

The Use of Neurotrophin Therapy in the Inner Ear to Augment Cochlear Implantation Outcomes

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ABSTRACT

Severe to profound deafness is most often secondary to a loss of or injury to cochlear mechanosensory cells, and there is often an associated loss of the peripheral auditory neural structures, specifically the spiral ganglion neurons and peripheral auditory fibers. Cochlear implantation is currently our best hearing rehabilitation strategy for severe to profound deafness. These implants work by directly electrically stimulating the remnant auditory neural structures within the deafened cochlea. When administered to the deafened cochlea in animal models, neurotrophins, specifically brain derived neurotrophic factor and neurotrophin-3, have been shown to dramatically improve spiral ganglion neuron survival and stimulate peripheral auditory fiber regrowth. In animal models, neurotrophins administered in combination with cochlear implantation has resulted in significant improvements in the electrophysiological and psychophysical measures of outcome. While further research must be done before these therapies can be applied clinically, neurotrophin therapies for the inner ear show great promise in enhancing CI outcomes and the treatment of hearing loss. *Anat Rec*, 295:1896–1908, 2012. © 2012 Wiley Periodicals, Inc.

Key words: neurotrophin; BDNF; NT3; cochlear implant

Deafness occurs in 0.1%–0.2% of newborns (Morton, 1991), and hearing loss affects up to 70% of the population over the age of 75 years (Campbell et al., 1999; Sprinzl and Riechelmann, 2010). Hearing loss may be genetic or acquired, and it is the end result of a variety of pathologies, including genetic mutations, congenital ear malformations, infection, ototoxic insult, aging, and noise exposure. Regardless of the underlying etiology, the majority of deafness is related primarily to loss of or damage to cochlear mechanosensory cells, and a secondary lesion in the neural network. The neural lesion may include regression of peripheral auditory fibers (PAF) and loss of spiral ganglion neurons (SGN) may also occur to a variable degree (Spoendlin, 1971a; Wright, 1976; Terayama et al., 1977; Bichler et al., 1983; Nadol et al., 1989). Cochlear implant (CI) devices are currently the best hearing rehabilitation strategy for the severely and profoundly deaf as the inner ear mechanosensory and neural elements do not spontaneously regenerate. The CI electrode array is surgically inserted into the scala tympani of the cochlea, and sounds are converted to electrical signals that are then delivered to the implanted electrodes. CIs function by directly stimulat-

ing the residual auditory neuronal structures in the deafened ear, thereby bypassing the defective or missing mechanosensory cells, and the status of these remnant neural structures likely directly impacts CI outcomes. Neurotrophin molecules play a critical role in the development and maintenance of many neural systems, and their application to the deafened inner ear has been found to promote SGN health and survival (Staecker et al., 1996b;

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Gillespie et al., 2003; Agterberg et al., 2008), as well as PAF regrowth toward the source of the neurotrophins (Wise et al., 2005, 2010; Shibata et al., 2010). Inner ear neurotrophin therapy shows promise in the augmentation of cochlear implantation outcomes by improving the health of the cochlear neural substrates and bringing neurons in closer proximity to the CI electrode array.

ANATOMY OF THE COCHLEA

The human cochlea is an anatomically and functionally complex structure that allows us to hear and distinguish sounds of many different frequencies and intensities, thereby allowing us to interact and communicate with our surrounding world. The membranous cochlea is a small spiral structure contained within the dense otic capsule of the temporal bone, and it is composed of three interrelated compartments: the scala vestibuli, scala tympani, and scala media. The organ of Corti, the sensory organ of hearing, resides on the basilar membrane (BM). The organ of Corti is composed of supporting cells and specialized mechanosensory cells, called hair cells due to the bundle of stereocilia located on their apical surface. It is the hair cells that initiate a neural signal in response to sound. The mechanical energy of sound causes movement and deflection of the BM in a tonotopic fashion, with high frequency sounds preferentially moving the basal BM and low frequency sounds moving the apical BM. This in turn causes a deflection of the stereocilia of the hair cells, leading to depolarization of the cells and release of neurotransmitter that stimulates the nerve endings. The hair cells are innervated by the PAF of SGNs, bipolar cells that provide afferent input to the cochlear nucleus (Merchant-Perez and Liberman, 1996). For more in-depth reviews of cochlear anatomy and histology please see these reviews: (Santi and Mancini, 1998; Raphael and Altschuler, 2003; Davis and Liu, 2011; Nayagam et al., 2011).

THE COCHLEAR PATHOLOGY OF HEARING LOSS

Various animal models have been used to simulate and investigate the morphologic and physiologic effects of insults to the inner ear. Many animal studies have utilized aminoglycoside antibiotics administered by various methods to induce severe ototoxic lesions. When administered directly to the cochlea, there is a loss of both cochlear sensory and supporting cells associated with a regression of the PAFs and a progressive loss of the majority of SGNs to the point where there are few remaining fibers and neurons within months of sustaining the lesion (Terayama et al., 1977, 1979; Bichler et al., 1983). Similarly, neomycin administered topically to the round window membrane has been demonstrated to induce degeneration of SGNs over a period of months (Fig. 1), though the effect is not as severe as that seen with direct cochlear infusion (Zappia and Altschuler, 1989). Other studies have utilized noise exposure to induce the loss of cochlear hair cells and the formation of a phalangeal scar (Spoendlin, 1971b; Hawkins, 1973; Johnsson and Hawkins, 1976; Abrashkin et al., 2006), and PAF degeneration is associated with and proportional to the damage seen in the organ of Corti (Wright, 1976; Bohne and Harding, 1992).

In mammalian animal models of deafness, spontaneous regeneration of hair cells has not been seen. However,

occasional spontaneous re-sprouting of PAFs has been seen following the administration of various otologic lesions. Months after severing the eighth cranial nerve and causing a near total loss of SGNs in Rosenthal's canal, Spoendlin and Suter (1976) found many large new fibers in the region between the habenula perforata and the inner hair cells. Similarly, spontaneously regenerated PAFs have been noted following noise-induced hearing loss (Wright, 1976; Bohne and Harding, 1992; Strominger et al., 1995; Lawner et al., 1997), as well as following aminoglycoside-induced ototoxicity (Johnsson and Hawkins, 1972; Terayama et al., 1977, 1979; Santi et al., 1982). However, this spontaneous re-sprouting of PAFs has not been accompanied by a concomitant increase in the number of SGNs in Rosenthal's canal (Spoendlin and Suter, 1976) and has primarily been seen in cochlea demonstrating only focal lesions with intact adjacent sensory and supporting cells in the organ of Corti (Bohne and Harding, 1992; Strominger et al., 1995). These findings have led to the conclusion that any spontaneous peripheral fiber regeneration that occurs arises from residual surviving SGNs and requires intact supporting and sensory cells to provide support and guidance to the re-growing fibers. Unfortunately, in human deafness, as well as in severe ototoxic lesions induced in animals, there is often a widespread loss of both sensory and supporting cells within the organ of Corti leading to the formation of a flat epithelium (Merchant et al., 2005; Kim and Raphael, 2007). Without surviving supporting and sensory cells to provide support to the surviving SGNs, no significant spontaneous fiber regrowth into the BM area has been seen in mammalian animal models following the induction of a severe ototoxic lesion (Shibata et al., 2010; Wise et al., 2010).

People with bilateral severe to profound deafness that cannot be adequately rehabilitated with conventional hearing aid amplification are candidates for cochlear implantation. CI candidates may be deaf for a variable duration of time and due to a variety of etiologies, and as such the histological and functional status of the remnant auditory neuronal structures within the cochlea is quite variable (Hinojosa and Lindsay, 1980; Nadol et al., 1989; Nadol, 1997; Pfingst et al., 2011). While the extent of auditory neural degeneration varies between species, and humans generally demonstrate a less severe loss of auditory neural structures following deafness as compared to animal models (Teufert et al., 2006; Linthicum and Fayad, 2009), human temporal bone studies have shown a decrease in SGNs in deaf individuals as compared to normal hearing controls (Kerr and Schuknecht, 1968; Hinojosa and Lindsay, 1980). Furthermore, human studies have demonstrated that particular etiologies of deafness, such as post-natal infectious labyrinthitis, are correlated with a severe loss of SGNs (Kerr and Schuknecht, 1968; Nadol et al., 1989). The extent of degeneration of inner ear sensorineural structures following deafness in humans may have implications for the success of cochlear implantation.

NEUROTROPHINS IN THE INNER EAR

Role of Neurotrophins in Inner Ear Development

Neurotrophins are a family of molecules critical to the development and maintenance of neural systems. Thus far four neurotrophins have been identified in mammals:

nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT3), and neurotrophin-4 (NT4). These four molecules share a similar sequence and structure as they all descend from a common ancestral gene, and all four are active in directing how neurons grow and differentiate during development and beyond (Huang and Reichardt, 2001). Each neurotrophin binds to a specific subtype of Trk receptor with high affinity (Lu et al., 2005), and some low affinity cross binding to other Trk receptors and the p75 receptor also occurs (see chapter by Davis and Green for more details). Within the auditory system, BDNF and NT3 are the two neurotrophins essential for normal auditory neural development (Pirvola et al., 1992). Both BDNF and NT3 are expressed at high levels in the cochlear sensory epithelium during development (Ylikoski et al., 1993; Sugawara et al., 2007), and *in vitro* studies of cochlear explants have demonstrated that BDNF and NT3 at physiologic levels promote both SGN survival and neurite outgrowth (Pirvola et al., 1992). Null mutant mouse studies have shown that NT3 is critical for the development of the majority of SGNs and cochlear afferent neurons, initially presumed to be the Type I afferent fibers to the inner hair cells (Fariñas et al., 1994; Ernfors et al., 1995). In contrast, BDNF is critical for the development of vestibular ganglion neurons and a minority of cochlear afferent neurons, initially presumed to be the Type II afferent fibers to the outer hair cells (Ernfors et al., 1995). Although these early studies suggested that NT3 and BDNF specifically corresponded to the development of Type I and Type II afferent auditory fibers respectively, additional studies utilizing immunocytochemistry to study the distribution of neurotrophin receptors during development have actually shown that all afferent cochlear neurons, both Type I and II, express receptors for both BDNF and NT3 (Mou et al., 1997; Fariñas et al., 2001).

Further, null mutant mouse studies have suggested that there is a gradient of expression within the cochlea for each neurotrophin during development. Through *in situ hybridization* techniques on developing, post-natal, and adult rats, NT3 and BDNF have been found to be robustly expressed throughout the cochlear inner and outer hair cells during embryonic development (Pirvola et al., 1992). During early embryonic development BDNF is expressed in the apical turns, and expression progresses toward the base later in development (Fariñas et al., 2001). In contrast, NT3 expression is more pronounced in the cochlear base in the early stages of development, and the expression gradient progresses more apically until it is primarily expressed in the apex of post-natal and adult mice (Fariñas et al., 2001; Sugawara et al., 2007). NT3 continues to be expressed in the inner hair cells and adjacent supporting cells of the post-natal and adult cochlea (Sugawara et al., 2007), and BDNF is primarily expressed within the vestibular system of the adult cochleovestibular apparatus (Pirvola et al., 1992).

This progression in the gradient of each neurotrophin's expression over the course of cochlear embryologic development explains the phenotypic appearance of null mutant mice. NT3 knockout mice have demonstrated an almost complete absence of innervation in the basal turns with a more moderate decrease in nerve fibers in the middle and apical turns (Fritzsche et al., 1997),

whereas BDNF deficient mice demonstrate a more subtle loss of neurons primarily in the apical turns (Ernfors et al., 1995). The critical time for development of basal innervation occurs early when NT3 is expressed in the organ of Corti while BDNF expression is absent in the base. Therefore, the normal endogenous expression of BDNF does not rescue the NT3 null mutation in the basal cochlea. However, the NT3 null mutation is completely rescued in the transgenic mouse expressing BDNF under the control of the NT3 promoter, thereby inducing expression of BDNF in the basal cochlea earlier in development (Fariñas et al., 2001). These findings suggest that each neurotrophin has a distinctive gradient of expression that evolves over the course of cochlear development, and that this gradient is critical to the normal development of cochlear innervation. Mice deficient for both neurotrophins do not develop either vestibular or SGN (Ernfors et al., 1995), further demonstrating that both BDNF and NT3 are essential for normal inner ear neural development.

In maturing spiral ganglion explants, each neurotrophin has been shown to direct the physiologic firing patterns of the neurons on which it acts, with NT3 promoting an apical firing pattern and BDNF promoting a firing pattern consistent with basal neurons (Adamson et al., 2002; Zhou et al., 2005). In this fashion, neurotrophins appear to continue to exert critical effects in the developing and mature cochlea through their influence on the tonotopic tuning of the neural elements within the cochlea.

Role of Neurotrophins in Inner Ear Maintenance

Neurotrophins may have primary effects on cell physiology at one time point and greater effects on survival and proliferation at another time point depending on the time of exposure to their target cell population (Adamson et al., 2002). In the mature mammal both BDNF and NT3 continue to play an important role in the survival and maintenance of the auditory neurons. Following the loss of cochlear hair cells in the adult mammalian ear there is a dramatic loss of both PAFs and SGNs, presumably secondary to the loss of neurotrophic support from the hair cells (Staecker et al., 1998). The remaining SGNs appear unhealthy with smaller soma (Kanzaki et al., 2002) and pyknotic, misshapen nuclei (Ernfors et al., 1996; Staecker et al., 1996b; Gillespie et al., 2003). Many studies have now demonstrated that when the deafened ear is treated with exogenous BDNF or NT3, there is a significant enhancement of SGN survival to near normal levels (Ernfors et al., 1996; Staecker et al., 1996b; Miller et al., 1997; Staecker et al., 1998; Altschuler et al., 1999; Nakaizumi et al., 2004) and the surviving SGNs appear larger and healthier with well-defined nuclei and nucleoli and abundant Nissl substance in the cytoplasm (Staecker et al., 1996a; Kanzaki et al., 2002). Furthermore, it is important to note that despite the limited endogenous expression of BDNF in the mature adult cochlea, it has comparable effects to NT3 in promoting auditory neural survival when administered exogenously (Staecker et al., 1996b, 1998). In addition to neurotrophins, other molecules, such as fibroblast growth factor and glial cell line-derived neurotrophic factor, have been shown to

influence the maintenance and growth of the auditory nerve (Altschuler et al., 1999; Kanzaki et al., 2002; Glueckert et al., 2008).

Regenerative Effects of Neurotrophins

In addition to the positive effects of exogenous neurotrophins on SGN survival, many studies have also demonstrated neurogenesis both *in vitro* and *in vivo* following exogenous neurotrophin administration (Table 1). Limited neurite outgrowth has been seen following exogenous neurotrophin administration to spiral ganglion neuronal cultures (Pirvola et al., 1992). More impressive neurite outgrowth has been seen *in vivo*. Following aminoglycoside deafening and neurotrophin administration into the guinea pig perilymph via a mini osmotic pump, peripheral neuronal fibers were seen growing in a robust yet diffuse fashion due to the elevated levels of the growth factors (Ernfors et al., 1996; Staecker et al., 1996b; Wise et al., 2005; Miller et al., 2007; Glueckert et al., 2008). Similarly, following aminoglycoside deafening and neurotrophin administration into the scala tympani via a viral vector, there was significant regrowth of PAFs into the cochlea (Shibata et al., 2010; Wise et al., 2010). The peripheral fiber regrowth seen with both administration methods was generally disorganized; however, administration of neurotrophin via viral vector promoted more directed growth of the fibers into the BM area toward transfected cells (Shibata et al., 2010; Wise et al., 2010). Such targeted growth is potentially important for enhancing the outcome of CI therapy because the nerve endings are more likely to be in proximity to the electrode array. Similar growth of PAFs into the BM area has been seen with inoculation by either adenovirus or adeno-associated viral vectors, as well as with either scala tympani or scala media inoculations (Shibata et al., 2010).

Neurotrophin Concentrations During Development and Beyond

NT3 and BDNF, administered at 1–5 ng/mL to develop rat ganglion explants in culture, are sufficient to elicit improved SGN survival and limited neurite outgrowth, and the more robust effect is seen in response to BDNF (Pirvola et al., 1992). However, maximal SGN survival *in vitro* is seen at higher concentrations of 50 ng/mL for BDNF and 10 ng/mL for NT3 (Lefebvre et al., 1994). Many groups have demonstrated improved SGN survival and neurite outgrowth when neurotrophins have been administered *in vivo* as well; however, the concentrations of neurotrophins required to elicit these effects appeared to be even higher based on early work using mini osmotic pumps for administration. These studies showed improved SGN survival with limited neurogenesis when either BDNF or NT3 were delivered to the scala tympani in supra-physiologic concentrations ranging from 50 to 100 μ g/mL (Ernfors et al., 1996; Yamagata et al., 2004). In the work by Shinohara et al. (2002) improved evoked auditory brainstem responses (eABR) thresholds following CI were seen with neurotrophins administered by mini osmotic pump at a concentration of 100 μ g/mL.

More recent *in vivo* work has demonstrated that improved SGN survival and neurite outgrowth can also

be achieved with lower, more physiologic levels of neurotrophins administered via alternative strategies (Table 1). Drug eluting CI devices are being developed. Physiologic concentrations of neurotrophins have been delivered subcutaneously over a period of weeks to the inner ear via NT3-loaded alginate beads and have led to improved SGN survival following otologic insult (Noushi et al., 2005). Similarly, physiologic levels of BDNF have been delivered to the scala tympani over a period of two weeks through a CI coated in a hydrogel soaked with BDNF (Chikar et al., 2012). Spiral ganglion survival has also been improved through the *ex vivo* gene transfer of BDNF to allogenic fibroblasts that were subsequently incorporated into agarose gel coated electrode arrays, thereby delivering BDNF at low levels over a period of weeks (Rejali et al., 2007). While drug eluting devices are attractive in their relative ease and safety of administration, potential disadvantages of this administration method include the lack of control over the rate of drug delivery and the relatively short time over which drug is eluted. Gene therapy holds the potential for the more directed and chronic administration of neurotrophins. Gene therapy with either BDNF or NT3 delivered via an adenoviral vector has achieved concentrations comparable to physiologic levels of neurotrophins when measured in cell culture and *in vivo*, and these levels have been sufficient to translate into improved SGN survival, neurite outgrowth (Shibata et al., 2010; Wise et al., 2010) (Fig. 2), and even decreased eABR thresholds elicited by CI stimulation (Chikar et al., 2008).

The Potential Role of Neurotrophins in Cochlear Implantation

Cochlear implantation has provided a highly effective means to rehabilitate deafness due to defects in the peripheral auditory system. CIs overcome most forms of deafness by bypassing the sensory cells of the cochlea and directly stimulating the remaining auditory neural structures. Although significant benefit is derived from our current CI technology in terms of restoring speech perception abilities in quiet, there continue to be major limitations when listening to complex sounds that are commonly encountered in day to day life, such as speech in noise and music. Current CIs have between 8 and 22 electrodes; however, likely due to current spread and diminished spiral ganglion neuronal populations, these configurations yield closer to 4–8 perceptual channels (Dorman et al., 1998; Friesen et al., 2001). In quiet only four channels are necessary to achieve adequate sentence understanding, but in noisy environments, as the signal to noise ratio becomes poorer, up to 20 perceptual channels are needed to achieve comparable speech understanding (Dorman et al., 1998). For music appreciation and perception of lexical tone as many as 32 or more perceptual channels are needed (Xu et al., 2002; Kong et al., 2004).

In order to diminish current spread and improve the number of perceptual channels achieved with CI electrode arrays, various strategies have been developed, including perimodiolar electrode arrays and concomitant neurotrophin treatment, to bring the electrode and auditory neural elements into closer proximity. Perimodiolar electrode arrays which are designed to lie on the medial side of the scala tympani in close proximity to the

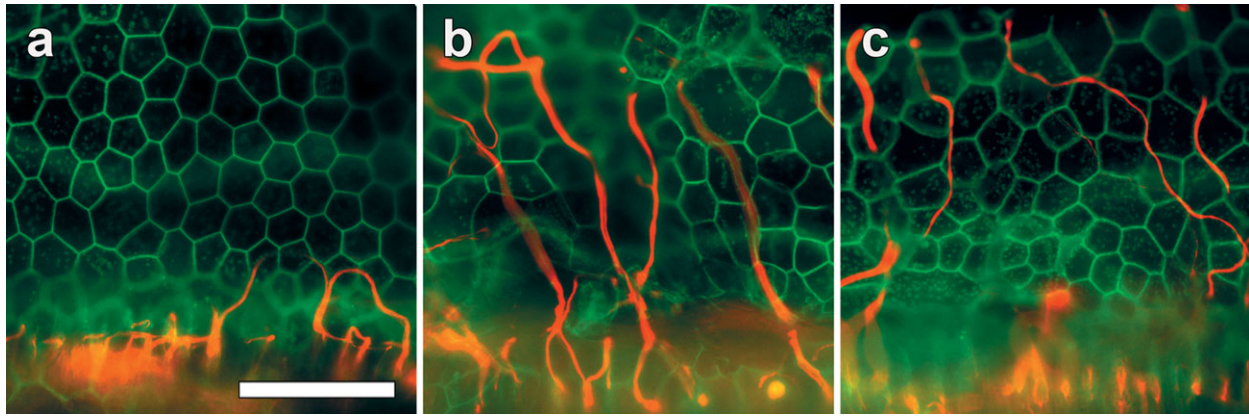


Fig. 1. Whole-mounts of the basal cochlear auditory epithelium stained for neurofilament (red) and actin (green), viewed with epifluorescence microscopy. (a) 90 days following deafening via administration of 5% neomycin directly into the scala tympani of the cochlea, all hair cells and supporting cells are absent and replaced by a flat epithelium. Neurofilament positive peripheral auditory fibers are absent with the exception of a few looping fibers extending into the basilar

membrane area. (b and c) Three months after inoculation of a deafened ear with AAV.NT3 (b) and AAV.BDNF (c), there is a persistent flat epithelium with no regeneration of supporting cells or hair cells. However, there are copious neurofilament positive fibers extending in a radial fashion into the basilar membrane area. Most of the fibers weave between the junctions of the flat epithelial cells. Scale Bar = 50 μ m.

modiolar wall and thus closer to the SGNs within the cochlear modiolus, were developed in an effort to decrease the electrical threshold required to stimulate the cochlear neural substrates. In computer modeling of the human cochlea, perimodiolar implants indeed appear to reduce current thresholds and improve frequency specificity in the basal turn of the cochlea (Frijns et al., 2001). In clinical studies examining the current thresholds required in individuals implanted with a perimodiolar array as compared to those implanted with a straight electrode array, which may lie more laterally in the scala tympani, it was found that people implanted with a perimodiolar array had lower stimulation thresholds (Cohen et al., 2005, 2006). Furthermore, individuals implanted with a perimodiolar array had a narrower forward masking profile, indicating that each perimodiolar electrode stimulates a more specific subpopulation of neurons (Cohen et al., 2006). However, unfortunately thus far these electrophysiological benefits of the perimodiolar array have not been translated into improved speech perception outcomes in the clinical population (Fitzgerald et al., 2007).

Only partial viability of the auditory nerve is required to derive some benefit from implantation and human temporal bone studies have not shown a link between SGN counts and CI speech recognition (Nadol et al., 2001; Khan et al., 2005; Fayad and Linthicum, 2006). However, studies have shown a correlation between psychophysical and electrophysiological outcomes and the histological status of the cochlear neural structures. Likely related to the degeneration seen in the PAFs and SGNs, eABRs are abnormal in the deafened inner ear (Shepherd and Javel, 1997). More damaged cochleae typically require a higher current threshold for stimulation with a CI and have a decreased dynamic range (Pfungst and Sutton, 1983; Kawano et al., 1998; Kang et al., 2010).

Although chronic electrical stimulation alone improves the survival of SGNs (Altschuler et al., 1999; Kanzaki et al., 2002), it does not promote regeneration or growth of PAFs toward the electrode array. However, in deafened animals treated with CI combined with an intracochlear

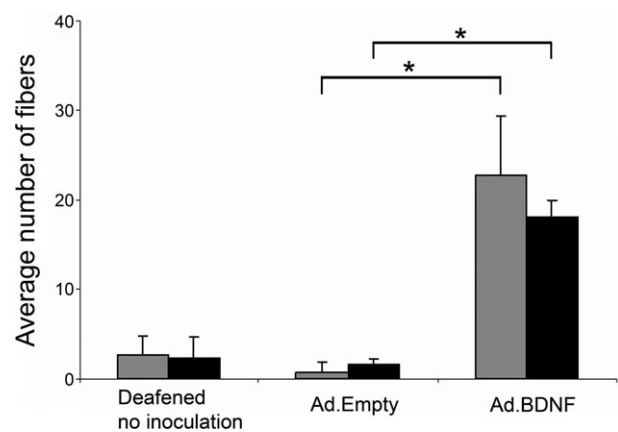


Fig. 2. Quantitative analysis of nerve fibers projecting into the basilar membrane area in deafened ears that have undergone either no treatment, or treatment with Ad.Empty or Ad.BDNF. Deafened animals inoculated with Ad.Empty had similar counts to untreated animals, whereas animals inoculated with Ad.BDNF had a significantly greater average nerve fiber count in the 1st and 2nd turns at 14 (gray bars) and 30 (black bars) days after deafening. (*) indicates $P < 0.01$. Error Bars are standard deviation and statistical comparison was done by Student t-test. (Reprinted with permission from Shibata et al., 2010).

administration of neurotrophins, there is both morphologic evidence of improved SGN survival beyond that seen with either electrical stimulation or neurotrophins alone (Kanzaki et al., 2002), as well as decreased post-implantation eABR thresholds (Shinohara et al., 2002). It is thought that an increase in frequency selectivity and the number of perceptual channels will be seen in connection with the diminished current thresholds associated with neurotrophin treatment.

Clinical Application of Neurotrophin Treatment

One obstacle to translating neurotrophin treatments to the clinical arena is in determining how to optimally

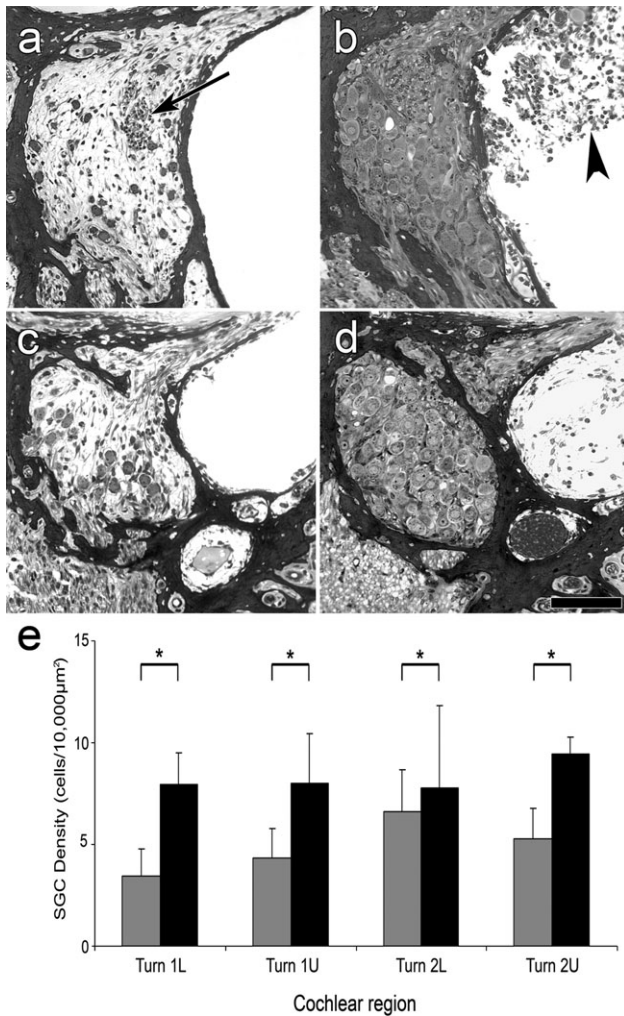


Fig. 3. Light microscope micrographs of plastic sections through Rosenthal's canal in the 1st (a and b) and 2nd (c and d) cochlear turns 14 days after inoculation of Ad.Empty (a and c), or Ad.BDNF (b and d), and quantification of SGNs (e). (a and c) SGNs are scarce. A presumed efferent fiber bundle is present (arrow in a). (b and d) Rosenthal's canal in Ad.BDNF inoculated ears exhibits a confluent population of neurons and no gliotic areas. In some of the Ad.BDNF inoculated ears, connective tissue could be observed in the scala tympani (arrow head in b). Scale bar = 100 μm. (e) Mean SGN density among the cochlear regions of basal and 2nd turn of Ad.Empty and Ad.BDNF inoculated animals. Comparison of SGN density in Ad.Empty (gray bars) and Ad.BDNF (black bars) inoculated groups in all regions showed significantly larger numbers of surviving SGN in the Ad.BDNF groups. Error bars are standard deviations. Statistical comparison was by one-way ANOVA. (*) indicates $P < 0.01$; L = Lower; U = Upper. (Reprinted with permission from Shibata et al., 2010).

deliver the molecules to the inner ear. The human auditory system poses unique advantages and disadvantages regarding the delivery of molecular therapies. Delivery of molecular therapies to the inner ear is made more difficult based on the fact that it is well protected from the systemic circulation by the blood-perilymphatic barrier and it is anatomically situated deep within the

dense otic capsule of the temporal bone, making direct access more difficult. However, these characteristics also present some interesting advantages, namely that therapies delivered to the inner ear will likely remain within the auditory system with little or no leakage into the systemic circulation. This limits the risk of inducing adverse side effects in other organ systems. Furthermore, the inner ear fluids allow distribution of neurotrophin treatment throughout the cochlea.

Various methods of administering molecular therapies to the inner ear have been used with success in animal models. For short-term effects, molecules can be delivered on a one-time basis to the inner ear by either diffusion across the round window membrane from the middle ear space or direct infusion into the perilymph through a surgical cochleostomy (Richardson et al., 2004, 2005, 2006; Noushi et al., 2005; Havenith et al., 2011; Pflugst et al., 2011). Conflicting results have been published regarding the duration of the effects of neurotrophin treatment after administration has been ceased (Table 1). While one study found durable benefits of BDNF treatment even after administration has ceased, both in terms of SGN counts and eABR thresholds (Agterberg et al., 2009), another found a severe decrease in SGN density following the removal of neurotrophin support (Gillespie et al., 2003). Given these conflicting findings it is unclear at this time whether neurotrophins need to be administered continuously to maintain their beneficial effects.

In animal models the two most commonly utilized methods for chronic neurotrophin administration have been: (1) surgical implantation of a mini osmotic pump, and (2) inoculation with a replication deficient viral vector carrying the gene for neurotrophin production. Each method of chronic administration has its own set of advantages and disadvantages. Mini osmotic pumps have been shown to be effective in the chronic administration of molecules directly into the scala tympani of small animals without inducing secondary cochlear damage as assessed by auditory brainstem response (ABR) testing (Brown et al., 1993). However, their application in the clinical world is limited by the facts that one must periodically replenish the reservoir of the agent being administered and that the therapeutic agent is delivered in a non-specific fashion into the perilymphatic space. Viral vectors have also been used with success in the inner ear. When inoculated directly into the cochlear perilymphatic space, replication deficient adenovirus has been shown to effectively transfect neural, epithelial, and connective tissue cells within the cochlea without inducing significant cytotoxic effects (Raphael et al., 1996; Weiss et al., 1997). While adenoviral vectors have been shown to effectively transfect inner ear cells, significant expression of the transgene is typically limited to a period of a few weeks as it is not incorporated into the target cell's genome (Lalwani and Mhatre, 2003). Adeno-associated viral (AAV) vectors have also been used with success in the inner ear (Figs. 1, 2 and 3). AAV has the advantage of being unable to replicate without the assistance of a helper virus and it is not associated with human disease. Furthermore, optimal expression is seen at approximately 2 weeks following transfection and significant levels of expression in the inner ear are seen up to 24 weeks following inoculation (Lalwani et al., 1998), and transgene expression has been seen as long as 6

TABLE 1. Effects of BDNF and NT3 in the Mammalian Inner Ear—Literature Review Highlights

Authors	Year of Pub.	NTF Used	Animal Model	Control	Method of Admin.	Timing of Admin.	Effects of Neurotrophin Treatment
Staecker et al.	1996	BDNF, NT3	Guinea pig	Deafened, untreated Normal hearing	Mini-osmotic pump Scala Tympani	Post-deafening 8 week infusion	-SGN: Increased SGN survival rate with BDNF, NT3, or both
Ernfors et al.	1996	NT3	Guinea pig	Deafened, untreated Normal hearing	Mini-osmotic pump Scala Tympani	Post-deafening 2 week infusion	SGN: Increased SGN survival rate PAP: Re-growth of neurofibers ABR: No significant change in eABR threshold
Miller et al.	1997	BDNF, NT3	Guinea pig	Deafened, untreated Deafened AP	Mini-osmotic pump Scala Tympani	Post-deafening 2 week infusion	SGN: Complete rescue of SGNs with BDNF; No statistically significant effect induced by NT3
Staecker et al.	1998	BDNF	Mouse	Deafened, untreated Normal hearing	Viral vector (HSV-1) Scala Tympani	Post-deafening 4 week cassette expression	SGN: Almost complete rescue of SGNs
Ruan et al.	1999	BDNF, NT3	Guinea pig	Deafened, AP	Mini-osmotic pump	Pre-deafening	IHC: Protected from ototoxic lesion with 30 days BDNF treatment or with NT3 treatment
Shoji et al.	2000	BDNF, NT3	Guinea pig	Deafened, AP	Scala Tympani Mini-osmotic pump	15, 30 or 60 days infusion Pre-deafening × 4 days OR	OHC: Protected from ototoxic lesion with NT3 treatment
Shinohara et al.	2002	BDNF, analog of CNTF	Guinea pig	Deafened, AP	Scala Tympani Mini-osmotic pump	Post-deafening × 1 week	OHC: Protected from noise induced lesion with NT3 treatment; Not protected with BDNF treatment ABR: eABR threshold lowered significantly for ototoxin exposed ear after neurotrophin treatment in a concentration dependent manner
Gillespie et al.	2003	BDNF	Guinea pig	Deafened, untreated Normal hearing	Scala Tympani	26 day infusion	SGN: Increased SGN survival rate after treatment with BDNF and after treatment with BDNF and CNTF analog ABR: Decrease in eABR threshold with BDNF + CNTF analog treatment
Nakaizumi et al.	2004	CNTF, BDNF	Guinea pig	Deafened, untreated Normal hearing	Mini-osmotic pump Scala Tympani Viral vector (AV) Scala Tympani	Post-deafening 4 week infusion	SGN: Increased SGN survival rate with continued therapy; 2 weeks following cessation of treatment led to decrease in SGN survival rates
							SGN: Increased SGN survival rate after treatment with BDNF or BDNF + CNTF (CNTF did not significantly enhance protective effect of BDNF)

TABLE 1. Effects of BDNF and NT3 in the Mammalian Inner Ear—Literature Review Highlights (continued).

Authors	Year of Pub.	NTF Used	Animal Model	Control	Method of Admin.	Timing of Admin.	Effects of Neurotrophin Treatment
Yamagata et al.	2004	CNTF, BDNF	Guinea pig	Deafened, empty vector	Mini-osmotic pump Scala Tympani	Post-deafening* 2 week infusion *Neurotrophin treatment initiated 2 or 6 weeks following deafening	SGN: Increased SGN density ABR: Decreased eABR threshold starting 7 days into treatment; Significant decrease in eABR threshold 14 days after BDNF treatment; eABR threshold for treated ears after 6 week delay is significantly higher than after 2 week delay
Shepherd et al.	2005	BDNF	Guinea pig	Deafened AP	Mini-osmotic pump	Post-deafening	SGN: Increased SGN survival rate; Electrical stimulation enhances protective effects of BDNF, but produces no significant trophic support alone ABR: Significant decrease in eABR threshold with BDNF treatment
Wise et al.	2005	BDNF, NT3	Guinea pig	Deafened untreated* Deafened untreated* Normal hearing *5 or 33 days post-deafening	Mini-osmotic pump Scala Tympani	Post-deafening* 4 week infusion Post-deafening* 4 week infusion	SGN: Increased SGN survival rate in ear 5 or 33 days after ototoxin exposure with BDNF or NT3 treatment PAF: Variable regrowth of peripheral processes with BDNF or NT3
Rejali et al.	2007	BDNF	Guinea pig	Deafened, electrode + empty vector	Viral vector (AV) treated fibroblast implant	Post-deafening 48 days casette expression	SGN: Increased SGN survival rate in basal turn adjacent to coated electrode
Miller et al.	2007	BDNF, FGF	Guinea pig	Deafened, AP + guinea pig serum albumin	Mini-osmotic pump	Post-deafening*	SGN: Increased SGN survival rate regardless of treatment delays, but longer delay led to decreased overall SGN density
Agterberg et al.	2008	BDNF	Guinea pig	Deafened, untreated	Mini-osmotic pump	26 day infusion *Neurotrophin treatment initiated 4 days, 3 or 6 weeks following deafening Post-deafening	PAF: Re-growth of peripheral processes with either neurotrophin, decreased fiber re-growth with increased treatment delay SGN: Increased SGN survival rate and density; SGNs in treated ears are larger than normal-hearing control ears PAF: Surrounded by thinner myelin sheath compared to normal hearing ears ABR: No change in aABR response

TABLE 1. Effects of BDNF and NT3 in the Mammalian Inner Ear—Literature Review Highlights (continued).

Authors	Year of Pub.	NTF Used	Animal Model	Control	Method of Admin.	Timing of Admin.	Effects of Neurotrophin Treatment
Chikar et al.	2008	BDNF	Guinea pig	Deafened, electrode implant + empty vector	Viral vector (AV) Scala Tympani	Post-deafening 80 day expression	SGN: Increased SGN survival rate ABR: Decrease in eABR threshold; Correlation of eABR threshold and SGN density; No significant correlation between SGN density and psychophysical detection thresholds
Agterberg et al.	2009	BDNF	Guinea pig	Deafened untreated Normal hearing	Mini-osmotic pump Scala Tympani	Post-deafening 4 week infusion	SGN: Increased SGN survival rate; Preserved SGN shape ABR: eABR response comparable to normal-hearing control, and did not decrease significantly after cessation of treatment; eABR latency in treated ears are comparable to deafened control animal
Wise et al.	2010	BDNF, NT3	Guinea pig	Deafened untreated	Viral vector (AV)	Post-deafening	Inoculation into scala media produced gene expression better localized to organ of Corti than into scala tympani SGN: Increased SGN survival rate and PAF: Re-growth of peripheral processes in basal turn
Shibata et al.	2010	BDNF	Guinea pig	Deafened untreated Deafened, empty vector (AV)	Scala Tympani or Scala Media Viral vector (AV or AAV) Scala Tympani	Post-deafening 14 or 30 day expression	SGN: Increased SGN survival rate PAF: Re-growth of peripheral processes beyond the habenula perforata towards BDNF expressing cells; Insignificant decline in number of peripheral process between 14 and 30 days post-inoculation
Havenith et al.	2011	BDNF	Guinea pig	Deafened untreated Deafened, gelfoam + PBS Normal hearing, gelfoam + PBS	Neurotrophin soaked gelfoam on intact round window membrane	Post-deafening 4 week exposure	SGN: Increased SGN survival rate in basal turn; No significant effect on SGN shape and size ABR: No significant effect on eABR peak-to-peak amplitudes

aABR, acoustically evoked auditory brainstem response; AAV, adeno-associated virus; AP, artificial perilymph; AV, adenovirus; BDNF, brain derived neurotrophic factor; CNTF, ciliary neurotrophic factor; eABR, electrically evoked auditory brainstem response; FGF, fibroblast growth factor; HSV-1, Herpes simplex virus-1; IHC, inner hair cell; NGF, nerve growth factor; NTF, neurotrophin factor; NT3, neurotrophin-3; OHC, outer hair cell; SGN, spiral ganglion neuron; PAF, peripheral auditory fiber; AP, artificial perilymph; GFP, green fluorescent protein; PBS, phosphate buffered saline.

years following AAV administration into the primate brain (Bankiewicz et al., 2006).

While molecular therapies using various administration methods in animal models have shown promise with regard to their potential for clinical application, as with any therapy, the risks and benefits of a treatment must be carefully weighed before being offered to patients. We must be particularly careful in the application of these therapies to the treatment of hearing loss, as hearing loss is not a life-threatening condition and therefore only the slightest treatment-associated risks can be tolerated. Currently, there are many unanswered questions regarding the effects of neurotrophins in the inner ear, and these will need to be addressed before proceeding with the development of clinical applications. Future work will need to be directed toward further characterizing the type and physiologic properties of these re-grown PAFs, as well as developing strategies to better direct the fibers to grow toward specific CI electrodes in a tonotopic fashion. Ultimately, we must prove that neurotrophin treatment in fact has a beneficial effect when administered in concert with cochlear implantation, and that there are no major long-term sequelae of treatment, such as the formation of neuromas or other tumors in the inner ear. Still, neurotrophin therapies for the inner ear, particularly in combination with CI, show great early promise and will hopefully come to clinical fruition after further research has been completed.

The Future of Neurotrophin Therapy

The development of the CI has revolutionized the world of hearing rehabilitation; however, severe limitations remain with this technology and it is likely CI will never be able to fully restore normal hearing. The ultimate goal of hearing research is to develop the means by which normal hearing can indeed be restored through the regeneration of an anatomically and physiologically functional inner ear. In the past this idea of rebuilding the inner ear seemed like an unrealistic dream; however, with our rapidly expanding knowledge and understanding of inner ear physiology and molecular biology this goal has for the first time appeared within reach. As discussed above, neurotrophin administration to the inner ear has been demonstrated on multiple occasions to induce regrowth of PAFs into the BM area. However, neurotrophins do not regenerate sensory hair cells, and alternative means for regenerating these mechanosensory cells are under development. *Atoh1* (formerly known as *Math1*) is a basic helix-loop-helix transcription factor involved in the Notch signaling pathway and is expressed specifically by cochlear sensory cells during inner ear development. Studies of *Atoh1* null mice have demonstrated that *Atoh1* is both necessary and sufficient for the development of cochlear hair cells (Bermingham et al., 1999; Fritzsche et al., 2011), and more recent work utilizing viral vectors carrying the *Atoh1* transgene have shown that *Atoh1* expression can be induced in non-sensory cells of the mature mammalian cochlea (Kawamoto et al., 2003). Furthermore, immature hair cells have been found in the region of the organ of Corti following the forced expression of *Atoh1* in these non-sensory cochlear supporting cells (Kawamoto et al., 2003; Izumikawa et al., 2005). Unfortunately, as of yet, efforts to regenerate hair cells in ears that have undergone a severe ototoxic lesion and

have a flat epithelium have not been successful, and it is hypothesized that differentiated supporting cells may be necessary for successful transdifferentiation to the hair cell phenotype to occur (Izumikawa et al., 2008). It remains unclear how to induce regeneration of specifically inner and outer hair cells in the normal regimented tonotopic pattern of the cochlea, and the molecular signaling that occurs during normal inner ear development clearly involves more complex pathways than the Notch signaling pathway alone (Fritzsche et al., 2011). Despite these current limitations, as our understanding of inner ear development improves, it is foreseeable in the years to come that we will be able to induce the regeneration of functional cochlear mechanosensory cells. These regenerated hair cells cannot restore hearing in isolation, but rather must be restored within the framework of an intact auditory neural circuit. The co-administration of neurotrophin therapy along with the regenerative treatment will be critical to preserving the SGN population until new hair cells are regenerated and connected to the neural circuit.

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