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Neurotrophic factors and neural prostheses: potential clinical applications based upon findings in the auditory system

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Abstract

Spiral ganglion neurons (SGNs) are the target cells of the cochlear implant, a neural prosthesis designed to provide important auditory cues to severely or profoundly deaf patients. The ongoing degeneration of SGNs that occurs following a sensorineural hearing loss is therefore considered a limiting factor in cochlear implant efficacy. We review neurobiological techniques aimed at preventing SGN degeneration using exogenous delivery of neurotrophic factors. Application of these proteins prevents SGN degeneration and can enhance neurite outgrowth. Furthermore, chronic electrical stimulation of SGNs increases neurotrophic factor-induced survival and is correlated with functional benefits. The application of neurotrophic factors has the potential to enhance the benefits that patients can derive from cochlear implants; moreover, these techniques may be relevant for use with neural prostheses in other neurological conditions.

Keywords

sensorineural hearing loss; neurotrophic factor; spiral ganglion neurons; cochlear implant; neural prosthesis

Introduction

Cochlear hair cells, which reside in the organ of Corti on the basilar membrane (Figure 1), are responsible for the transduction of mechanical sound energy into neural impulses. Spiral ganglion neurons (SGNs), the primary afferent neurons of the cochlea, have their cell bodies located in Rosenthal's canal in the central core, or modiolus, of the cochlea. SGNs form synaptic connections with hair cells via their peripheral processes, and neural impulses generated by the hair cells are transmitted by the SGNs to the central auditory pathway where they are decoded, leading to the perception of sound. Damage to, or destruction of, the sensory hair cells leads to a permanent sensorineural hearing loss (SNHL), which subsequently leads to pathological changes to the SGNs. Initially, loss of hair cells results in the loss of the synaptic terminals between the SGN peripheral processes and the hair cells. This is followed by demyelination and degeneration of the peripheral processes as they recede from the damaged organ of Corti, eventually leading to degeneration of the SGNs. These degenerative changes are ongoing, and ultimately result in small numbers of surviving SGNs after long periods of deafness [1-4].

In addition to the morphological changes observed following SNHL, physiological changes are also apparent. For example, there is a loss of driven activity and a significant reduction in

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the level of spontaneous activity in deafferented SGNs [5]. In response to electrical stimulation, action potentials recorded from SGNs from long-term deaf ears exhibit reduced temporal resolution [5] and prolonged refractory periods [6], while central auditory neurons show significantly increased response latencies [1]. Elevated electrically evoked auditory brainstem responses (EABRs) are also typically observed following a SNHL in experimental animals, and significantly, more extensive changes are reported with increased periods of deafness [1].

The cochlear implant is used by more than 100,000 people worldwide and is currently the only therapeutic intervention for patients with a severe-profound SNHL. These devices provide auditory cues by bypassing the damaged or missing hair cells to electrically stimulate residual SGNs directly. Since SGNs are the target cells of the cochlear implant, their ongoing loss, as well as the other pathological changes that occur in deafness, may reduce the benefits that patients can derive from these devices. Indeed, there are indications from animal studies that the efficacy of a cochlear implant may be compromised by ongoing SGN degeneration [7-10]. The maintenance of a viable SGN population is likely to enhance the benefits of the cochlear implant and lead to improved outcomes in terms of language acquisition and speech perception in patients.

Neurotrophic factors are naturally-occurring proteins that are released by neuronal target tissues to regulate neuronal survival and differentiation during development, and are also essential for maintenance of neurons and neural circuitry throughout adulthood [11-13]. The neurotrophins are the best characterised family of neurotrophic factors. In particular, members of the neurotrophin family, brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) have been shown to play an important role in the auditory system, and can support SGN survival following injury or trauma. As such, neurotrophins are considered potential therapeutic agents for improving the efficacy of the cochlear implant by enhancing SGN survival in deafness [14].

This paper will review experimental findings from the auditory system of neurotrophin treatment, both alone and in combination with chronic electrical stimulation via a cochlear implant, and will consider the potential application for these techniques in other neurological disorders using neural prostheses.

Neurotrophins are important for cochlear development

A primary factor in SGN degeneration in response to deafness is the loss of neurotrophic support which is normally provided by the hair cells [15-17]. In the auditory system, BDNF and NT-3 are known to be important for cochlear development and SGN survival and maintenance. The neurotrophins are localised in the developing organ of Corti, while their respective receptors, trkB and trkC, are concurrently expressed by the SGNs [15,16,18-21]. The roles of BDNF and NT-3 in cochlear development have also been substantiated with gene knockout studies. Mice lacking the gene for NT-3 have a significant reduction in the number of SGNs [17,22], and deletion of the trkC receptor gene resulted in a loss of more than half of the normal complement of SGNs [23]. A small loss of SGNs is also observed in BDNF or trkB knockout mice [17,23]. Studies utilising deletion of both the BDNF and NT-3 genes, or both the trkB and trkC genes, show almost total loss of all SGNs and a complete loss of innervation to the inner ear [17,24]. Cumulatively, these findings highlight the importance of neurotrophic support during the development of the cochlea. Continued expression of neurotrophins and their receptors in the mature cochlea indicate that neurotrophins are also required throughout life for the maintenance of SGNs [15,21].

Neurotrophins support the survival of SGNs in animal models of deafness

In addition to their role in development and maintenance, neurotrophins can also rescue SGNs from the degeneration that is typically observed following damage to or loss of the sensory hair cells.

In vitro animal models of deafness isolate the SGN-containing modiolus from the organ of Corti, thereby severing synaptic connections, removing any intrinsic neurotrophic support and initiating neural degeneration. As such, these models have been used extensively to test the effectiveness of exogenous neurotrophin application in supporting SGN survival, resulting in the identification of numerous neurotrophins with survival-promoting capacities. For example, in cultures of rat SGNs, BDNF and NT-3 have been reported to promote SGN survival in comparison to neurotrophin-free controls [25-27], and provide protection against ototoxic agents [28].

Neurotrophins also support SGN survival in animal models of deafness *in vivo*. In these models, guinea pigs are typically deafened either acoustically or via ototoxic drugs; in both forms of pathology, the hair cells are destroyed and there is a resulting degeneration of SGNs. It is now well established that exogenous application of neurotrophins can prevent this degeneration. For example, following intracochlear BDNF delivery to deafened guinea pigs for between two and eight weeks, approximately 80% of SGNs survived, in comparison to less than 30% survival in contralateral, untreated cochleae (Figure 2) [29-32]. In addition to enhanced survival, the soma area of SGNs in BDNF-treated cochleae were similar to or greater than that observed in normal hearing controls [32]. Similarly, NT-3 treatment also promoted SGN survival, with survival rates greater than 90% in the deaf guinea pig cochlea [29,33]. Combined neurotrophic factor administration, using two or more neurotrophic factors, has also been reported to lead to enhanced SGN survival in comparison to deaf controls, and is typically more effective than individual treatments [29,34-36].

Although these studies have provided evidence that exogenously applied neurotrophic factors can rescue SGNs following the loss of hair cells, they have all been performed in the guinea pig. When evaluating the efficacy of exogenous neurotrophic factor delivery for potential clinical application, it is important to establish whether neurotrophic factors exhibit neuroprotective effects across a broad range of mammalian species. Two studies have examined this issue in species other than guinea pig. BDNF gene therapy, in which the introduction of the BDNF gene into the cochlea of ototoxically deafened mice caused cochlear cells to produce BDNF, resulted in 95% SGN survival, as compared to only 35% survival in deafened controls [37]. In a second study using deafened rats, intracochlear BDNF infusion via a mini-osmotic pump resulted in a significant increase in SGN density compared with control cochleae that received artificial perilymph treatment or deafened cochleae that were left untreated [38]. Similar to a number of the guinea pig studies described above, chronic delivery of exogenous BDNF into the rat cochlea also prevented the SGN soma shrinkage that typically follows SNHL [38]. Taken together, these studies offer confidence that the exogenous delivery of neurotrophins to the human cochlea may provide a significant level of trophic support to residual SGNs.

Specific questions have also been asked pertaining to the time-course of neurotrophin treatment following deafness, as well as the longevity of the survival effects. In particular, since there is generally a significant time delay between the onset of a SNHL and the time when a patient receives a cochlear implant, the ongoing loss of SGNs may impinge upon the success of the implant. Indeed, the duration of deafness prior to cochlear implantation is a key variable affecting post-operative performance [39,40]. Therefore, if neurotrophic factors are to be clinically applicable, it is important to know if there is a critical period following the onset of

deafness in which such treatments will be most beneficial. Neurotrophic factors can support the survival of the remaining SGN population when applied following extended periods of deafness, when the degenerative processes are further advanced. Specifically, after a two-week period of deafness, when approximately 17% of the SGNs had degenerated, each of BDNF, NT-3, nerve growth factor (NGF) and neurotrophin-4/5 (NT-4/5) prevented further degeneration [41]. Furthermore, BDNF plus NT-3 effectively prevented ongoing SGN death when applied four weeks after the onset of deafness [36], and combined administration of BDNF and the cytokine ciliary-derived neurotrophic factor (CNTF) had protective effects when treatment commenced up to six weeks post-deafening [42].

It is reasonable to assume that in humans, the longer the period of deafness prior to intervention, then the greater the extent of degeneration, and thus a smaller population of SGNs would be available for protection and/or rescue. Therefore, while SGNs can be rescued from deafness-induced degeneration, early intervention would be recommended in order to maintain a robust population of SGNs and maximise the benefits of the cochlear implant.

Another important factor relating to the time-course of neurotrophin treatment is the longevity of the survival effects, particularly if the exogenous support is withdrawn. Although BDNF treatment in deaf guinea pigs can protect SGNs from degeneration, the survival effects were not maintained beyond the treatment period. In fact, cessation of BDNF treatment led to a rapid decline in SGN survival, such that survival rates as early as two weeks following the completion of BDNF treatment were not significantly different to contralateral, untreated controls, as shown in Figure 3 [31]. Similar findings have also been reported in other neuronal classes. For example, NGF administration was not sufficient to permanently rescue cholinergic neurons following lesion of the septohippocampal pathway [43]. In addition, although BDNF treatment supported the survival of retinal ganglion cells (RGCs) following optic nerve transection, most of the rescued cells died soon after the treatment stopped [44].

Therefore, in order for neurotrophic treatments to be clinically viable, a means to permanently rescue SGNs from SNHL-induced degeneration is necessary. Such therapies may include techniques for continuous neurotrophic factor delivery, or the combined use of neurotrophic agents and electrical stimulation, as discussed below.

Neurotrophins enhance neuritic outgrowth from SGNs

In addition to the importance of maintaining a viable SGN population for improved cochlear implant efficacy, a means to stimulate and control growth of peripheral processes from SGNs may also prove beneficial. Although SGNs can not currently be replaced once they have degenerated, surviving SGNs are able to spontaneously resprout and regrow their peripheral processes *in vivo* following deafferentation. Resprouting of SGN peripheral processes has been observed in a number of species, such as chinchillas, guinea pigs and cats, and after different forms of cochlear damage, including acoustic trauma, ototoxicity and nerve transection [45-52]. Resprouting processes were identified morphologically on the basis of their abnormal projections, which were substantially different to the well structured and uniform innervation profile that is characteristic of a normal (undamaged) cochlea (Figure 4a). The resprouting processes were observed to loop back upon themselves (i.e. they reversed their direction and projected towards their cell body), and were also observed to project onto the basilar membrane and course their way laterally, sometimes in a disorganised tangle of many processes (Figure 4b). A common observation was that resprouting processes were associated with regions of the cochlea that had sustained significant damage to the organ of Corti [36,45,46,48], which suggests that the signals associated with the degeneration of SGNs might also provide important cues in the resprouting procedure.

The administration of neurotrophins has been shown to enhance the resprouting of auditory peripheral processes. In ototoxically deafened guinea pigs the SGN peripheral processes were observed over a significantly greater area following treatment with BDNF plus NT-3, with the increased resprouting observed in the basal turn of the cochlea, in close proximity to the site of neurotrophin application (Figure 4c) [36]. Neurotrophic factor treatment has also been reported to lead to the regrowth of SGN peripheral processes in the tissue spaces of the damaged organ of Corti, and on the underside of the basilar membrane within the scala tympani [29, 34]. Although the origin of these resprouted fibres remains to be determined, acetylcholinesterase immunohistochemistry (AChE) has previously been used to characterise regenerating fibres within the noise-damaged chinchilla cochlea. Specifically, the regenerated fibres did not display AChE immunopositivity, but normal AChE-positive fibres were observed in the undamaged apical turn of the same cochlea [48]. Since SGN afferent fibres and their synaptic terminals on hair cells do not express AChE, and nerve fibres belonging to the efferent cochlear system are reported to be immunopositive for AChE [53], it was concluded that the regenerated fibres were not efferent and therefore were most likely afferent [48]. Future studies will need to confirm that resprouted fibres following ototoxin-induced deafening and neurotrophin treatment are afferent, in order to ensure these fibres are relevant to improved functioning of the cochlear implant.

The long-term fate of resprouted auditory peripheral processes remains unknown. In one study, peripheral processes that spontaneously regrew onto the basilar membrane after noise-induced deafening were still present more than two years later, with some of the processes appearing to terminate on or near cells located within the damaged organ of Corti [45]. However, a second study showed that although resprouting processes were observed up to one month following ototoxin-induced deafening, processes were seldom observed after approximately four months [50]. Since ototoxicity generally causes widespread cochlear damage, while noise-induced deafening results in more localised areas of damage, long-term survival of resprouted processes may rely upon a close interaction with viable hair cells or supporting cells within the organ of Corti. Therefore, if it becomes possible to regenerate auditory hair cells in humans, the growth of peripheral processes in an organised manner towards this target may lead to at least partial restoration of hearing.

Regrowth of peripheral auditory processes also has implications for enhancing the efficacy and benefits of the cochlear implant. Specifically, growth of peripheral processes towards a cochlear implant electrode array may lead to an improved electro-neural interface, resulting in decreased excitation thresholds and decreased power consumption. However, due to the tonotopic organisation of the cochlea, neuritic outgrowth from SGNs *in vivo* would need to be highly structured in order to achieve meaningful outcomes. In contrast, ectopic neurite growth would prove counter-productive as it would adversely affect the place-dependent cochleotopic organization cochlear implants use to encode pitch.

Chronic electrical stimulation enhances the survival effects of neurotrophins on SGNs

From the perspective of neural prostheses it is important to determine whether the trophic effects of exogenous neurotrophin delivery on SGNs, as described above, will be affected by simultaneous chronic electrical stimulation (ES) via a cochlear implant. This question is particularly important given the requirement for long-term neurotrophin delivery in order to maintain deafferented SGNs [31].

Two studies have addressed this issue using complimentary techniques to deliver the neurotrophic factor. In the first study, gene therapy using glial cell-line derived neurotrophic factor (GDNF) was coupled with chronic ES via a monopolar ball electrode placed in the scala

tympani of deaf guinea pigs. The animals were stimulated for 36 days using charge-balanced biphasic current pulses at levels above threshold, as determined electrophysiologically via EABRs. Individually, both chronic ES and GDNF exhibited significant rescue of SGNs compared with deafened controls, with GDNF being more effective than chronic ES. Importantly, combining treatments was significantly more effective than either factor alone [54].

In a second study, BDNF was delivered to the deafened guinea pig cochlea via a cochlear implant electrode array incorporating a mini-osmotic pump drug delivery system. The bipolar electrode array was inserted into the scala tympani five days after deafening, and drug delivery continued for a 28-day period. The animals were stimulated for 23 days at 6 dB above EABR threshold. While chronic ES alone showed no evidence of SGN rescue compared to deafened controls, animals treated with BDNF exhibited significantly greater numbers of SGNs. The combination of BDNF and chronic ES produced significantly greater SGN rescue compared with BDNF alone, suggesting that an interaction may exist between the ES and BDNF treatment. Moreover, functionally, both BDNF plus ES and BDNF alone cohorts demonstrated significant reductions in EABR thresholds compared with deafened cohorts that did not exhibit SGN rescue [32].

The mechanism(s) underlying the significant reduction in threshold of BDNF-treated cochleae remains unclear, but could be associated with the distribution and conductance of ion channels [55,56]; an increase in the diameter of the neurotrophin treated neurons [57]; and/or neurotrophin-induced neurite growth towards the electrode array [29,36]. Irrespective of the underlying mechanism(s), techniques that lead to reductions in threshold at the electrode-neural interface offer significant reductions in power consumption for neural prostheses using transcutaneous radio-frequency links, which are inherently inefficient [58]. Longer battery life, smaller external components, increased dynamic range and even the potential for smaller, more numerous electrode contacts may be realized through such reductions in threshold.

The additive trophic effects of neurotrophic factors and ES described in these studies hold promise for similar trophic and functional advantages in other pathologies where neural prosthesis are used for restoration of function.

Clinical considerations for neurotrophin application in the inner ear

Experimental findings of the effects of neurotrophins in animal models of deafness have highlighted the potential of neurotrophins to rescue SGNs in severely to profoundly deaf patients. However, such information can not be directly extrapolated to human application, and therefore appropriate delivery techniques and treatment regimes need to be established before these trophic agents can be used clinically. In particular, based upon the indications that neurotrophin-induced survival effects are not maintained beyond the treatment period [31], techniques for neurotrophin treatment need to be aimed at providing long-term or permanent SGN maintenance following deafening. Such techniques may include long-term neurotrophin administration. However, current experimental models have only delivered neurotrophins to the cochlea for periods of up to eight weeks. It therefore remains to be confirmed if long-term neurotrophin administration will provide ongoing, improved SGN survival. Any side effects relating to prolonged neurotrophin delivery will also need to be ascertained, especially considering that neurotrophin receptors are not specific to SGNs and neurotrophins may therefore elicit effects on other cell types within the cochlea, or potentially, throughout the nervous system. Alternatively, long-term SGN survival may be achieved by combining initial, short-term neurotrophin treatment with ongoing electrical stimulation via a cochlear implant. Preclinical trials are required to determine the appropriate time-course of neurotrophin treatment and concurrent treatment conditions, such as electrical stimulation from a cochlear

implant, as well as optimal dosing regimes to maximise efficacy and minimise toxicity. Furthermore, the various delivery methods available need to be assessed.

Delivery techniques for neurotrophin administration in the cochlea

The anatomy of the cochlea presents several options for neurotrophin delivery; direct infusion into the scala tympani or scala vestibuli (perilymph) or scala media (endolymph); indirect infusion via the vestibular organs which are connected with the cochlea via these fluids; or delivery across the round window membrane. There are also a number of options for the mode of neurotrophin delivery, whether it is the pure neurotrophin protein in solution, neurotrophin captured within a polymer, or expression of neurotrophins via cell-based or gene-based therapies.

Neurotrophin diffusion through the cochleaâ Neurotrophins in solution may be infused into the cochlea via a cochleostomy made in either the cochlear bony wall or the round window membrane. This places the neurotrophins directly into the perilymph and is very efficacious for SGN protection [29,30,32,33,36,41]. However, tracer studies â which use visually-detectable markers â have revealed that much of the introduced substances bound non-specifically to non-neural tissues such as the basilar membrane, osseous spiral lamina, spiral ligament and organ of Corti, with only minor quantities of tracer detected in the cell bodies of SGNs [59,60]. Additionally, neurotrophins, as well as gene transfer vectors and transplanted cells, have been shown to spread beyond the cochlea to the vestibular apparatus, the central nervous system (CNS) and the contralateral cochlea [61-65]. The cerebrospinal fluid (CSF) provides a direct link to these organs via the cochlear aqueduct â a bony channel which connects the perilymphatic space of the basal turn of the cochlea with the subarachnoid space of the posterior cranial cavity. The implications of this are two-fold; firstly, the non-specific binding dictates that a far greater quantity of neurotrophin is required to produce a therapeutic effect than if neurotrophins were only targeted to neurons; and secondly, safety studies must include the evaluation of potential side-effects of neurotrophins in the vestibular system and the CNS.

Neurotrophins are commonly delivered to the basal turn of the cochlea, as this is the most surgically accessible region. Protective effects on SGNs in the apical turns therefore requires basal to apical diffusion of neurotrophins through the perilymph. In the sealed cochlea, as is the case during chronic neurotrophin delivery, passive diffusion of neurotrophins through the cochlea may be facilitated by the perilymphatic flow, albeit at a very slow rate of 4.4 nL/minute [66]. Although maximal SGN survival is commonly observed adjacent to the infusion site in the cochlear basal turn, significant SGN protection is typically observed throughout the cochlea, implying that the infused neurotrophins are distributed to regions of the cochlea beyond the basal turn [30,32,36,38].

An alternative delivery technique, allowing diffusion throughout the cochlea, could involve the capture of one or more neurotrophins within a polymer that can then be incorporated into the design of the cochlear implant electrode array. Slow release via diffusion or controlled-release techniques have been demonstrated using such technologies to date [67,68]. Of particular relevance is an *in vitro* study in which a material known as polypyrrole was polymerised onto electrodes and released NT-3 under the control of electrical stimulation, promoting neurite outgrowth from SGNs. Importantly, polypyrrole did not alter the impedance of the electrodes, ensuring normal electrode function if used in cochlear implants [69,70].

Round window delivery methodsâ The round window membrane offers an alternative site for atraumatic delivery of pharmacological agents to the cochlea, based upon its permeability to a variety of substances [71-75]. The application of a neurotrophin-soaked alginate polymer bead to the round window membrane resulted in SGN protection throughout

the cochlea [74]. Round window delivery of steroids and anti-oxidant agents also proved effective for protecting the inner ear from metabolic stressors such as exposure to noise or ototoxins [76]. However, the effectiveness of some pharmacological agents may be compromised by their non-uniform distribution within the cochlea, with relatively high concentrations detected in the basal turn near the round window, and little evidence of the drug reaching the apical turn [77,78]. The permeability of the human round window may also differ from experimental animals, as well as between individuals due to cochlear pathologies, suggesting that the effectiveness of this route may be variable [79].

Gene-based therapiesâ Gene therapy provides an alternative vehicle for delivering neurotrophins to the inner ear. Gene therapy involves the insertion of genes into cells *in situ* and may be used to replace defective genes, or to induce or increase expression of a desired gene, such as a neurotrophin. Five main types of vectors, or vehicles, have been used to drive gene expression in the cochlea; adeno-associated virus, adenovirus, herpes simplex virus, vaccinia virus and liposomes, the latter being the only non-viral vector tested [80,81]. Reporter gene expression studies have demonstrated that, amongst other cells and tissues, transgene expression in SGNs and the organ of Corti is commonly obtained [62,82-85]. Persistence of transgene expression depends greatly on the mode of delivery and can range from days to months [84,86-88].

Transfer of BDNF, GDNF or NT-3 genes into the cochlea has resulted in SGN protection comparable to that achieved with neurotrophic factors delivered to the cochlea as a protein solution [37,89-92]. However, gene therapy has the potential benefit of enabling cell-specific expression of genes, whilst leaving other cells unaffected. For example, directed expression of the reporter gene green fluorescent protein could be achieved exclusively in neurons, hair cells, supporting cells, blood vessels or cells of the spiral limbus using promoters specific for each cell type [93,94].

Cell-based therapiesâ Cell transplantation is another method for neurotrophic factor delivery into the cochlea. Some cells, such as Schwann cells, are known to naturally produce small quantities of neurotrophic factors [95,96], and transplantation of these cells into the cochlea of deaf guinea pigs has demonstrated a small but significant protective effect on SGNs [97]. Alternatively, *ex vivo* gene transfer may lead to even greater survival effects. Such a technique would involve the genetic modification of a host population of cells with the gene (s) of interest â in our case, neurotrophin(s) â followed by transplantation of the cells into the cochlea. *In vitro* findings have shown that Schwann cells that were genetically modified to over-express BDNF or NT-3 produced significantly greater amounts of the respective neurotrophin than normal Schwann cells [98]. In addition, co-culture of these neurotrophin over-expressing Schwann cells with dissociated rat SGNs resulted in significantly greater SGN survival than was observed using normal Schwann cells [98]. Future investigations will determine whether these cells can elicit similar survival effects in different species *in vivo*, and if concurrent cochlear implantation can provide additive benefits.

Cell transplantation studies may also utilise stem cells, for the *replacement* of damaged or degenerated SGNs or hair cells. Previous studies have reported that transplanted stem cells survived within the cochlear environment for periods of 3-4 weeks, and that the transplanted cells migrated throughout all turns of the cochlea [99-101], into the modiolus [100,102,103], and to the vestibular organs [64,104]. While the spread of stem cells may be beneficial in terms of replacing lost or damaged SGNs and/or hair cells within the cochlea, it would not be desirable for the cells to spread beyond the cochlea. However, as previously indicated, the patency of the cochlear aqueduct with the CSF means there is the potential for any agent delivered to the cochlea to enter the CNS, which may ultimately induce adverse side effects.

Prospective cell-based therapies are therefore likely to include encapsulation techniques, whereby the cells are incorporated into a biocompatible matrix that will prevent cellular spread from the cochlea. Such techniques would allow for continued molecular exchange through the matrix, providing essential nutrients to the enclosed cells. In the case of neurotrophin-producing cells this would also allow release of neurotrophins from those cells into the surrounding environment. Other forms of encapsulation could include biodegradable matrices that would enable neuronally-differentiated stem cells to extend neurites beyond the implantation site for establishment of synaptic connections with desired targets. Previous studies have successfully demonstrated that cells can survive and remain contained within biocompatible capsules, and that neurotrophin-producing cells maintained expression of the neurotrophin and elicited neuroprotective effects [105-110].

Safety considerations

Experimental studies commonly use mini-osmotic pumps for delivery of neurotrophins to support SGN survival in deafness. However, in addition to the limited delivery period of these devices, infusion via an intracochlear cannula is not considered a clinically viable technique. Such cannulae are niduses for infection, which may lead to labyrinthitis and potentially meningitis [111]. In comparison, as a delivery system, the bolus delivery of a therapeutic substance to the cochlea at the time of surgery can be considered reasonably safe, provided that the seal is adequate and the rate of delivery does not cause mechanical trauma to the cochlea. However, the longevity of the survival effects on SGNs using a single bolus delivery remains unknown.

Safety issues are also apparent in relation to gene- and cell-based therapies. For example, high virus loading with gene therapy can cause cell toxicity and immune responses [81,93,112]. There is also concern of the spread of the viral vector to other sites via the cochlear fluid pathways, with gene-based studies demonstrating that unilateral viral inoculation of the inner ear also leads to gene expression within the contralateral cochlea and the CNS [61,62]. The safety issues posed by viral vectors could be prevented through the use of non-viral vectors, such as lipid-based liposomes, despite their low transfection efficiency. In addition, the actual duration of transgene expression â be it via viral or nonviral vectors â can be quite short, making gene therapy suitable for some treatments, such as transforming organ of Corti supporting cells into new hair cells [113], although such techniques are not suitable for neurotrophin delivery for SGN preservation because of the need for ongoing expression of neurotrophins.

In terms of cell transplantation techniques, careful consideration needs to be given to cell type (s) used, in order to avoid cells that may have a predisposition to form tumours, as well as the type of transplantation. Autologous transplantation â where the cells or tissue used for transplantation are taken from the patients own body â would minimise the immune response and the risk of rejection. For *ex vivo* gene transfer, host cells could be taken from the patient, genetically modified to over-express neurotrophins and then transplanted into the cochlea, providing benefits as a result of the increased neurotrophic support, as discussed previously. Encapsulation technologies are likely to prove beneficial in preventing migration or dispersal of cells from the transplantation site, as well as immunologically isolating the modified cells from the host, further preventing inflammatory responses.

Application of neurotrophic factors and neural prostheses in other sensory systems

Significant levels of research are currently being directed to the development of bionic systems that link, via neural interfaces, the human nervous system with electronic or robotic prostheses. Such ventures aim to restore motor and/or sensory functions in patients with spinal cord

injuries, CNS or peripheral nerve pathologies, or degenerative diseases. Therefore, in addition to the application of neurotrophins to enhance outcomes for cochlear implant patients, such techniques may prove useful in other systems incorporating neural prostheses, although the precise neurotrophic factor(s) required for maximal benefit may differ across neuronal classes.

For example, the development of retinal implants is a major subject of investigation in the field of visual prostheses. One concept behind retinal implants is to stimulate surviving RGCs following the loss of photoreceptor cells in retinal degenerative and dystrophic diseases [114]. It has also been suggested that more focal stimulation could theoretically be achieved if the neurons of the visual system can be encouraged to grow onto an array of stimulating electrodes [114]. However, a major issue associated with the development of a bionic eye is that the degree of RGC degeneration in the latter stages of retinal disorders is unknown [115]. Importantly, RGCs have been shown to respond to neurotrophins, with BDNF, NT-3 and NGF described as target-derived trophic factors for developing RGCs [116,117]. In addition, neurotrophic factors support the survival of RGCs and stimulate neurite outgrowth *in vitro* [118-123], and intraocular administration of either BDNF or CNTF has been shown to enhance RGC survival after axotomy [44,124-126].

Similarly, neural prostheses which use electrical activation of the nervous system for the restoration of functions such as limb movement, bladder function and sensation following spinal cord injury, and in motor neuron diseases such as Amyotrophic Lateral Sclerosis, may benefit from the use of neurotrophic factors to prevent neural degeneration. Furthermore, a device that uses electrical stimulation to induce regeneration of neural fibres for the formation of functional connections, and aims to restore tactile sensation and movement for patients with acute spinal cord injuries, is being developed for use with a variety of neurotrophic factors [127].

Therefore, any technique that uses neurotrophin administration to support SGN survival or induce neurite outgrowth and enhance the benefits of the cochlear implant may also be applicable to neural prostheses for other neurological impairments. Importantly, evidence from the auditory system suggests that neuronal survival is potentiated with the combined use of neurotrophins and electrical stimulation; concurrent techniques may provide similar benefits in other neural prosthetic applications.

Conclusion

Neurotrophins play an important role in the formation of functional neural connections between SGNs and adjacent hair cells within the developing mammalian cochlea. Moreover, it is also apparent that endogenous neurotrophins â supplied by inner hair cells and supporting cells of the organ of Corti â play a vital role in the maintenance of SGNs in the mature cochlea; the loss of the intrinsic neurotrophins following SNHL is a major factor leading to the gradual degeneration of SGNs.

Exogenously applied neurotrophins are highly effective at protecting SGNs from degeneration, and results from studies combining neurotrophic factor treatment with chronic depolarization via a cochlear implant are particularly promising. The ability of neurotrophins to promote neurite outgrowth is also very attractive, provided mechanisms to achieve highly organized and directed growth to target electrodes can be achieved.

In terms of clinical application, the side effects and risks associated with neurotrophic factor administration must be carefully considered, especially in view of the free communication between the cochlea and the CSF and vestibular system. Furthermore, the development of appropriate delivery techniques must be explored carefully as evidence suggests that exogenous neurotrophic factors must be delivered continuously to maintain a trophic

advantage. While the delivery of neurotrophic factors to the cochlea via a cannula and pump system is, in our opinion, not clinically viable, cell-based therapies, perhaps in conjunction with gene transfer, are likely to provide a safer and more efficient means of delivering neurotrophic factors to the cochlea at physiologically relevant levels, and over long periods of time.

Finally, the application of neurotrophic factors with cochlear implants, as described here, is an example of a potentially broader application of combining neurobiology with biomedical engineering in new areas of neural prosthetic development.

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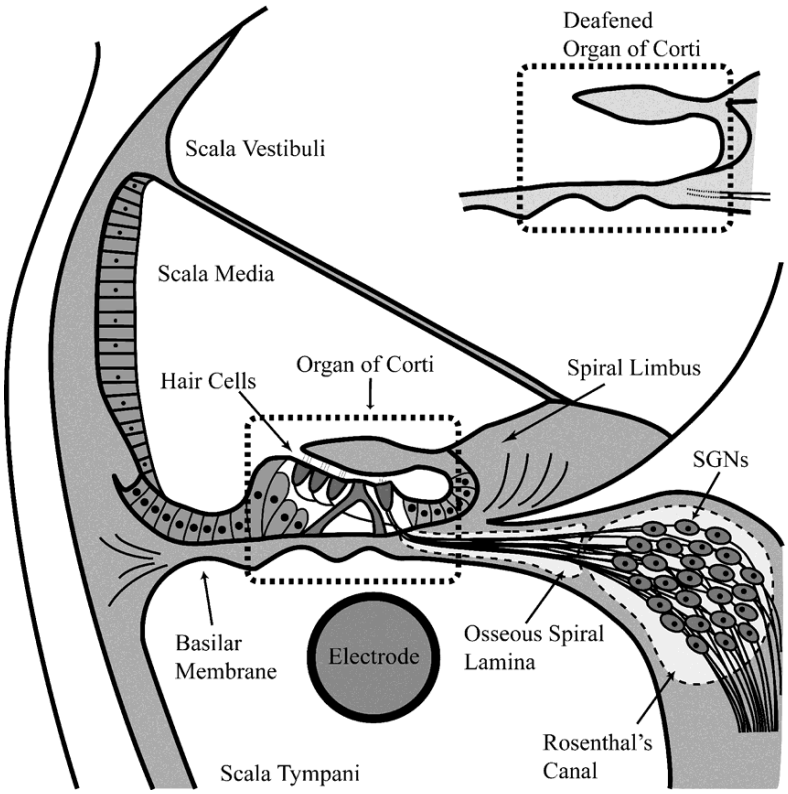

Figure 1.

Schematic diagram of a cross-section through the cochlea showing the three fluid-filled chambers, scala vestibuli, scala media and scala tympani. The round window membrane (not shown) is located at the basal end of the scala tympani. The cell bodies of the SGNs reside centrally in Rosenthal's canal and their peripheral processes project towards the organ of Corti (dotted box) and synapse with the sensory hair cells. In a deafened cochlea (inset), damage to the organ of Corti causes loss of the hair cells and surrounding support cells. The cochlea implant electrode is implanted into the scala tympani to electrically excite the residual SGNs.

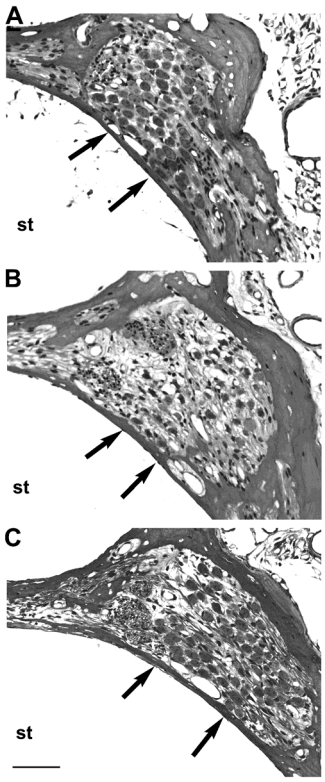

Figure 2.

Photomicrographs of Rosenthal's canal (arrows) showing SGN survival in the upper basal turn of a guinea pig cochlea (A) deafened and treated with BDNF and chronic ES via a cochlear implant; (B) deafened and untreated; and (C) from a normal hearing animal. The SGN density in the BDNF/ES treated cochlea was similar to that of the normal hearing control, while the deafened control exhibited a â50% loss. The cochleae illustrated in (A) and (B) were deaf for a period of four weeks. Scale bar = 50Îm; st = scala tympani.

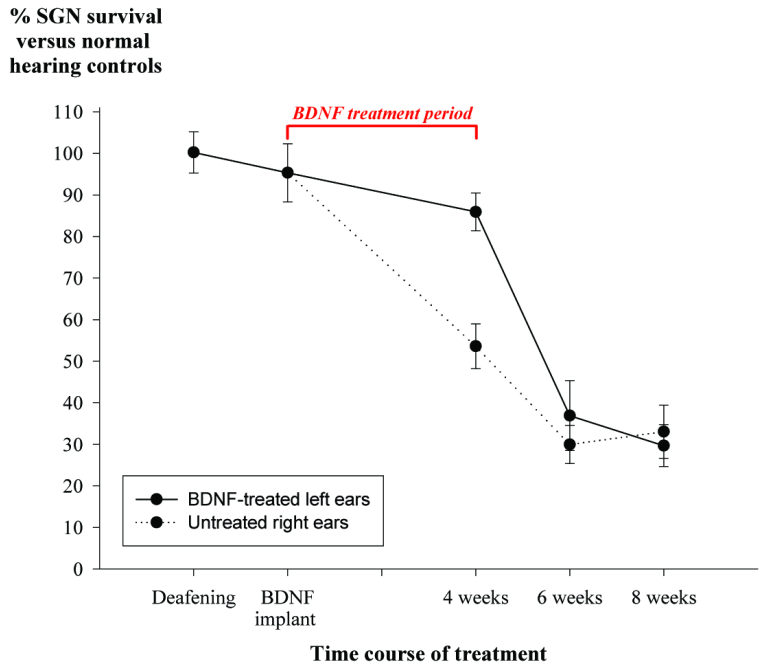

Figure 3.

Longevity of the survival effects of intracochlear BDNF infusion on SGNs in deaf guinea pigs. Guinea pigs were ototoxically deafened, implanted with a mini-osmotic pump five days later, and then received four weeks of BDNF treatment. At the end of the treatment period, a significantly greater proportion of surviving SGNs were present in the BDNF-treated cochleae as compared to contralateral, untreated cochleae. However, the survival effects did not extend beyond the treatment period, with survival rates as early as two weeks following the cessation of BDNF treatment not significantly different to untreated controls (Adapted from Gillespie *et al.* 2003 [31]).

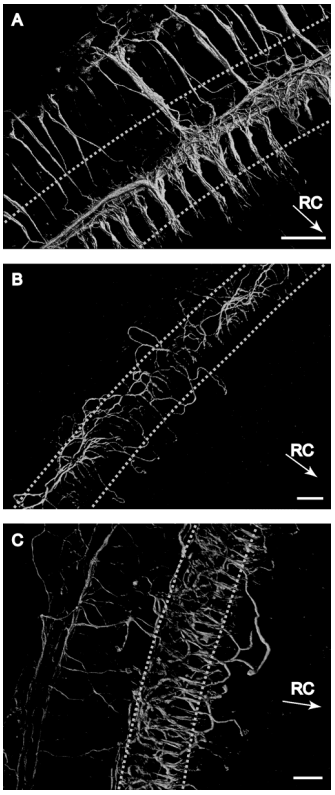

Figure 4.

Whole mount preparation showing a top down view of the SGNs in the guinea pig organ of Corti (see box in Figure 1). The dotted lines indicate the approximate location of the implanted electrode that would be positioned below the organ of Corti in the scala tympani. The arrows indicate the direction of the location of Rosenthal's canal (RC). (A) SGN peripheral processes in the normal organ of Corti, projecting towards and synapsing with the base of the hair cells. The sensory hair cells are not visible in this image. (B) Resprouting SGN peripheral processes in a deafened cochlea; although there were fewer neurons following deafening, some remaining neurons resprouted onto the organ of Corti. (C) Resprouting SGN peripheral processes in a deafened cochlea that received neurotrophin treatment; resprouting processes were observed over a greater area in the neurotrophin treated cochleae. Scale bars = 20Îm (Adapted from Wise *et al.* 2005 [36]).