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Neurotrophin gene therapy to promote survival of spiral ganglion neurons after deafness

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Abstract

Hearing impairment is a major health and economic concern worldwide. Currently, the cochlear implant (CI) is the standard of care for remediation of severe to profound hearing loss, and in general, contemporary CIs are highly successful. But there is great variability in outcomes among individuals, especially in children, with many CI users deriving much less or even marginal benefit. Much of this variability is related to differences in auditory nerve survival, and there has been substantial interest in recent years in exploring potential therapies to improve survival of the cochlear spiral ganglion neurons (SGN) after deafness. Preclinical studies using osmotic pumps and other approaches in deafened animal models to deliver neurotrophic factors (NTs) directly to the cochlea have shown promising results, especially with Brain-Derived Neurotrophic Factor (BDNF). More recent studies have focused on the use of NT gene therapy to force expression of NTs by target cells within the cochlea. This could provide the means for a one-time treatment to promote long-term NT expression and improve neural survival after deafness. This review summarizes the evidence for the efficacy of exogenous NTs in preventing SGN degeneration after hearing loss and reviews the animal research to date suggesting that NT gene therapy can elicit long-term NT expression in the cochlea, resulting in significantly improved SGN and radial nerve fiber survival after deafness. In addition, we discuss NT gene therapy in other non-auditory applications and consider some of the remaining issues with regard to selecting optimal vectors, timing of treatment, and place/method of delivery, etc. that must be resolved prior to considering clinical application

Keywords

Neurotrophin gene therapy; Cochlear spiral ganglion neurons; Profound hearing loss; Brain-derived neurotrophic factor (BDNF); Adeno-associated viral vectors (AAV); Cochlear implant (CI)

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1. Introduction

Worldwide, more than 432 million adults and 34 million children are living with a disabling hearing loss, making it a major health and economic concern (WHO, 2019). Hearing impairment often leads to adverse educational, social and vocational consequences that significantly affect quality of life. In addition, hearing loss is also a potential risk factor for cognitive impairment (see Chern and Golub, 2019 for review) and depression (Jayakody et al., 2018), especially in the elderly. Although the gene therapy approaches described in previous articles in this Special Issue may improve these statistics in the future, currently the cochlear implant(CI)is the standard of care for remediation of severe to profound hearing loss. In general, contemporary CIs are highly successful. Average CI recipients using the latest technology score around 80% correct on speech recognition for high-context sentences and are able to use a telephone (Zeng et al., 2008). The most fortunate may even enjoy music (Drennan and Rubinstein, 2008; Won et al., 2010; Jiam et al., 2019). But there is still great variability in outcomes among individual CI recipients (Firszt et al., 2004; Holden et al., 2013) and especially in children using a CI (Svirsky et al., 2000; Ortmann et al., 2017; Zhao et al., 2019), and many CI users derive much less or even marginal benefit. Much of this variability is likely related to differences in auditory nerve survival, and the number of surviving cochlear SGNs in individual CI recipients has been shown to be an important factor influencing performance (Kamakura and Nadol, 2016; Seyyedi et al., 2014). Moreover, numerous studies have reported correlations of indirect functional measures of neural survival to CI outcomes (Holden et al., 2013; Scheperle, 2017; Schvarts-Leyzac and Pfingst, 2018), providing additional evidence for the importance of auditory nerve survival. Consequently, there has been great interest in recent years in exploring potential therapies that can improve auditory nerve survival by ameliorating the degeneration of the SGNs after deafness. In preclinical studies, osmotic pumps and other approaches have been used in deafened animal models to deliver a number of NTs directly to the cochlea, and these studies have shown promising results, especially with BDNF. However, use of osmotic pumps is not a good option for widespread clinical application due to concerns about infection and duration of efficacy, and other approaches are not yet fully developed (see Ma et al., 2019 for review). More recent studies have focused on the possibility of using neurotrophin gene therapy to force expression of NTs by target cells within the cochlea. This would provide a possible means for a one-time treatment to promote long-term NT expression and improved survival of SGNs after deafness.

In this review, we will first summarize the evidence for the efficacy of exogenous NTs in promoting improved survival of SGNs within the cochlea after hearing loss. In addition, we will review the animal research completed to date using NT gene therapy in the inner ear and in other non-auditory applications. Finally, we will outline some of the remaining issues with regard to selection of optimal vectors, treatment timing, and place/method of delivery, etc. That must be resolved prior to considering clinical application.

2. Exogenous neurotrophins promote improved SGN survival after deafness

There has been considerable interest over the past 2 decades in neurotrophic agents that might enhance SGN and auditory nerve survival and thereby improve outcomes with CIs (see Staecker et al., 2010; Leake et al., 2013). Of particular interest are the NTs, which belong to the nerve growth factor (NGF) family of proteins and include NGF, BDNF, neurotrophin-3 (NT-3), and NF-4/5, each of which binds to specific high-affinity receptors of the Trk family. BDNF and NT-3, in particular, are known to have major roles in both the development and maintenance of SGNs. During cochlear development, neurotrophins regulate neuronal differentiation and survival (Farinas et al., 2001; Fritzsch et al., 1999; Rubel and Fritzsch, 2002; Yang et al., 2011). Neurotrophic support to SGNs is provided by hair cells, supporting cells of the organ of Corti and neurons of the cochlear nucleus (Fritzsch et al., 1999; Schecterson and Bothwell, 1994; Stankovic et al., 2004), and SGNs express the receptors for BDNF (TrkB) and NT-3 (TrkC) (Schecterson and Bothwell, 1994; Ylikoski et al., 1993). Moreover, BDNF and TrkB have recently been identified in the developing human cochlea, suggesting a similar role in human SGNs (Johnson Chacko et al., 2017)

Both BDNF and NT-3 also play a role in the maintenance of SGNs in the adult cochlea (Qun et al., 1999; Ylikoski et al., 1993), and loss of this neurotrophic support after deafness leads to the gradual degeneration of SGNs through apoptotic cell death (Alam et al., 2007; Fritzsch et al., 1999). Moreover, numerous studies have demonstrated that exogenous NTs delivered directly to the cochlea by an osmotic pump over several weeks can protect SGNs and promote improved neuronal survival after deafness due to various insults (Ramekers et al., 2012; Leake et al., 2013; Ma et al., 2019). Highly significant neurotrophic effects of BDNF on SGN survival have been reported in deafened guinea pigs (Agterberg et al., 2008; Glueckert et al., 2008; Miller et al., 2007; Shepherd et al., 2008; Wise et al., 2005; Ramekers et al., 2015), and also in neonatally deafened cats (Leake et al., 2011). Neurotrophic effects also have been reported with other NTs such as glial-cell-line-derived neurotrophic factor (GDNF) (Kanzaki et al., 2002; Maruyama et al., 2008; Yagi et al., 2000; Ylikoski et al., 1998) and Fibroblast growth factor (FGF) (Glueckert et al., 2008). Moreover, although a single study reported precipitous SGN loss after NT delivery was terminated (Gillespie et al., 2003), several other more recent studies have shown that neurotrophic effects can persist long after terminating delivery of exogenous NTs (Agterberg et al., 2009; Leake et al., 2011; Shepherd et al., 2008), especially when combined with electrical stimulation (Shepherd et al., 2005; Leake et al., 2013). Importantly, when BDNF infusion was combined with implantation of a CI, highly significant improvement in SGN survival (>50% increase re: contralateral) was maintained when electrical stimulation from the CI continued 3–4 months after termination of BDNF delivery (Fig. 1; Leake et al., 2013).

Several labs have demonstrated that exogenous NT infusion also elicits significant improvement in survival of the radial nerve fibers in the osseous spiral lamina (the peripheral dendrites of the SGNs), as compared with deafened controls (Glueckert et al., 2008; Leake et al., 2011, 2013; Pettingill et al., 2007; Wise et al., 2005). Improved fiber

survival was associated with reduced thresholds and increased dynamic ranges for electrical stimulation delivered by a CI (Leake et al., 2013; Landry et al., 2013), which could improve CI function. However, BDNF infusion also commonly resulted in extensive ectopic and disorganized sprouting of the radial nerve fibers down into the scala tympani (Fig. 2) and taking a spiral course over hundreds of micrometers in the connective tissue encapsulating the implanted CI (Glueckert et al., 2008; Leake et al., 2011, 2013; Staecker et al., 1996). The typical ectopic fibers shown in Fig. 2 include both myelinated and unmyelinated profiles and clearly demonstrate ectopic sprouting (i.e., normally, SGN peripheral axons never appear in the scala tympani, but are limited to the osseous spiral lamina and organ of Corti). Moreover, although these animals received BDNF at one month of age, the anatomy of the cat cochlea is mature at this time, and the sprouted fibers persisted when the animals were examined as young adults at about 6 months of age. Importantly, Glueckert et al. (2008) also utilized immunolabeling after combined BDNF and FGF treatment to demonstrate that both the fibers within the osseous spiral lamina and the ectopic fibers elicited by BDNF treatment were afferent peripheral processes of SGNs, making them relevant to CI stimulation; whereas efferent fiber survival was not affected. Finally, electrophysiological studies recording from the inferior colliculus in deafened, BDNF-treated animals have shown that such sprouting can be deleterious to the optimal function of the CI, by degrading the normally precise cochleotopic organization of the radial nerve fibers and thereby degrading the selectivity of CI stimulation channels in the auditory midbrain (Leake et al., 2013). Recent advances in CI technologies such as current focusing and virtual channel stimulation rely on highly spatially restricted activation of the SGNs and would be undermined by such sprouting.

It has also been reported that exogenous NTs delivered to the cochlea can promote the survival of cochlear hair cells after trauma, in particular, several reports have demonstrated that neurotrophins can protect hair cells and ameliorate hearing loss after noise trauma (Keithley et al., 1998; Shoji et al., 2000; Shibata et al., 2007; Le et al., 2017). Improved hair cell survival could certainly contribute to enhanced SGN survival (and improved CI function). However, in most of the preclinical animal studies of the effects of NTs on SGN survival cited in the preceding section, careful study of cochlear histology demonstrated that very few hair cells survived in the deafened animals studied (e.g., Leake et al., 2011, 2013) and this was not a factor.

Finally, recent studies have shown that the synapses of SGNs on inner hair cells (IHCs) are more susceptible to acoustic trauma than the hair cells, and this synaptopathy may lead to functional impairment and SGN loss in ears with normal hair cells (Kujawa and Liberman, 2009). Thus, it is important to note that exogenous delivery of neurotrophin NT-3 can induce regeneration of the SGN peripheral fibers and reconnection to their IHC synapses and can rescue hearing function in adult animals exposed to acoustic trauma (Sly et al., 2016; Suzuki et al., 2016; Wan et al., 2014; Wang et al., 2011).

3. Cochlear neurotrophin gene therapy

Recent advances in cochlear molecular therapies, reviewed in several previous articles of this Special Issue, are showing exciting progress towards the development of clinical

therapies for hearing loss, by targeting the repair of genetic defects that result in loss of hair cells. As we have outlined above, preclinical studies have demonstrated a potentially important opportunity for NT therapy for preventing SGN degeneration after deafness and improving the outcomes with Cis. Virally-mediated gene therapy offers the potential advantage of administering a one-time injection to elicit safe and sustained expression of NTs by cells within the target tissue.

3.1. Studies in rodents

Numerous studies in deafened rodent models have reported that virally-mediated NT cochlear gene therapy using several different NTs (BDNF, GDNF, NT3, CNTF and others) is effective in reducing SGN degeneration and improving survival after insult. Most of the earlier studies employed Ad vectors (Table 1). Several studies in deafened guinea pigs have documented greater SGN survival compared to contralateral after cochlear administration of vectors forcing expression of NTs, particularly BDNF (Atkinson et al., 2012, 2014; Chikar et al., 2008; Nakaizumi et al., 2004; Rejali et al., 2007; Shibata et al., 2010; Wise et al., 2010, 2011). Further, studies in other deaf animal models, including deaf mutant mice (Fukui et al., 2012) and rats subjected to blast exposure (Wu et al., 2011), have demonstrated improved SGN survival after virally-mediated NT delivery to the cochlea, supporting the cross-species assumption. Several research groups have also reported improved survival of the radial nerve fibers (the peripheral processes of the SGNs) or evidence of their resprouting (Atkinson et al., 2012, 2014; Chen et al., 2018; Fukui et al., 2012; Shibata et al., 2010; Wise et al., 2010) after NT gene therapy. This could be beneficial to CI function by reducing thresholds to electrical stimulation and improving spatial selectivity and temporal coding, due to improving the proximity of the fibers to the CI electrodes. And some studies have reported evidence of functional improvements with an implanted CI (Chikar et al., 2008; Budenz et al., 2015; Pfingst et al., 2017) following cochlear NT gene therapy.

Most of the more recent studies have employed AAV vectors (Budenz et al., 2015; Pfingst et al., 2017; Chen et al., 2018) to force expression of NT-3 and or BDNF and have also shown impressive efficacy in improving both SGN and radial fiber survival. This transition to AAV likely occurred because AAV has been shown to efficiently transfer trangenes to the inner ear and is not ototoxic (Ballana et al., 2008; Konishi et al., 2008; Lustig and Akil, 2012; Gyorgy et al., 2017; Pfingst et al., 2017; Suzuki et al., 2017; Tao et al., 2018). Further, AAV already has been applied clinically without adverse effects (see section 3, below). Pfingst et al. (2017) reported long-term (albeit variable) efficacy of AAV-mediated NT-3 gene therapy in deafened, implanted guinea pigs and also showed that psychophysical and electrophysiological measures may be useful for monitoring SGN density in the implanted cochlea. Interestingly, Budenz et al. (2015) demonstrated that BDNF was more effective than NT-3 in preventing SGN degeneration and promoting long-term neural survival after deafness, but NT-3 had a greater effect in eliciting re-growth of radial nerve fibers. These authors suggested that a combined over-expression of both BDNF and NT-3 may be optimal for enhancing overall neural survival.

3.2. Studies in cats

The promising results in deafened rodents with NT gene therapy led to a recent study assessing the potential for applying gene therapy in the much larger feline cochlea, making results more relevant to the human cochlea (Leake et al., 2019). Another novel aspect of this study was that animals were deafened as neonates *prior to hearing onset* (systemic neomycin injections) to model congenital deafness. Gene therapy was delivered when animals were about a month old, to facilitate comparison to earlier studies in which exogenous BDNF was delivered by osmotic pumps at this age (Leake et al., 2011, 2013), and with the rationale that long-term SGN survival and improved CI outcomes are particularly important for the pediatric population. Two AAV vectors that had shown efficacy in other systems were compared, AAV2 encoding for BDNF (under control of the CGA promotor) and AAV5-GDNF (CBA promotor). Both vectors elicited modest neurotrophic effects, with about 6% of the normal SGN population rescued as compared to contralateral at 3 months post-injection. However, GDNF expression also elicited unwanted fiber sprouting into the scala tympani, and also failed to improve the number of surviving fibers within the osseous spiral lamina. In contrast, AAV2-mediated BDNF expression resulted in more than double the number of surviving radial nerve fibers as compared to untreated ears, with no ectopic or disorganized sprouting observed. Given the promising results with AAV2-BDNF, a follow-up study sought to determine if neurotrophic effects would persist when the post-injection survival period was extended to 6 months. The substantial neurotrophic effects seen in this long term study are illustrated in Fig. 3, with greater SGN neuronal survival maintained throughout the cochlea as compared to the contralateral control. Six months after AAV2-BDNF injections, the overall mean SGN survival was 53% of normal vs 39% contralateral, representing rescue of about 14% of the normal SGN population. Expressed as percentage increase normalized to contralateral survival, this represents an improvement in SGN survival of more than 35% relative to control. It is interesting that SGN survival at the time of vector injections (1 month of age) in these early-deafened cats was expected to be about 75% of normal (Leake et al., 2011). Thus, although AAV2-BDNF elicited a highly significant neurotrophic effect, some further degeneration of the SGNs in the injected ears still occurred. Immunohistochemistry performed 2 weeks after virus injections demonstrated that transfection occurs rapidly, but only moderate numbers of cells were transduced and peak expression of NT appears to take much longer. The survival of the radial nerve fibers was also quantified in these deafened animals by counting them in sections cut orthogonal to the radial plane at 3 cochlear locations. In the 6-month AAV2-BDNF group, SGN peripheral fiber survival was consistently higher in the injected ears in all 3 cochlear locations examined as compared to contralateral. Overall, fiber survival averaged 47% of normal after AAV2-BDNF treatment, double the value measured on the opposite side (24% of normal).

Together, the findings in a number of deafened animal models and using several different viral vectors and NTs delivered to the cochlea, suggest that NT cochlear gene therapy may offer a viable strategy for ameliorating the degeneration of SGN and radial nerve fibers following deafness. Thus, a treatment that requires only a single injection shows great promise as a means of promoting long-term improvement in the cochlear neural substrate and thereby enhancing CI outcomes.

3.3. NT gene therapy for cochlear synaptopathy

As mentioned earlier (Section 1), animal studies of noise-induced hearing loss have shown that exposures causing only reversible threshold shifts and no hair cell loss can result in permanent loss of the SGN synapses on IHCs (cochlear synaptopathy) and when followed long-term, can result in functional deficits and ultimately lead to SGN degeneration (Kujawa and Liberman, 2009). Further, several studies have shown that NT-3 can protect or even regenerate the IHC synapses and rescue hearing function after such acoustic trauma (Sly et al., 2016; Suzuki et al., 2016; Wan et al., 2014; Wang et al., 2011). Thus, it is noteworthy that recent animal studies have shown that AAV-mediated NT-3 overexpression also can protect against and repair noise-induced cochlear synaptopathy (Chen et al., 2018; Hashimoto et al., 2019). Moreover, recent research in both noise-exposed and aging human ears has shown that degeneration of cochlear synapses commonly precedes both hair cell loss and threshold elevations (Sereyenko et al., 2013; Kujawa and Liberman, 2015; Liberman, 2015, 2017; Liberman and Kujawa, 2017). The silencing of affected neurons significantly alters information processing and is likely a contributor to many common perceptual abnormalities such as speech-in-noise difficulties, tinnitus and hyperacusis. Accordingly, NT gene therapy ultimately might be useful for eliciting resprouting of SGN peripheral axons and regeneration of synapses to innervate residual (or regenerated) hair cell populations in such cases of “hidden” hearing loss.

4. Neurotrophin gene therapy in non-auditory applications

As in the auditory system, NTs and the members of the neurotrophin family also play critical roles in the development, maintenance and repair of the central nervous system (CNS) (see Review by Huang and Reichardt, 2001). Over the past three decades, multiple gene therapy approaches have been investigated to modulate neurotrophin receptor signaling in animal models and in clinical trials of several neurodegenerative diseases, including retinal and optic nerve degeneration, spinal cord injury, Alzheimer disease, Parkinson’s disease, Huntington’s Disease, and amyotrophic lateral sclerosis (see Review by Blesch et al., 1998; Khalin et al., 2015; Daly et al., 2018; Hardcastle et al., 2018; Hodgetts and Harvey, 2017; Mestre and Sampaio, 2017). Although significant progress has been made and mounting evidence from animal studies supports the efficacy of neurotrophin gene therapy in preventing neuronal degeneration and promoting neural repair, several technical limitations must be fully addressed before these applications can be successfully applied to human patients. This section focuses on both the efficacy and current challenges of these gene therapy applications. The discussion below summarizes our current knowledge about how to optimize delivery procedures to enhance efficacy and specificity in NT gene therapy in other neurodegenerative disorders and implications for the potential application in auditory nerve degeneration and hearing loss.

4.1. Other neurodegenerative disorders

Given the anatomical accessibility of the retinal ganglion cells, gene therapy (such as intravitreal gene transfer) has been an attractive strategy for inherited optic neuropathies and other retinal diseases (Thanos and Emerich, 2005; Yu-Wai-Man et al., 2014). It is important to note that although experimental data are encouraging, these gene therapy approaches are

still at an early stage of development, and further evidence of their efficiency, specificity and safety is needed before application to human patients. The NGF family of NTs such as NGF, BDNF and NT3 play several critical roles in retinal ganglion cell survival. For example, BDNF is generated locally by retinal ganglion cells (Herzog and von Bartheld, 1998). GDNF, a member of the transforming growth factor superfamily, shows retinal protection in several animal models of retinal degeneration (Thanos and Emerich, 2005). Previous studies support the notion that NT deprivation and dysfunction contributes to the pathogenesis of glaucoma (Bringmann et al., 2006; Johnson et al., 2011). AV vectors have been applied to induce Müller glial expression of BDNF in a rat model of optic nerve transection (Di Polo et al., 1998). Gauthier et al. (2005) documented that AAV-mediated BDNF transfer to Müller cells protected photoreceptors from light-induced retinal degeneration. Applying AAV mediated gene delivery to an animal model of human retinitis pigmentosa (S334ter-4 rhodopsin transgenic rat), AAV-GDNF prevented photoreceptor loss for at least 45 days (McGee Sanftner et al., 2001). These studies suggest that it may be possible to slow or prevent retinal and optic nerve degeneration by using viral vectors to increase endogenous retinal production of select NTs.

The most frequently studied NT protein in experimental spinal cord injury (SCI) is BDNF, which plays a critical role in axonal sprouting, neuroprotection, myelination, adaptive synaptic plasticity, synaptic transmission and potential antioxidative effects (Kovalchuk et al., 2004; Weishaupt et al., 2012). Ziemlinska et al. (2014) reported a significant improvement in the treadmill locomotor capabilities in a rat model of complete spinal cord transection treated with local BDNF overexpression mediated by AAV1/2 vectors under control of the neuron-specific human SYN 1 promoter. Intraspinal injections of AAV-BDNF were performed within 30 min after spinal cord trans-section and locomotor function improvement was identified as early as two weeks after treatment and lasted for at least 7 weeks. An upregulation of molecules related to excitatory neurotransmission was seen in the AAV-BDNF treated animals. However, an altered balance of excitatory and inhibitory neural activity was also detected around the injured site. These changes may have contributed to the motoneuron hyperexcitability that was often seen after injury. In a chronic SCI model (*twy/twy* mice), a retrograde adenovirus (Ad-mediated) BDNF gene transfection resulted in reduced cell death in neurons and oligodendrocytes (Uchida et al., 2012). This neuroprotective effect was reported 4 weeks after Ad-BDNF delivery, but no functional evaluation was performed in the treated animals. Two earlier studies also demonstrated that virus-mediated BDNF gene transfection reduced neural cell death and promoted axonal regeneration and locomotor functional recovery for at least 6 weeks following the injections in rat models of spinal cord injury (Koda et al., 2004; Nakajima et al., 2010). AAV-mediated BDNF + GDNF transfection promoted the survival of motor neurons but failed to show functional recovery following ventral root avulsion (Blits et al., 2003). A recent study using a cervical spinal cord injury model showed that the delivery of AAV7-mediated gene transduction for TrkB, a high-affinity receptor of BDNF, enhanced recovery of respiratory function (Martínez-Gálvez et al., 2016). Efficacy of NT gene therapy has also been investigated in numerous studies using other NT family members, combinations of multiple NTs, or combined NT overexpression with cell-based treatment (e.g., implants of stem cells or Schwann cells) (see review by Blesch et al., 1998; Hendriks et al., 2004; Harvey et al.,

2015; Hodgetts and Harvey, 2017). Injection of an AAV-BDNF and AAV-NT-3 mixture into the spinal gray matter, caudal to a Schwann cell graft transplanted in injured animals, resulted in improvement of locomotor function, however no evidence of regeneration of axons from the Schwann cell implant was identified (Blits et al., 2003). It is important to note that the virus-mediated gene therapy was given immediately or shortly (e.g., within 3 days after the injury) after injury in all studies mentioned above.

Dysregulation of NTs, such as BDNF and NGF, is a major factor in neuropathological disorders including Alzheimer's disease, Parkinson's disease, Huntington's disease and schizophrenia (Phillips et al., 1990; Sampaio et al., 2017; Simmons, 2017). Alzheimer's disease is a progressive neurodegenerative disease and the main cause of dementia in older adults (see review by Loera-Valencia et al., 2019). Studies have suggested that decreased expression of NTs and dysregulation of their related signaling pathways contribute to Alzheimer's disease and Parkinson's disease (Alves et al., 2016; Ventriglia et al., 2013). Various levels of success in NT gene therapy using a viral-based delivery system have been reported in several animal studies (Nagahara et al., 2009, 2013; Alves et al., 2016; Jiao et al., 2016) as well as in clinical trials (Rafii et al., 2014; Tuszynski et al., 2015; Malkki, 2015). Application of lentivirus-mediated BDNF into the cortex of an animal model of Alzheimer's disease (APP amyloid-transgenic mice) enhanced the expression of the synaptic protein, reversed synapse degeneration and improved learning and memory function (Nagahara et al., 2009). In this study, similar lentivirus-mediated BDNF treatment was administered to both aged rats and primates. Improvement of cognitive function, along with reduced neuronal atrophy, was reported by 2–4 weeks after treatment in these animals. This beneficial neuroprotective effect, however, appeared independently with the presence of amyloid plaques. Jiao et al. (2016) applied BDNF gene therapy in P301L mice, an animal model of Alzheimer's disease that is characterized by age-related tau pathology and memory impairment. The mice were given intraventricular injections of AAV8-BDNF at 3 months of age before the occurrence of significant tau pathology and cognitive impairment. Restoration of neural structures and improvement of cognitive function, but no changes in the tau pathological condition, were reported 9 months after treatment.

NGF is another important NT, which is necessary to prevent the loss of basal forebrain cholinergic neurons that often undergo early pathological alterations in Alzheimer's disease. Investigations from several Phase I clinical trials suggested that virus-mediated NGF therapy might prevent or reduce the degeneration of cholinergic neurons in Alzheimer's disease patients. In one report, AAV2-NGF was administered to 10 Alzheimer's disease patients through bilateral injections into the basal forebrain regions (Rafii et al., 2014). The brains of all treated patients exhibited a trophic response to NGF transduction and a reduced rate of cognitive decline. Examination of brain-autopsy tissues in this investigation identified bioactivity of AAV2-mediated NGF expression; the longest post-treatment assessment time in this study was about 24 months, suggesting that this approach may be feasible and capable of producing relatively long-term and biologically active NT expression. In an earlier report, NGF was delivered through transplantation of autologous fibroblasts transfected with NGF-leukemia viruses into the basal forebrain region that contained cholinergic neurons (Tuszynski et al., 2005). This *ex vivo* study reported a structural and functional improvement over a 2-year observation period. The second phase I study by the

same group reported that degenerated neurons responded to NGF gene therapy in all treated patients (Tuszynski et al., 2015). The observation periods for the patients in this study varied from 11 months to 10 years. Again, these data suggest that the virus-mediated neurotrophin gene via either *ex vivo* or *in vivo* delivery is a potential means of treating Alzheimer's disease and other neurodegenerative diseases.

4.2. Lessons learned from NT gene therapy studies in non-auditory systems

A growing body of evidence has demonstrated an important linkage between age-related deafness, blindness and dementia (see review by Mancino et al., 2018; Chern and Golub, 2019), suggesting some common mechanisms of neurodegeneration in the peripheral auditory nerve, retinal and optic nerve and brain. It is also possible that the loss of sensory information and social communication is detrimental to neurocognitive function and leads to acceleration of cognitive decline. As evidence for this hypothesis, a recent study of cochlear implantation in older adults noted improvement in attention and working memory such as with the operation span task following implantation (Volter et al., 2018). A better understanding of the challenges and issues identified in the studies of NT gene therapy in retinal and optical nerve degeneration, spinal cord injury, Alzheimer's disease and other neurodegenerative disorders has important implications in designing and refining the investigations of these applications in the auditory system and hearing loss. Three major issues discussed here delineate how to optimize the efficacy of NT gene therapy approaches. *First*, the efficacy of NT gene therapy is very much time-dependent such that the earlier the disease stage when treatment occurs, the better the outcome. As mentioned above in CNS trauma such as spinal cord injury, the treatments were given either immediately or shortly after injury in most of these animal studies. Similarly, in Alzheimer's disease and other neurodegenerative disorders, late-stage patients had little response to neurotrophic gene therapy (Bartus and Johnson, 2017b). A greater understanding of the dynamic changes in the endogenous NF expression features in different populations of neural cells and conditions of inflammation after CNS injury or at different stages of neurodegenerative disease is crucial for optimizing the timing of the administration. *Second*, it is critical to determine the best locations to administer the gene therapy reagents. Virus-mediated gene therapy has emerged as a promising therapeutic approach for SCI, Alzheimer's disease, and other neurodegenerative disease because the technology is able to provide controlled, long-term biologically active NTs in the targeted sites or cells. However, developing an effective and safe administration approach is still a challenge for many of these applications. For instance, in the phase I clinical trial of Alzheimer's disease mentioned above, the *ex vivo* delivery approach was developed for NGF gene transduction in the cholinergic neurons of the basal forebrain region (Tuszynski et al., 2005). *Finally*, experimental effort is still needed to determine the best approach to administer the treatment and whether a specific dose or volume of virus is adequate (Harvey et al., 2015; Bartus and Johnson, 2017a, 2017b). Careful consideration is required with respect to potential implications of the exogenous neurotrophin gene expression on the function of the host neural cells, such as dysregulation of the neurotransmitter or disruption of neural plasticity. For example, treatment with overdosage of neurotrophic factors in some cases of spinal cord injury may produce side effects such as increased sensitivity to pain or epilepsy (Cunha et al., 2009; Weishaupt et al., 2012; Hodgetts and Harvey, 2017). Continued high expression level of BDNF led to

negative feedback of the BDNF/TrkB signaling pathway and 'trapping' of regenerated axons (Eaton et al., 2002; Blits, 2003).

5. Future goals and challenges for clinical application

Preservation of SGNs has been the focus of multiple preclinical cochlear gene therapy studies designed to either preserve/restore hearing, or improve the function of cochlear implants. As reviewed above, numerous studies have demonstrated that Ad or AAV mediated delivery of NTs (BDNF, GDNF and NT-3) can ameliorate the degeneration of the cochlear SGNs after deafness. Unlike conventional drug treatments, cochlear gene therapy is a complex biological treatment, and its efficacy may depend on a multitude of factors. These include, but are not limited: 1) optimization of the viral vector design (safety, toxicity and target specificity) and high quality vector production (efficacy at the lowest possible dose; ability to reproduce long lasting and stable levels of expression); 2) site and method of viral delivery; and 3) intervention at an appropriate stage of the hearing disorder.

5.1. Selection of optimum viral vector(s)

A number of engineered replication-deficient viral vectors have been described in previous gene therapy studies, particularly the Ad and AAV vectors, which have emerged as the most widely used tools for virally-mediated delivery of NTs to the cochlea (Table 1).

5.1.1. Adenovirus—Over the last two decades, Ad has become quite a popular choice for gene therapy (Lee et al., 2017). Thus, it is not surprising that it has also been one of the two principle viral vectors used for cochlear gene therapy (Praetorius et al., 2009; Staecker et al., 2014). Some of the advantages of Ads for gene delivery are their relatively high cloning capacity, more than 10 kb, and tropism for a number of cochlear cell types. The cloning capacity is important because numerous diseases are caused by mutations in genes with coding sequences exceeding the AAV capacity. Thus, the cloning capacity of Ads vectors comprises a critical advantage over AAV. The duration of transgene expression is typically a few weeks to months, which is advantageous for applications in which a relatively short period of expression is required. This is also one of its principle disadvantages for NT gene therapy or for genetic forms of hearing loss, where long-term gene expression may be essential. Another disadvantage of Ad is its tendency to elicit an immune response. Although newer generations of Ad have been engineered to reduce this likelihood, if repeated delivery is required it could potentially aggravate immune-related side effects. The more complicated nature of Ad and the increased potential to elicit unwanted immune responses may limit their application in the ear despite their high cloning capacity.

5.1.2. Adeno-associated virus—Numerous studies have documented partial or complete rescue of hearing loss in mouse models of genetic deafness using AAV as a vector for gene therapy (Akil et al., 2012; Askew et al., 2015; Emptoz et al., 2017; Geng et al., 2017; Isgrig et al., 2017; Pan et al., 2017; Akil et al., 2019a,b; see other papers in this Special Issue). AAV vectors have the ability to efficiently transduce post mitotic cells (Colella et al., 2018). They also have excellent safety profiles (low immunogenicity), which is an advantage over Ad. Moreover, it has been demonstrated specifically that AAV is not

ototoxic (Ballana et al., 2008; Konishi et al., 2008; Lustig and Akil, 2012; Gyorgy et al., 2017; Pfingst et al., 2017; Suzuki et al., 2017; Tao et al., 2018). Importantly, AAV vectors have demonstrated efficacy in proof-of-concept studies and clinical trials for gene delivery to the eye and other tissues (Simonelli et al., 2010; Flotte et al., 2011; Nathwani et al., 2011; Bowles et al., 2012; Le Meur et al., 2018). Further, recombinant AAV vectors are non-replicating, can efficiently transfer transgenes to different cochlear cell types, including non-dividing neurons and hair cells. AAV is not incorporated into the host genome; rather, the virus remains episomal and results in stable, long-term expression of the transgene (Xia et al., 2012). Because long-term expression is very important for many human applications, the long-term expression elicited by AAV vectors comprises a critical advantage over Ad. This suggests that AAV vectors may be the best option for delivery of NTs to the inner ear, since NTs expression is likely to be required over the long term. Finally, the cochlea is a favorable target organ for gene transfer because it is relatively isolated from surrounding tissues, limiting viral spread and exposure to the immune system.

5.1.3. Promoters and serotypes—Specificity for gene delivery can be achieved through the use of tissue-specific promoters. Although directing the expression of a transgene with a cochlear cell-specific promotor may minimize unwanted off-target effects, whether the promotor actually increases the transduction efficiency for the cochlear cells remains unclear. Toxicity and inflammation have also been seen with broadly active promoters (CMV, CBA., etc), but not with cell-type–specific promoters, in some tissues including the heart (Ai et al., 2008; Merentie et al., 2016) and the central nervous system (Klein et al., 2006; Watakabe et al., 2015). One mechanism that might explain this toxicity is that broadly active promoters tend to drive higher expression of transgenes than cell-type–specific promoters. The use of cell-specific promoters can reduce the transgene expression in off-target cells and increase the specificity of the gene delivery to the cell type of interest. There are a number of possible candidates for cochlear cell-type-specific promoters to choose from. Such promotors include myosin VIIA promoter, elongation factor 1α promoter, neuron-specific enolase promoter and glial fibrillary acidic protein promoter. These promoters have been cloned and well-characterized. Boeda et al. (2001) characterized the myosin VIIA promoter, which exhibits strong, selective expression in hair cells of the cochlea and vestibule. Lui et al. (2007) further demonstrated that the myosin VIIA promoter provided selective expression of eGFP within hair cells following cochlear injection in adult rats and mice. These same authors also showed that the neuron-specific enolase promoter and the elongation factor 1α promoter both provided selective eGFP expression within SGN and cells of the spiral ligament (Lui et al., 2007). Earlier, Rio et al. (2002) observed glial fibrillary acid protein promoter selective activity in all cochlear supporting cells early after birth.

Transfection specificity also can be accomplished through retargeting of the AAV to alternate cellular receptors, by using different AAV serotypes that have different binding sites (Nam et al., 2011). The effectiveness of NT gene therapy is likely to depend on the viability of the cochlear cells targeted for viral transfection. In cases where the organ of Corti (OC) is selected as the target (Wise et al., 2010), ongoing degeneration as a consequence of deafness pathology may limit the capacity of NT gene therapy to provide

neurotrophic support necessary for the protection of SGNs. Viral transfection of cells within the scala media was still possible even after severe degeneration of the OC (Wise et al., 2011). Among these cells are the supporting cells (e.g., pillar and Deiters'cells), cells within the stria vascularis and the spiral ligament of the cochlear lateral wall, endosteal cells coving the scala compartments and interdental cells within the spiral limbus (Wise et al., 2011). The optimization of the transduction efficiency is fundamental to maximizing the treatment effect of NT gene therapy; however, the AAV serotype, viral load, and promoter combinations that efficiently transduce specific cochlear cell types are largely unknown. Various AAVs serotypes haven been used for neurotrophin delivery in animal studies, including AAV2 (Budenz et al., 2015; Pfingst et al., 2017; Leake et al., 2019), AAV8 (Chen et al., 2018) and AAV5 (Leake et al., 2019) (Table 1). Although all these studies demonstrated improvement in SGN survival after deafness, no direct comparison of the efficacy of the various AAV serotypes used for NT gene therapy can be made due to differences in other variables, such as different animal models, sites and methods of delivery, concentration and dose of virus, etc.. One study by Kilpatrick et al. (2011) study directly investigated the transduction efficiency and cellular specificity of several available AAV vectors (serotypes 1, 2, 5, 6, and 8) in normal and drug-deafened ears. This study demonstrated that all five serotypes of AAV vectors successfully transduced the common cochlear cell types mentioned above, which suggests that any of the AAV serotypes can be used for efficient NT SGN gene therapy. However, the recombinant AAV2 serotype appears to be the best choice for two reasons: 1) It efficiently transduces the targeted cochlear cells. 2) It is the most common viral vector adopted in clinical trials for other organs (e.g., ocular gene therapy), and is currently being used for treatment of Leber's congenital amaurosis (Cideciyan et al., 2013; Bainbridge et al., 2015; Russel et la. 2017) and choroideremia (MacLaren et al. 2014; Edwards et al. 2016).

Studies conducted to date highlight the need to develop sensitive assays, specific to the organ and cell types that are being targeted, for each viral construct. Such assays will enable the design of vectors that can be applied safely to deliver optimum doses of vectors, potentially leading to both greater safety and efficacy (Xiong et al., 2019). If it is possible to develop safer vectors, a greater number of cochlear cells may be transduced, likely leading to greater efficacy and reduced safety concerns.

5.2. Determining optimum concentration/dosage; implications for clinical application

Whereas inner ear gene therapy may ultimately prove to be a useful therapeutic tool, safety issues for viral gene delivery must be carefully considered relative to the expected benefits of the procedure. In order to optimize virus-mediated gene therapy for clinical application, it will be necessary to identify a viral titer that will provide the expected benefit, while eliciting minimal or no toxicity. The two factors contributing to the transduction efficiency of a viral vector are the viral dose and the promoter, two variables that also show the strongest association with toxicity. It is likely that other variables also can contribute to toxicity (e.g., stocks with a high degree of endotoxin, or non-viral protein contamination). This highlights the importance of optimizing the viral load and promoter construct, which are critical to maximizing the treatment effect in the development of cochlear-targeted gene therapy.

5.2.1. Vector dose—The current cochlear local delivery approaches sometimes lead to transfection of only cells that are near the injection site, which comprise a small percentage of target cells. A more complete infection would likely lead to improvement in the desired effect(s), but would require a larger viral load which can lead to toxicity. Toxicity associated with higher doses has been seen in animal studies in the eye and other tissues (Mingozzi and High, 2013; Hinderer et al., 2018; Vandenberghe et al., 2011; Ramachandran et al., 2017; Khabou et al., 2018). A recent cochlear gene therapy study by Akil et al., 2019a reported that the overexpression of hGDNF elicited by AAV5-hGDNF in newborn mice resulted in severe neurological symptoms and hearing loss due to Purkinje cell loss and cochlear nucleus pathology. Thus, extremely high levels of transgene protein expression should be avoided, particularly for proteins that may have neurological functions (Akil et al., 2019a). This can be achieved by reducing the amount of the virus injected into the ear to minimize toxicity while still providing a good level of expression of the protein of interest in the targeted cells.

5.3. Route of administration - scala tympani vs scala media

A number of animal studies have reported the successful application of various delivery routes used for gene transduction in sensory hair cells, spiral ganglion neurons and the cells in the stria vascularis (see review by Lustig and Akil, 2012; Géléoc and Holt, 2009; Chien et al., 2015; Ma et al., 2019). Perhaps the most predictable of all the variables is the surgical delivery to the inner ear space, which must minimize trauma to the inner ear but maximize viral transduction of the target cells throughout the cochlea turns while preserving hearing function. Reflux of the vector outside of the cochlea may reduce the dose administered to target cells. Furthermore, the injected vector particles may stimulate an immune response against the viral capsids, which may further degrade the number of effective vector particles in the cochlear space. Safe and reproducible delivery of gene therapy vector into the inner ear is essential for successful targeting of the cochlear cells. The discussion here focuses on the comparison of two main delivery routes: 1) into the perilymph within scala tympani, and 2) into the endolymph within the scala media. The scala tympani procedures, which can be performed via the round window membrane, oval window, or direct cochleostomy through the bony otic capsule, have been the most frequently used methods for cochlear gene therapy. Among these, the route via the round window membrane has favored as the best procedure with respect to protecting residual hearing, and several studies have demonstrated effective NT gene therapy utilizing this approach (Table 1). However, the scala tympani method generally showed lower transduction efficacy in cochlear cells compared to the scala media approach. The scala media route is accessed by a cochleostomy through the cochlear lateral wall or by direct injection through the basilar membrane (Shibata et al., 2009; Wise et al., 2010: Kilpatrick et al., 2011; Chang et al., 2015). The surgical procedures for this route have a higher likelihood of causing hair cell damage and hearing loss due to the complexity of the organ of Corti and the importance of the endolymphatic barrier and ion homeostasis for normal hearing function. Kilpatrick et al. (2011) reported an efficient AAV inoculation approach via the scala media performed in mouse ears with limited injection trauma. In this study, a microinjection system (WPI) capable of delivering volumes in the nanoliter (nl) *to* microliter (μl) range was employed to precisely control the delivered amount (<350 nl) and speed of injection into the scala media via the cochlear lateral wall. This approach, in adult

mice that were deafened with kanamycin and furosemide, achieved a high transduction efficiency for AAV8 in the auditory nerve. Finally, the optimum injection location will also depend upon the specific cells targeted by the gene therapy. For example, if the SGNs are the targeted cells, the scala tympani is likely to be a better choice because it is directly adjacent to Rosenthal's canal, whereas the scala media is much more distant from the ganglion. Moreover, the scala media is also isolated from the remainder of the cochlear structures by tight junctional complexes among all the cells limiting this specialized fluid space.

5.4. Timing of administration; consideration of the host cochlear microenvironment, glial cell activation and inflammatory responses after deafness

The timing of the administration of the therapeutic agent is an essential factor for the efficacy of neurotrophin gene therapy. As noted in the discussions above, gene therapy studies involving Alzheimer's disease and CNS trauma such as spinal cord injury suggest that the earlier the treatment (e.g., gene therapy given immediately after injury) the better the outcome (Harvey et al., 2015; Bartus and Johnson, 2017a, 2017b). It is believed that early administration may be helpful to reducing axonal retraction, protecting myelination, and modulating the phenotype and activation of microglia/macrophages recruited from circulation. In the peripheral auditory nervous system, loss of the auditory nerve occurs through either primary degeneration or secondary degeneration after hair cell loss as the result of cochlear insults (Spoendlin, 1984; Leake and Hradek, 1988; Kujawa and Liberman, 2009; Lang, 2015a,b; Liberman, 2015). As shown in Fig. 4, several pathological alterations appear in non-neuronal cells of the degenerated auditory nerve following cochlear insult resulting from noise exposure and administration of ototoxic drugs. These pathological changes include demyelination, disruption of the node of Ranvier, and activation of glial cells, along with increased recruitment and activation of macrophages (or other immune cells). Importantly, all these pathological alterations appear in a time-dependent sequence, as reported in several animal studies (Lang et al., 2011, 2015; Tagoe et al., 2014; Panganiban et al., 2018; Kohrman et al., 2019). Previous observations have suggested that the NT treatment must be applied in relatively early stages of disease to be effective, i.e., when there are still enough healthy neurons to respond to therapy (Harvey et al., 2015; Quintino et al., 2019). However, the timing of SGN degenerative alterations varies greatly among different animal models (Wise et al., 2011; Leake et al., 2019). Careful choice of the timing of administration based on consideration of the cochlear pathophysiological conditions following deafness, in particular, the pathologies of these non-neuronal cells, should be included into the experimental design in applications of NT gene therapy.

5.5. Site of administration: tonotopic gradients in endogenous cochlear NT expression

Neurotrophins BDNF and NT3 play pivotal roles in the development and early maintenance of the tonotopic orgnization of the auditory nerve, both structurally and functionally (Pirvola et al., 1992; Davis, 2003; Flores-Otero et al., 2007; Fritzsch et al., 2015). Embryonic elimination of the *Bdnf* gene results in a reduction of auditory nerve innervation to hair cells in the apical turn, while NT-3 gene knockout causes a significant loss of basal turn spiral ganglion neurons (Fritzsch et al., 1997). Moreover, BDNF has a higher expression in the basal region, whereas NT-3 appears to have a higher concentration in the apex in both the postnatal and adult auditory nerve (Fariñas et al., 2001; Sugawara et al., 2007). Using a

novel *in vitro* system, Adamson et al. (2002) showed that BDNF and NT3 have opposite effects on the firing patterns of SGNs. Specifically, exposure to BDNF enhanced the activity of the SGNs in the apex, while the impact on the neurons was limited in the basal turn. In contrast, exposure to NT-3 resulted in firing patterns of neurons in both the apex and base being similar to those of the apical control. Similar to the current challenges we discussed above in the investigations of spinal cord injury and Alzheimer's disease, it is imperative to consider the proper location and cells in the cochlea that the gene therapy will target. How to limit or avoid the disruption of the tonotopic firing properties of the surviving SGNs may be a crucial question to address when developing novel clinical applications of NT gene therapy. For the long-term goal of restoring hearing by regeneration of hair cells or reconnecting surviving hair cells to the cochlear nerve, the gene therapy must be designed to maintain/restore the normal intrinsic firing attributes of the SGN. On the other hand, in the case of the potential use of NT gene therapy to maintain improved survival of SGN for application of a CI, it is possible that intrinsic firing properties of SGNs may be overwhelmed by the relatively crude direct electrical stimulation delivered by a CI, and thus may not be as important.

6. Conclusions

If the numerous challenges outlined above can be addressed adequately and safety concerns allayed by further research in animal models, NT cochlear gene therapy could potentially provide a single treatment that might significantly improve neural survival and enhance outcomes in CI recipients. This opportunity is particularly important for pediatric CI recipients who must depend on electrical hearing for an entire lifetime, and in whom the outcomes are extremely variable (Svirsky et al., 2000; Ortmann et al., 2017; Zhao et al., 2019).

Moreover, in the future, NT gene therapy may also offer the potential of remediating hearing loss in patients with noise-induced hearing loss or neural presbyacusis (e.g., age-related cochlear synaptopathy), in whom primary neuronal loss is a key contributor to hearing loss. Specifically, animal studies of noise-induced hearing loss have shown that exposures causing only reversible threshold shifts and no hair cell loss can result in permanent loss of the SGN synapses on hair cells, resulting in functional deficits and ultimately leading to SGN degeneration (Kujawa and Liberman, 2009). Similarly, recent research in human ears has shown that degeneration of cochlear synapses commonly precedes both hair cell loss and threshold elevations (Sereyenko et al., 2013; Kujawa and Liberman, 2015; Liberman, 2015, 2017). Cochlear synaptopathy can be widespread in ears with intact hair cell populations and normal audiograms, where it has been called "hidden" hearing loss (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5438769/; Schaette and McAlpine, 2011). The silencing of affected neurons significantly alters information processing and likely underlies many common perceptual abnormalities such as speech-in-noise difficulties, tinnitus and hyperacusis. Thus, NT gene therapy ultimately might be useful for eliciting resprouting of SGN peripheral axons and regeneration of synapses to innervate residual (or even regenerated) hair cell populations. In this review, we have outlined substantial evidence that BDNF gene therapy can improve survival of SGNs and elicit maintenance or resprouting of their peripheral axons. And NT-3 has been shown to promote synaptic regeneration of these

fibers, reconnecting them to the hair cells and their ribbon synapses and to rescue hearing function in adult animals exposed to acoustic trauma (Sly et al., 2016; Suzuki et al., 2016; Wan et al., 2014; Wang et al., 2011;Hashimoto et al., 2019). Thus, as earlier suggested by Budenz et al. (2015) the combination of BDNF and NT-3 gene therapy may be optimal for maintaining/restoring a more normal cochlear neural substrate.

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Abbreviations

AAV	Adeno-associated viral vector
Ad	Adenoviral viral vector
BDNF	Brain-derived neurotrophic factor
CI	Cochlear implant
FGF	Fibroblast growth factor
GDNF	Glial-cell-line-delivered neurotrophic factor
IHC	inner hair cells
NGF	Nerve growth factor
NTs	Neurotrophic factors
NT-3	Neurotrophin-3
NT-4/5	Neurotrophin-4/5
SGN	Spiral ganglion neuron
SCI	Spinal cord injury
SYN 1	Synapsin 1

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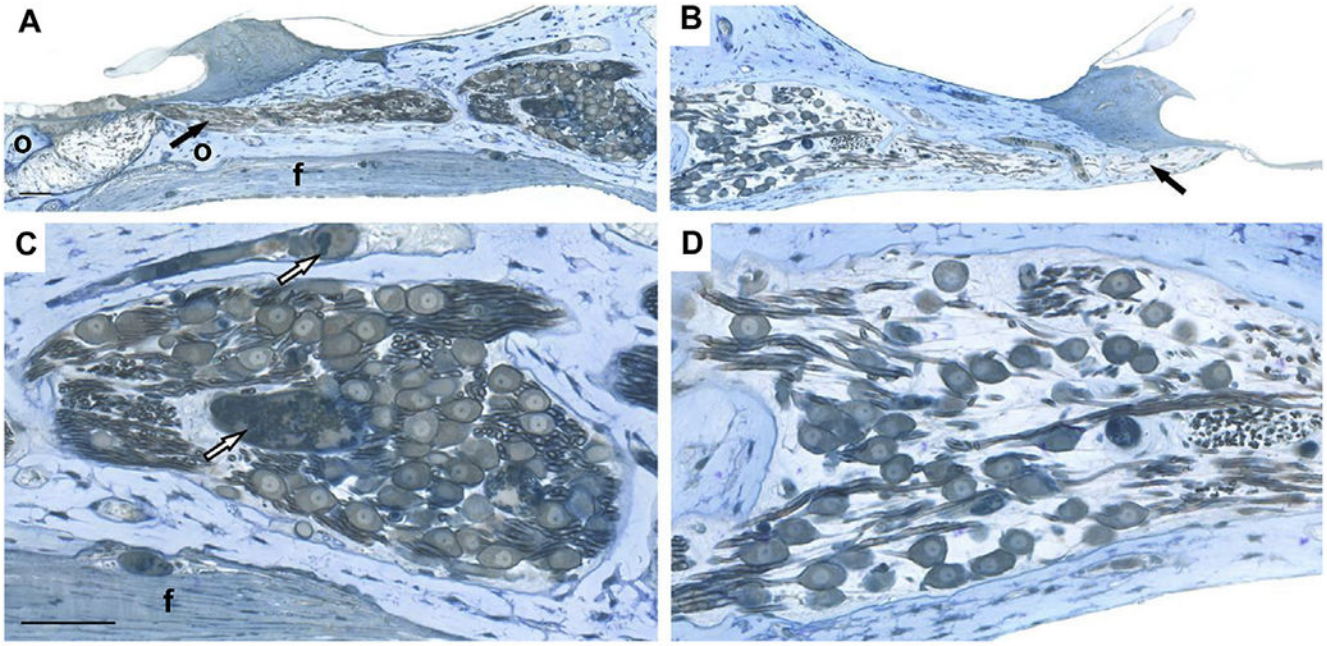

Fig. 1.

Histological sections of the organ of Corti and spiral ganglion from a cat that was neonatally deafened (daily systemic injections of neomycin sulfate) and examined after 10 weeks of BDNF infusion (via osmotic pump) combined with several months of electrical stimulation delivered by a cochlear implant. Left panels (A,C) show implanted cochlea with neo-osteogenesis (o) and fibrosis (f) that encapsulated the CI electrode within the scala tympani; right panels (B,D) show the same cochlear region of the contralateral ear. Highly significant neurotrophic effects persisted when CI stimulation was continued for 3 months after BDNF infusion was completed. On average, for a group of 5 animals, SGN survival was 70% of normal after BDNF and ES as compared to 45% of normal in the contralateral untreated ears. The greater density of SGN cell bodies and radial nerve fibers (filled arrows) is evident in the implanted cochlea. Larger vessels compared to normal (open arrows in C) indicate neo-angiogenesis. Scale bar = 50 μm. Modified and reprinted from Leake et al. (2013), Fig. 5 with permission from JARO, Springer Science + Business Media, LLC.

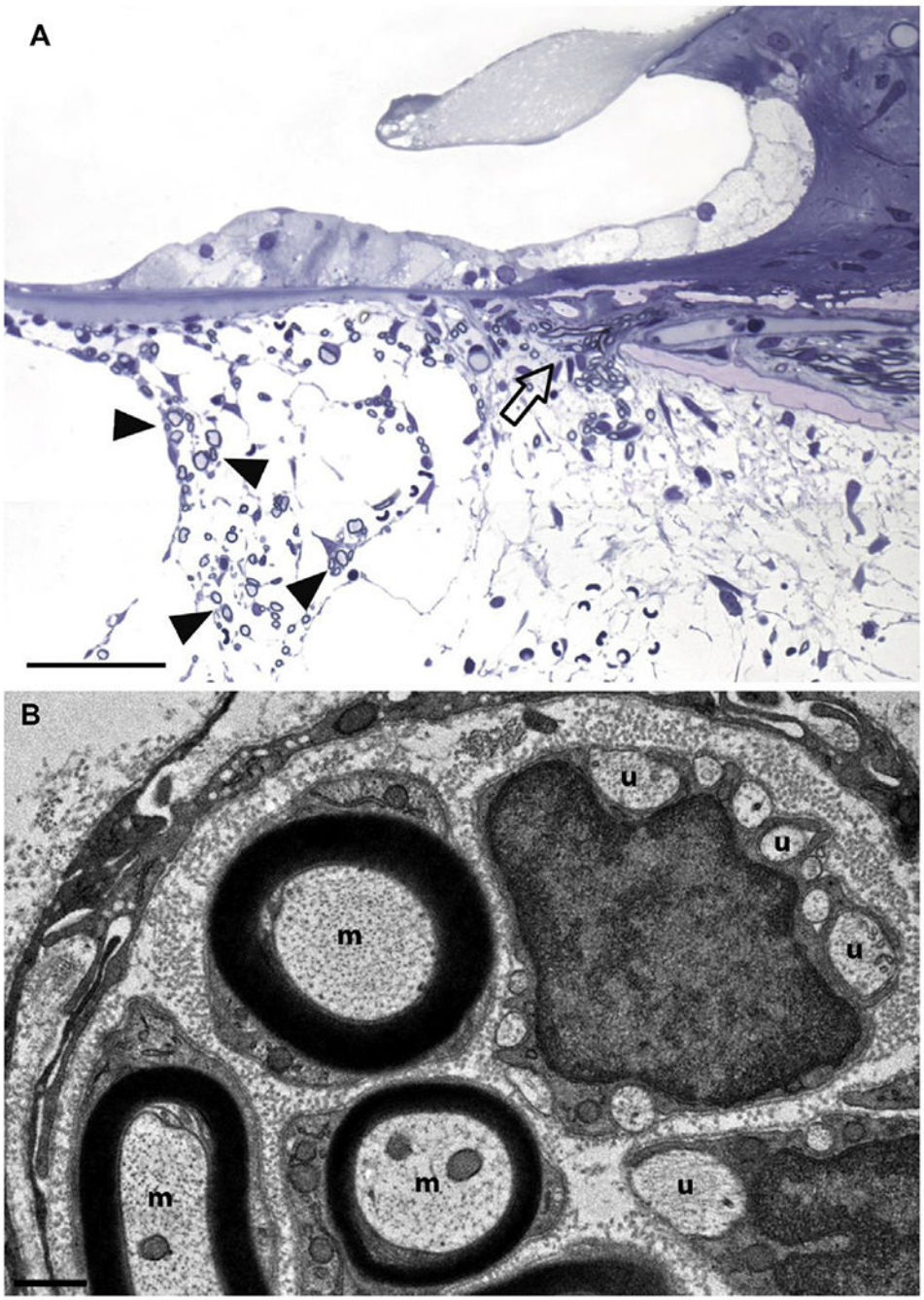

Fig. 2.

A. Light microscopic image of the organ of Corti in the cochlear base from a neonatally deafened cat after combined BDNF + ES. Disorganized and ectopic sprouting of radial nerve fibers is illustrated, with fibers exiting the osseous spiral lamina, passing down into the scala tympani (open arrow) and forming small bundles (arrowheads) that take a spiral course within the fibrotic tissue matrix above the CI electrode. Scale bar = 50 μm. B. Transmission electron micrograph showing the ectopic fibers in the scala tympani, same cochlea shown in A. Several large, well-myelinated axonal profiles (m) as well as numerous unmyelinated

fibers (u) are present. Scale bar = 0.5 μm. Reprinted from Leake et al. (2013), Fig. 8, with permission from J. Assoc. Res. Otolaryngol., Springer Science + Business Media, LLC.

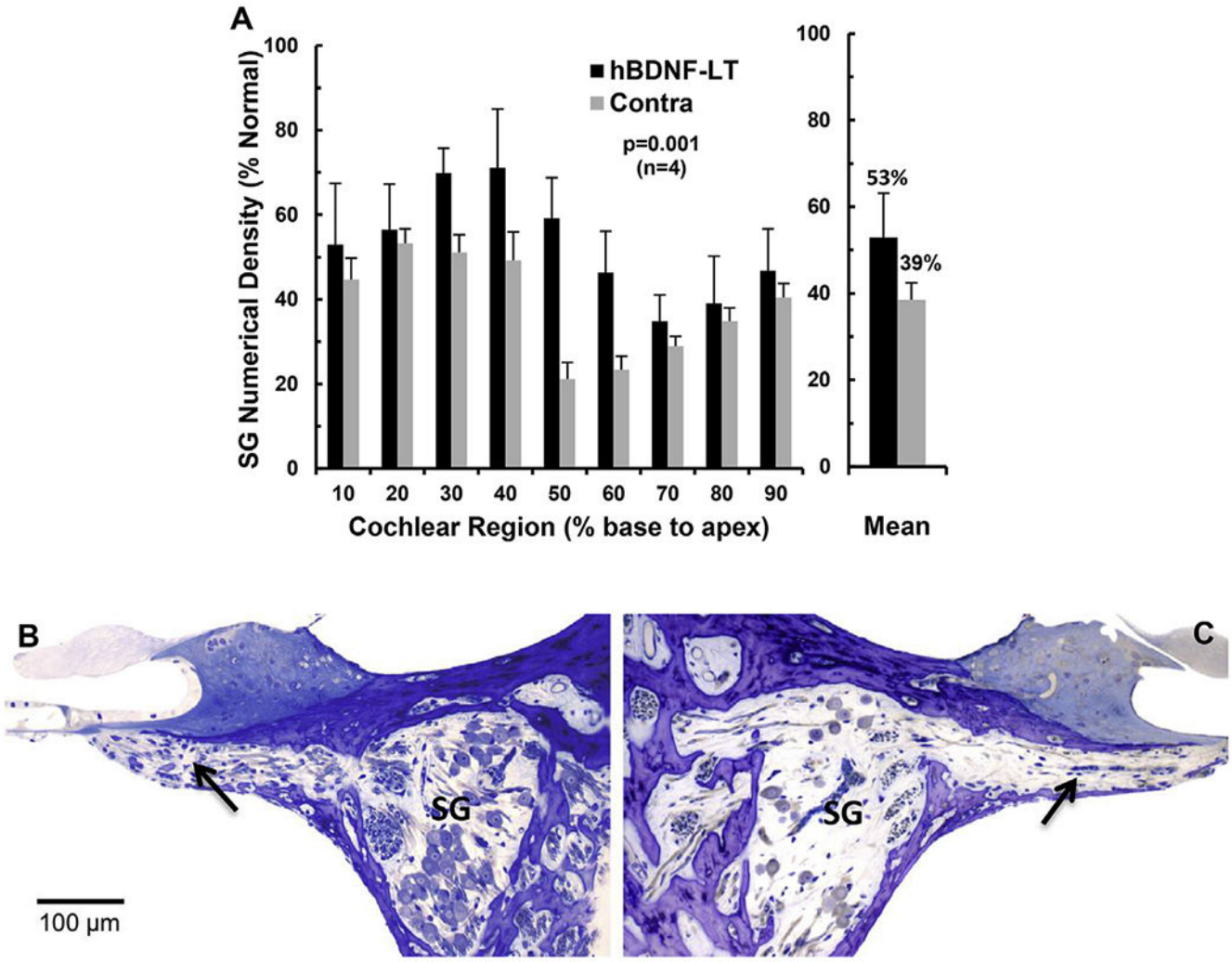

Fig. 3.

A. Morphometric data show the mean density of SGN cells in 10% sectors from base to apex of the cochlea for a group of early-deafened cats examined ~6 months after AAV2-hBDNF injections into the scala tympani. The injected ears show consistently higher SGN densities throughout the cochlea as compared to the paired data for the opposite side. Overall, SGN cell survival was markedly improved, averaging 53% of normal after AAV2-BDNF transfection, compared to 39% of normal in the opposite ear ($p = 0.001$). (Error bars indicate standard errors of the means.) B,C. Light microscopic histology from the cochleae of one subject in the group shown in A, illustrating neurotrophic effects of AAV2-hBDNF. The 40–50% sector of the injected cochlea (B, 48% of normal) and the paired region from the contralateral ear (C, 12% of normal) illustrate the higher density of SGN cells in Rosenthal's canal in this sector. Improved survival of radial nerve fibers (arrows) is also evident with AAV2-hBDNF; fiber survival was quantified in cross-sections through the osseous spiral lamina. Importantly, no ectopic sprouting of the radial nerve fibers was seen in any of the ears after AAV2-BDNF. Also, note the lack of inflammatory reaction in the

scala tympani. Scale bar = 100 μm. Reprinted from Leake et al. (2019), Fig. 6, with permission from J. Assoc. Res. Otolaryngol., Springer Science + Business Media, LLC.

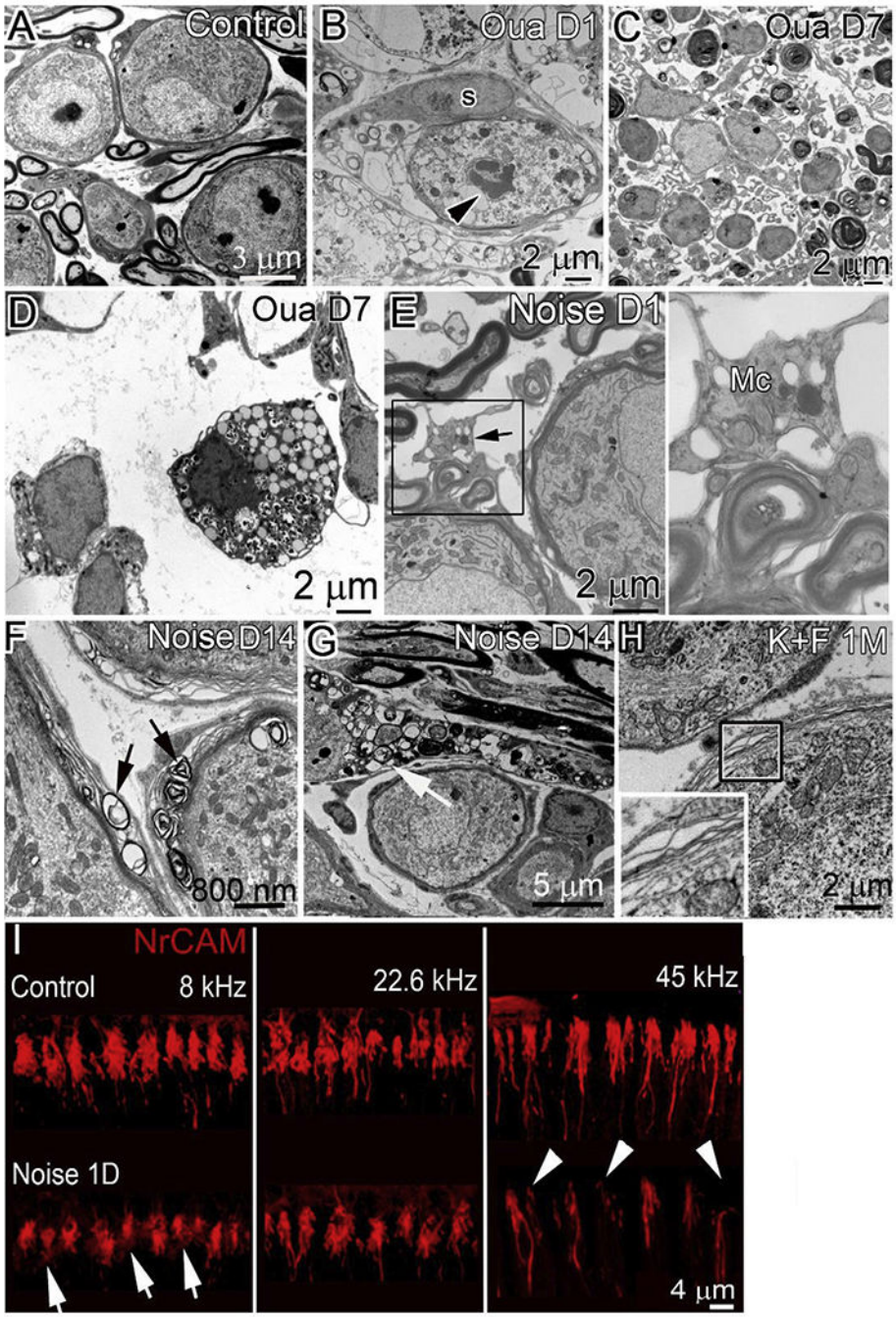

Fig. 4.

Auditory nerve responses to cochlear insults in several mouse models of sensorineural hearing loss. A: Normal SGNs and their myelinating satellite cells from a young adult CBA/CaJ mouse. B: Type I SGNs after ouabain (Oua) exposure (See details in Lang et al., 2011), a mouse model of selective SGN loss. Apoptotic-like degeneration (arrowhead) appears in the auditory nerve from the basal turn of a young adult mouse 1 day (D1) after Oua exposure. A satellite (s) cell enclosed the dying SGN with a relatively normal appearance. C: Seven days (D7) after Oua exposure. Glia cells in Rosenthal's canal of a

treated mouse show profiles of nuclei with prominent euchromatin and less dense heterochromatin, suggesting active transcription in these cells. D: A macrophage seen within Rosenthal's canal with numerous vesicles and cellular debris suggesting active phagocytosis. E: One day after exposure to an octave-band (8–16 kHz) noise at 106 dB SPL for 2 h (See details in Panganiban et al., 2018, a mouse model of noise-induce hearing loss), compact myelin lamellae are disrupted. Macrophages appear to be recruited to and activated in regions of noise-induced demyelination. Images in E show a macrophage (arrow) within the boxed area containing numerous inclusion bodies with electron-dense contents and is often seen closely opposed to segments of the myelin sheath. The panel to the right of E shows an enlarged image of the macrophage in E. F, G: 14 days (D14) after noise exposure, demyelination is seen around SGNs (F; black arrows; G; white arrow). H: One month (1 M) after exposure to kanamycin and furosemide (K + F) (See details in Kilpatrick et al., 2011; a mouse model of ototoxic drug-induced hair cell loss), reduction of compact myelin lamellae was still present. I: A reduction (arrows) and loss (arrowheads) of $NrCAM^{+}$ nodal structures at the habenula opening D1 after noise exposure (Some images modified from Lang et al., 2011 and Panganiban et al., 2018 with permission from J. Assoc. Res. Otolaryngol. and J. Neurosci., respectively.).

Table 1

Studies of virally mediated neurotophin gene therapy in deafened animals.

Animal	Viral vector	Neurotrophins	Route of administration	Morphological improvement	References
Guinea pig	Ad 5	GDNF	RWM/ST	Significantly enhanced SGN survival	Yagi et al. (2000)
Guinea pig	Ad 5	BDNF, CNTF	RWM/ST	BDNF significantly improved SGN survival	Nakaizumi et al. (2004)
Guinea pig	Ad5/fibroblast	BDNF	RWM/ST	Significantly greater SGN survival, basal turn	Rejali et al. (2007)
Guinea pig	Ad	BDNF	RWM/ST	Improved SGN survival & lower CI thresholds	Chikar et al. (2008)
Guinea pig	Ad/AAV	BDNF	SM or ST	Robust regrowth of nerve fibers & significant preservation of SGN	Shibata et al. (2010)
Guinea pig	Ad 5	BDNF & NT-3	ST & SM	Greater SGN and radial nerve fiber survival	Wise et al. (2010)
Guinea pig	Ad 5	BDNF & NT-3	Cochleostomy/SM	Greater SGN survival in basal cochlea after longer duration of deafness	Wise et al. (2011)
Rat	Ad	β-NGF	Cochleostomy/ST	Greater SGN survival	Wu et al. (2011)
Guinea pig	Ad 5	NT-3 & BDNF	Cochleostomy/SM	Greater SGN survival and fiber regrowth	Atkinson et al. (2012)
Mutant mice	Ad	BDNF	Cochleostomy/SM	Enhanced survival of SGN & radial nerve fibers	Fukui et al. (2012)
Guinea pig	Ad 5	NT-3 & BDNF	Cochleostomy/SM	Long term protection of SGN	Atkinson et al., 2014a
Guinea pig	AAV2	NT-3 & BDNF	Cochleostomy/ST	Better SGN survival with BDNF than NT-3	Budenz et al. (2015)
Guinea pig	AAV2	NT-3	Cochleostomy/ST	Variable degree of SGN preservation	Pfingst et al. (2017)
Guinea pig	rAAV8	NT-3	Cochleostomy/ST	IHCs and SGN synaptic protection/repair	Chen et al. (2018)
Cat	AAV2 & 5	BDNF & GDNF	RWM/ST	Significant long-term improvement in SGN and radial nerve fiber survival with BDNF	Leake et al. (2019)

Studies in which neurotophins (BDNF, GDNF, NT-3 and others) were administered to deafened animals (guinea pig, rats and cats) and to transgenic mice. All these studies demonstrated 1) improved preservation of SGNs or 2) IHCs and SGN synaptic protection/repair. RWM: round window membrane; ST: Scala Tympani; SM: Scala Media; NF: Nerve Fibers; AN: Auditory nerve; SGN: Spiral ganglion neuron; CI: Cochlear implant; IHC: Inner Hair Cells; Ad: adenovirus; AAV: adeno-associated virus.