid peroxidation (iv) Oxidative modifications of proteins (v) Nucleic acids damage by caspase system activation (vi) S-Nitrosylation of cochlear proteins

3. Results

3.1. Mechanisms of Cisplatin Ototoxicity. Cisplatin inflicts injuries mainly to outer hair cells, progressing from the third to the first row and to some extent to inner hair cells of the organ of Corti in the basal turn of the cochlea followed by alterations in sensorial cells situated in the apex. Cisplatin also targets supporting cells, marginal cells of the stria vascularis, and the spiral ligament [4]. The vestibular organs are not spared, nor are spiral ganglion cells in experimental conditions [5]. The ensuing hearing loss may be very disabling to patients whose communication is already impaired due to cancers of the head and neck. Hearing loss is also a common cause for depression and reduction of the quality of life [6].

Molecular mechanisms of cisplatin-induced hearing loss involve the creation of reactive oxygen species and depletion of antioxidant glutathione and its regenerating enzymes as well as increased rate of lipid peroxidation, oxidative modifications of proteins, nucleic acids damage by caspase system activation [3], and S-nitrosylation of cochlear proteins and resulting apoptosis of inner ear cells [7].

Oxygen free radicals in the cochlea are produced principally in the wake of nicotinamide adenine dinucleotide phosphate oxidase 3 isoform (NOX3) activation. NOX3 is known to be upregulated by cisplatin. Cochlear tissues fight the oxidative stress by means of antioxidant defence systems including glutathione, glutathione reductase, superoxide dismutase, and catalase [1]. Cisplatin-induced disturbance of potassium uptake and secretion in the stria vascularis has also been suggested, leading to impairment of the function of outer and inner hair cells in the organ of Corti, with alteration of the endocochlear potential and subsequent hearing loss [1]. A summary of mechanisms of cisplatin associated ototoxicity is displayed in Table 1.

The extent of ototoxicity due to cisplatin administration can be assessed clinically through measurements of hearing loss by means of pretreatment and follow-up serial audiologic tests and experimentally through histological examinations [8]. The former include pure tone (normal frequency and extended high-frequency range), speech and impedance audiometry, auditory brainstem responses, and Distortion Product Otoacoustic Emissions (DPOAEs) testing. DPOAEs reflect early injuries to outer hair cells in the organ of Corti thereby allowing monitoring and early detection of cisplatin ototoxicity during cancer treatment [9, 10]. Cisplatin early ototoxic effects cause hearing impairment in the high frequencies at 6 kHz, 8 kHz, and above as measured by conventional or extended high-frequency pure tone audiometry [11]. Multiple doses of cisplatin worsen hearing, ultimately affecting the speech frequency range (500–4000 Hz). Since DPOAEs measurement is based on the integrity of outer hair cells which is affected by cisplatin therapy before elevation of auditory threshold as measured by pure tone audiometry, DPOAE testing is more sensitive than the latter for the detection of cisplatin-induced ototoxicity. Moreover, the results provided by DPOAEs, as an objective testing, are not influenced by the ability of the deteriorating cancerous patient to respond to the sound stimulus [9]. In small children who undergo cisplatin administration for cancer treatment and who cannot cooperate for pure-tone testing because of their younger age and poor cognitive ability, either sound field behavioural testing or DPOAEs can be performed [12].

In experimental animals, hearing loss after cisplatin treatment can be assessed by audiological study of the auditory brainstem responses where threshold measurement defines the lowest intensity of sound stimulus that evokes a clear, visually detectable, reproducible waveform [4].

Histologic examinations of inner ear after cisplatin administration reveal destruction primarily of outer hair cells and to some extent inner hair cells and associated nerves, degeneration of the vestibular organs, stria vascularis edema, and detachment of the myelin sheath of the spiral ganglion cells [5]. Light microscopy of cochlear samples obtained from animals receiving cisplatin shows loss of hair cells with collapse of the tunnel of Corti and Nuel's space. In cisplatin treated animals' cochlea the scanning electron microscopy detects damage and loss of stereocilia of the hair cells as well as rupture of the cuticular plate [13]. The damage is especially noticed in the high frequency region of the cochlea (i.e., the basal turn) probably due to a base to apex gradient of the cisplatin ototoxicity [14]. Tumour necrosis factor-alpha (TNF-α) and other inflammatory cytokines (IL-8, IL-6) were also detected by immunostaining in the outer hair cells, stria vascularis, spiral ligament, and spiral ganglion neurons in cisplatin exposed cochleae [4].

Cisplatin pharmacokinetics in the inner ear after intravenous injection is influenced by its strong binding to the plasma proteins rendering a large part of it nonactive and by the barrier systems in the cochlea, the blood-perilymph barrier, separating blood from perilymph, and the intrastrial fluid-blood barrier, separating blood from endolymph [15]. The amount of free chemotherapeutic agent reaching targets in the cochlea is responsible for the ototoxic effect and consequent hearing loss. High frequency audiometric

Table 2: Treatment of cisplatin ototoxicity.

Preventive treatment for cisplatin ototoxicity	Restorative treatment for cisplatin ototoxicity
Treatment of hypoalbuminemia, anemia, renal failure Intratympanic dexamethasone Transtympanic L-N-acetylcysteine Resveratrol	Thiol compounds Sertraline Hyperbaric oxygen therapy

thresholds are initially affected. When doses in excess of 100 mg/m^2 are used the hearing impairment may progress from high frequencies to involve the middle frequencies. Reducing the total amount of cisplatin by limitation of the total dose per cycle, dose intensity and the cumulative dose would diminish the antitumor effect which is not desirable. Various otoprotective compounds have been tested both in experimental animals and humans. If given systemically, they should be nontoxic, must attain efficient concentrations in the inner ear to protect labyrinthine tissues from cisplatin ototoxicity, and should not hamper the antitumor effect of cisplatin. Higher concentrations of otoprotective molecules can be achieved by intratympanic administration. This latter route provides direct access of the protective agents to inner ear structures while avoiding systemic side effects and interference with the antineoplastic activity of cisplatin [16].

3.2. Experimental Studies. Several otoprotective molecules and strategies against cisplatin-induced ototoxicity have been tested in experimental animals. Some, like diethyldithiocarbamate, exerted important side effects in humans [16]. A summary of preventative and restorative treatment options is presented in Table 2.

Hyperbaric oxygen therapy consists of intermittent inhalations of 100% oxygen at a pressure higher than 1 atm and is used as adjuvant therapy in pathological processes like soft tissue infections, radiation injury, gas gangrene, and decompressive disease. Its main effect is tissue hyperoxygenation through plasma dissolved oxygen and was successfully tested in guinea pigs as a protective agent against cisplatin ototoxicity. Efficacy of otoprotection by hyperbaric oxygen therapy after cisplatin administration was evaluated by otoacoustic emissions following hyperbaric treatment and by scanning electron microscopy examination. The study confirmed that cisplatin induces dose-dependent cochlear alterations consisting of cellular lesions and significant hair damage in outer hair cells. Analysis of anatomical changes in cochlear outer hair cells indicated signs of otoprotection against cisplatin in animals treated with hyperbaric oxygen therapy although in functional studies distortion product acoustic emission were absent reflecting a certain degree of hearing loss, most probably reversible and related to experimental artefacts. The study concluded that hyperbaric oxygen therapy has otoprotective effects against cisplatin-induced ototoxicity. However, further studies are necessary to test other effects of high pressure oxygen on the cochlea [17].

Another study investigated the effect of epigallocatechin gallate (EGCG) on the transcription factor STAT1, an important mediator of cell death. STAT1 phosphorylation is involved in both hair cells and support cells transformation after experimental exposure of mouse utricule to cisplatin. EGCG proved its efficiency as an otoprotective agent against cisplatin ototoxicity due to its inhibition of STAT1. The hypothesis was further supported by the failure of EGCG to provide protection against cisplatin in STAT1-deficient mice [18].

Former studies showed that lactate injected intratympanically in guinea pigs treated with ototoxic levels of cisplatin allowed near total preservation of otoacoustic emissions [19]. Since lactate is a part of Ringer solution, safely used in human subjects, and has the smallest molecular weight among other antioxidants which facilitates transport across the round window membrane, a further study was conducted to prove its otoprotective effect against cisplatin ototoxicity when injected intratympanically, before intraperitoneal cisplatin administration. The molecular protective mechanism is based on the enzyme lactate dehydrogenase located in the mitochondria of outer hair cells. The conversion of lactate to pyruvate in the presence of the enzyme leads to the formation of nicotinamide adenine dinucleotide (NADH) which is a natural antioxidant that may be involved in reducing toxic effects of oxygen reactive species resulted at cellular level following cisplatin therapy. Electron microscopy examinations of guinea pig cisplatin-insulted inner ears pretreated with lactate showed partial preservation of outer hair cells stereocilia, more significant at midfrequencies (2000–4000 HZ) but not statistically significant at higher frequencies. The study used auditory brainstem responses recordings which is a more sensible method than otoacoustic emissions testing, a fact that explains the differences in otoprotective effect reported by previous studies. The same study investigated the otoprotective effect of intratympanic N-acetylcysteine injections as well as its systemic diffusion following the administration. The results showed that high concentration of intratympanic N-acetylcysteine is not reliable for otoprotection against cisplatin ototoxicity since it caused more middle and inner ear damage than cisplatin alone. Yet, N-acetylcysteine did not diffuse systemically when applied to the middle ear. This was confirmed by high-performance liquid chromatography testing of blood samples taken from the venous system of the experimental animals after intratympanic injections of N-acetylcysteine. This latter outcome proves that the intratympanic route of administration would be safe and prevent inactivation of antitumor effect of cisplatin by binding between the thiol moiety of N-acetylcysteine and the platinum-containing molecule of the chemotherapeutic drug [20].

Most of the existing studies focus on exogenous administration of antioxidants. Pharmacological activation of intrinsic defence mechanisms against oxidative stress in the inner

ear caused by cisplatin therapy also proved helpful as showed by an experimental animal study using systemic administration of thiamine pyrophosphate (TPP). Thiamine pyrophosphate functions as coenzyme for peroxisomes being a crucial factor for energy metabolism, antioxidation, and myelinisation of nerve cells. Its intraperitoneal injection increased the level of natural antioxidants like glutathione and antioxidant enzymes (superoxide dismutase, glutathione peroxidase and glutathione reductase) and reduced the content of malonildialdehyde, an indicator of lipid peroxidation following increased levels of oxygen reactive species resulting from cisplatin toxicity. The histologic evaluation of cochleae harvested from TPP treated animals showed preservation of the morphology of the organ of Corti and outer hair cells and no destruction of spiral ganglion cells and stria vascularis following cisplatin therapy [5].

The wide range of therapeutic molecules studied for their otoprotective effect against cisplatin-induced ototoxicity also includes sertraline, an antidepressant with neuroprotective effects in rats [6]. The selective serotonin reuptake inhibitor also has antioxidant effects, stimulates neurogenesis, and increases antiapoptotic protein levels [21]. It has been documented by distorsion product otoacoustic emissions recordings that oral administration of sertraline in cisplatin-treated rats prevented hearing loss above 5000 Hz, in a statistically significant manner. Besides, sertraline would be beneficial to patients whose communication abilities are already deteriorated either by the cancer itself or by the treatment modalities and, therefore, feel depressed [6].

An experimental single dose model of cisplatin ototoxicity in guinea pigs showed the otoprotective effect of systemic histone deacetylase inhibitor sodium butyrate. Distortion product otoacoustic emissions testing were chosen to provide a sensitive assay of the functional state of outer hair cells after systemic cisplatin insult on the cochlea. The systemic administration of the otoprotective agent avoided the side effects of the more invasive tympanic local route of administration. Moreover, sodium butyrate did not interfere with the tumoricidal effect of cisplatin providing both protections from reactive oxygen species and a certain degree of antitumor activity according to former reports. Acetylation of different cell proteins, including histones, is responsible both for the protective effect against oxidative stress and for the cell division inhibition and subsequent anticancer activity. The experiment's weak point is that it only showed the effect of sodium butyrate in a single dose of cisplatin model whereas in clinical practice cisplatin is typically given repeatedly at a couple of weeks intervals for several months [22].

The unique isoform of NADPH oxidase, NOX3, found in the cochlea and its involvement in the generation of reactive oxygen species was at the base of an animal study showing the efficacy of short interfering RNA in preventing cisplatin ototoxicity by reducing the expression of NOX3 in outer hair cells, spiral ganglion cells, and stria vascularis in the rat. Auditory brainstem responses were used to certify reduced threshold shifts in cisplatin treated animals who received transtympanic NOX3 siRNA. Since cisplatin administration has been previously associated with upregulation of NOX3 in the inner ear, Nox3 is thought to be a major source of free radicals in the cochlea following cisplatin exposure. The resulting free radicals initiate the inflammatory process in the cochlea by activating signal transducer and activator of transcription-1 (STAT1), followed by activation of p53 and increase in inflammatory mediators like TNF-alpha and interleukin-1β [23]. A single transtympanic injection of siRNA attenuated cisplatin ototoxicity by suppressing inflammation in a dose-related manner. It hampered cisplatin-induced auditory brainstem responses threshold shift and higher doses allowed for complete morphological preservation of outer hair cells as proven by scanning electron microscopy examinations of the rat cochleae [24].

Among various strategies that have been devised in experimental settings to prevent cisplatin ototoxicity, minocycline, a tetracycline derivative, proved its partial efficacy in vivo and in vitro. The anti-inflammatory and neuroprotective properties of minocycline have been previously reported. The biochemical mechanisms involve caspase-1 and caspase-3 inhibition, which decreases the amount of interleukin-1 and prevents apoptosis. The protective effect of minocycline has been tested both on cisplatin treated cell cultures and in experimental animals which underwent cisplatin intraperitoneal therapy after systemic administration of the otoprotective agent. Cell viability assays showed that minocycline had a protective effect against cisplatin toxic action. Yet, minocycline failed to protect cells at higher concentrations of cisplatin. Recordings of auditory brainstem responses and evaluation of the scanning electron microscopy sections of inner ears harvested from minocycline plus cisplatin treated animals indicated a partial preservation of the function and morphology of the outer hair cells as compared to those from animals treated with cisplatin alone [25].

The effect of intraperitoneal administration of erdosteine on cisplatin-induced ototoxicity in a guinea pig model was also studied. Erdosteine is a thiol derivative with established antioxidant properties due to its active sulfhydryl groups following liver first-pass metabolism. Pre- and posttreatment auditory brainstem responses measurements were performed in living animals while outer hair cell counts were analyzed by scanning electron microscopy of the cochleae removed from euthanized animals. Although the study had limitations (i.e., minimal number of animals included, lack of enzymatic activity detection for the main antioxidant enzymatic systems of the inner ear), the results outlined the systemic administration of erdosteine as a promising therapeutic strategy for cisplatin-induced ototoxicity [26].

In a first murine model for cisplatin-induced ototoxicity, it was shown that intratympanic dexamethasone prevents hearing loss in a frequency related manner. Evoked brainstem responses audiometry indicated that 8 kHz and 16 kHz stimulus elicited responses in cisplatin plus dexamethasone treated mice while high frequency stimulus (32 kHz) perception was affected. Apparently, cisplatin had deleterious effects on outer and inner ear cells situated in the basal turn of the cochlea despite intratympanic administration of protective dexamethasone [27]. Further experimental studies supported the finding that cisplatin exerts its damaging effect in a base to apex gradient, lower frequencies being spared for long.

Higher doses of dexamethasone also seem to be more protective than lower doses. Moreover, lower doses of cisplatin allow the naturally present antioxidants to annihilate the resulting reactive oxygen species, explaining the spontaneous hearing threshold recovery even in the absence of protective dexamethasone administration [28].

The intratympanic administration of dexamethasone avoids diminishing the tumoricidal activity of cisplatin. Downregulating apoptosis genes in tumour cells are responsible for this common side effect of systemic steroid therapy [29]. An experimental study conducted on cisplatin treated guinea pigs asserted the safety of intratympanic dexamethasone based on audiologic and histologic results. Auditory brainstem responses testing, optic microscopic and scanning electron microscopic examinations of cochleae showed no significant differences between intratympanic dexamethasone-treated animals and saline-treated controls. Dexamethasone administered intratympanically proved efficacious in protecting the labyrinth against cisplatin-induced ototoxicity as shown by reduced auditory brainstem responses threshold shifts and unaltered histological inner ear structures. The molecular mechanisms involve increased expression of Na/K channels and aquaporins in the endolymphatic sac and the tissues around the endolymphatic spaces. The study's results also suggest that giving the intratympanic dexamethasone one hour before the cisplatin administration provides the best protection (total protection) against the ototoxic insult by the alkylating agent compared to dexamethasone injections one day prior to cisplatin administration (partial protection) [14]. According to another study, in order for the dexamethasone to exert a protective effect against cisplatin ototoxicity, the timing of administration of the two drugs should be highly synchronized so that the peak concentration of dexamethasone in the perilymph should correlate with the peak concentration of the chemotherapeutic agent [3].

Otoprotection with dexamethasone against cisplatin-induced age-related hearing loss was investigated in a guinea pig model following observations that persons older than 65 years account for more than half of the newly diagnosed malignancies. Hearing loss due to the aging process shares the same cause (i.e., oxidative stress) with hearing loss due to ototoxic chemotherapeutic agents. A single dose of cisplatin was administered intraperitoneally in old mice preceded and followed by dexamethasone injected intratympanically to counteract the cisplatin toxic effect on inner ear hair cells. Pre- and posttreatment auditory brainstem responses were recorded to evaluate hair cell function. The results of the study pointed out that no synergistic action between age related hearing loss and cisplatin-induced hearing loss exists since threshold shifts were smaller in older animals than those in young mice. Another finding of the study was that the protective effect of dexamethasone against cisplatin-induced ototoxicity was a function of stimulus frequency in old mice. Susceptibility to otoprotective effect of dexamethasone was higher in mid to basal cochlear regions (at and above 24 kHz) in old mice, whereas in young mice, dexamethasone bestowed more protection in apical regions of the cochlea (at 16 kHz and below). Age-related changes of the mechanism of distribution of dexamethasone in scala tympani perilymph after round window membrane application in guinea pigs seem to account for the frequency dependent otoprotective effect [30].

Intratympanic dexamethasone failed to protect against cisplatin-induced ototoxicity in a multidose cisplatin ototoxicity mouse model. The study was prompted by typical clinical protocols of cancer treatments which require administration of multiple, smaller cisplatin doses, exerting their curative effect through cumulative dosing. Contrary to previous experimental studies, the mice received five doses of cisplatin throughout five days, mimicking the cumulative exposure seen in malignant tumours treatment. Intratympanic dexamethasone was administered on the same days as the intraperitoneal cisplatin. The results mirrored by auditory brainstem responses threshold measurements demonstrated continued change in hearing thresholds several weeks after cisplatin exposure and no protective effect of intratympanic dexamethasone against cisplatin ototoxicity [31].

An experimental study focused on systemic administration of steroid for protection against cisplatin-induced ototoxicity showed no otoprotection following several days' prophylaxis with a high dose dexamethasone treatment. Only a slight decrease of TNF-alpha expression in the cochlea was demonstrated by immunohistochemical staining of anatomical samples harvested from systemic cisplatin plus dexamethasone treated animals. Dexamethasone also seemed to protect stria vascularis from morphological alterations, probably owing this effect to higher concentrations of steroid in the lateral cochlear wall following increased cochlear flow and a naturally highly vascularised stria vascularis. Still, a functional otoprotective effect of systemic dexamethasone against cisplatin-induced hearing loss was not observed [4].

Among naturally occurring molecules, Rosmarinic acid, a water-soluble polyphenolic compound extracted from Dansam-Eum, was tested for its protective effect against cisplatin-induced ototoxicity in laboratory settings. The results of the study showed that Rosmarinic acid inhibited cisplatin-induced caspase-1 activation providing protection against stereocilia loss in the primary organ of Corti explants [32].

Another natural remedy, the Maytenus ilicifolia aqueous extract, was evaluated for its possible otoprotection in guinea pigs. Despite the well-known South America plant's antioxidant effects (due to the presence of flavonoids and alkaloids), functional tests did not demonstrate any protective action on the cisplatin exposed cochleae. Yet, the extract improved the clinical status and weight of guinea pigs and diminished mortality after cisplatin exposure [33].

Resveratrol, a polyphenol found in grape skin and seed, has antioxidant, neuroprotective, and dose dependent anti-apoptotic properties [34]. Recent experimental research pointed out the preventive effect of resveratrol against cisplatin induced ototoxicity. An in vitro study on House Ear Institute-Organ of Corti 1 cell line showed that resveratrol in low doses prevented ototoxicity mainly influencing apoptotic gene expression but proved cytotoxic effect in high doses [34]. Two other studies showed conflicting results. Thus, high doses of oral resveratrol administered to mice seem to

Table 3: Clinical trials currently underway (http://clinicaltrials.gov/ct2/results?term=cisplatin+ototoxicity).

Trial title	Trial status
Protection from cisplatin ototoxicity by lactated Ringers	Completed
Alpha-Lipoic acid in preventing hearing loss in cancer patients undergoing treatment with cisplatin	Completed
The protective effect of Ginkgo Biloba extract on cisplatin-induced ototoxicity in humans	Completed
Preventing nephrotoxicity and ototoxicity from osteosarcoma therapy	Recruiting
Sodium thiosulfate in preventing hearing loss in young patients receiving cisplatin for newly diagnosed germ cell tumor, hepatoblastoma, medulloblastoma, neuroblastoma, osteosarcoma, or other malignancies	Active, not recruiting
SPI-1005 for prevention and treatment of chemotherapy induced hearing loss	Not yet recruiting

enhance cisplatin ototoxicity [35] whereas systemic administration of lower doses of resveratrol provided significant protection to the cochlea against cisplatin [36].

3.3. Clinical Studies. Intratympanic dexamethasone was clinically tested for its otoprotective effect in patients suffering from neoplastic diseases for which the treatment protocol included cisplatin. Intratympanic dexamethasone has already been tested clinically for the treatment of idiopathic sudden sensorineural hearing loss and Meniere disease [37]. Intratympanic administration of steroids avoids significant systemic side effects like hyperglycaemia, peptic ulcers, hypertension, osteoporosis, and psychosis. The intratympanic route also provides higher concentrations of drug in the inner ear fluids and prevents significant interference between dexamethasone, which is known to reduce efficacy of chemotherapeutic agents, and cisplatin. Patients enrolled in the study underwent unilateral intratympanic dexamethasone administration prior to every cisplatin treatment session, with the contralateral ear used as a control. Serial follow-up audiometry and distorsion product otoacoustic emissions testing were performed to check the functional state of both study and control ears. The statistically significant results showed that intratympanic dexamethasone is slightly protective against cisplatin-induced hearing loss at 6000 Hz and decreases the outer hair cells dysfunction in the frequency range of 4000 to 8000 Hz. The conclusion of the study is that intratympanic dexamethasone has minimal effect towards reducing cisplatin ototoxicity. Further studies using different concentrations of dexamethasone and a perfect timing of administration are necessary to investigate its role in preventing hearing loss after cisplatin therapy [3].

Transtympanic L-N-acetylcysteine was also clinically tested in head and neck cancer patients undergoing cisplatin therapy. Thiol compounds are known to either directly bind cisplatin or act as free radical scavengers. Based on that, their intratympanic administration was suggested to avoid the decrease of oncologic effectiveness of cisplatin and to reduce the oxidative stress caused by it. Intratympanic L-N-acetylcysteine was well tolerated by patients receiving multiple doses of cisplatin as part of their oncologic treatment. The relation between dose and otoprotection was not taken into account. Higher concentrations may have yielded better otoprotection. The study protocol required the L-NAC injection to be approximately 1 hour before systemic administration of cisplatin, for the sake of better timing. The outcome of the pure tone audiometry testing at 1 and 2 months after the last cycle of cisplatin showed that L-N-acetylcysteine was overall not significantly otoprotective. Still, hearing loss was reduced in two patients out of eleven who completed the study. The study protocol had several challenges like the difficulty in maintaining high enough concentrations of aqueous solution of L-N-acetylcysteine in the middle ear due to the technique of administration. Another study flaw was the different initial hearing thresholds due to preexisting hearing loss [38].

Few clinical studies tested systemic otoprotective molecules for preventing cisplatin-induced hearing loss. Amifostine, a phosphorylated aminothiol designed to protect against radiation damage, was known to counteract the toxic effect of different anticancer treatments without interfering with the tumoricidal effect. Although significant protection of amifostine against haematological toxicity after high dose carboplatin therapy in a child with medulloblastoma was reported [39], a clinical study considering systemic administration of amifostine failed to prove any otoprotective effect against cisplatin-induced hearing loss in a group of pediatric patients treated with cisplatin associated with other chemotherapeutic agents [40]. Clinical trials currently underway as documented by their registration in a public database (http://clinicaltrials.gov/ct2/results?term=cisplatin+ototoxicity/) are listed in Table 3.

3.4. Otopharmacogenetics. The well-isolated inner ear organ makes it prone to targeted genetic therapies. Viral or nonviral gene vectors can be delivered through a transtympanic route without the risk of dispersing them and reaching other tissues with subsequent undesirable genetic alteration. Long term effects after single administration, cellular selectivity, and replacement of genetically flawed nucleic acid sequences are the main benefits of gene therapy. Common viral vectors include herpes simplex virus, recombinant adenoassociated virus, recombinant adenovirus, and adenovirus, used to amplify the expression of targeted genes. Cells are infected with the vectors (transfection) which transfer genes whose expressed proteins influence important processes like growth, oxidative stress, and apoptosis. Chemical transfection can also be achieved with plasmid vectors. Short interfering RNA can be used to shut down target genes.

Among inner ear target genes dealt with by the gene therapy studies are ATOH1 (Math1), CAT (catalase), SOD1 (Cu/Zn superoxide dismutase), SOD2 (Mn superoxide dismutase), BDNF (brain-derived neurotrophic factor), HGF

(hepatocyte growth factor), GJB2 (gap junction protein), Bcl-xL (B-cell lymphoma-extra large), FGF2 (basic fibroblast growth factor). The gene therapy modifies the synthesis of a wide range of proteins including neurotrophic factors (NTF3, GDNF), apoptosis mediators (XIAP, BCL2), oxidases (NADPH, NOX1, NOX3, NOX4), an antioxidant response regulator (Nfe2l2), a cytoprotective enzyme (HO-1), copper transporters (Ctr1), a nonselective cation channel (Trpv1), and protein Otospiralin (Otos).

Cisplatin-exposed tissues can benefit from genetically induced upregulation of neurotrophic factors, inhibition of apoptosis, and generation of endogenous antioxidant enzymes.

Experimental animal studies and in vitro experiments show the efficacy of gene therapy for cisplatin-induced ototoxicity. Clinical applications require further studies regarding safety, immunogenicity, and consequences of genetic manipulation [41].

Another strategy to avoid cisplatin-induced hearing loss would be the pretreatment genotyping to find out patients at risk for the ototoxic effect of cisplatin [42]. Genetic variants (polymorphism) of different protein systems (Thiopurine S-methyltransferase, Catechol-O-methyl transferase, Glutathione-S-transferase with its subclasses M1/T1/P1, Magalia) can stand for the interindividual variability in cisplatin ototoxicity [43].

4. Discussion

Forty-three publications were reviewed concerning prevention or treatment of cisplatin induced ototoxicity. Publications were devised in either experimental or clinical studies. Experimental studies sustained the efficiency of hyperbaric oxygen therapy, epigallocatechin therapy, and intratympanic lactate. The latter two therapies provide exogenous antioxidants while pharmacologic activation of endogenous antioxidants by means of intratympanic thiamine pyrophosphate was consistent with higher levels of natural antioxidants. Oral sertraline, besides its otoprotective effect against cisplatin induced ototoxicity, also has therapeutic value concerning the depression occurring frequently in oncologic patients. Sodium butirate proved its efficiency against cisplatin induced hearing loss in a monodose cisplatin model. Yet, in clinical practice the patient receives multiple doses of cisplatin. The production of endogenous radicals of oxygen species was reduced after intratympanic administration of short interfering RNA which reduces the expression of NOX3 in the cochlea. Minocycline appeared to be efficient only at low doses of cisplatin while systemic erdosteine showed promising results. Dexamethasone in experimental studies combined efficiency against cisplatin induced ototoxicity with preservation of the tumoricidal activity of cisplatin. When older mice were treated, the dexamethasone was more otoprotective at higher frequencies compared to experiments including younger subjects. Rosmarinic acid also proved to be otoprotective while the Maytenus ilicifolia aqueous extract was not. Resveratrol had contradictory effects, systemic low doses, and in vitro administration preventing ototoxicity, whereas high oral doses seem to enhance cisplatin ototoxicity. There also were experimental studies which showed inefficiency of intratympanic N-acetylcysteine and intratympanic and systemic dexamethasone. N-Acetylcysteine also had a damaging effect on middle and inner ear structures.

Clinical studies proved a minor otoprotective effect of intratympanic dexamethasone and no effect of systemic amifostine and intratympanic L-N-acetylcysteine. New perspectives are brought about by genetic therapy using viral vectors and genotyping to anticipate interindividual variability in cisplatin ototoxicity.

5. Conclusion

Hearing loss prevention and treatment during cisplatin therapy for cancer needs further research to find new strategies and optimize old ones. The intratympanic route of administration along with the gene therapy appears to be the most attractive objective for further experimental and clinical studies.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] M. S. Gonçalves, A. F. Silveira, A. R. Teixeira, and M. A. Hyppolito, "Mechanisms of cisplatin ototoxicity: theoretical review," *Journal of Laryngology and Otology*, vol. 127, no. 6, pp. 536–541, 2013.

[2] J. Schacht, A. E. Talaska, and L. P. Rybak, "Cisplatin and aminoglycoside antibiotics: hearing loss and its prevention," *The Anatomical Record*, vol. 295, no. 11, pp. 1837–1850, 2012.

[3] T. Marshak, M. Steiner, M. Kaminer, L. Levy, and A. Shupak, "Prevention of cisplatin-induced hearing loss by intratympanic dexamethasone: a randomized controlled study," *Otolaryngology—Head and Neck Surgery*, vol. 150, no. 6, pp. 983–990, 2014.

[4] S. Waissbluth, P. Salehi, X. He, and S. J. Daniel, "Systemic dexamethasone for the prevention of cisplatin-induced ototoxicity," *European Archives of Oto-Rhino-Laryngology*, vol. 270, no. 5, pp. 1597–1605, 2013.

[5] O. Kuduban, C. Kucur, E. Sener, H. Suleyman, and F. Akcay, "The role of thiamine pyrophosphate in prevention of cisplatin ototoxicity in an animal model," *The Scientific World Journal*, vol. 2013, Article ID 182694, 5 pages, 2013.

[6] M. Ozturk, S. Ucar, F. Sari, S. Erdogan, M. Topdag, and M. Iseri, "Possible protective effect of sertraline against cisplatin-induced ototoxicity: an experimental study," *The Scientific World Journal*, vol. 2013, Article ID 523480, 5 pages, 2013.

[7] S. Jamesdaniel, S. Manohar, and S. Hinduja, "Is S-nitrosylation of cochlear proteins a critical factor in cisplatin-induced ototoxicity?" *Antioxidants & Redox Signaling*, vol. 17, no. 7, pp. 929–933, 2012.

[8] S. A. Alam, K. Ikeda, T. Oshima et al., "Cisplatin-induced apoptotic cell death in *Mongolian gerbil* cochlea," *Hearing Research*, vol. 141, no. 1-2, pp. 28–38, 2000.

[9] P. Eiamprapai, N. Yamamoto, H. Hiraumi et al., "Effect of cisplatin on distortion product otoacoustic emissions in Japanese patients," *Laryngoscope*, vol. 122, no. 6, pp. 1392–1396, 2012.

[10] K. R. Knight, D. P. Kraemer, C. Winter, and E. A. Neuwelt, "Early changes in auditory function as a result of platinum chemotherapy: Use of extended high-frequency audiometry and evoked distortion product otoacoustic emissions," *Journal of Clinical Oncology*, vol. 25, no. 10, pp. 1190–1195, 2007.

[11] C. L. Zuur, Y. J. W. Simis, P. E. M. Lansdaal et al., "Audiometric patterns in ototoxicity of intra-arterial cisplatin chemoradiation in patients with locally advanced head and neck cancer," *Audiology and Neurotology*, vol. 11, no. 5, pp. 318–330, 2006.

[12] R. Warrier, A. Chauhan, M. Davluri, S. L. Tedesco, J. Nadell, and R. Craver, "Cisplatin and cranial irradiation-related hearing loss in children," *The Ochsner Journal*, vol. 12, no. 3, pp. 191–196, 2012.

[13] J. R. García-Berrocal, J. Nevado, R. Ramírez-Camacho et al., "The anticancer drug cisplatin induces an intrinsic apoptotic pathway inside the inner ear," *British Journal of Pharmacology*, vol. 152, no. 7, pp. 1012–1020, 2007.

[14] A. G. Shafik, R. H. Elkabarity, M. T. Thabet, N. B. Soliman, and N. K. Kalleny, "Effect of intratympanic dexamethasone administration on cisplatin-induced ototoxicity in adult guinea pigs," *Auris Nasus Larynx*, vol. 40, no. 1, pp. 51–60, 2013.

[15] V. Hellberg, I. Wallin, H. Ehrsson, and G. Laurell, "Cochlear pharmacokinetics of cisplatin: an in vivo study in the guinea pig," *The Laryngoscope*, vol. 123, no. 12, pp. 3172–3177, 2013.

[16] L. P. Rybak, D. Mukherjea, S. Jajoo, and V. Ramkumar, "Cisplatin ototoxicity and protection: clinical and experimental studies," *Tohoku Journal of Experimental Medicine*, vol. 219, no. 3, pp. 177–186, 2009.

[17] C. Yassuda, A. Righetti, M. Cury, M. Hyppolito, J. A. de Oliveira, and O. Féres, "The role of hyperbaric oxygen therapy (hot) as an otoprotection agent against cisplatin ototoxicity," *Acta Cirurgica Brasileira*, vol. 23, supplement 1, pp. 72–76, 2008.

[18] N. C. Schmitt, E. W. Rubel, and N. M. Nathanson, "Cisplatin-induced hair cell death requires STAT1 and is attenuated by epigallocatechin gallate," *Journal of Neuroscience*, vol. 29, no. 12, pp. 3843–3851, 2009.

[19] W. Choe, N. Chinosornvatana, and K. W. Chang, "Prevention of cisplatin ototoxicity using transtympanic N-acetylcysteine and lactate," *Otology and Neurotology*, vol. 25, no. 6, pp. 910–915, 2004.

[20] M. Nader, Y. Théorêt, and I. Saliba, "The role of intratympanic lactate injection in the prevention of cisplatin-induced ototoxicity," *Laryngoscope*, vol. 120, no. 6, pp. 1208–1213, 2009.

[21] J. E. Malberg and J. A. Blendy, "Antidepressant action: to the nucleus and beyond," *Trends in Pharmacological Sciences*, vol. 26, no. 12, pp. 631–638, 2005.

[22] M. Drottar, M. C. Liberman, R. R. Ratan, and D. W. Roberson, "The histone deacetylase inhibitor sodium butyrate protects against cisplatin-induced hearing loss in guinea pigs," *The Laryngoscope*, vol. 116, no. 2, pp. 292–296, 2006.

[23] T. Kaur, D. Mukherjea, K. Sheehan, S. Jajoo, L. P. Rybak, and V. Ramkumar, "Short interfering RNA against STAT1 attenuates cisplatin-induced ototoxicity in the rat by suppressing inflammation," *Cell Death and Disease*, vol. 2, article e180, 2011.

[24] D. Mukherjea, S. Jajoo, T. Kaur, K. E. Sheehan, V. Ramkumar, and L. P. Rybak, "Transtympanic administration of short interfering (si)RNA for the NOX3 isoform of NADPH oxidase protects against cisplatin-induced hearing loss in the rat," *Antioxidants and Redox Signaling*, vol. 13, no. 5, pp. 589–598, 2010.

[25] C. Lee, J. Shin, and Y. Cho, "Protective effect of minocycline against cisplatin-induced ototoxicity," *Clinical and Experimental Otorhinolaryngology*, vol. 4, no. 2, pp. 77–82, 2011.

[26] S. Waissbluth, I. Dupuis, and S. J. Daniel, "Protective effect of erdosteine against cisplatin-induced ototoxicity in a guinea pig model," *Otolaryngology—Head and Neck Surgery*, vol. 146, no. 4, pp. 627–632, 2012.

[27] G. W. Hill, D. K. Morest, and K. Parham, "Cisplatin-induced ototoxicity: effect of intratympanic dexamethasone injections," *Otology & Neurotology*, vol. 29, no. 7, pp. 1005–1011, 2008.

[28] D. Murphy and S. J. Daniel, "Intratympanic dexamethasone to prevent cisplatin ototoxicity: a guinea pig model," *Otolaryngology: Head and Neck Surgery*, vol. 145, no. 3, pp. 452–457, 2011.

[29] I. Herr, E. Ucur, K. Herzer et al., "Glucocorticoid cotreatment induces apoptosis resistance toward cancer therapy in carcinomas," *Cancer Research*, vol. 63, no. 12, pp. 3112–3120, 2003.

[30] K. Parham, "Can intratympanic dexamethasone protect against cisplatin ototoxicity in mice with age-related hearing loss?" *Otolaryngology—Head and Neck Surgery*, vol. 145, no. 4, pp. 635–640, 2011.

[31] A. L. Hughes, N. Hussain, R. Pafford, and K. Parham, "Dexamethasone otoprotection in a multidose cisplatin ototoxicity mouse model," *Otolaryngology—Head and Neck Surgery*, vol. 150, no. 1, pp. 115–120, 2014.

[32] H.-J. Jeong, Y. Choi, M.-H. Kim et al., "Rosmarinic acid, active component of dansam-eum attenuates ototoxicity of cochlear hair cells through blockage of caspase-1 activity," *PLoS ONE*, vol. 6, no. 4, Article ID e18815, 2011.

[33] C. A. Kasse, O. L. M. Cruz, L. C. N. Iha, H. O. Costa, E. C. Lopes, and F. Coelho, "The use of *Maytenus ilicifolia* to prevent cisplatin-induced ototoxicity," *Brazilian Journal of Otorhinolaryngology*, vol. 74, no. 5, pp. 712–717, 2008.

[34] Y. Olgun, Z. Altun, S. Aktas et al., "Molecular mechanisms of protective effect of resveratrol against cisplatinium induced ototoxicity," *Journal of International Advanced Otology*, vol. 9, no. 2, p. 415, 2013.

[35] Y. Olgun, G. Kirkim, E. Kolatan, and M. Kiray, "Friend or foe? Effect of oral resveratrol on cisplatin ototoxicity," *Laryngoscope*, vol. 124, no. 3, pp. 760–766, 2014.

[36] A. C. Yumusakhuylu, M. Yazici, M. Sari et al., "Protective role of resveratrol against cisplatin induced ototoxicity in guinea pigs," *International Journal of Pediatric Otorhinolaryngology*, vol. 76, no. 3, pp. 404–408, 2012.

[37] A. A. McCall, E. E. L. Swan, J. T. Borenstein, W. F. Sewell, S. G. Kujawa, and M. J. McKenna, "Drug delivery for treatment of inner ear disease: current state of knowledge," *Ear and Hearing*, vol. 31, no. 2, pp. 156–165, 2010.

[38] J. Yoo, S. Hamilton, D. Angel et al., "Cisplatin otoprotection using transtympanic L-N-Acetylcysteine: a pilot randomized study in head and neck cancer patients," *Laryngoscope*, vol. 124, pp. E87–EE94, 2014.

[39] J. D. Borsi, C. Csaki, T. Ferencz, and W. Oster, "Administration of Ethyol (amifostine) to a child with medulloblastoma to ameliorate hematological toxicity of high dose carboplatin," *Anti-Cancer Drugs*, vol. 7, no. 1, pp. 121–126, 1996.

[40] N. Marina, K. W. Chang, M. Malogolowkin et al., "Amifostine does not protect against the ototoxicity of high-dose cisplatin combined with etoposide and bleomycin in pediatric germ-cell tumors," *Cancer*, vol. 104, no. 4, pp. 841–847, 2005.

[41] S. Waissbluth, J. Pitaro, and S. J. Daniel, "Gene therapy for cisplatin-induced ototoxicity: a systematic review of in vitro and experimental animal studies," *Otology & Neurotology*, vol. 33, no. 3, pp. 302–310, 2012.

[42] L. Wyatt and M. Jayne, "Cisplatin-induced ototoxicity and the role of pharmacogenetic testing," *Journal of Pediatric Pharmacology and Therapeutics*, vol. 17, no. 4, pp. 395–399, 2012.

[43] D. Mukherjea and L. P. Rybak, "Pharmacogenomics of cisplatin-induced ototoxicity," *Pharmacogenomics*, vol. 12, no. 7, pp. 1039–1050, 2011.

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Review Article

Cognitive Spare Capacity and Speech Communication: A Narrative Overview

Mary Rudner[1] and Thomas Lunner[1,2,3]

[1] *Linnaeus Centre HEAD, Swedish Institute for Disability Research, Department of Behavioural Sciences and Learning, Linköping University, 581 83 Linköping, Sweden*
[2] *Department of Clinical and Experimental Medicine, Linköping University, 581 83 Linköping, Sweden*
[3] *Eriksholm Research Centre, Oticon A/S, 3070 Snekkersten, Denmark*

Correspondence should be addressed to Mary Rudner; mary.rudner@liu.se

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Background noise can make speech communication tiring and cognitively taxing, especially for individuals with hearing impairment. It is now well established that better working memory capacity is associated with better ability to understand speech under adverse conditions as well as better ability to benefit from the advanced signal processing in modern hearing aids. Recent work has shown that although such processing cannot overcome hearing handicap, it can increase cognitive spare capacity, that is, the ability to engage in higher level processing of speech. This paper surveys recent work on cognitive spare capacity and suggests new avenues of investigation.

1. Introduction

Speech is the main mode of communication for most people. If speech understanding is compromised by noise or hearing impairment, communication may become harder, leading to limitations in social participation. Technical compensation is available in the form of hearing aids. However, although the amplification provided by hearing aids can improve speech understanding in quiet, persons with hearing impairment still have disproportionately large difficulties understanding speech in noise. One of the reasons for this may be that when the cognitive resources required for speech comprehension are engaged in the lower level processes of deciphering the signal, fewer resources may be available for higher level language processing. In other words, cognitive spare capacity is reduced.

1.1. Speech Comprehension. Speech comprehension requires the auditory ability to hear the signal and the cognitive ability to relate this information to the existing knowledge stored in semantic long-term memory [1, 2]. The role of cognition in speech comprehension is reflected in the hierarchical nature of its cortical representation [3, 4].

Speech processing engages a clearly defined cortical network involving the classical language areas in the left inferior frontal cortex and superior temporal gyrus [3, 4]. The primary auditory cortex is sensitive to most sounds and is the first cortical region to be activated during speech perception [4]. Listening to words activates the middle and superior temporal gyri bilaterally and listening to sentences engages regions involved in processing semantics and syntax in the left prefrontal cortex [3]. It has been possible to trace the pathways linking these regions by using animal models [4–6]. These pathways represent different functional streams that take either a ventral route through superior temporal regions to ventrolateral prefrontal cortex or a dorsal route through posterior parietal cortex and dorsolateral prefrontal cortex [6, 7]. One ventral route seems to deal more with conceptual or semantic processing, while there is a dorsal route that is more related to phonological or articulatory processing [6, 7]. Ventral and dorsal routes for syntactic processing have also been proposed [8].

1.2. Hearing Impairment. Around 25% of the population in developed countries has a hearing impairment severe enough to interfere with speech communication [9]. Hearing sensitivity decreases with age such that although only about 2% of individuals in their early twenties have a hearing loss, the prevalence of significant hearing impairment is 40–45% in persons over the age of 65 and exceeds 83% in persons over the age of 70 [10, 11]. Hearing difficulties are associated with long-term absence from work in the working age population [12, 13] and loneliness in the older population [14]. Further, individuals with better cognitive abilities report more hearing difficulties [15, 16], possibly because they have higher expectations of their communication. Even moderate degrees of hearing impairment lead to decrease in neural activity during speech processing and may contribute to grey matter loss in primary auditory cortex [17, 18].

Types of hearing loss are traditionally categorized according to site of lesion: impairment of sound transmission in the external or middle ear is referred to as conductive hearing loss, while other types of hearing loss are referred to as sensorineural. Sensorineural hearing loss can be further subdivided into sensory loss, resulting from impairment of cochlear function, retrocochlear loss, resulting from impairments relating to conduction in the auditory nerve or brainstem, and central losses, resulting from impairments in cortical processing of the auditory signal. Sensorineural hearing loss is the major diagnostic category and includes age-related hearing loss or presbyacusis. These categories are relatively coarse and it has been suggested that they may be inadequate for pinpointing the contribution of hearing loss to communication difficulties under adverse listening conditions [19].

The primary diagnostic tool in audiology is the pure tone audiogram. This method of determining frequency-specific hearing thresholds is based on delivering sine waves of different intensities to each ear and asking the patients to respond by pressing a button each time they hear a sound. The resulting resolution is poor, and since this procedure requires the processes of intention and attention that characterize listening as opposed to simply hearing and thus tap into cognitive processes that may also be declining with age, diagnosis may be confounded. Other diagnostic tools include measures of auditory brainstem response and otoacoustic emissions which may be more independent of high-level cognitive contribution, although it has recently been shown that cognitive load influences brainstem responses [20] and otoacoustic emissions may also be influenced by attention through efferent innervation [21]. Assessment of speech intelligibility in quiet and in noise is also part of hearing evaluation.

1.3. Hearing Aids. The most important objective for hearing aid signal processing is to make speech audible [22]. This is not a trivial problem. Over 30 years ago, Plomp [23] proposed a model of hearing aid benefit that classed hearing impairment in terms of attenuation and distortion showing that while the hearing aids of the day could compensate well for the former by providing amplification, they were poorer at tackling the latter. As distortion is a characteristic of even the mildest hearing losses, it is important that hearing aids address this issue and the industry has taken on this challenge [24]. Distortion can be simply characterized as a decrease in the ability to distinguish speech from noise. It is not only due to decreased frequency and temporal resolution, as well as impaired ability to discriminate pitch and localize sound sources, but also due to abnormal growth of loudness [25], such that if all sounds are amplified the same way, some may become uncomfortably loud. Thus, modern digital hearing aids include technologies that tackle some of these problems [26]. Wide dynamic range compression systems restore audibility by amplifying weaker sounds more than loud sounds to compensate for the abnormal growth of loudness. The regulation of the compression system may be fast (syllabic) or slow (automatic volume control). Fast-acting wide dynamic range compression (fast WDRC) provides different gain-frequency responses for adjacent speech sounds with different short-term spectra on a syllabic level. On the assumption that communication partners look at each other, directional microphones may be used to attenuate sounds not coming from the front. Of course, if the attended signal does not come from the front, directional microphones may make communication harder. Single-channel noise reduction schemes (NR) may reduce background sounds by identifying portions of the signal as nonspeech and attenuating these. This does not improve speech intelligibility per se, but it may reduce the annoyance from background sounds. Notwithstanding the benefits of signal processing, there is no getting away from the fact that it may also degrade the auditory signal, which may make listening harder. This applies in particular to aggressive signal processing algorithms that may be used experimentally but are not generally prescribed to patients. Aggressive processing is characterized by substantial spectral alteration of the signal within the space of a few milliseconds. For example, some aggressive NR algorithms generate audible artifacts [27] and WDRC distorts individual speech sounds in ways that influence the phonological or sublexical structure of the incoming speech signal [22, 28–30].

1.4. Noise. Acoustic noise impacting speech perception can be categorized as signal degradation, energetic masking, and informational masking [31]. Signal degradation reduces the amount of information in the signal. As we have seen, this is the result of hearing aid signal processing. Other examples relate to processing for data transmission. Energetic masking is a competing signal that partially obscures the target signal. Air conditioning fans are a good example. Informational masking also obscures the target signal but in addition has a fluctuating structure that in some circumstances may distract the listener but in others may allow the listener to systematically glimpse parts of the signal. An informational masker may consist of tonal patterns, for example, or one or more competing speakers. As regards the neural networks underpinning speech comprehension in noise, a pattern is starting to emerge involving widespread frontal and parietal

activation as well as increased temporal activation [32]. There is also some evidence that the brain tracks target and competing speech streams in a manner that is modulated by attention [33] with selective attention networks for pitch and location [34].

Persons with hearing impairment have particular difficulties listening in noise which may be reflected in recruitment of neural networks supporting compensatory processing [35, 36] whereas persons with normal hearing are generally better at coping with informational than energetic masking [37]; the same may not always be true for persons with hearing impairment [38–40]. An informational masker includes cues in terms of pitch or temporal fine structure that may help segregation and dips in the masker may reveal portions of the target signal. This may result in the listener perceiving fragments of a target signal that need to be pieced together to achieve understanding. An informational masker may also include semantic information that distracts the listener from the target signal and thus needs to be inhibited. Such processes rely on cognitive functions.

2. Working Memory and Speech Comprehension

2.1. The Role of Cognition in Listening. Cognitive processes are required to focus on the speech signal and match its contents to stored knowledge [1, 2]. When listening takes place in adverse conditions, for example, when there is background noise or the listener has a hearing impairment, high-level cognitive functions such as working memory and executive processes are implicated [41, 42]. Working memory (WM) is the capacity to perform task-relevant processing of information kept in mind [42, 43] and is supported by a frontoparietal network [44, 45] that is sensitive to stimulus quality and memory load [46, 47]. Many different models of WM have been proposed [48], and one of the most influential of them is the component model originating in the seminal 1974 paper by Baddeley and Hitch [43]. This model was characterized by a central executive controlling two slave buffers for processing verbal and visuospatial information, respectively. It elegantly accounted for a host of empirical data from dual task paradigms, that is, tasks requiring two different kinds of processing at the same time. However, it could not easily account for evidence of multimodal information binding, for example, use of visual cues during speech understanding. A new generation of WM models including an episodic buffer filling just such a function saw the light of day around the turn of the 21st century. These include an updated version of the original component model [49] and a model specifically describing the role of WM in language understanding: the WM model for ease of language understanding (ELU) [41, 50]. Although early work placed the episodic buffer among executive functions organized in the frontal lobes [51], later work has shown that multimodal information binding does not necessarily load on executive functions. For example, visual binding has been shown to take place without executive involvement [52] and multimodal semantic binding has been shown to have its locus in the temporal lobes [53, 54].

The ELU model [41] links in with a parallel line of conceptual development represented by the individual differences approach to WM. This approach focuses on the large variance in individual ability to perform WM tasks rather than characterizing different components of WM [55–57]. According to the ELU model [41], language understanding proceeds rapidly and smoothly under optimal listening conditions, facilitated by an episodic buffer which matches phonological information in the incoming speech stream with the existing representations stored in long-term memory. Because this buffer deals with the rapid, automatic multimodal binding of phonology, it is known by the acronym RAMBPHO. Adverse listening conditions hinder RAMBPHO processing. This may result in a mismatch between auditory signal and information in the mental lexicon in long-term memory. Under such circumstances, explicit or conscious processing resources need to be brought into play to unlock the lexicon. The ELU model proposes that this occurs in a slow processing loop. Processing in the slow loop may include executive functions such as shifting, updating, and inhibition [58]. Inhibition may be required to suppress irrelevant interpretations, while updating may bring new information into the buffer at the expense of discarding older information. Shifting may come into play to realign expectations [30, 59]. All these functions are linked to the frontal lobes [44] and there is evidence that they are supported by anatomically distinct substrates [60]. Their role in speech communication under adverse conditions may be bringing together ambiguous signal fragments with relevant contextual information. There is a constant interplay between predictive kinds of priming of what is to come in a dialogue and postdictive reconstructions of what was missed through mismatches with the lexicon in semantic long-term memory [41]. There is no doubt that such processing is effortful and increases cognitive load [61, 62] and modulates the neural networks involved in speech processing under adverse conditions [63]. From an individual difference perspective, it makes sense that individuals with high WM capacity would perform better on tasks requiring speech understanding under adverse conditions, and this is indeed the case [64–66].

More than a decade ago, it was established that there is a relation between cognitive ability, in particular WM capacity, and the benefit obtained from hearing aid signal processing [64, 67–69]. In particular, it was shown that any benefit of fast-acting WDRC in terms of the ability to understand speech in noise was contingent on cognitive ability [64, 68]. Since then, it has been shown that this relationship is influenced by type of background noise [70–72] and the type of target speech material [30, 70, 73]. Cognitive resources are especially important when modulated noise is combined with fast-acting WDRC [30, 61, 71–73] above all when the target speech is unpredictable [30]. These complex relations change over time [30, 73, 74].

The capacity of WM can be increased by training, suggesting an inherent plasticity in the system [75, 76]. Training effects may generalise to similar nontrained tasks, for example, a different WM task [75]. This is known as near

transfer. However, generalization to other cognitive abilities, known as far transfer, has been elusive [77]. Recent work, however, has shown that for older adults, cognitive training requiring multitasking can result in sustained reduction in multitasking costs and improvement in WM [78]. As we have noted, WM is about simultaneous storage and processing, in other words a form of multitasking. The results of Anguera et al. [78] suggest that in order to improve WM, it may be more efficient to target multitasking abilities as such. Since WM capacity is related to the ability to understand speech in noise, it is tempting to speculate that increasing WM capacity may also improve the ability to understand speech in noise. However, published evidence for the efficacy of individual computer-based auditory training for adults with hearing loss is not robust [79]. We suggest that cognitive training that targets the multitasking abilities inherent in speech understanding under adverse conditions may improve WM capacity and result in better speech understanding in adverse conditions. This is an important avenue for future research.

3. Cognitive Spare Capacity for Communication

3.1. Cognitive Spare Capacity. When listening takes place in adverse conditions, it is clear that the cognitive resources available for higher level processing of speech will be reduced [80]. In other words, the listener has less cognitive spare capacity (CSC) [59, 69, 81, 82].

CSC is closely related to WM in that it is concerned with short-term maintenance and processing of information [59]. Work to date suggests that the storage functions of CSC and WM are similar [83] but that once executive processing demands are introduced, there no longer seems to be a simple relationship between the two concepts [69, 82, 84]. Thus, in order to understand the role of cognition in speech understanding under adverse conditions, it is important to measure not only WM capacity but also CSC. The concept of CSC is related to, although distinct from, other concepts in the literature. For example, differences in susceptibility to functional impairment as a result of brain damage have been explained in terms of "cognitive reserve," that is, individual differences in cognitive function [85], or "brain reserve," that is, individual differences in brain size [86]. CSC is similar to these concepts in that it is based on individual differences in cognitive function and may explain differences in speech communication and underlying mechanisms that may be related to functional changes at any level of the auditory system [69, 81].

Recent work has shown that noise reduction (NR) in hearing aids can enhance CSC by improving retention of heard speech [83, 87]. This applies to both adults with normal hearing thresholds [87] and adults with sensorineural hearing impairment [83]. In the study by Ng et al. [83], experienced hearing aid users listened to sets of highly intelligible, ecologically valid sentences from the Swedish hearing in noise test (HINT) [88, 89]. The HINT sentences were presented in noise and the participants were asked to memorize the final word of each sentence. The participants repeated all the target words to ensure that they were intelligible. At the end of each set, participants were prompted to recall all the sentence-final words. Although they were capable of repeating the sentence-final words, irrespective of the presence of background noise, noise did disrupt recall performance [83]. Being able to retain heard information is an integral part of speech communication. Thus, the findings of Ng et al. [83] demonstrate that, for individuals with hearing impairment, background noise reduces the cognitive resources available for performing the kind of cognitive processing involved in communication. This is in line with the work showing that extra effort expended simply in order to hear comes at the cost of processing resources that might otherwise be available for encoding the speech content in memory [90, 91]. However, when NR was implemented, the negative effect of noise on recall was reduced, even though the ability to repeat sentence-final words remained the same [83]. This demonstrates that hearing aid signal processing can enhance memory processes underpinning speech communication. Informational masking was more disruptive of memory processing than energetic masking and was also more susceptible to the positive effect of NR [83]. However, it remains to be determined whether it is the semantic content or phonological structure of the informational masker that interacts with the ability of NR to improve memory for highly intelligible speech.

Speech communication under adverse conditions is likely to draw on cognitive functions other than simply memory retention [30, 59]. In order to investigate the ability to perform executive processing of heard speech at different memory loads, the cognitive spare capacity test (CSCT) [82, 84] was developed. In the CSCT, sets of spoken two-digit numbers are presented and the participant reports back certain numbers according to instructions. Two executive functions are targeted at two different memory loads. The executive functions in question are updating and inhibition, both of which are likely to be engaged during speech understanding in adverse conditions. Updating ability may be required to strategically replace the contents of WM with relevant material while inhibition ability may be brought into play to keep irrelevant information out of WM. Memory load depends on how many numbers need to be reported. In everyday communication, seeing the face of your communication partner can enhance speech perception by several dB [92]. Thus, in order to determine how visual cues influence CSC, the CSCT manipulates availability of visual cues. The CSCT can be administered in quiet or in noise and other manipulations introducing different kinds of signal processing are also possible.

Across three different studies including persons with and without hearing loss, an interesting pattern of results has emerged [69, 82, 84, 93]. Adults with normal hearing who perform the CSCT in quiet conditions have lower scores when they see the talker's face [82, 84]. This is probably because when target information is highly intelligible, visual cues provide superfluous information that causes distraction during performance of the executive tasks [82, 84]. Although this finding is contrary to the literature on speech perception, which demonstrates better performance in noise when

the talker's face is visible, for individuals with normal hearing [94] and individuals with hearing impairment [95–97], it is in line with other lines of evidence showing that visual cues may increase listening effort [98, 99]. In particular, dual task performance is lower for audiovisual compared to auditory stimuli when intelligibility is equated across modalities [98, 99].

Adults with normal hearing who perform CSCT in noisy conditions do not show this pattern [82] and nor do older adults with raised hearing thresholds, even in quiet [93]. In these conditions, visual cues probably help segregate the target signal from internal or external noise, resulting in richer cognitive representations [82, 100]. Older adults with hearing loss demonstrate lower CSC than young adults, even with better SNR, adapted to provide high intelligibility [101] and individualised amplification, and this effect is most notable in noise and when memory load is high [69]. Although CSC and WM do not seem to be strongly related, there is evidence that age-related differences in WM and executive function do influence CSC [69, 93]. It remains to be seen how different kinds of hearing aid signal processing will interact with executive processing of speech with and without visual cues and whether training CSC can counteract age-related decline in its capacity or even improve CSC. Adaptive training based on CSCT processing may provide a means of improving the ability to understand speech under adverse conditions.

3.2. Phonological Representation. The ELU model describes the way in which the mapping of phonological structure of target speech onto phonological representations in the mental lexicon [102] is mediated by WM during speech understanding under adverse conditions [41]. We have seen that fast-acting WDRC distorts the speech signal in a way that may influence its phonological characteristics [22, 28–30]. In the short term, this may make it harder to match speech to representations, thus requiring more cognitive engagement to achieve speech understanding [41, 70, 73]. However, in the long term, when hearing aid users have had the opportunity to become accustomed to the way in which speech sounds different, phonological representations may alter to match incoming information. Some evidence of this has been found in cochlear implantees [103] and hearing aid users [30]. It is even possible that the new phonological representations based on processed speech may be more mutually distinct than the representations they replace based on less appropriate signal processing. The neural correlates of such changes in phonological representation due to habitual use of WRDC have yet to be investigated.

Lexical access is faster when phonological representations are easier to distinguish from each other [102, 104]. However, long-term severe acquired hearing impairment may lead to less distinct phonological representations [103]. This makes it harder to determine whether printed words rhyme with each other [105], especially when orthography is misleading [106]. For example, individuals with poor phonological representations due to severe long-term hearing impairment may be more unsure than their peers with normal hearing whether "pint" rhymes with "lint" or whether "blue" rhymes with "through." However, good WM capacity can compensate for this deficit, albeit at the cost of long-term memory representations [106]. Compensatory processing by individuals with hearing impairment during visual rhyme judgment is associated with larger amplitude of the N2 component [107], indicating use of a compensatory strategy, possibly involving increased reliance on explicit mechanisms such as articulatory recoding and grapheme-to-phoneme conversion.

In summary, phonological structure of target speech material is not only influenced by speaker characteristics but also by distortion due to hearing aid signal processing. Phonological representations in the mental lexicon may be influenced by long-term effects of both hearing impairment and signal processing. Further, both of these may have distinct neural signatures. Measures designed to improve phonological distinctiveness of both target speech and phonological representations are likely to enhance CSC and support speech communication under adverse conditions. This deserves further investigation.

3.3. Semantic Context. Provision of semantic context can facilitate speech understanding under adverse conditions. This process engages language networks in left posterior inferior temporal cortex and inferior frontal gyri bilaterally [108]. Studies investigating the role of WM capacity in the benefit obtained from WDRC have indicated that the semantic content of the materials delivered for speech recognition may influence this relationship. For example, Rudner et al. [30] found that WM capacity was associated with speech understanding for individuals with hearing impairment using WDRC listening to matrix-type sentences [109, 110], but not Swedish HINT sentences [88, 89]. The Hagerman sentences are semantically coherent, but the five-word syntactic structure is always the same and each word comes from a closed set of ten appropriate items. Thus none of the items can be accurately predicted. The HINT sentences, by contrast, are diverse in length, syntactic structure and semantic coherence. It is likely that the constrained structure and content of the Hagerman sentences make guessing harder and thus increase reliance on the bottom-up information provided by the speech signal. However, it has been found that the benefit of having access to the temporal fine structure of the speech signal was greater for open set materials than for closed-set materials [111], indicating that the regular structure and closed set of matrix-like sentences can facilitate guessing . Future work should systematically investigate the interaction between the semantic coherence of the speech signal, hearing aid signal processing, and individual cognitive characteristics such as WM and CSC.

Text cues can facilitate speech understanding in noise when they match the semantic content of the auditory signal [112–116] and inhibit it when they are misleading [116]. Cue integration is supported by language networks including the inferior frontal gyrus and temporal regions [115]. Matching text cues also enhance the perceived clarity

of degraded speech [63] and recently it was shown that this effect may be modulated by both lexical access speed and WM capacity [117]. WM capacity modulates the activation of networks involved in semantic processing [115] and also predicts the ability to inhibit misleading text cues during speech understanding in steady state noise [116] as well as the facilitation of speech understanding against a single talker background [112]. Recently, it has been shown that coherence and cues can have separate facilitatory effects on perceived clarity of degraded speech [117]. Future work should focus on determining the benefit of providing text cues for hearing aid users, for example, using automatic speech recognition [114] and how this interacts with the semantic coherence of the target speech, the availability of semantic content in the noise background, and individual cognitive skills. Imaging studies are likely to provide important information about the neurocognitive systems supporting these complex interactions.

3.4. Aging and Communication. Sensory and cognitive functions decline with age [118, 119]. Sensory decline can be traced to physiological change, but the mechanisms behind cognitive change are more elusive, although both genetic and lifestyle factors have been implicated [118]. Several different theories attempt to explain the relation between sensory and cognitive decline. The common cause hypothesis [119] proposes that a general reduction in processing efficiency drives both phenomena. The information degradation hypothesis [120], on the other hand, claims than when sensory input is degraded, cognitive processing becomes less efficient as a result. Reserve theories suggest that the ability to cope with brain damage is related to premorbid brain size or cognitive ability [86]. The compensation-related utilization of neural circuits hypothesis [35] suggests that older adults compensate for less efficient processing by engaging more neural resources than younger adults when task load is still relatively low while brain maintenance theory [118] proposes that individual differences in the manifestation of age-related brain changes and pathology allow some people to show little or no age-related cognitive decline. All these theories are more or less sophisticated in their attempts to capture the relationship between physiological, sensory, and cognitive function in an aging perspective. The relations they describe suggest that keeping the brain healthy and providing it with better sensory input will facilitate speech understanding for individuals of advancing age. The theories that focus on a special role for cognition suggest that lowering cognitive load and enhancing CSC during speech communication may have special importance in later adulthood and even allow some older adults to function communicatively just as successfully as their younger counterparts.

Recent work has shown that older adults show less activation in auditory cortex than younger adults while listening to speech in noise, especially at poor signal to noise ratios and compensate by recruiting prefrontal and parietal areas associated with WM [36]. Epidemiological studies show that individuals with hearing loss are at increased risk of cognitive impairment and that rate of cognitive decline and risk of cognitive impairment are associated with severity of hearing loss [121]. Thus, hearing loss may result in decreasing CSC. No study has yet specifically addressed this issue. However, analysis of data from the Betula study of cognitive aging [122] demonstrated that hearing aid users with poorer hearing also had poorer long-term memory [123]. This applied even when the long-term memory task had no auditory component. However, degree of hearing loss was not associated with decline in WM. Importantly, there was no significant association between loss of vision and cognitive function. These results suggest that although hearing loss and cognitive decline are related, even in hearing aid users, the association may not apply across all cognitive domains. The challenge is to uncover the specific mechanisms behind age-related sensory and cognitive decline so that speech communication can be preserved into old age by optimizing cognitive capacity. This may involve a range of different interventions that target hearing through appropriate hearing aid fitting, enhance the role of other sensory modalities that can be exploited in communication, and capitalize on cognitive abilities by seeking to maintain and extend them.

4. Conclusion

Speech communication in adverse conditions makes specific demands on cognitive resources. In particular, WM capacity and executive function are engaged in unravelling the speech signal. This depletes CSC and leaving fewer resources for higher level processing of speech. CSC is influenced by cognitive load, noise, visual cues, and aging and can be enhanced by appropriate hearing aid signal processing. The phonological structure and semantic content of speech influence processing mechanisms and engagement of cognitive resources. Optimizing CSC is an important aim for preserving speech communication into old age. We have reviewed evidence suggesting that CSC may be enhanced by a number of means including cognitive training and providing the optimal balance between visual, phonological, and semantic information. Future research should focus on finding ways to optimize CSC.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

[1] J. Kiessling, M. K. Pichora-Fuller, S. Gatehouse et al., "Candidature for and delivery of audiological services: special needs of older people," *International Journal of Audiology*, vol. 42, supplement 2, pp. S92–S2101, 2003.

[2] M. K. Pichora-Fuller and G. Singh, "Effects of age on auditory and cognitive processing: implications for hearing aid fitting and audiologic rehabilitation," *Trends in Amplification*, vol. 10, no. 1, pp. 29–59, 2006.

[3] J. E. Peelle, "The hemispheric lateralization of speech processing depends on what "speech" is: a hierarchical perspective," *Frontiers in Human Neuroscience*, vol. 6, article 309, 2012.

[4] S. K. Scott and I. S. Johnsrude, "The neuroanatomical and functional organization of speech perception," *Trends in Neurosciences*, vol. 26, no. 2, pp. 100–107, 2003.

[5] J. H. Kaas and T. A. Hackett, "Subdivisions of auditory cortex and processing streams in primates," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 22, pp. 11793–11799, 2000.

[6] J. P. Rauschecker and S. K. Scott, "Maps and streams in the auditory cortex: nonhuman primates illuminate human speech processing," *Nature Neuroscience*, vol. 12, no. 6, pp. 718–724, 2009.

[7] G. Hickok and D. Poeppel, "The cortical organization of speech processing," *Nature Reviews Neuroscience*, vol. 8, no. 5, pp. 393–402, 2007.

[8] A. D. Friederici and S. M. Gierhan, "The language network," *Current Opinion in Neurobiology*, vol. 23, no. 2, pp. 250–254, 2013.

[9] A. Davis and G. Britain, "Acceptability, benefit and costs of early screening for hearing disability: a study of potential screening tests and models," Tech. Rep., National Coordinating Centre for Health Technology Assessment, University of Southampton, 2007.

[10] A. Davis and K. A. Davis, "Epidemiology of aging and hearing loss related to other chronic illnesses," in *Proceedings of the 2nd International Adult Conference Hearing Care for Adults—The Challenge of Aging*, L. Hickson, Ed., pp. 23–32, Chicago, Ill, USA, 2009.

[11] S. Gordon-Salant, "Hearing loss and aging: new research findings and clinical implications," *Journal of Rehabilitation Research and Development*, vol. 42, no. 4, pp. 9–24, 2005.

[12] J. Nachtegaal, J. M. Festen, and S. E. Kramer, "Hearing ability in working life and its relationship with sick leave and self-reported work productivity," *Ear and Hearing*, vol. 33, no. 1, pp. 94–103, 2012.

[13] P. V. Pierre, A. Fridberger, A. Wikman, and K. Alexanderson, "Self-reported hearing difficulties, main income sources, and socio-economic status; a cross-sectional population-based study in Sweden," *BMC Public Health*, vol. 12, no. 1, article 874, 2012.

[14] M. Pronk, D. J. Deeg, and S. E. Kramer, "Hearing status in older persons: a significant determinant of depression and loneliness? Results from the Longitudinal Aging Study Amsterdam," *American Journal of Audiology*, vol. 22, no. 2, pp. 316–320, 2013.

[15] E. H. N. Ng, M. Rudner, T. Lunner, and J. Rönnberg, "Relationships between self-report and cognitive measures of hearing aid outcome," *Speech, Language and Hearing*, vol. 16, no. 4, pp. 197–207, 2013.

[16] A. A. Zekveld, E. L. George, T. Houtgast, and S. E. Kramer, "Cognitive abilities relate to self-reported hearing disability," *Journal of Speech, Language, and Hearing Research*, vol. 56, no. 5, pp. 1364–1372, 2013.

[17] M. A. Eckert, S. L. Cute, K. I. Vaden Jr., S. E. Kuchinsky, and J. R. Dubno, "Auditory cortex signs of age-related hearing loss," *Journal of the Association for Research in Otolaryngology*, vol. 13, no. 5, pp. 703–713, 2012.

[18] J. E. Peelle, V. Troiani, M. Grossman, and A. Wingfield, "Hearing loss in older adults affects neural systems supporting speech comprehension," *The Journal of Neuroscience*, vol. 31, no. 35, pp. 12638–12643, 2011.

[19] S. Stenfelt and J. Rönnberg, "The Signal-Cognition interface: interactions between degraded auditory signals and cognitive processes," *Scandinavian Journal of Psychology*, vol. 50, no. 5, pp. 385–393, 2009.

[20] P. Sörqvist, S. Stenfelt, and J. Rönnberg, "Working memory capacity and visual-verbal cognitive load modulate auditory-sensory gating in the brainstem: toward a unified view of attention," *Journal of Cognitive Neuroscience*, vol. 24, no. 11, pp. 2147–2154, 2012.

[21] S. Srinivasan, A. Keil, K. Stratis, K. L. Woodruff Carr, and D. W. Smith, "Effects of cross-modal selective attention on the sensory periphery: cochlear sensitivity is altered by selective attention," *Neuroscience*, vol. 223, pp. 325–332, 2012.

[22] T. Lunner, M. Rudner, and J. Rönnberg, "Cognition and hearing aids," *Scandinavian Journal of Psychology*, vol. 50, no. 5, pp. 395–403, 2009.

[23] R. Plomp, "Auditory handicap of hearing impairment and the limited benefit of hearing aids," *Journal of the Acoustical Society of America*, vol. 63, no. 2, pp. 533–549, 1978.

[24] B. Edwards, "The future of hearing aid technology," *Trends in Amplification*, vol. 11, no. 1, pp. 31–45, 2007.

[25] B. C. J. Moore, "Perceptual consequences of cochlear hearing loss and their implications for the design of hearing aids," *Ear and Hearing*, vol. 17, no. 2, pp. 133–161, 1996.

[26] H. Dillon, *Hearing Aids*, Boomerang Press, Turramurra, Australia, 2nd edition, 2012.

[27] D. Wang, U. Kjems, M. S. Pedersen, J. B. Boldt, and T. Lunner, "Speech perception of noise with binary gains," *Journal of the Acoustical Society of America*, vol. 124, no. 4, pp. 2303–2307, 2008.

[28] L. M. Jenstad and P. E. Souza, "Quantifying the effect of compression hearing aid release time on speech acoustics and intelligibility," *Journal of Speech, Language, and Hearing Research*, vol. 48, no. 3, pp. 651–667, 2005.

[29] S. Bor, P. Souza, and R. Wright, "Multichannel compression: effects of reduced spectral contrast on vowel identification," *Journal of Speech, Language, and Hearing Research*, vol. 51, no. 5, pp. 1315–1327, 2008.

[30] M. Rudner, J. Rönnberg, and T. Lunner, "Working memory supports listening in noise for persons with hearing impairment," *Journal of the American Academy of Audiology*, vol. 22, no. 3, pp. 156–167, 2011.

[31] S. L. Mattys, M. H. Davis, A. R. Bradlow, and S. K. Scott, "Speech recognition in adverse conditions: a review," *Language and Cognitive Processes*, vol. 27, no. 7-8, pp. 953–978, 2012.

[32] S. K. Scott and C. McGettigan, "The neural processing of masked speech," *Hearing Research*, vol. 303, pp. 58–66, 2013.

[33] E. M. Zion Golumbic, N. Ding, S. Bickel et al., "Mechanisms underlying selective neuronal tracking of attended speech at a 'cocktail party'," *Neuron*, vol. 77, no. 5, pp. 980–991, 2013.

[34] A. K. C. Lee, S. Rajaram, J. Xia et al., "Auditory selective attention reveals preparatory activity in different cortical regions for selection based on source location and source pitch," *Frontiers in Neuroscience*, vol. 6, article 190, 2013.

[35] P. A. Reuter-Lorenz and K. A. Cappell, "Neurocognitive aging and the compensation hypothesis," *Current Directions in Psychological Science*, vol. 17, no. 3, pp. 177–182, 2008.

[36] P. C. M. Wong, J. X. Jin, G. M. Gunasekera, R. Abel, E. R. Lee, and S. Dhar, "Aging and cortical mechanisms of speech perception in noise," *Neuropsychologia*, vol. 47, no. 3, pp. 693–703, 2009.

[37] A. J. Duquesnoy, "Effect of a single interfering noise or speech source upon the binaural sentence intelligibility of aged persons," *Journal of the Acoustical Society of America*, vol. 74, no. 3, pp. 739–743, 1983.

[38] J. M. Festen and R. Plomp, "Effects of fluctuating noise and interfering speech on the speech-reception threshold for impaired and normal hearing," *Journal of the Acoustical Society of America*, vol. 88, no. 4, pp. 1725–1736, 1990.

[39] E. L. J. George, A. A. Zekveld, S. E. Kramer, S. T. Goverts, J. M. Festen, and T. Houtgast, "Auditory and nonauditory factors affecting speech reception in noise by older listeners," *Journal of the Acoustical Society of America*, vol. 121, no. 4, pp. 2362–2375, 2007.

[40] C. Lorenzi, G. Gilbert, H. Carn, S. Garnier, and B. C. J. Moore, "Speech perception problems of the hearing impaired reflect inability to use temporal fine structure," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 49, pp. 18866–18869, 2006.

[41] J. Rönnberg, T. Lunner, A. Zekveld et al., "The Ease of Language Understanding (ELU) model: theory, data, and clinical implications," *Frontiers in Systems Neuroscience*, vol. 7, article 31, 2013.

[42] P. Sörqvist, "The role of working memory capacity in auditory distraction: a review," *Noise and Health*, vol. 12, no. 49, pp. 217–224, 2010.

[43] A. D. Baddeley and G. Hitch, "Working memory," *Psychology of Learning and Motivation—Advances in Research and Theory*, vol. 8, pp. 47–89, 1974.

[44] R. Cabeza and L. Nyberg, "Imaging cognition II: an empirical review of 275 PET and fMRI studies," *Journal of Cognitive Neuroscience*, vol. 12, no. 1, pp. 1–47, 2000.

[45] E. E. Smith and J. Jonides, "Working memory: a view from neuroimaging," *Cognitive Psychology*, vol. 33, no. 1, pp. 5–42, 1997.

[46] D. M. Barch, T. S. Braver, L. E. Nystrom, S. D. Forman, D. C. Noll, and J. D. Cohen, "Dissociating working memory from task difficulty in human prefrontal cortex," *Neuropsychologia*, vol. 35, no. 10, pp. 1373–1380, 1997.

[47] T. S. Braver, J. D. Cohen, L. E. Nystrom, J. Jonides, E. E. Smith, and D. C. Noll, "A parametric study of prefrontal cortex involvement in human working memory," *NeuroImage*, vol. 5, no. 1, pp. 49–62, 1997.

[48] A. Miyake and P. Shah, *Models of Working Memory: Mechanisms of Active Maintenance and Executive Control*, Cambridge University Press, 1999.

[49] A. Baddeley, "The episodic buffer: a new component of working memory?" *Trends in Cognitive Sciences*, vol. 4, no. 11, pp. 417–423, 2000.

[50] J. Rönnberg, "Cognition in the hearing impaired and deaf as a bridge between signal and dialogue: a framework and a model," *International Journal of Audiology*, vol. 42, supplement 1, pp. S68–S76, 2003.

[51] V. Prabhakaran, K. Narayanan, Z. Zhao, and J. D. E. Gabriel, "Integration of diverse information in working memory within the frontal lobe," *Nature Neuroscience*, vol. 3, no. 1, pp. 85–90, 2000.

[52] R. J. Allen, A. D. Baddeley, and G. J. Hitch, "Is the binding of visual features in working memory resource-demanding?" *Journal of Experimental Psychology: General*, vol. 135, no. 2, pp. 298–313, 2006.

[53] M. Rudner, P. Fransson, M. Ingvar, L. Nyberg, and J. Rönnberg, "Neural representation of binding lexical signs and words in the episodic buffer of working memory," *Neuropsychologia*, vol. 45, no. 10, pp. 2258–2276, 2007.

[54] M. Rudner and J. Rönnberg, "The role of the episodic buffer in working memory for language processing," *Cognitive Processing*, vol. 9, no. 1, pp. 19–28, 2008.

[55] M. A. Just and P. A. Carpenter, "A capacity theory of comprehension: individual differences in working memory," *Psychological Review*, vol. 99, no. 1, pp. 122–149, 1992.

[56] M. Daneman and P. A. Carpenter, "Individual differences in integrating information between and within sentences," *Journal of Experimental Psychology: Learning, Memory, and Cognition*, vol. 9, no. 4, pp. 561–584, 1983.

[57] M. Daneman and P. A. Carpenter, "Individual differences in working memory and reading," *Journal of Verbal Learning and Verbal Behavior*, vol. 19, no. 4, pp. 450–466, 1980.

[58] A. Miyake, N. P. Friedman, M. J. Emerson, A. H. Witzki, A. Howerter, and T. D. Wager, "The unity and diversity of executive functions and their contributions to complex "frontal lobe" tasks: a latent variable analysis," *Cognitive Psychology*, vol. 41, no. 1, pp. 49–100, 2000.

[59] M. Rudner, H. N. Ng, N. Rönnberg et al., "Cognitive spare capacity as a measure of listening effort," *Journal of Hearing Science*, vol. 1, no. 2, pp. 47–49, 2011.

[60] P. C. Fletcher and R. N. A. Henson, "Frontal lobes and human memory insights from functional neuroimaging," *Brain*, vol. 124, no. 5, pp. 849–881, 2001.

[61] M. Rudner, T. Lunner, T. Behrens, E. S. Thorén, and J. Rönnberg, "Working memory capacity may influence perceived effort during aided speech recognition in noise," *Journal of the American Academy of Audiology*, vol. 23, no. 8, pp. 577–589, 2012.

[62] A. A. Zekveld, S. E. Kramer, and J. M. Festen, "Cognitive load during speech perception in noise: the influence of age, hearing loss, and cognition on the pupil response," *Ear and Hearing*, vol. 32, no. 4, pp. 498–510, 2011.

[63] C. J. Wild, A. Yusuf, D. E. Wilson, J. E. Peelle, M. H. Davis, and I. S. Johnsrude, "Effortful listening: the processing of degraded speech depends critically on attention," *The Journal of Neuroscience*, vol. 32, no. 40, pp. 14010–14021, 2012.

[64] T. Lunner, "Cognitive function in relation to hearing aid use," *International Journal of Audiology*, vol. 42, supplement 1, pp. S49–S58, 2003.

[65] M. A. Akeroyd, "Are individual differences in speech reception related to individual differences in cognitive ability? A survey of twenty experimental studies with normal and hearing-impaired adults," *International Journal of Audiology*, vol. 47, supplement 2, pp. S53–S71, 2008.

[66] J. Besser, T. Koelewijn, A. A. Zekveld, S. E. Kramer, and J. M. Festen, "How linguistic closure and verbal working memory relate to speech recognition in noise—a review," *Trends in Amplification*, vol. 17, no. 2, pp. 75–93, 2013.

[67] M. K. Pichora-Fuller, "How cognition might influence hearing aid design, fitting, and outcomes," *Hearing Journal*, vol. 62, no. 11, pp. 32–35, 2009.

[68] S. Gatehouse, G. Naylor, and C. Elberling, "Benefits from hearing aids in relation to the interaction between the user and the environment," *International Journal of Audiology*, vol. 42, supplement 1, pp. S77–S85, 2003.

[69] M. Rudner and T. Lunner, "Cognitive spare capacity as a window on hearing aid benefit," *Semin Hear*, vol. 34, no. 4, pp. 298–307, 2013.

[70] C. Foo, M. Rudner, J. Rönnberg, and T. Lunner, "Recognition of speech in noise with new hearing instrument compression release settings requeres explicit cognitive storage and processing capacity," *Journal of the American Academy of Audiology*, vol. 18, no. 7, pp. 618–631, 2007.

[71] T. Lunner and E. Sundewall-Thorén, "Interactions between cognition, compression, and listening conditions: effects on speech-in-noise performance in a two-channel hearing aid," *Journal of the American Academy of Audiology*, vol. 18, no. 7, pp. 604–617, 2007.

[72] M. Rudner, C. Foo, E. Sundewall-Thorén, T. Lunner, and J. Rönnberg, "Phonological mismatch and explicit cognitive processing in a sample of 102 hearing-aid users," *International Journal of Audiology*, vol. 47, supplement 2, pp. S91–S98, 2008.

[73] M. Rudner, C. Foo, J. Rönnberg, and T. Lunner, "Cognition and aided speech recognition in noise: specific role for cognitive factors following nine-week experience with adjusted compression settings in hearing aids," *Scandinavian Journal of Psychology*, vol. 50, no. 5, pp. 405–418, 2009.

[74] R. M. Cox and J. Xu, "Short and long compression release times: speech understanding, real-world preferences, and association with cognitive ability," *Journal of the American Academy of Audiology*, vol. 21, no. 2, pp. 121–138, 2010.

[75] E. Dahlin, A. S. Neely, A. Larsson, L. Bäckman, and L. Nyberg, "Transfer of learning after updating training mediated by the striatum," *Science*, vol. 320, no. 5882, pp. 1510–1512, 2008.

[76] T. Klingberg, E. Fernell, P. J. Olesen et al., "Computerized training of working memory in children with ADHD—a randomized, controlled trial," *Journal of the American Academy of Child and Adolescent Psychiatry*, vol. 44, no. 2, pp. 177–186, 2005.

[77] A. M. Owen, A. Hampshire, J. A. Grahn et al., "Putting brain training to the test," *Nature*, vol. 465, no. 7299, pp. 775–778, 2010.

[78] J. Anguera, J. Boccanfuso, J. Rintoul et al., "Video game training enhances cognitive control in older adults," *Nature*, vol. 501, no. 7465, pp. 97–101, 2013.

[79] H. Henshaw and M. A. Ferguson, "Efficacy of individual computer-based auditory training for people with hearing loss: a systematic review of the evidence," *PLoS ONE*, vol. 8, no. 5, Article ID e62836, 2013.

[80] M. K. Pichora-Fuller, "Audition and cognition: what audiologists need to know about listening," in *Hearing Care for Adults*, C. Palmer and R. Seewald, Eds., pp. 71–85, Stäfa, Switzerland, 2007.

[81] S. Mishra, M. Rudner, T. Lunner, and R. Rönnberg, "Speech understanding and cognitive spare capacity," in *Proceedings of the International Symposium on Auditory and Audiological Research (ISAAR '10)*, pp. 305–313, Elsinore, Denmark, 2010.

[82] S. Mishra, T. Lunner, S. Stenfelt, J. Rönnberg, and M. Rudner, "Seeing the talker's face supports executive processing of speech in steady state noise," *Frontiers in Systems Neuroscience*, vol. 7, article 96, 2013.

[83] E. H. N. Ng, M. Rudner, T. Lunner, M. S. Pedersen, and J. Rönnberg, "Effects of noise and working memory capacity on memory processing of speech for hearing-aid users," *International Journal of Audiology*, vol. 52, no. 7, pp. 433–441, 2013.

[84] S. Mishra, T. Lunner, S. Stenfelt, J. Rönnberg, and M. Rudner, "Visual information can hinder working memory processing of speech," *Journal of Speech, Language, and Hearing Research*, vol. 56, no. 4, pp. 1120–1132, 2013.

[85] D. Barulli and Y. Stern, "Efficiency, capacity, compensation, maintenance, plasticity: emerging concepts in cognitive reserve," *Trends in Cognitive Sciences*, vol. 17, no. 10, pp. 502–509, 2013.

[86] P. Satz, M. A. Cole, D. J. Hardy, and Y. Rassovsky, "Brain and cognitive reserve: mediator(s) and construct validity, a critique," *Journal of Clinical and Experimental Neuropsychology*, vol. 33, no. 1, pp. 121–130, 2011.

[87] A. Sarampalis, S. Kalluri, B. Edwards, and E. Hafter, "Objective measures of listening effort: effects of background noise and noise reduction," *Journal of Speech, Language, and Hearing Research*, vol. 52, no. 5, pp. 1230–1240, 2009.

[88] M. Hällgren, B. Larsby, and S. Arlinger, "A Swedish version of the Hearing In Noise Test (HINT) for measurement of speech recognition," *International Journal of Audiology*, vol. 45, no. 4, pp. 227–237, 2006.

[89] M. Nilsson, S. D. Soli, and J. A. Sullivan, "Development of the hearing in noise test for the measurement of speech reception thresholds in quiet and in noise," *Journal of the Acoustical Society of America*, vol. 95, no. 2, pp. 1085–1099, 1994.

[90] S. L. McCoy, P. A. Tun, L. C. Cox, M. Colangelo, R. A. Stewart, and A. Wingfield, "Hearing loss and perceptual effort: downstream effects on older adults' memory for speech," *Quarterly Journal of Experimental Psychology Section A: Human Experimental Psychology*, vol. 58, no. 1, pp. 22–33, 2005.

[91] D. R. Murphy, F. I. M. Craik, K. Z. H. Li, and B. A. Schneider, "Comparing the effects of aging and background noise on short-term memory performance," *Psychology and Aging*, vol. 15, no. 2, pp. 323–334, 2000.

[92] W. H. Sumby and I. Pollack, "Visual contribution to speech intelligibility in noise," *Journal of the Acoustical Society of America*, vol. 26, article 212, 1954.

[93] S. Mishra, S. Stenfelt, T. Lunner, J. Rönnberg, and M. Rudner, "Cognitive spare capacity in older adults with hearing loss," *Frontiers in Aging Neuroscience*, vol. 6, article 96, 2014.

[94] N. P. Erber, "Interaction of audition and vision in the recognition of oral speech stimuli," *Journal of Speech and Hearing Research*, vol. 12, no. 2, pp. 423–425, 1969.

[95] J. G. W. Bernstein and K. W. Grant, "Auditory and auditory-visual intelligibility of speech in fluctuating maskers for normal-hearing and hearing-impaired listeners," *Journal of the Acoustical Society of America*, vol. 125, no. 5, pp. 3358–3372, 2009.

[96] K. W. Grant, B. E. Walden, and P. F. Seitz, "Auditory-visual speech recognition by hearing-impaired subjects: consonant recognition, sentence recognition, and auditory-visual integration," *Journal of the Acoustical Society of America*, vol. 103, no. 5, pp. 2677–2690, 1998.

[97] K. W. Grant and P.-F. Seitz, "The use of visible speech cues for improving auditory detection of spoken sentences," *Journal of the Acoustical Society of America*, vol. 108, no. 3 I, pp. 1197–1208, 2000.

[98] S. Fraser, J.-P. Gagné, M. Alepins, and P. Dubois, "Evaluating the effort expended to understand speech in noise using a dual-task paradigm: the effects of providing visual speech cues," *Journal of*

Speech, Language, and Hearing Research, vol. 53, no. 1, pp. 18–33, 2010.

[99] P. A. Gosselin and J.-P. Gagné, "Older adults expend more listening effort than young adults recognizing audiovisual speech in noise," *International Journal of Audiology*, vol. 50, no. 11, pp. 786–792, 2011.

[100] J. B. Frtusova, A. H. Winneke, and N. A. Phillips, "ERP Evidence that auditory-visual speech facilitates working memory in younger and older adults," *Psychology and Aging*, vol. 28, no. 2, pp. 481–494, 2013.

[101] M. K. Pichora-Fuller, B. A. Schneider, and M. Daneman, "How young and old adults listen to and remember speech in noise," *Journal of the Acoustical Society of America*, vol. 97, no. 1, pp. 593–608, 1995.

[102] P. A. Luce and D. B. Pisoni, "Recognizing spoken words: the neighborhood activation model," *Ear and Hearing*, vol. 19, no. 1, pp. 1–36, 1998.

[103] D. S. Lazard, H. J. Lee, M. Gaebler, C. A. Kell, E. Truy, and A. L. Giraud, "Phonological processing in post-lingual deafness and cochlear implant outcome," *NeuroImage*, vol. 49, no. 4, pp. 3443–3451, 2010.

[104] S. Moradi, B. Lidestam, and J. Rönnberg, "Gated audiovisual speech identification in silence vs. noise: effects on time and accuracy," *Frontiers in Psychology*, vol. 4, article 359, 2013.

[105] U. Andersson, "Deterioration of the phonological processing skills in adults with an acquired severe hearing loss," *European Journal of Cognitive Psychology*, vol. 14, no. 3, pp. 335–352, 2002.

[106] E. Classon, M. Rudner, and J. Rönnberg, "Working memory compensates for hearing related phonological processing deficit," *Journal of Communication Disorders*, vol. 46, no. 1, pp. 17–29, 2013.

[107] E. Classon, M. Rudner, M. Johansson, and J. Rönnberg, "Early ERP signature of hearing impairment in visual rhyme judgment," *Frontiers in Psychology*, vol. 4, article 241, 2013.

[108] J. M. Rodd, M. H. Davis, and I. S. Johnsrude, "The neural mechanisms of speech comprehension: fMRI studies of semantic ambiguity," *Cerebral Cortex*, vol. 15, no. 8, pp. 1261–1269, 2005.

[109] B. Hagerman and C. Kinnefors, "Efficient adaptive methods for measuring speech reception threshold in quiet and in noise," *Scandinavian Audiology*, vol. 24, no. 1, pp. 71–77, 1995.

[110] B. Hagerman, "Sentences for testing speech intelligibility in noise," *Scandinavian Audiology*, vol. 11, no. 2, pp. 79–87, 1982.

[111] T. Lunner, R. K. Hietkamp, M. R. Andersen, K. Hopkins, and B. C. Moore, "Effect of speech material on the benefit of temporal fine structure information in speech for young normal-hearing and older hearing-impaired participants," *Ear and Hearing*, vol. 33, no. 3, pp. 377–388, 2012.

[112] A. A. Zekveld, M. Rudner, I. S. Johnsrude, and J. Rönnberg, "The effects of working memory capacity and semantic cues on the intelligibility of speech in noise," *Journal of the Acoustical Society of America*, vol. 134, no. 3, pp. 2225–2234, 2013.

[113] E. Sohoglu, J. E. Peelle, R. P. Carlyon, and M. H. Davis, "Predictive top-down integration of prior knowledge during speech perception," *The Journal of Neuroscience*, vol. 32, no. 25, pp. 8443–8453, 2012.

[114] A. A. Zekveld, S. E. Kramer, M. S. M. G. Vlaming, and T. Houtgast, "Audiovisual perception of speech in noise and masked written text," *Ear and Hearing*, vol. 29, no. 1, pp. 99–111, 2008.

[115] A. A. Zekveld, M. Rudner, I. S. Johnsrude, D. J. Heslenfeld, and J. Rönnberg, "Behavioral and fMRI evidence that cognitive ability modulates the effect of semantic context on speech intelligibility," *Brain and Language*, vol. 122, no. 2, pp. 103–113, 2012.

[116] A. A. Zekveld, M. Rudner, I. S. Johnsrude, J. M. Festen, J. H. M. Van Beek, and J. Rönnberg, "The influence of semantically related and unrelated text cues on the intelligibility of sentences in noise," *Ear and Hearing*, vol. 32, no. 6, pp. e16–e25, 2011.

[117] C. Signoret, I. Johnsrude, E. Classon, and M. Rudner, "Lexical access speed determines the role of working memory in pop-out," in *Proceedings of the Cognitive Hearing Science for Communication*, Linköping, Sweden, June 2013.

[118] L. Nyberg, M. Lövdén, K. Riklund, U. Lindenberger, and L. Bäckman, "Memory aging and brain maintenance," *Trends in Cognitive Sciences*, vol. 16, no. 5, pp. 292–305, 2012.

[119] P. B. Baltes and U. Lindenberger, "Emergence of a powerful connection between sensory and cognitive functions across the adult life span: a new window to the study of cognitive aging?" *Psychology and Aging*, vol. 12, no. 1, pp. 12–21, 1997.

[120] B. A. Schneider, M. Daneman, and M. K. Pichora-Fuller, "Listening in aging adults: from discourse comprehension to psychoacoustics," *Canadian Journal of Experimental Psychology*, vol. 56, no. 3, pp. 139–152, 2002.

[121] F. R. Lin, K. Yaffe, J. Xia et al., "Hearing loss and cognitive decline in older adults," *JAMA Internal Medicine*, vol. 173, no. 4, pp. 293–299, 2013.

[122] L.-G. Nilsson, L. Bäckman, K. Erngrund et al., "The betula prospective cohort study: memory, health, and aging," *Aging, Neuropsychology, and Cognition*, vol. 4, no. 1, pp. 1–32, 1997.

[123] J. Rönnberg, H. Danielsson, M. Rudner et al., "Hearing loss is negatively related to episodic and semantic long-term memory but not to short-term memory," *Journal of Speech, Language, and Hearing Research*, vol. 54, no. 2, pp. 705–726, 2011.