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Expression of Brain-Derived Neurotrophic Factor in Human Spiral Ganglia Neurons Following Cochlear Implantation

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Abstract

Background: Brain-derived neurotrophic factor (BDNF) is an important factor in the development and neuroprotection of afferent auditory pathways. In this study, we investigated the expression of BDNF in the afferent auditory pathway following cochlear implantation (CI), hypothesizing that electrical stimulation following CI stimulates BDNF expression in the afferent auditory pathway.

Methods: Archival human temporal bones from eight patients with a history of CI and five patients with normal hearing (ages 65–93 years old) were studied. Temporal bone specimens were immunoreacted with rabbit polyclonal antibodies against BDNF and mouse monoclonal antibodies against pan-neurofilaments. In cases of unilateral CI, the BDNF expression was compared with the contralateral unimplanted ear and normal temporal bones without hearing loss.

Results: BDNF immunoreactivity (IR) localized to the spiral ganglion neurons (SGNs) somata and the surrounding satellite cells. BDNF-IR in the spiral ganglia was similar in the apical, middle, and basal hook regions. Neurofilament-IR localized to SGN nerve fibers in both implanted and unimplanted cochleae. BDNF-IR in the SGN and satellite cells was significantly increased in the implanted specimens compared with the unimplanted specimens ($p<0.05$) and the normal hearing specimens ($p<0.05$). BDNF-IR expression was similar in the unimplanted cochlea and in the normal cochlea. BDNF protein expression was increased despite complete loss of the organ of Corti hair cells and supporting cells. Even in the cases of CI with a 6 mm first-generation electrode, BDNF expression was upregulated throughout the cochlea.

Conclusions: BDNF expression in the SGN appears to be upregulated by the electrical stimulation from CI. This study provides evidence that the electrical stimulation from CI may stimulate expression of BDNF, playing a neuroprotective role in the rehabilitation of hearing in the deafened ear.

Introduction

Sensorineural hearing loss (SNHL) results from damage to inner ear structures including inner ear hair cells, the vestibulocochlear nerve, and the afferent auditory pathway.[1] For

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patients with profound SNHL, cochlear implantation (CI) remains the only therapeutic option for recovery of hearing. CI provides electrical stimulation directly to spiral ganglion neurons (SGNs), bypassing the mechanism for converting acoustic inputs to electrical signals.[2] Hearing outcomes vary widely following CI, but studies consistently demonstrate improvement in word perception and speech perception ability.[3]

CI candidacy has broadened significantly in the past three decades, and patients with residual hearing are now candidates for CI. Among those patients with residual hearing who undergo CI, some develop progressive, delayed hearing loss following implantation.[4,5] The cause of this is not well understood, but studies have postulated etiologies including surgical trauma resulting in inflammatory responses, development of fibrotic tissue, and trauma or degeneration of SGN, with animal studies suggesting a strong correlation between SGN preservation and overall hearing.[6] Archival human temporal bone studies have also noted that the number of surviving cochlear SGNs in the CI recipient is associated with better speech outcomes.[7]

Brain-derived neurotrophic factor (BNDF) is a factor that promotes the differentiation, maturation, and survival of neurons, and plays an important role in cochlear development, regulating neuronal differentiation and survival.[8,9] The absence of BDNF has been shown to result in hearing loss and SGN loss in mouse models, suggesting the critical role of BDNF within the cochlea. Indeed, in early mouse development, BDNF expression is upregulated in SGNs prior to the onset of hearing.[10,11] BDNF and its receptor, TrkB, are expressed in the developing human cochlea; however, there are no studies to date that demonstrate BDNF expression in the human SGN in normative or deafened human cochleae following CI.[12]

Animal studies have demonstrated a protective effect of electrical stimulation on SGNs.[13] Electrical stimulation has been shown to promote BDNF and TrkB mRNA expression and nerve regeneration in rat motor and spinal cord neurons.[14,15] Within the cochlea, there is an increase in its transcription factor, P-CREB, opening up the possibility of increased BNDF expression due to electrical stimulation within the rat cochlea.[16] Many experiments using animal models including guinea pigs and cats have shown that the application of BDNF into to the cochlea following CI—studied using osmotic pumps or BDNF-eluting implants—can preserve, and even regenerate, SGNs.[17–20] However, there are no studies localizing BDNF within the human cochlea after CI. This study aims to examine the immunoreactive pattern of BDNF in human temporal bones among patients who underwent cochlear implantation.

Materials and Methods

Archival Temporal Bone Specimens

All methods and protocols used in this study were approved by the Institutional Review Board (IRB) at our institution (IRB protocols #10–001449 and #22–001587). The temporal bone donors were part of the National Institutes of Health-funded National Temporal Bone Laboratory at UCLA through the National Institute on Deafness and Other Communication Disorders (NIDCD). Seventeen archival human temporal bones (HTBs) from thirteen patients (ages 65–93 years, average age = 76.5) were examined in this study (Table 1a–b). Five of these patients had normal hearing, and four patients underwent unilateral CI but

had the contralateral, unimplanted ear available to study for within-subject comparison of BDNF-IR expression within the cochlea (see Table 1a).

Celloidin removal and Antigen retrieval:

The methodology for celloidin removal and antigen retrieval has been described in detail (Lopez et al., 2016). In brief, celloidin sections were immersed in sodium-ethoxide (saturated solution) diluted in 100% ethylic alcohol (1:3, 60 minutes), 100% ethanol (2×5 minutes), and distilled water (3×5 minutes). Sections were immersed in antigen retrieval solution heated to 100°C (diluted 1:500 in double-distilled water, Vector antigen unmasking acidic solution, Vector Labs, Burlingame, CA). Sections were allowed to cool for 30 minutes, washed with phosphate-buffered saline (PBS) for 2×5 minutes, and immediately incubated for 8 minutes in a diluted trypsin solution (1:3, Abcam Trypsin Kit) and washed for 4×10 minutes in PBS before immunohistochemistry.

Immunofluorescence (IF):

Sections were incubated for two hours with a blocking solution containing 1% bovine serum albumin (BSA) fraction-V (Sigma, St. Louis, MO) and 0.5% Triton X-100 (Sigma) in PBS, followed by incubation with rabbit antibodies against BDNF (1:500) and mouse monoclonal anti pan-neurofilaments (Zymed, Cat# 13–1300, San Francisco, CA) diluted in PBS for 72 hours at 4°C in a humid chamber. BDNF rabbit polyclonal antibody (IgG) was affinity purified (Millipore Cat. # AB1779SP, Temecula, CA). The immunogen used to produce this antibody is recombinant human BDNF. This antibody recognizes only human BDNF, by dot blot, with less than 1% cross-reactivity against NGF, NT3, and NT4. Following a 3×15-minute PBS washing step, sections were incubated in goat anti-rabbit antibody labeled with Alexa 488 and goat-anti mouse labelled with Alexa 594 (1:1000 in PBS, Invitrogen) for two hours. The tissue sections were then washed with PBS (3×15 minutes) and a coverslip with aqua soluble mounting media containing DAPI was used to visualize cell nuclei (Vectashield, Vector).

Immunohistochemistry (IHC):

IHC staining was used to corroborate BDNF-IF staining. Secondary antibodies against rabbit labelled with HRP (ABC kit, Vector Labs) were used, and the antigen-antibody reaction was visualized with diaminobenzidine (ImmPact™ DAB Chromogen, Vector Labs). The IHC protocol has been described in detail.[21]

Immunohistochemical controls:

As a positive control, cryostat sections from mouse cochlea were incubated with antibodies against BDNF and NF. These sections were subjected to the described protocol. As a negative control, the primary antibodies against BDNF were omitted and the immunoreaction was performed in the human cochlea sections as described above, and no immunoreaction was observed.

Microscopic observation and documentation:

Digital fluorescent and light microscopic images were obtained using a Leica (SP8) high-resolution light-sheet laser confocal microscope, a Leica inverted microscope (Thunder system) coupled to a high-resolution light, and a fluorescence camera.

Image analysis:

A. Qualitative analysis: BDNF-IR in the temporal bones with CI was compared with BDNF-IR in the contralateral unimplanted temporal bones. IR was assessed by two independent observers to minimize bias in the analysis. One observer was "blinded" to the identity of the immunostained celloidin sections. Qualitative assessment was categorized as mild (+), moderate (++), or strong (+++) presence of BDNF immunoreactive cells for all the sections. A third person, who was not blinded to the immunostained sections, coded each sample. Systematic observations were made at the apical, middle, and basal portions of the cochlea spiral ganglia (X400) to determine whether there were regional variations in the localization of BDNF immunoreactive cells (See Table 1a).

B. Quantitative analysis: BDNF immunoreactivity (IR) in the areas of the SGNs of implanted cochleae, contralateral unimplanted cochleae, and normal hearing cochleae were evaluated using ImageJ free software (https://imagej.net/ij/) as described by Matsui et al.[22] Each micrograph was opened in the ImageJ program and converted to gray scale (image/type 8 bit). The threshold of IR was set, and the background IR was measured in an area outside of BDNF-IR cells and subtracted from the BDNF-IR area values. The image was converted to black and white and the IR was selected using the drawing tool. To determine the BDNF-IR area within the region of interest, "command analyze/analyze particles" was selected and the "mask tool" was selected. The resulting measurements represent the area fraction, which is the proportion of the region of interest with BDNF-IR. BDNF-IR area measurements were made in the spiral ganglia at the apical, middle, and basal-hook region in each specimen. For each specimen, IR areas were averaged and standard errors of the mean (SEM) were calculated (Table 2).

Statistical analysis:

Statistical comparisons between groups were made using a non-parametric Mann-Whitney *U* test. A value of $p < 0.05$ was denoted as statistically significant different. The IBM SPSS statistics software program version 25 was used for the statistical analysis (IBM corporation, Armonk, NY, USA). BDNF-IR comparisons between the groups at the different SGN regions were made as follows:

A. BDNF-IR in the implanted cochlea vs. the contralateral, unimplanted cochlea with hearing loss;

B. BDNF-IR in the implanted cochlea vs. the normal hearing cochlea; and

C. BDNF-IR in the unimplanted cochlea with hearing loss vs. a normal hearing cochlea.

Results

BDNF immunoreactivity (IR) in the normal hearing cochlea

Figure 1 demonstrates BDNF expression in the unimplanted cochlea of a 67-year-old male subject with normal hearing. BDNF-IR was found in the cytoplasm of spiral ganglia neurons (SGNs) in the normal cochlea (dark amber color). Figure 1A shows BDNF-IR expression in the mid-cochlea. BDNF-IR was found to be uniformly expressed in neurons from the basal region to the apical region. Fig 1B demonstrates a high-magnification view, which enables the visualization of the satellite cells that surround the SGN. These satellite cells also demonstrate strong BDNF expression.

BDNF immunoreactivity (IR) in the implanted cochlea

A 92-year-old female with a history of bilateral cochlear otosclerosis underwent right sided CI with a 6mm first-generation Sigma electrode at age 82 (TB 7L, 7R). She used the implant for 10 years. Subjectively, she reported an improvement in noise and music perception with CI. Figure 2A shows the SGNs at the mid basal cochlea of the implanted ear (right side). BDNF-IR was present in both the SGNs and the surrounding satellite cells. Figure 2B shows the SGNs from at the mid basal cochlea on the contralateral unimplanted ear (left) from the same patient. There are significantly fewer SGNs, and the degree of expression of BDNF is lower in the unimplanted ear.

Similarly, a 72-year-old male with bilateral symmetric hereditary progressive hearing loss underwent CI with a 16mm electrode Nucleus C-124 on the left side at age 67, and his post-implantation hearing test showed significant improvement in warbletone thresholds in the implanted ear (TB 5L, 5R). On histopathologic examination of bilateral temporal bones, there was decreased SGN density in the unimplanted (right) side compared with the implanted (left) side. Figure 3A–A1 shows a representative sample of SGNs at the middle and base region of the cochlea on the implanted side (left). BDNF-IR was present in both the SGNs and satellite cells in the middle and basal implanted cochlea. Figure 3B–3B1 shows the SGNs from at the middle and basal cochlea of the contralateral unimplanted ear (right) from the same patient. There is decreased BDNF-IR in SGN in the unimplanted cochlea compared with the implanted cochlea.

A 93-year-old male diagnosed with cochlear otosclerosis underwent CI on the left side with an 18mm Nucleus 22 electrode at an unknown age. Figure 4A–A1 shows the SGNs at the middle and base region of the cochlea. BDNF-IR was present in both the SGNs and satellite cells. Figure 4B–4B1 shows the SGNs from at the middle and base of the cochlea from the contralateral side unimplanted side (right) from the same patient. BDNF-IR is decreased in the unimplanted cochlea compared with the side which had been implanted.

Quantitative analysis

There were statistically significant increases in BDNF expression at the base, middle, and apical portions of the spiral ganglia in the implanted cochlea compared with the contralateral, unimplanted cochlea with hearing loss. Interestingly, there was also a similar

statistically significant increase in BDNF-IR expression in all portions of the spiral ganglia in the implanted cochlea when compared with the normal hearing cochlea (Table 2).

Regional cochlear BDNF expression with differential lengths of implantation electrodes.

Length of CI electrode and pattern of BDNF expression was examined. As demonstrated in Figure 5 A1–3, BDNF is expressed similarly in the SGN and the satellite cells within the basal region (A1), middle region (A2) and the apical region (A3) of the cochlea of an 87-year-old male (TB 3L, 3R) with a CI with a 20 mm electrode length placement. Figure 5 B1–3 similarly shows that BDNF-IF in the SGNs and satellite cells of a 67-year-old male (TB 8R) with a 6 mm electrode was similarly strong in the basal (5B1), middle (5B2), and apical (5B3). It is notable that even with the placement of a short, first-generation CI of 6 mm length, which does not extend beyond the first turn, there is apparent upregulation of expression of BDNF within not only the basal and middle cochlear SGN, but also in the apical SGN and satellite cells.

Discussion

The importance of neurotrophins in the protection of the neurons of the cochlea has been proposed with BDNF believed to play a critical role. *In situ hybridization* in animal models has shown that BDNF and the specific receptor TrkB are expressed in the inner ear and in the innervating sensory neurons, and *in vitro* studies using dissociated neonatal rat SGN cell cultures performed by Hansen et al. demonstrate that electrical activity drives BDNF expression in SGNs.[23] No prior study has demonstrated the presence of BDNF in the human cochlea organ of Corti and the SGN. The present study is the first to confirm the expression of BDNF within the SGN and the surrounding satellite cells of patients with normal hearing (Figure 1). The satellite cell surrounding the SGN is activated in the setting of cochlear damage and is believed to play a role in the rehabilitation and survival of the SGN. The presence of BDNF in the satellite glial cell is of interest. Previous studies have demonstrated connexin 43 gap junction subunits in the satellite cell, and that these cells tightly envelop the primary afferent SGN in the human.[24] These satellite cells may be critical in the phenomenon of monopolar SGNs surviving with central projections, despite the loss of afferent peripheral axons and loss of the organ of Corti cochlear hair cells and supporting cells. Notably, spiral ganglion glial cells as well as spiral ganglion Schwann cells both express neurotrophins which can be concomitantly regulated by neuregulins, suggesting that glia could provide neurotrophic support in the injured or developing spiral ganglia neuron.[25] This provides a possible mechanism for the manner by which BDNF expression within the satellite cell might contribute to overall spiral ganglia neuronal health. In TBs 4, 7, and 8, there was the near complete loss of both cochlear hair cells as well as supporting cells. The satellite cells and the SGN in the present study exhibit increased expression of BDNF in the setting of electrical stimulation with CI despite the absence of supporting cells and cochlear hair cells.

In the present study, archival human temporal bone specimens from patients with a history of CI for profound hearing loss of varied causes were studied for BDNF expression. There was a relative increase in the expression of BDNF, both in the number of SGN expressing

BDNF and in the intensity of expression. Varied causes of hearing loss were studied including cochlear otosclerosis and hereditary hearing loss, and despite the differential causes of hearing loss, the pattern of increased expression of BDNF in both SGN and satellite cells in the implanted side as compared with the unimplanted side demonstrated increased expression in all cases. The apparent upregulation of BDNF expression in the implanted side suggests a possible neuroprotective role provided by electrical stimulation of the SGNs. A second noted phenomenon was the spread of increased expression of BDNF throughout the cochlea despite the shorter length of some electrodes. Even among patients with early generation 6 mm CI electrodes, there was an upregulation of BDNF expression seen throughout the cochlea up to and within the apical SGN, suggesting that electrical stimulus may provide a beneficial effect on neuroprotection beyond the locally located SGN. It is notable that Zha et al. found in animal models that BDNF was constitutively expressed in the presence of CREB family transcription factors, independent of the activity of depolarization.[26] This finding taken with our study suggests that electrical stimulation may play an indirect and additive effect on the BDNF transcription pathway.

The present study findings corroborate multiple animal studies showing improved preservation of SGNs following electrical stimulation. Shepherd et al. treated experimentally deafened guinea pig with differential length of time of electrical stimulation following BDNF infusion cessation, demonstrating that chronic electrical stimulation via cochlear implantation was associated with the preservation of SGNs following withdrawal of exogenous BDNF neurotrophin.[27] Shepherd et al. implies that chronic stimulation via cochlear implants may enhance SGN survival. Although previous animal studies have demonstrated increased neuronal growth and reduced neuronal death with BDNF-eluting electrode arrays or continuous infusion of BDNF into the cochlea, these experiments were all conducted in the acute setting. In the present study, many of the patients had used the implant and electrical stimulation for years, and in two cases for greater than 10 years. In both cases, the unimplanted ear exhibited greatly diminished BDNF expression. The present study findings suggest that the human cochlea may derive protective effects from chronic electrical stimulation via cochlear implantation. Given that animal models show that earlier treatment with neurotrophins leads to better outcomes, earlier implantation with cochlear implant and early electrical stimulation may be associated with better preservation of the SGN and thus better outcome. Furthermore, a study performed by Leake et al. demonstrated that the additional sprouting induced by BDNF in addition to electrical stimulation caused ectopic and disorganized sprouting with potential blurring of the precise cochlear frequency map.[20] Hartshorn et al. demonstrate that SGN survival is increased with electrical stimulation in a dose-dependent manner, further corroborating our findings.[13] While multiple studies have examined the effect of exogenous BDNF application to the cochlear microenvironment via drug-eluting implants, exogenously delivered BDNF may have undesirable effects compared with the apparent beneficial effects of electrical stimulation-induced increased BDNF expression. It is interesting that the present study demonstrates that the unimplanted ear with no hearing has a similar degree of expression of the BDNF to a normal hearing ear. This would imply that the increased BDNF is directly related to electrical stimulation. Given the apparent maintenance of healthy SGN and satellite cells compared with the contralateral unimplanted ear in the deaf patient

and given the maintenance of functional hearing in the implanted ear, it implies that the increased BDNF expression is beneficial for the cochlear health in the deafened ear.

There are several limitations to this study. There are limitations in the interpretation of the cellular localization of BDNF by immunohistochemical techniques using celloidin embedded human inner ear sections.[21] The morphology is dependent on the postmortem time before the temporal bones are harvested, and differential postmortem times may affect the antigenicity of the specimen. Comparing the ipsilateral implanted cochlea with the contralateral unimplanted cochlea provided reasonable controls for differential post-mortem times between patients. Lastly, the celloidin embedding protocol includes the use of decalcifying agents such as EDTA and solvents such as ethanol and ether, all of which can affect the immunochemical staining. The study is retrospective in nature, and we therefore relied on the available clinical data and therefore did not always have complete audiometric data available to quantify audiologic performance. Still, this study represents an important contribution to understanding the role of BDNF in the afferent auditory pathway, and provides avenues for future investigation in improving hearing outcomes following cochlear implantation.

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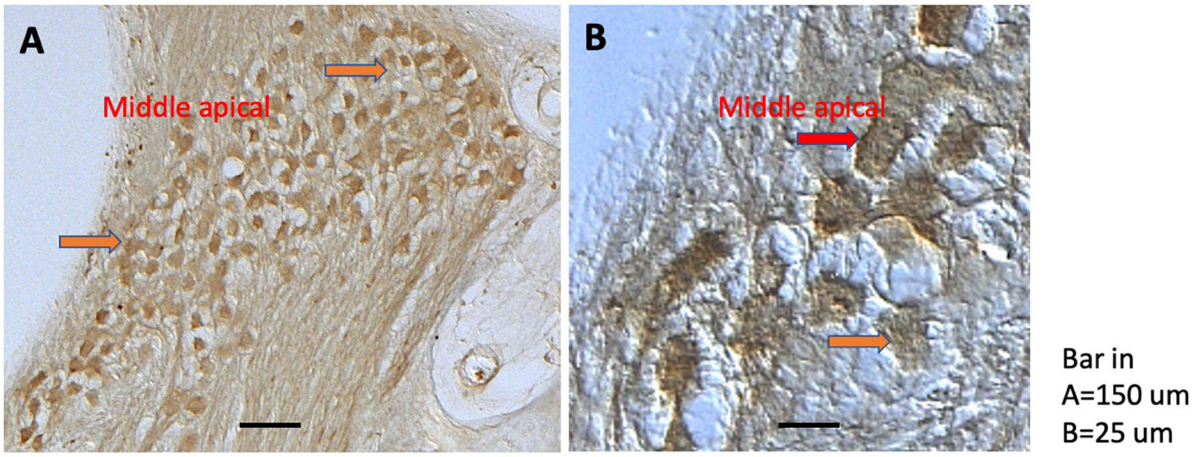

Figure 1.

BDNF expression in the cochlea from a 67-year-old male with normal hearing. BDNF-IR is identified in spiral ganglia neurons (SGNs) within the cytoplasm in the normal cochlea (orange arrows). Figure 1A shows uniform BDNF-IR expression from the basal region to the apical region. Figure 1B is a high-magnification view which shows that the satellite cells surrounding the SGN are also BDNF-positive (red arrows).

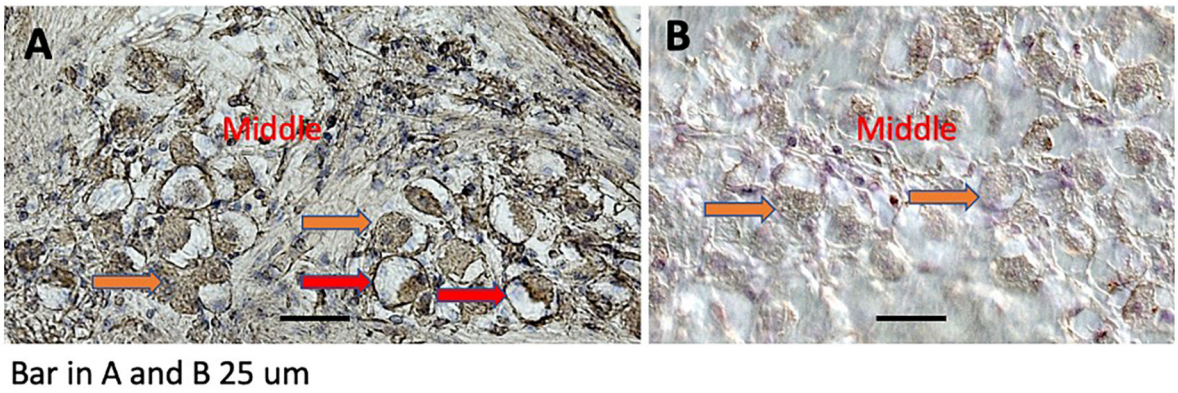

Figure 2.

An 82-year-old female (TB 7R, 7L) with a history of bilateral cochlear otosclerosis who underwent unilateral right-sided implantation of a 6mm first-generation Sigma electrode 10 years prior. **2A:** The basal cochlea of the implanted (right) ear demonstrates BDNF-IR present in both the SGNs and the surrounding satellite cells. **2B:** The unimplanted (left) ear of the same patient shows significantly fewer SGNs, and the degree of expression of BDNF is lower in the unimplanted ear.

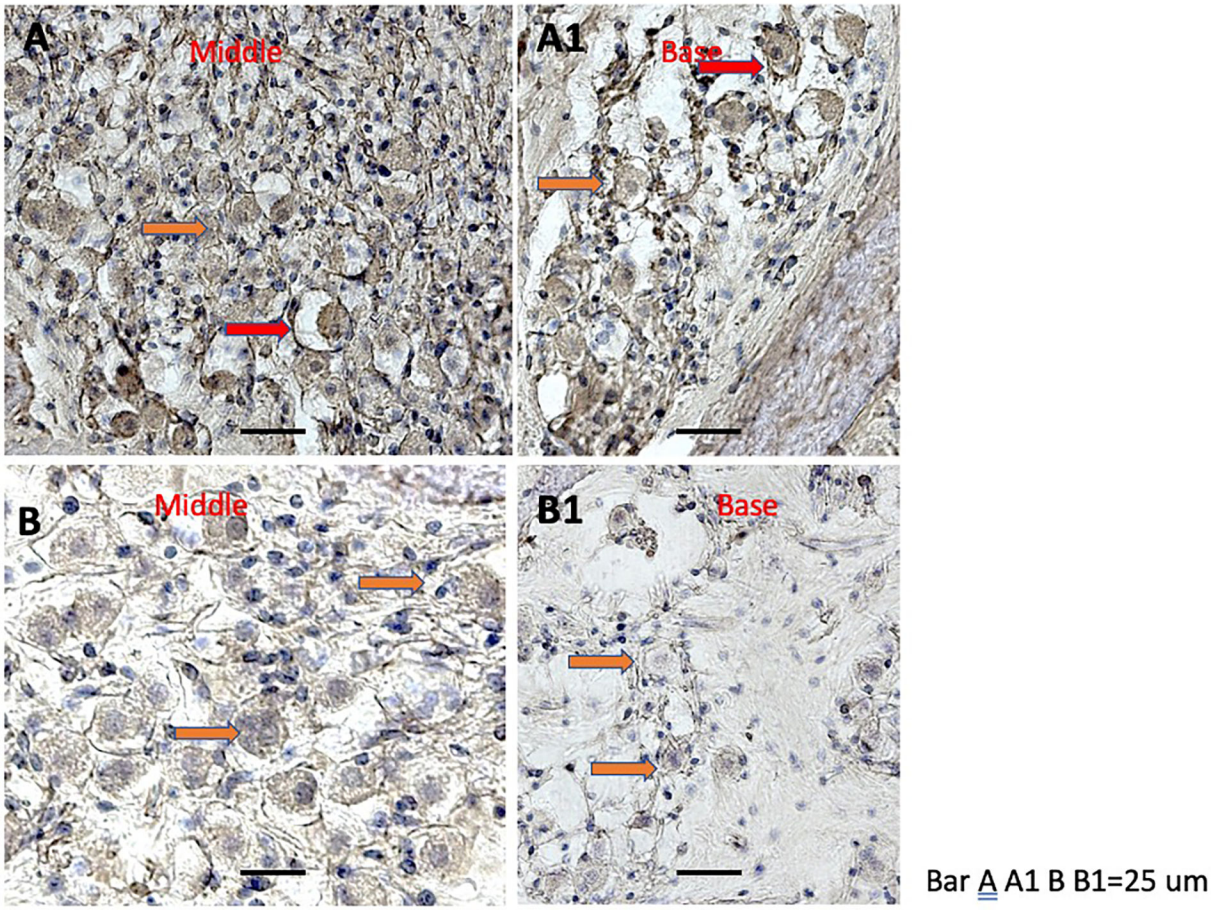

Figure 3.

A 72-year-old male (TB 5L, 5R) diagnosed with hereditary progressive hearing loss on the left ear. He underwent implantation on the left ear with a 16mm electrode 5 years prior. **3A and 3A1** demonstrate SGNs at the middle and base regions of the cochlea on the implanted side, respectively. **3B and 3B1** demonstrate SGNs from the middle and basal regions of the cochlea on the unimplanted side, respectively demonstrating weaker BDNF-IR expression in both the SGN and the satellite cells.

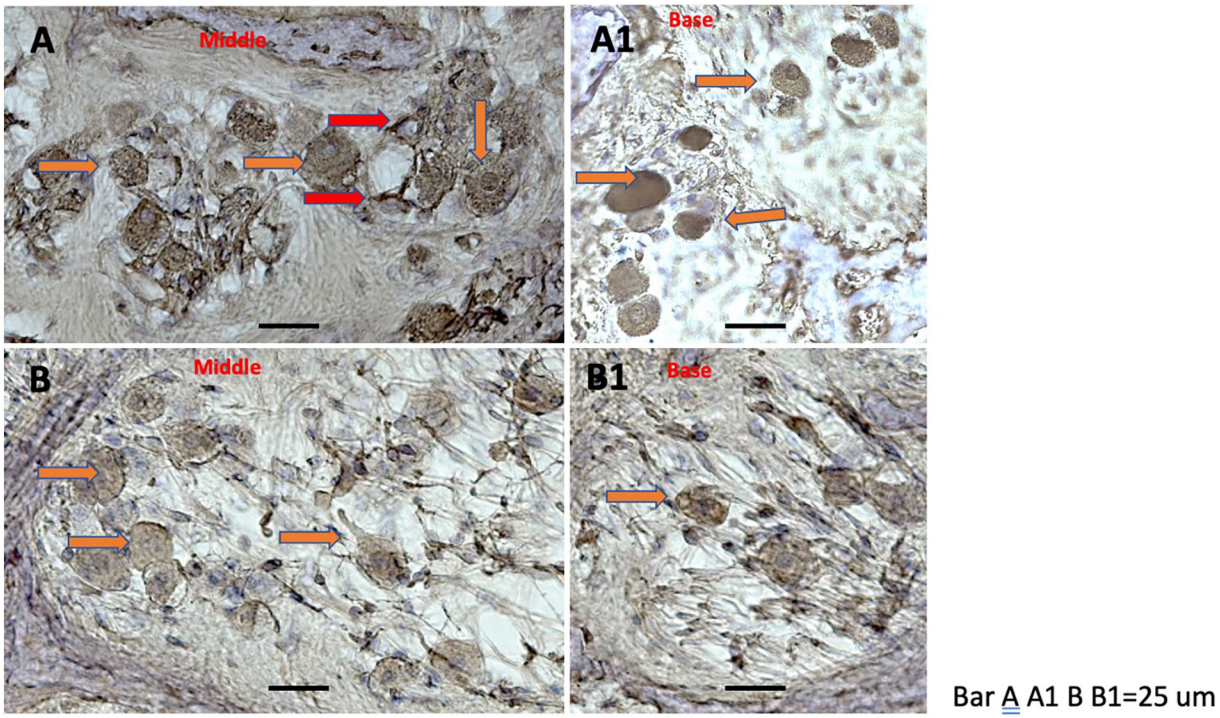

Figure 4.

A 93-year-old male (TB 6L) was diagnosed with cochlear otosclerosis and underwent left-sided cochlear implantation Nucleus 22 inserted 18 mm at unknown age. **4A-A1** shows the SGNs in the middle and base regions of the cochlea on the implanted side. **4B-B1** demonstrates the SGNs from the contralateral side. BDNF-IR is decreased in the unimplanted cochlea compared with the side which had been implanted, but there is more expression than that noted for the unimplanted contralateral cochlea in 2B and 3B.

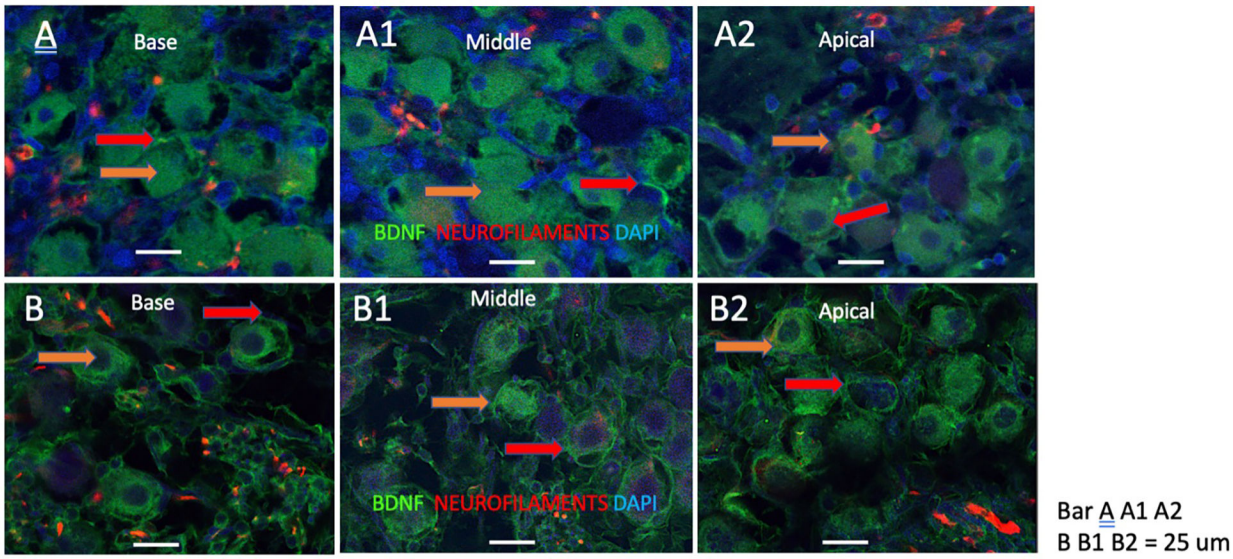

Figure 5A.

An 87-year-old male (TB 3R, 3L) underwent CI on the left with a 20mm electrode Nucleus 22. BDNF-IF is expressed similarly in the SGN and the satellite cells within the basal region (A1), middle region (A2), and apical region (A3) of the cochlea. Figure 5B. A 67-year-old male (TB 8R) with bilateral otosclerosis with a history of 6mm electrode placed 2 years prior demonstrates strong BDNF-IF expression in the SGNs and satellite cells in the basal region (B1), middle region (B2), and apical region (B3).

Table 1a.

Temporal bone specimens from implanted cochleae and their unimplanted counterparts

Temporal bone specimen	Age (years) at death	Sex	Diagnosis	Age (years) at CI	Type of Electrode (length in mm)	Significant Clinical History	BDNF-IR in SGN	BDNF-IR in satellite cells	Presence of HC/SC in organ of Corti
TB 1L	85	F	SNHL	79	Nucleus 22 (13)	Used implant + HA post-operatively	+++	+++	Yes
TB 2L	75	M	otosclerosis	73	3M (6)	N/A	+++	+++	No
TB 3L	87	M	SNHL, otosclerosis	Unk	Nucleus 22 (20)	Not available	+++	+++	Yes
TB 3R	87	M	SNHL, otosclerosis	Unk	Unimplanted	Not available	---	---	---
TB 4R	67	F	non-hereditary SNHL	55	Sigma (18)	Improvement in noise perception, not speech intelligibility	+++	+++	No
TB 5L	72	M	hereditary progressive HL	67	Nucleus C-124 (16)	Improvement of warbletone thresholds from 90 dB to 30–40 dB SPL	+++	+++	Mainly SC
TB 5R	72	M	hereditary progressive HL	67	Unimplanted	N/A	+	+	Mainly SC
TB 6L	93	M	otosclerosis	82	Nucleus 22 (18)	Prior mastoidectomy; average post-implantation improvement was less than average	+++	+++	Mainly SC
TB 6R	93	M	otosclerosis	82	Unimplanted	Prior mastoidectomy	+	+	Yes
TB 7R	92	F	otosclerosis	82	Sigma (6)	Subjectively, CI didn't improve speech intelligibility but improved noise and music perception	+++	+++	Mainly SC
TB 7L	92	F	otosclerosis	82	Unimplanted	Prior L stapedectomy (at age 65)	+	+	No
TB 8R	67	M	otosclerosis	65	3M House (6)	Post-implantation, improvement from no response to SPL of ~50 dB	+++	+++	No

Table 1b.

Temporal bone specimens from normal hearing cochleae

Temporal bone specimen	Age (years) at death	Sex	Diagnosis
TB 9	65	M	Carcinoma of breast with metastases
TB 10	75	M	Carcinoma of lung
TB 11	70	F	Lymphosarcoma
TB 12	73	F	Multiple myeloma
TB 13	72	M	Carcinoma of stomach

TB = temporal bone, L = left, R = right, Unk = unknown, SNHL = sensorineural hearing loss, M = male, F = female, HC = cochlear hair cell, SC = supporting cell, HA = hearing aid.

Triple line - - - indicates no immunoreactivity.

Table 2.

Average area measurements (as a percentage compared to background non-IR) of BDNF immunoreactivity seen in the spiral ganglia of the (1) implanted cochlea, (2) contralateral non-implanted cochlea, and (3) normal hearing cochlea.

Spiral ganglia Region	CI cochlea (n=8)	Contralateral non-CI (n=4)	Normal hearing (n=5)
Apical	53.3 ± 2.33	44.32 ± 1.75 *	42.92 ± 1.94 *
Middle	59.97 ± 2.07	43.33 ± 1.76 *	45.3 ± 1.33 *
Base	53.27 ± 0.82	38.54 ± 2.33 *	42.07 ± 0.46 *

* = $p < 0.05$

± standard error of the mean