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Publication Date

2016-12-01

DOI

10.1016/j.heares.2016.10.011

Peer reviewed

Published in final edited form as:

Hear Res. 2016 December ; 342: 134–143. doi:10.1016/j.heares.2016.10.011.

Effects of Brain-Derived Neurotrophic Factor (BDNF) on the Cochlear Nucleus in Cats Deafened as Neonates

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Abstract

Many previous studies have shown significant neurotrophic effects of intracochlear delivery of BDNF in preventing degeneration of cochlear spiral ganglion (SG) neurons after deafness in rodents and our laboratory has shown similar results in developing cats deafened prior to hearing onset. This study examined the morphology of the cochlear nucleus (CN) in a group of neonatally deafened cats from a previous study in which infusion of BDNF elicited a significant improvement in survival of the SG neurons. Five cats were deafened by systemic injections of neomycin sulfate (60mg/kg, SQ, SID) starting one day after birth, and continuing for 16–18 days until auditory brainstem response (ABR) testing demonstrated profound bilateral hearing loss. The animals were implanted unilaterally at about 1 month of age using custom-designed electrodes with a drug-delivery cannula connected to an osmotic pump. BDNF (94μg/ml; 0.25μl/hr) was delivered for 10 weeks. The animals were euthanized and studied at 14–23 weeks of age. Consistent with the neurotrophic effects of BDNF on SG survival, the total CN volume in these animals was significantly larger on the BDNF-treated side than on the contralateral side. However, total CN volume, both ipsi- and contralateral to the implants in these deafened juvenile animals, was markedly smaller than the CN in normal adult animals, reflecting the severe effects of deafness on the central auditory system during development. Data from the individual major CN subdivisions (DCN, Dorsal Cochlear Nucleus; PVCN, Posteroventral Cochlear Nucleus; AVCN, Anteroventral Cochlear Nucleus) also were analyzed. A significant difference was observed between the BDNF-treated and control sides only in the AVCN. Measurements of the cross-sectional areas of spherical cells showed that cells were significantly larger in the AVCN ipsilateral to the implant than on the contralateral side. Further, the numerical density of spherical cells was significantly lower in the AVCN ipsilateral to the implant than on the contralateral side, consistent with the larger AVCN volume observed with BDNF treatment. Together, findings indicate significant neurotrophic effects of intracochlear BDNF infusion on the developing CN.

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1. INTRODUCTION

The family of proteins called the neurotrophins includes nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5(NT-4/5). These neurotrophic factors bind to specific high affinity Tyrosine Kinase (Trk) receptors. Neurotrophic factors regulate survival and differentiation of neurons throughout the nervous system during embryonic and postnatal development (Farinas et al., 2001; Fritzsch et al., 1999; Gao et al., 1995; Korsching, 1993; Rubel et al., 2002) and are also important for maintenance of synaptic connectivity and plasticity in the adult nervous system (Fritzsch et al., 2005; Rubel et al., 2002). Among the neurotrophic factors, BDNF and NT-3 have been found to be important for the development and maintenance of the auditory system, specifically the SG neurons (Fritzsch et al., 2004; Hegarty et al., 1997; Lefebvre et al., 1992; Malgrange et al., 1996; Miller et al., 2007; Mou et al., 1997; Ramekers et al., 2012; Rubel et al., 2002; Schecterson et al., 1994; Staecker et al., 1996; Stankovic et al., 2004; Vieira et al., 2007).

Many previous animal studies have shown highly significant neurotrophic effects of exogenous BDNF delivered to the cochlea following deafness, including larger SG cell size and substantially improved neuronal survival (Agterberg et al., 2009; Agterberg et al., 2008; Chikar et al., 2008; Glueckert et al., 2008; Leake et al., 2011; Leake et al., 2013; McGuinness et al., 2005; Miller et al., 2007; Nakaizumi et al., 2004; Ramekers et al., 2012; Shepherd et al., 2008; Shepherd et al., 2005; Song et al., 2009; Staecker et al., 1998; Staecker et al. 2010; Wise et al., 2005). It has been suggested that improved SG survival resulting from the administration of exogenous BDNF may be important for optimizing the function of a cochlear implant (CI) (Landry et al., 2013; Sameer Mallick et al., 2013; Sly et al., 2012; Staecker et al., 2010; Wise et al., 2012). The efficacy of BDNF in preventing SG degeneration after deafness may be of clinical relevance, particularly in pediatric CI users who must use the CI for many decades of life. Intracochlear drug delivery has gained importance recently as a potential modality of treatment in the effort to ameliorate the effects of deafness, and human CI electrodes have already been developed to deliver drugs through the implanted array (Hochmair et al., 2006).

Despite the encouraging findings in animal studies with the effects of BDNF in the cochlea, to date there have been no studies of the potential effects of delivery of neurotrophic factors (and the resulting improvement in SG survival) upon the central auditory system in deafened animal models. The present study was designed to evaluate the potential neurotrophic effects of direct intracochlear delivery of BDNF (10 weeks infusion) on the morphology of the CN complex. The CN is of particular interest because it comprises the first and obligatory synaptic relay center in the ascending auditory pathway in the brainstem. Within the CN, each cochlear nerve fiber bifurcates into ascending and descending branches and forms distinctive terminals on numerous cells in each of the three major subdivisions; the anteroventral, posteroventral and dorsal cochlear nuclei. Moreover, the Tyrosine kinase B receptors (TrkB), which are high affinity receptors for BDNF, are present in CN neurons and have been suggested to contribute to neuronal plasticity seen after cochlear ablation (Suneja et al., 2002). For the present study, cats were deafened by daily ototoxic drug administration beginning 1 day after birth and continuing for 16–18 days until profound hearing loss was

observed. Since the feline auditory system is very immature at birth (kittens are deaf at birth and begin to hear at about day 10 of post-natal life), these animals became deaf prior to having any normal auditory experience and are considered to model congenital deafness, likely corresponding to a hearing loss during the perinatal period in human infants. It should be noted that four of the five animals included in this study were part of a prior study that demonstrated significant neurotrophic effects of intracochlear delivery of BDNF in promoting improved survival of cochlear spiral ganglion (SG) neurons (Leake et al., 2011). Based on these previous findings, we hypothesized that the detrimental effects of deafness on the CN would also be lessened by BDNF infusion in these same animals. Specifically we hypothesized that both CN volume and spherical cell size would be larger ipsilateral to the BDNF treatment as compared to contralateral.

2. METHODS

All procedures involving animals were approved by the Institutional Animal Care and Use Committee at the University of California, San Francisco and conformed to all NIH guidelines. The animals included in the study were bred in a closed colony maintained at the University.

2.1 Experimental groups

A total of five animals were included in this study. Figure 1 illustrates the overall timeline and treatment details of the study, and Table 1 summarizes the experimental histories for the individual animals. Animals were deafened as neonates and implanted at 4–5 weeks of age with a cochlear implant and an osmotic pump containing BDNF, which flowed through a cannula in the implant that terminated at the tip of the intracochlear electrode array. All the animals except for one (K215) were studied immediately after 10 weeks of BDNF infusion at an age of 14–15 weeks. K215 was studied later, 8 weeks after the 10 weeks of BDNF infusion, at 23 weeks of age. Four of the 5 animals in the present study (K210, K214, K217, K310) were part of a prior study investigating the effects of BDNF on the spiral ganglion neurons (Leake et al., 2011).

2.2. Deafening procedure

All five animals were deafened as described in previous studies from this laboratory (Stakhovskaya et al 2008; Leake et al., 2011; Leake et al., 2013). Daily injections of the ototoxic aminoglycoside, neomycin sulfate (60 mg/kg body weight; SQ, SID) were initiated one day after birth and continued for 16–18 days, at which time click-evoked auditory brainstem response (ABR) testing was performed. Acoustic stimuli (100 μs clicks, 20/s) were delivered through a canister headphone (STAX, model SMR-1/MK-2) coupled to the ear by a hollow bar inserted into the external ear canal. If a profound hearing loss was documented by an absence of an ABR at 90dB peak SPL in both the ears, neomycin injections were discontinued. If residual hearing was observed, neomycin injections were continued in increments of 2 to 3 days and ABR testing was repeated until bilateral profound hearing loss was confirmed. The period of neomycin administration ranged from 16 to 18 days. After profound hearing loss was established, the kittens were unilaterally implanted (left ear) at 4–5 weeks of age.

2.3. BDNF and osmotic pump preparation

Cochlear implant electrodes with a miniature cannula for intracochlear drug delivery were used in the study (Rebscher et al., 2007). Details of the electrodes, osmotic pumps and implantation procedures were as described in previous studies from this laboratory (Leake et al., 2011; Leake et al., 2013). The devices were six-wire scala tympani electrodes, which contained a drug-delivery cannula (0.13mm/0.005 inch ID) with the port at the apical tip of the electrode. In practice, the electrode tip was positioned approximately 360° from the round window or 12–13 mm from the base of the cochlea, at about the middle of the cochlear spiral. The intracochlear electrode was secured in place by a dacron cuff that was fixed to the inferior aspect of the round window using tissue adhesive (Tissuemend II™, Veterinary Products Laboratories, Phoenix AZ). The drug delivery cannula within the cochlear implant was connected to vinyl tubing (0.069mm/0.027 inch ID; 1.143mm/0.008 inch OD) that was attached to the regulator of the osmotic pump, which was implanted behind the right pinna.

Human recombinant BDNF for these studies was supplied by Amgen, Inc. of Thousand Oaks, CA. Osmotic pumps (Alzet model # 1002 or 2004; infusion rate − 0.25 μl/hr) were filled and primed with artificial perilymph (125mM NaCl, 4.3 mM KCL, 1.3 mM $CaCl_2$, 21 mM NaCHO3, pH7.4, osmolarity 285–295 mOsm). Blood was drawn from each subject, centrifuged and the supernatant serum added to the artificial perilymph (final concentration of 200 mg/dl) to provide normal autologous protein. Pumps were handled and filled in a UV sterile environment. BDNF (94 μg/ml) was added to the sterile perilymph and the solution loaded into the pumps according to the manufacturer's instructions. Both the pump, which was then primed in a sterile saline bath, and the remaining BDNF/perilymph solution were placed in an incubation oven (37°C, 100% CO_2) for at least 24 hours until implantation. The extra solution was used to pre-fill the vinyl tubing and cannula before attaching the osmotic pump during the surgical implantation procedure. At implantation, since the animals were small (mean body weight 490g, range 395–590g), a smaller osmotic pump (model#1002) that delivered 14 days of BDNF was implanted initially. At the end of 14 days, the animals underwent a brief surgical procedure to replace the first pump with a larger one (model #2004) that delivered 28 days of BDNF. The second pump was replaced 4 weeks later, allowing continuous infusion for a total of 10 weeks. The pumps were examined upon removal from each animal and found to be fully depleted with the exception of one animal, K210 in which about 60 μl of BDNF remained in the final pump after 28 days (the pump was initially filled with 220 μl, and after pumping at 0.25 μl/hr for 28 days, a residual volume of less than 50 μl should have been present; thus, this subject might not have received the full amount of BDNF over the last few days). The pump, cannula and the electrode components were intact in all subjects at the time of euthanasia and there was no evidence that of leakage.

As described in our previous study (Leake et al., 2011), the biological activity of the residual BDNF remaining in the osmotic minipumps after 28 days of implantation in 2 animals was assessed for its ability to promote survival of cultured SG neurons by Dr. Steven Green at the University of Iowa. The samples of BDNF removed from the two depleted pumps retained survival-promoting activity, but the effective concentrations were reduced to

approximately 20μg/ml and 30μg/ml, respectively (about 20–30% of the original concentrations).

2.4. Histological preparation

At the end of the experiment, all the animals were studied in terminal acute electrophysiological experiments recording from the inferior colliculus (for a separate study). After these recordings were completed, the animals were maintained deeply anesthetized with sodium pentobarbital, I.V, the stapes was removed, the round window opened and the cochleae were perfused with mixed aldehyde fixative (1.5% glutaraldehyde and 2.5% paraformaldehyde in 0.1M phosphate buffer, pH7.4). An overdose of sodium pentobarbital was then administered, and transcardiac perfusion was performed with normal saline solution followed by the same fixative solution. The temporal bones were removed and placed in fixative overnight, then transferred to buffer for dissection, subsequent embedding in epoxy resin and study of the SG neurons. The brain was removed from the skull, placed in the same fixative overnight, then transferred into a 40% sucrose solution in phosphate buffer (0.1M, pH 7.4) for at least 72 hours. The caudal midbrain, pons and the rostral medulla were separated from the rest of the brain and the right side was marked. Specimens were rapidly frozen with liquid nitrogen, and the brainstem was sectioned serially on a sliding microtome in the coronal plane at a thickness of 50 μm. Two sets of sections were selected and utilized for the analyses as described below.

2.5. Estimation of cochlear nucleus volume

The CN volumes were measured on both the BDNF–treated side and the control deaf side in the 5 deafened animals and the data compared. First, every third section through the CN was selected. These sections were mounted on glass slides (2% gelatin coated), stained with 0.25% toluidine blue and used to measure the individual volumes of all three major CN subdivisions, DCN, PVCN and AVCN. Digital images of sections were captured using a Zeiss Axioskop 2, a 2.5× objective and a Zeiss AxioCam MRc5 digital camera. Cytoarchitectural criteria according to Kiang et al., (1975); Leake et al., (2002); Leake et al., (2008); Stakhovskaya et al., (2008) were utilized to outline the three individual CN subdivisions in each imaged section (excluding the cochlear nerve root). The boundary between the DCN and the PVCN was defined by the intermediate acoustic stria in caudal sections and using the granule cell layer as a landmark in more rostral sections. The interstitial nucleus (IN), where the auditory nerve bifurcates into the AVCN and PVCN, was used to define the boundary between these CN subdivisions. The area ventral to the IN was considered to be part of AVCN and the area dorsal to the IN was considered to be part of the PVCN. The subdivision outlines were measured using NIH Image J (ver1.44p, Bethesda, MD). Measurements were made in both CN, ipsilateral and contralateral to the cochlear implant/BDNF infusion. All measurements of individual subdivisions were done with the observer blinded to the experimental manipulations (Images of the right CN were digitally reversed so that the observer was blinded to side). The total CN volume was calculated by multiplying the total area (sum of the areas of the three individual subdivisions) of the sections measured (ΣA) by section thickness (T=0.05) and the sampling step (s=3):

$$\text{Total Volume} = \sum_{A*T*S}$$

Figure 2 (a–e) illustrates representative coronal sections of the CN delineating perimeters of the three individual subdivisions arranged in a rostral-caudal direction, as utilized for volume estimations. The individual CN subdivision areas from four normal adult cats were also measured using identical methods, and their total CN volumes were calculated..

2.6. Measurements of spherical cell

A second set of every third section (only in the AVCN) was post-fixed with osmium tetroxide, embedded in epoxy resin, sectioned at 5μm on an ultramicrotome and then counter–stained with 0.25% toluidine blue (Fig. 3). Three pairs of consecutive overlapping sections, one each from the dorsal, middle and ventral regions of the rostral AVCN were chosen from the set. Multiple 40× images were captured using a Zeiss Axioskop 2 and a Zeiss 40× Plan-Apochromat lens with a numerical aperture of 0.95 (optical resolution <1μm) and montaged with the 'Auto-align/blend' feature in Adobe Photoshop™ CS5. The cross-sectional areas of all spherical cells containing a clear nucleus and nucleolus were traced in the images and measurements were made using NIH Image J (ver 1.44p, Bethesda, MD). The areas of the cell somata were calculated directly in the software application after calibration. Cross-sectional areas of spherical cells from each section of the AVCN were averaged and the mean values obtained.

2.7 Unbiased stereology for assessment of spherical cell numerical density

For this analysis, we used a physical dissector method applied to the same 3 pairs of overlapping sections that were utilized for estimating spherical cell size. The dissector method requires comparing adjacent serial sections and counting the number of "new" nucleoli of spherical cells that appear in the second section (i.e. that are non–overlapping with those in the first section). Adobe Photoshop™ was used to align the sections precisely. For the physical dissector estimates of cell density, all cells with a visible nucleolus in the first serial section were selected using the Photoshop 'wand' tool, preserving cytoplasmic boundaries but excluding the nucleus and the cell soma. Additional reference structures were also selected. The selected structures were copied and pasted into a file with the second serial section, creating a template which was precisely aligned with the second section by matching the cells and the reference structures. The "new" nucleoli appearing only in the second section were then counted. Three pairs of serial sections were analyzed for each region of the AVCN. The magnetic Lasso tool was utilized to outline the area of the section that was analyzed. The images of the cells with the outline of the sample were imported into the NIH Image J (ver 1.44p, Bethesda, MD), the cell areas and as well as the sample area were determined. All the image selection procedures for these analyses were performed with the observer blinded to experimental conditions.

3. Statistical analyses

To test the hypotheses that the CN volume and the spherical cell size would be larger ipsilateral to the BDNF treated side, four within-animal statistical tests were performed between the BDNF-treated CN and the contralateral side. First, for the data from the frozen sections, the total CN volumes (sum of AVCN, PVCN and DCN) were compared using a paired student's t-test. Second, the individual subdivision volumes on the BDNF-treated and the contralateral sides were compared using two-way repeated measures analysis of variance (ANOVA) with BDNF treatment as the first factor and CN subdivision as the second factor (Tukey test). Third, for the data obtained from the plastic sections, the AVCN spherical cell sizes sampled in the caudal, middle and rostral part of the AVCN were compared on the BDNF-treated and the contralateral sides using two-way repeated measures ANOVA. Finally, comparisons of the mean AVCN spherical cell density between the two sides were also made using a two-way repeated measures ANOVA. All statistical analyses were performed using Sigma Plot (version 12.0). P values below 0.05 were considered to be statistically significant. All data had equal variance among the samples and all data were also normally distributed and thus did not violate the assumptions of the test.

4. Results

4.1 Effects of BDNF on cochlear SG neurons

All five neonatally deafened animals included in the study showed substantial degeneration of the SG at the time of study, 4–6 months of age (Table 2). The control data from the contralateral, non-implanted cochleae indicate substantial intersubject variability in the cochlear SG cell densities in the individual subjects, ranging from 49% to 75% of normal. However, the SG densities for the implanted/BDNF-treated cochleae in all five animals were consistently higher than on the contralateral side, ranging from 66% to 92% of normal. Thus, as reported previously for 4 of these 5 animals (Leake et al., 2011), BDNF infusion over 10 weeks resulted in a highly significant improvement in SG survival (two way ANOVA on SG numerical density at 9 cochlear sectors from base to apex; $F_{(1,3)}$=150.582. p<0.001. A logarithmic transformation was used to adjust for heterogenous variances in the data;, and there was a significant interaction between BDNF treatment and SG sector ($F_{(8,32)}$=2.647, p=0.024)). A mean increase of roughly 16% of the normal neuronal population was maintained ipsilateral to the implant compared to the contralateral side. This represents an average improvement in SG survival of about 26% when normalized to the deafened control cochleae. Our previous report also demonstrated that the mean cross-sectional areas of SG neurons were larger in BDNF-treated ears across all cochlear sectors, as compared to the contralateral side (Leake et al., 2011). Because of this difference in cell size, SG cell density was evaluated using a physical dissector stereological method that estimated the number of cells independently of cell size. It is interesting to note that although K215 had a somewhat longer duration of deafness than the other animals (23 weeks vs 14–15 in the other subjects), the SG degeneration was not more severe in this subject; two other animals (K217, K310) had greater SG cell loss than K215. On the other hand, this subject demonstrated the greatest difference in SG survival (17% higher SG

survival in the BDNF-treated ear compared to contralateral), which may be explained by a longer survival time over which SG degeneration progressed in the contralateral ear.

4.2 Effects of BDNF on the cochlear nucleus

4.2.1 Cochlear nucleus volume—CN volume data for the individual BDNF-treated animals are presented in Table 3. All 5 BDNF-treated animals showed larger total CN volumes on the BDNF-treated side (ipsilateral to the implant) than on the contralateral side. The group mean data comparing total CN volume on the two sides are shown graphically in Figure 4, and statistical analysis demonstrated that the difference elicited by BDNF was statistically significant ($t(4) = 3.99$, $p = 0.016$; student's t-test, paired). For reference, the data collected from the group of normal adult cats are presented in Table 4. All the individual CN volumes measured in the deafened group (both sides) were markedly smaller than the values obtained in the normal group, with no overlap between the 2 groups, indicating the severe effects of early deafness. Both groups showed considerable intersubject variability in total CN volume, which ranged from 8.6 to 14 mm3 in the deafened group and from 16.2 to 19.6 mm3 in normal cats. Total CN volumes in the deafened cats, when expressed as % of normal, averaged 66% of normal on the BDNF side and 59% normal on the contralateral CN.

Data for the volumes of the 3 individual major CN subdivisions are also presented in Table 3 for the BDNF-treated group. Comparison of the values for BDNF-treated and contralateral sides reveals that a substantial difference was observed only in AVCN (5.09 mm^3 BDNF vs 4.17 mm^3 contralateral), whereas values for DCN (3.8 mm^3 vs. 3.6 mm^3) and PVCN (2.6, were similar on both sides. The group data for the individual subdivisions are presented in Figure 5. Statistical comparison of the BDNF-treated and contralateral subdivision volumes in ANOVA showed a significant main effect of BDNF treatment ($F_{(1,4)}$ 15.6, $p= 0.017$) and a significant interaction between BDNF treatment and CN subdivision ($F_{(2,8)}$ 12.54, $p= 0.003$). Post hoc comparisons showed that the difference elicited by BDNF was significant only for the AVCN ($p<0.001$); Tukey test). To provide the reader with a context for the extent of the BDNF effects observed, Figure 5 also presents the CN subdivision volumes calculated as percentage of normal, based on the values from Table 4. AVCN measured roughly 60% of normal after BDNF treatment, as compared to only 50% of normal for the contralateral, deafened control AVCN. The mean volume of the DCN ipsilateral to BDNF treatment was about 69% of normal vs 65% of normal on the contralateral side; and PVCN was estimated to be about 73% of normal as compared to 72% normal on the control side.

4.2.2 Cross-sectional areas and density of spherical cells in the AVCN—The spherical cell areas, as measured in CN sections that were post-fixed in osmium tetroxide, embedded in epoxy and sectioned at 5 μm (Fig. 6), were found to be significantly larger in the AVCN ipsilateral to the implant/BDNF-treatment than on the contralateral side (~400 vs ~330μm^2). Expressed as percent increase relative to the contralateral side, this represents an increase of approximately 20% on the BDNF-treated side over the control deafened condition. This difference was determined to be highly significant ($F_{(1,4)}=31.61$, $p=0.005$, two-way repeated measures ANOVA) with no significant effect of the location within the AVCN. Post-hoc comparisons (Tukey test) showed a significant difference in cell size

between the BDNF-treated and the deaf side in the rostral (p=0.008) and caudal (p=0.008) parts of the AVCN. No significant interaction was observed between the two factors (BDNF treatment and location within the AVCN). In addition, the numerical density of spherical cells in the AVCN (Fig. 7) was significantly lower in the AVCN ipsilateral to the BDNF implant than on the contralateral side ($F_{(1,4)}$=19.15, p=0.012, two-way repeated measures ANOVA) with a significant main effect of the location within the AVCN ($F_{(2,8)}$=13.17, p=0.003) from which the sections were selected. Post-hoc comparisons (Tukey test) showed a significantly larger difference in the numerical density between the BDNF-treated and contralateral untreated sides in the middle part of the AVCN (p=0.012).

We chose to focus on the AVCN spherical cells to make these additional cell area and density measurements because these cells are one of the primary targets of inputs from the cochlear spiral ganglion, and also because our data showed a significant increase only in the AVCN volume (not in DCN or PVCN) following BDNF treatment as compared to the contralateral untreated side. It should be noted that post-fixation with osmium tetroxide in the preparation of the epoxy sections used for these measurements helps to preserve the lipids in the cell membranes of the spherical cells, better maintaining their structural integrity. This minimizes shrinkage of the cell somata during the further processing required for embedding. The osmium tetroxide also turns the cell membrane black, providing better contrast and making the cell perimeter more visible in these sections (compared to traditional frozen sections). These characteristics allowed us to make accurate measurements of the spherical cell areas and to use a statistically unbiased (physical dissector) density measurement in the AVCN. Use of the physical dissector method was required because it accurately determines the number of objects (spherical cell nucleoli) in a given volume of tissue, regardless of the size of the objects. That is, because the spherical cells were larger in the BDNF-treated AVCN, their larger size would otherwise have resulted in a bias in simple counts of the cells.

5. Discussion

It is well known that normal auditory experience during the postnatal period is critical for the normal maturation and development of the immature auditory system, and that sensorineural hearing loss in neonatal animals elicits much more profound degenerative effects in the CN than hearing loss in adults. Because kittens are deaf at birth, due to the immaturity of their auditory system, these neonatally deafened animals are completely deprived of normal auditory experience and are considered to model congenital profound hearing loss in human infants. Stakhovskaya et al. (2008) previously reported a study of the CN in cats that were neonatally deafened by the same neomycin protocol used in the present study. This report showed that the total auditory deprivation in these neonatally deafened animals resulted in a severe reduction in the total CN volume to 67% of normal when the animals were studied at 8 months of age. The data from the present study also clearly demonstrated the severe effects of early deafness. The total CN volumes measured in the BDNF-treated animals in our study, both ipsi- and contralateral to the implants, were markedly smaller than normal and comparable to data in the prior study (Stakhovskaya et al., 2008) with ipsilateral CN measuring 66% of normal and the contralateral CN at 59% of normal. These marked reductions in CN volumes are presumably a direct result of the

substantial decrease in the SG neuron population, which averaged roughly 78% of normal in the BDNF-treated cochleae and 62% of normal on the control side, after ototoxic deafening during the neonatal period. The reduction in CN volume is assumed to be at least partly attributable to the loss of neuropil resulting from the loss of the central axons of the degenerated SG neurons (auditory nerve). Among the individual subdivisions of the CN, the AVCN showed the most severe degenerative change (mean volume 61% of normal ipsilateral to the BDNF implant; 50% of normal for the control deafened CN), which is consistent with the previous report of Stakhovskaya et al, (2008) that also showed that AVCN was more severely altered than the other CN subdivisions after early deafness.

The larger CN volume observed ipsilateral to the BDNF implant compared to the contralateral side in these animals suggests a relatively modest but significant neurotrophic effect of BDNF that led to improved CN growth and significantly less severe degenerative effects of the ototoxic insult and auditory deprivation. It is important to note that among the individual CN subdivisions, a significant difference was observed only in the AVCN, and not in the DCN or PVCN. The significant improvement in SG survival ipsilateral to the implant, compared to the contralateral side and the consequent better maintenance of auditory nerve inputs likely accounts for the increase in CN volume ipsilateral to the implant observed in the BDNF-treated animals. In addition, our data indicate that BDNF treatment elicited a robust neurotrophic effect on the size of spherical cells in the AVCN, with the cross-sectional areas of cells ipsilateral to the implant found to be about 20% larger than those on the control side. Again, it is most likely that this difference is due to the improved SG and auditory nerve survival ipsilateral to the BDNF treatment. However, we certainly cannot rule out the possibility of a direct effect of BDNF diffusion into the CN from the cochlea during the 10-week period of intracochlear infusion of BDNF in these animals.

BDNF is known to play an important functional role in the neonatal development of the inner ear (Bianchi et al., 1996; Davis, 2003; Ernfors et al., 1995; Farinas et al., 2001; Fritzsch et al., 1997; Fritzsch et al., 2004; Pirvola et al., 1992; Ramekers et al., 2012; Schimmang et al., 1997; Singer et al., 2014; Tessarollo et al., 2004). Although, the neurotrophic effects of BDNF in the peripheral auditory pathways have been illustrated in numerous studies, as mentioned earlier, a full understanding of the role and effects of BDNF in both peripheral and central auditory pathways is still lacking. Recently, Singer et al., (2014) summarized our current knowledge of the expression patterns and functional roles of BDNF in the peripheral and central auditory systems. BDNF expression is absent from all auditory nuclei in the brainstem at birth (Hafidi, 1999), but BDNF and NT-3 positive neurons are widely distributed in the CN beginning in the early post-natal period at about P6 (Hafidi, 1999; Tierney et al., 2001; Wiechers et al., 1999). Further, *in vitro* studies of the CN, showed an upregulation of BDNF expression in the AVCN and PVCN 3 days after unilateral cochlear ablation in the guinea pig (Suneja et al., 2005), and a study of dissociated neurons of neonatal rat CN (Rak et al., 2014) demonstrated that BDNF stimulated neuronal survival and axonal outgrowth. The results of our current study showing that the total CN volume and spherical cell size were significantly larger on the BDNF-treated side than on the contralateral side, are consistent with these previously reported findings of neurotrophic effects of BDNF in the CN. Moreover, the present results demonstrate for the first time, significant neurotrophic effects of BDNF on the morphology of the CN in deafened animals.

It is also encouraging that we saw no evidence of pathologic changes in the CN elicited by prolonged intracochlear infusion of BDNF (e.g., inflammation, gliosis, tumor formation).

Many earlier studies demonstrated that exogenous intracochlear administration of neurotrophins can promote increased survival of the SG neurons over a prolonged period (Leake et al., 2011; Leake et al., 2013; Ramekers et al. 2012; Staecker et al., 1996; Staecker et al., 1998; Staecker et al. 2010). Several studies have reported neuritogenesis following exogenous neurotrophin administration both *in vitro* (Hansen et al., 2001; Hansen et al., 2003; Hegarty et al., 1997; Lefebvre et al., 1994; Malgrange et al., 1996; Mou et al., 1998; Mou et al., 1997; Roehm et al., 2005; Vieira et al., 2007) and *in vivo* (following an ototoxic insult) (Ernfors et al., 1996; Farinas et al., 2001; Fritzsch et al., 1999; Fritzsch et al., 2005; Fritzsch et al., 2004; Rubel et al., 2002; Stankovic et al., 2004). Various studies have specifically shown improved maintenance or regrowth of peripheral neuronal fibers (Ernfors et al., 1996; Glueckert et al., 2008; Miller et al., 2007; Shibata et al., 2010; Staecker et al., 1996; Wise et al., 2005; Wise et al., 2010; Leake et al., 2011, 2013). This effect of neurotrophins to enable sprouting of the peripheral neuronal fibers may promote closer coupling of a CI electrode and adjacent neural elements, thereby potentially resulting in a more favorable outcome in CI patients (Budenz et al., 2012), although disorganized ectopic sprouting might also compromise the precise tonotopic organization of these fibers. Further, the capacity of BDNF to elicit neuronal sprouting also may be expected to affect the central axons of the SG neuron and thus could also potentially account, at least in part, for the increase in total CN volume that was observed ipsilateral to the implant in the BDNF treated animals.

In humans, the central auditory system is known to reach adult dimensions only by the third year of life, whereas the inner ear completes its morphological development by about 25 weeks of gestation (Anson, 1981). In congenital deafness, the deprivation of initial auditory experience during the critical developmental period prevents the normal development and maturation of the auditory system. In such individuals, cochlear implants have proven to be highly effective for hearing rehabilitation. Advances in the field of cochlear implants have significantly improved speech reception abilities in these individuals. More recently, cochlear implant based drug delivery systems have been garnering much interest as a potential modality of treatment, e.g., for reduction of inflammation and tissue reaction to the implant, and to improve auditory nerve survival (Aliuos et al., 2016; Ayoob et al., 2015; El Kechai et al., 2015; Gillespie et al., 2015; Lee et al., 2015; Rivera et al., 2012). Once inserted, the presence of the CI electrode within the scala tympani, which is connected to the cerebrospinal fluid of the subarachnoid space via the cochlear aqueduct (Gopen et al., 1997), may limit the specific drugs that can be utilized and also may comprise an increased risk of infection, e.g., meningitis. Although the findings of our study are encouraging and suggest a potential role for neurotrophins in promoting SG and auditory nerve survival for improved CI outcomes, many issues must be addressed before use in a clinical setting can be considered. A better understanding of all of the potential effects is required, and methods must be devised for optimal delivery to the auditory system without affecting adjacent structures (Hendricks et al., 2008). Long term studies are necessary to determine whether beneficial effects persist after cessation of treatment, to establish optimum duration of

treatment, etc. and more importantly, to provide assurance that there are no long term negative sequelae.

Acknowledgments

This research was supported by the National Institutes of Health; National Institute on Deafness and other Communicative Disorders, Contract# HHS-N-263-2007-00054-C, NIDCD Grant # R01DC013067 and the Epstein Endowment Fund. Human recombinant BDNF utilized for these studies were generously donated by AMGEN INC., Thousand Oaks, CA.

The authors would like to thank Steve Rebscher for his insightful comments on the manuscript.

References

Agterberg MJ, Versnel H, van Dijk LM, de Groot JC, Klis SF. Enhanced survival of spiral ganglion cells after cessation of treatment with brain-derived neurotrophic factor in deafened guinea pigs. J Assoc Res Otolaryngol. 2009; 10:355–67. [PubMed: 19365690]

Agterberg MJ, Versnel H, de Groot JC, Smoorenburg GF, Albers FW, Klis SF. Morphological changes in spiral ganglion cells after intracochlear application of brain-derived neurotrophic factor in deafened guinea pigs. Hear Res. 2008; 244:25–34. [PubMed: 18692557]

Anson BJ. The ear: developmental anatomy. Surgical Anatomy of the Temporal Bone. 1981:23–57.

Aliuos P, Schulze J, Schomaker M, Reuter G, Stolle SR, Werner D, Ripken T, Lenarz T, Warnecke A. Magnetic Beads Enhance Adhesion of NIH 3T3 Fibroblasts: A Proof-of-Principle In Vitro Study for Implant-Mediated Long-Term Drug Delivery to the Inner Ear. PLoS One. 2016; 11:e0150057. [PubMed: 26918945]

Ayoob AM, Borenstein JT. The role of intracochlear drug delivery devices in the management of inner ear disease. Expert Opin Drug Deliv. 2015; 12:465–79. [PubMed: 25347140]

Bianchi LM, Conover JC, Fritzsch B, DeChiara T, Lindsay RM, Yancopoulos GD. Degeneration of vestibular neurons in late embryogenesis of both heterozygous and homozygous BDNF null mutant mice. Development. 1996; 122:1965–73. [PubMed: 8674435]

Budenz CL, Pfingst BE, Raphael Y. The use of neurotrophin therapy in the inner ear to augment cochlear implantation outcomes. Anat Rec (Hoboken). 2012; 295:1896–908. [PubMed: 23044834]

Chikar JA, Colesa DJ, Swiderski DL, Di Polo A, Raphael Y, Pfingst BE. Over-expression of BDNF by adenovirus with concurrent electrical stimulation improves cochlear implant thresholds and survival of auditory neurons. Hear Res. 2008; 245:24–34. [PubMed: 18768155]

Davis RL. Gradients of neurotrophins, ion channels, and tuning in the cochlea. Neuroscientist. 2003; 9:311–6. [PubMed: 14580116]

El Kechai N, Agnely F, Mamelle E, Nguyen Y, Ferrary E, Bochot A. Recent advances in local drug delivery to the inner ear. Int J Pharm. 2015; 494:83–101. [PubMed: 26260230]

Ernfors P, Van De Water T, Loring J, Jaenisch R. Complementary roles of BDNF and NT-3 in vestibular and auditory development. Neuron. 1995; 14:1153–64. [PubMed: 7605630]

Ernfors P, Duan ML, ElShamy WM, Canlon B. Protection of auditory neurons from aminoglycoside toxicity by neurotrophin-3. Nat Med. 1996; 2:463–7. [PubMed: 8597959]

Farinas I, Jones KR, Tessarollo L, Vigers AJ, Huang E, Kirstein M, de Caprona DC, Coppola V, Backus C, Reichardt LF, Fritzsch B. Spatial shaping of cochlear innervation by temporally regulated neurotrophin expression. J Neurosci. 2001; 21:6170–80. [PubMed: 11487640]

Fritzsch B, Pirvola U, Ylikoski J. Making and breaking the innervation of the ear: neurotrophic support during ear development and its clinical implications. Cell Tissue Res. 1999; 295:369–82. [PubMed: 10022958]

Fritzsch B, Gregory D, Rosa-Molinar E. The development of the hindbrain afferent projections in the axolotl: evidence for timing as a specific mechanism of afferent fiber sorting. Zoology (Jena). 2005; 108:297–306. [PubMed: 16351978]

Fritzsch B, Silos-Santiago I, Bianchi LM, Farinas I. The role of neurotrophic factors in regulating the development of inner ear innervation. Trends Neurosci. 1997; 20:159–64. [PubMed: 9106356]

Fritzsch B, Tessarollo L, Coppola E, Reichardt LF. Neurotrophins in the ear: their roles in sensory neuron survival and fiber guidance. Prog Brain Res. 2004; 146:265–78. [PubMed: 14699969]

Gao WQ, Zheng JL, Karihaloo M. Neurotrophin-4/5 (NT-4/5) and brain-derived neurotrophic factor (BDNF) act at later stages of cerebellar granule cell differentiation. J Neurosci. 1995; 15:2656–67. [PubMed: 7722620]

Gillespie LN, Zanin MP, Shepherd RK. Cell-based neurotrophin treatment supports long-term auditory neuron survival in the deaf guinea pig. J Control Release. 2015; 198:26–34. [PubMed: 25481440]

Glueckert R, Bitsche M, Miller JM, Zhu Y, Prieskorn DM, Altschuler RA, Schrott-Fischer A. Deafferentation-associated changes in afferent and efferent processes in the guinea pig cochlea and afferent regeneration with chronic intrascalar brain-derived neurotrophic factor and acidic fibroblast growth factor. J Comp Neurol. 2008; 507:1602–21. [PubMed: 18220258]

Gopen Q, Rosowski JJ, Merchant SN. Anatomy of the normal human cochlear aqueduct with functional implications. Hear Res. 1997; 107:9–22. [PubMed: 9165342]

Hafidi A. Distribution of BDNF, NT-3 and NT-4 in the developing auditory brainstem. Int J Dev Neurosci. 1999; 17:285–94. [PubMed: 10479064]

Hansen MR, Zha XM, Bok J, Green SH. Multiple distinct signal pathways, including an autocrine neurotrophic mechanism, contribute to the survival-promoting effect of depolarization on spiral ganglion neurons in vitro. J Neurosci. 2001; 21:2256–67. [PubMed: 11264301]

Hansen MR, Bok J, Devaiah AK, Zha XM, Green SH. Ca2+/calmodulin-dependent protein kinases II and IV both promote survival but differ in their effects on axon growth in spiral ganglion neurons. J Neurosci Res. 2003; 72:169–84. [PubMed: 12671991]

Hegarty JL, Kay AR, Green SH. Trophic support of cultured spiral ganglion neurons by depolarization exceeds and is additive with that by neurotrophins or cAMP and requires elevation of [Ca2+]i within a set range. J Neurosci. 1997; 17:1959–70. [PubMed: 9045725]

Hendricks JL, Chikar JA, Crumling MA, Raphael Y, Martin DC. Localized cell and drug delivery for auditory prostheses. Hear Res. 2008; 242:117–31. [PubMed: 18573323]

Hochmair I, Nopp P, Jolly C, et al. Med-El Cochlear implants: state of the art and a glimpse into the future. Trends Amplif. 2006; 10:201–219. [PubMed: 17172548]

Kiang NY, Godfrey DA, Norris BE, Moxon SE. A block model of the cat cochlear nucleus. J Comp Neurol. 1975; 162:221–45. [PubMed: 1150920]

Korsching S. The neurotrophic factor concept: a reexamination. J Neurosci. 1993; 13:2739–48. [PubMed: 8331370]

Landry TG, Fallon JB, Wise AK, Shepherd RK. Chronic neurotrophin delivery promotes ectopic neurite growth from the spiral ganglion of deafened cochleae without compromising the spatial selectivity of cochlear implants. J Comp Neurol. 2013; 521:2818–32. [PubMed: 23436344]

Leake PA, Snyder RL, Hradek GT. Postnatal refinement of auditory nerve projections to the cochlear nucleus in cats. J Comp Neurol. 2002; 448:6–27. [PubMed: 12012373]

Leake PA, Hradek GT, Bonham BH, Snyder RL. Topography of auditory nerve projections to the cochlear nucleus in cats after neonatal deafness and electrical stimulation by a cochlear implant. J Assoc Res Otolaryngol. 2008; 9:349–72. [PubMed: 18574634]

Leake PA, Hradek GT, Hetherington AM, Stakhovskaya O. Brain-derived neurotrophic factor promotes cochlear spiral ganglion cell survival and function in deafened, developing cats. J Comp Neurol. 2011; 519:1526–45. [PubMed: 21452221]

Leake PA, Stakhovskaya O, Hetherington A, Rebscher SJ, Bonham B. Effects of brain-derived neurotrophic factor (BDNF) and electrical stimulation on survival and function of cochlear spiral ganglion neurons in deafened, developing cats. J Assoc Res Otolaryngol. 2013; 14:187–211. [PubMed: 23392612]

Lee MY, Lee JH, Lee HS, Choi JJ, Jang J, Choi H, Oh SH, Jang JH. Continuous topical drug delivery using osmotic pump in animal cochlear implant model: Continuous steroid delivery is effective for hearing preservation. Acta Otolaryngol. 2015; 135:791–8. [PubMed: 25813859]

Lefebvre PP, Weber T, Rigo JM, Staecker H, Moonen G, Van De Water TR. Peripheral and central target-derived trophic factor(s) effects on auditory neurons. Hear Res. 1992; 58:185–92. [PubMed: 1568940]

Lefebvre PP, Malgrange B, Staecker H, Moghadass M, Van de Water TR, Moonen G. Neurotrophins affect survival and neuritogenesis by adult injured auditory neurons in vitro. Neuroreport. 1994; 5:865–8. [PubMed: 8061284]

Malgrange B, Lefebvre PP, Martin D, Staecker H, Van de Water TR, Moonen G. NT-3 has a tropic effect on process outgrowth by postnatal auditory neurones in vitro. Neuroreport. 1996; 7:2495–9. [PubMed: 8981411]

McGuinness SL, Shepherd RK. Exogenous BDNF rescues rat spiral ganglion neurons in vivo. Otology & neurotology : official publication of the American Otological Society, American Neurotology Society [and] European Academy of Otology and Neurotology. 2005; 26:1064–72.

Miller JM, Le Prell CG, Prieskorn DM, Wys NL, Altschuler RA. Delayed neurotrophin treatment following deafness rescues spiral ganglion cells from death and promotes regrowth of auditory nerve peripheral processes: effects of brain-derived neurotrophic factor and fibroblast growth factor. J Neurosci Res. 2007; 85:1959–69. [PubMed: 17492794]

Mou K, Hunsberger CL, Cleary JM, Davis RL. Synergistic effects of BDNF and NT-3 on postnatal spiral ganglion neurons. J Comp Neurol. 1997; 386:529–39. [PubMed: 9378849]

Mou K, Adamson CL, Davis RL. Time-dependence and cell-type specificity of synergistic neurotrophin actions on spiral ganglion neurons. J Comp Neurol. 1998; 402:129–39. [PubMed: 9831050]

Nakaizumi T, Kawamoto K, Minoda R, Raphael Y. Adenovirus-mediated expression of brain-derived neurotrophic factor protects spiral ganglion neurons from ototoxic damage. Audiol Neurootol. 2004; 9:135–43. [PubMed: 15084818]

Pirvola U, Ylikoski J, Palgi J, Lehtonen E, Arumae U, Saarma M. Brain-derived neurotrophic factor and neurotrophin 3 mRNAs in the peripheral target fields of developing inner ear ganglia. Proc Natl Acad Sci U S A. 1992; 89:9915–9. [PubMed: 1409719]

Rak K, Volker J, Frenz S, Scherzad A, Schendzielorz P, Radeloff A, Jablonka S, Hagen R, Mlynski R. Effects of the neurotrophic factors BDNF, NT-3, and FGF2 on dissociated neurons of the cochlear nucleus. Neuroreport. 2014; 25:960–4. [PubMed: 24978398]

Ramekers D, Versnel H, Grolman W, Klis SF. Neurotrophins and their role in the cochlea. Hear Res. 2012; 288:19–33. [PubMed: 22465680]

Rebscher SJ, Hetherington AM, Snyder RL, Leake PA, Bonham BH. Design and fabrication of multichannel cochlear implants for animal research. J Neurosci Methods. 2007; 166:1–12. [PubMed: 17727956]

Rivera T, Sanz L, Camarero G, Varela-Nieto I. Drug delivery to the inner ear: strategies and their therapeutic implications for sensorineural hearing loss. Curr Drug Deliv. 2012; 9:231–42. [PubMed: 22283653]

Roehm PC, Hansen MR. Strategies to preserve or regenerate spiral ganglion neurons. Curr Opin Otolaryngol Head Neck Surg. 2005; 13:294–300. [PubMed: 16160524]

Rubel EW, Fritzsch B. Auditory system development: primary auditory neurons and their targets. Annu Rev Neurosci. 2002; 25:51–101. [PubMed: 12052904]

Sameer Mallick A, Qureishi A, Pearson R, O'Donoghue G. Neurotrophins and cochlear implants: a solution to sensorineural deafness? Cochlear Implants Int. 2013; 14:158–64. [PubMed: 22889496]

Schecterson LC, Bothwell M. Neurotrophin and neurotrophin receptor mRNA expression in developing inner ear. Hear Res. 1994; 73:92–100. [PubMed: 8157510]

Schimmang T, Represa J. Neurotrophins gain a hearing. Trends Neurosci. 1997; 20:100–2. [PubMed: 9061861]

Shepherd RK, Coco A, Epp SB. Neurotrophins and electrical stimulation for protection and repair of spiral ganglion neurons following sensorineural hearing loss. Hear Res. 2008; 242:100–9. [PubMed: 18243608]

Shepherd RK, Coco A, Epp SB, Crook JM. Chronic depolarization enhances the trophic effects of brain-derived neurotrophic factor in rescuing auditory neurons following a sensorineural hearing loss. J Comp Neurol. 2005; 486:145–58. [PubMed: 15844207]

Shibata SB, Cortez SR, Beyer LA, Wiler JA, Di Polo A, Pfingst BE, Raphael Y. Transgenic BDNF induces nerve fiber regrowth into the auditory epithelium in deaf cochleae. Exp Neurol. 2010; 223:464–72. [PubMed: 20109446]

Singer W, Panford-Walsh R, Knipper M. The function of BDNF in the adult auditory system. Neuropharmacology. 2014; 76(Pt C):719–28. [PubMed: 23688926]

Sly DJ, Hampson AJ, Minter RL, Heffer LF, Li J, Millard RE, Winata L, Niasari A, O'Leary SJ. Brain-derived neurotrophic factor modulates auditory function in the hearing cochlea. J Assoc Res Otolaryngol. 2012; 13:1–16. [PubMed: 22086147]

Song BN, Li YX, Han DM. Delayed electrical stimulation and BDNF application following induced deafness in rats. Acta Otolaryngol. 2009; 129:142–54. [PubMed: 18607918]

Staecker H, Garnham C. Neurotrophin therapy and cochlear implantation: translating animal models to human therapy. Exp Neurol. 2010; 226:1–5. [PubMed: 20654616]

Staecker H, Gabaizadeh R, Federoff H, Van De Water TR. Brain-derived neurotrophic factor gene therapy prevents spiral ganglion degeneration after hair cell loss. Otolaryngol Head Neck Surg. 1998; 119:7–13. [PubMed: 9674508]

Staecker H, Kopke R, Malgrange B, Lefebvre P, Van de Water TR. NT-3 and/or BDNF therapy prevents loss of auditory neurons following loss of hair cells. Neuroreport. 1996; 7:889–94. [PubMed: 8724667]

Stakhovskaya O, Hradek GT, Snyder RL, Leake PA. Effects of age at onset of deafness and electrical stimulation on the developing cochlear nucleus in cats. Hear Res. 2008; 243:69–77. [PubMed: 18590947]

Stankovic K, Rio C, Xia A, Sugawara M, Adams JC, Liberman MC, Corfas G. Survival of adult spiral ganglion neurons requires erbB receptor signaling in the inner ear. J Neurosci. 2004; 24:8651–61. [PubMed: 15470130]

Suneja SK, Potashner SJ. TrkB levels in the cochlear nucleus after unilateral cochlear ablation: correlations with post-lesion plasticity. Brain Res. 2002; 957:366–8. [PubMed: 12445980]

Suneja SK, Yan L, Potashner SJ. Regulation of NT-3 and BDNF levels in guinea pig auditory brain stem nuclei after unilateral cochlear ablation. J Neurosci Res. 2005; 80:381–90. [PubMed: 15795930]

Tessarollo L, Coppola V, Fritzsch B. NT-3 replacement with brain-derived neurotrophic factor redirects vestibular nerve fibers to the cochlea. J Neurosci. 2004; 24:2575–84. [PubMed: 15014133]

Tierney TS, TPD, Xia G, Moore DR. Development of brain-derived neurotrophic factor and neurotrophin-3 immunoreactivity in the lower auditory brainstem of the postnatal gerbil. Eur J Neurosci. 2001; 14:785–93. [PubMed: 11576182]

Vieira M, Christensen BL, Wheeler BC, Feng AS, Kollmar R. Survival and stimulation of neurite outgrowth in a serum-free culture of spiral ganglion neurons from adult mice. Hear Res. 2007; 230:17–23. [PubMed: 17521837]

Wiechers B, Gestwa G, Mack A, Carroll P, Zenner HP, Knipper M. A changing pattern of brain-derived neurotrophic factor expression correlates with the rearrangement of fibers during cochlear development of rats and mice. J Neurosci. 1999; 19:3033–42. [PubMed: 10191320]

Wise AK, Gillespie LN. Drug delivery to the inner ear. J Neural Eng. 2012; 9:065002. [PubMed: 23186937]

Wise AK, Richardson R, Hardman J, Clark G, O'Leary S. Resprouting and survival of guinea pig cochlear neurons in response to the administration of the neurotrophins brain-derived neurotrophic factor and neurotrophin-3. J Comp Neurol. 2005; 487:147–65. [PubMed: 15880560]

Wise AK, Hume CR, Flynn BO, Jeelall YS, Suhr CL, Sgro BE, O'Leary SJ, Shepherd RK, Richardson RT. Effects of localized neurotrophin gene expression on spiral ganglion neuron resprouting in the deafened cochlea. Mol Ther. 2010; 18:1111–22. [PubMed: 20216530]

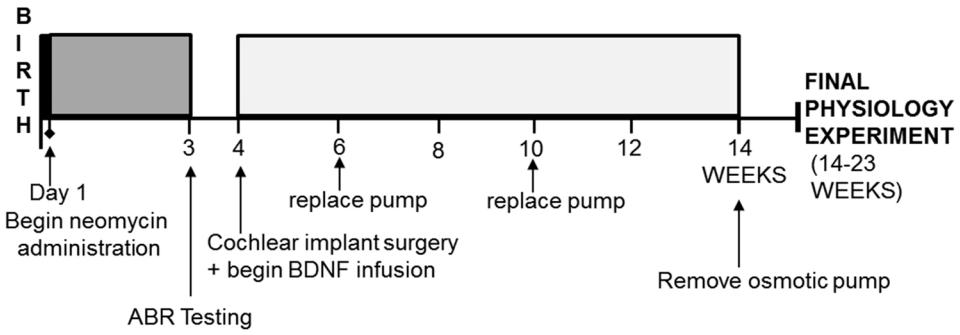

Figure 1. Timeline

Daily neomycin sulfate injections were administered beginning the day after birth and continuing for up to 18 days, until ABR testing confirmed deafness. Custom 6-wire scala tympani electrodes were implanted unilaterally at 4–5 weeks of age, and BDNF from an osmotic pump was infused through a drug–delivery cannula within the electrode. The pumps were replaced twice in order to continue BDNF treatment for 10 weeks, and animals were studied at 14–23 weeks of age.

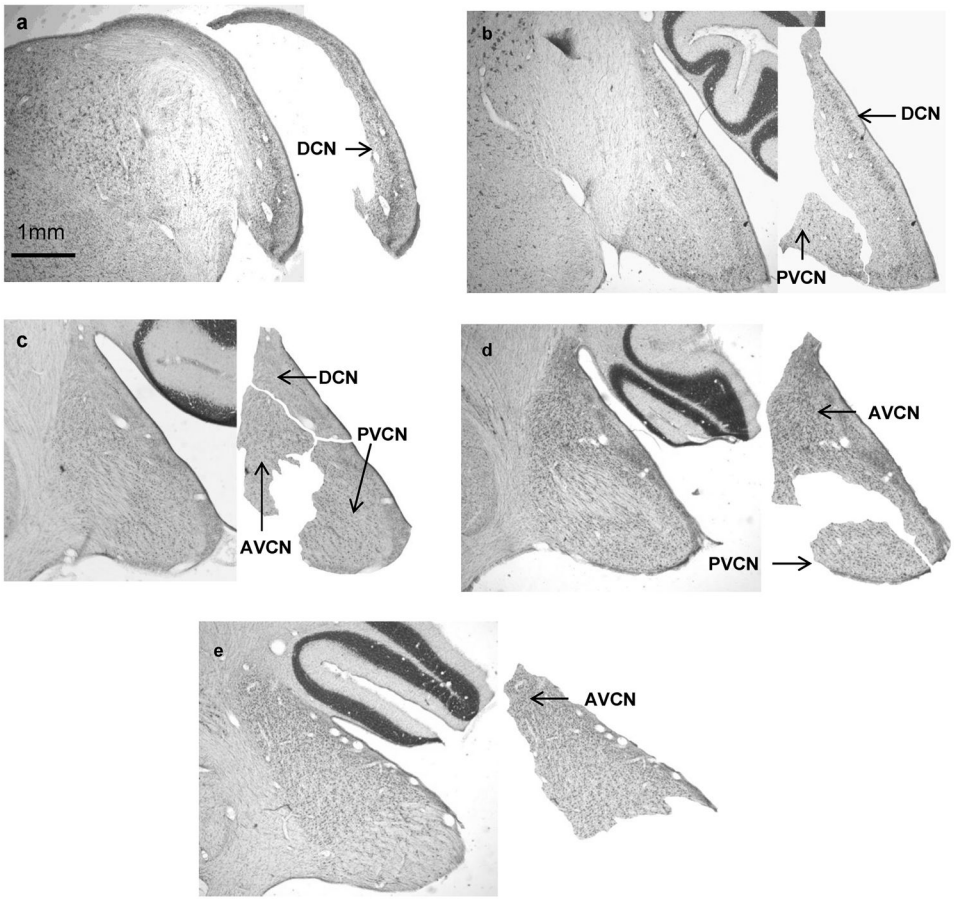

Figure 2. CN Subdivisions

Coronal sections through the CN in a normal hearing animal (arranged rostral to caudal) illustrate the three subdivisions (DCN, PVCN and AVCN) used to determine volumes. CN subdivisions were delineated according to cytoarchitectural criteria (Kiang et al., 1975). The scale bar in (a) applies to all the five images.

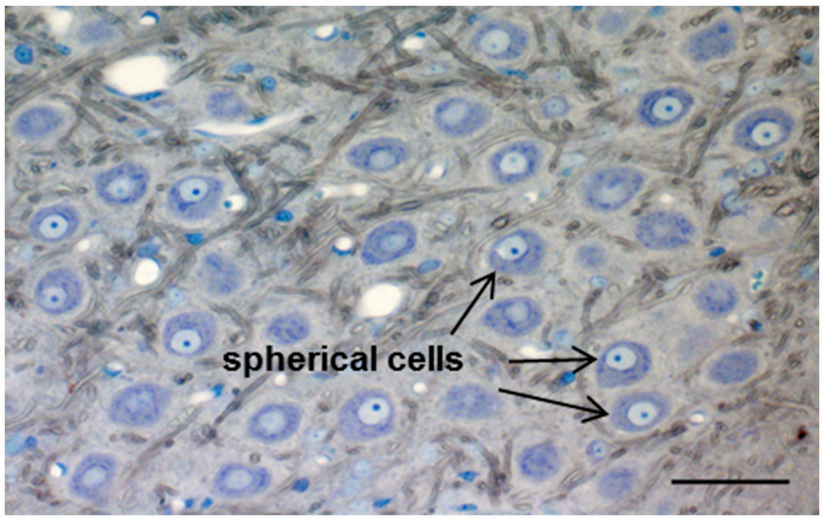

Figure 3. Spherical Cells in the AVCN

Example of the histology after post-fixation with osmium tetroxide in a 5 μm plastic section taken from the AVCN ipsilateral to the BDNF-treated cochlea of one of the deafened cats (K214) included in the study. These sections were used for spherical cell area and numerical density measurements. Scale bar is 50 μm.

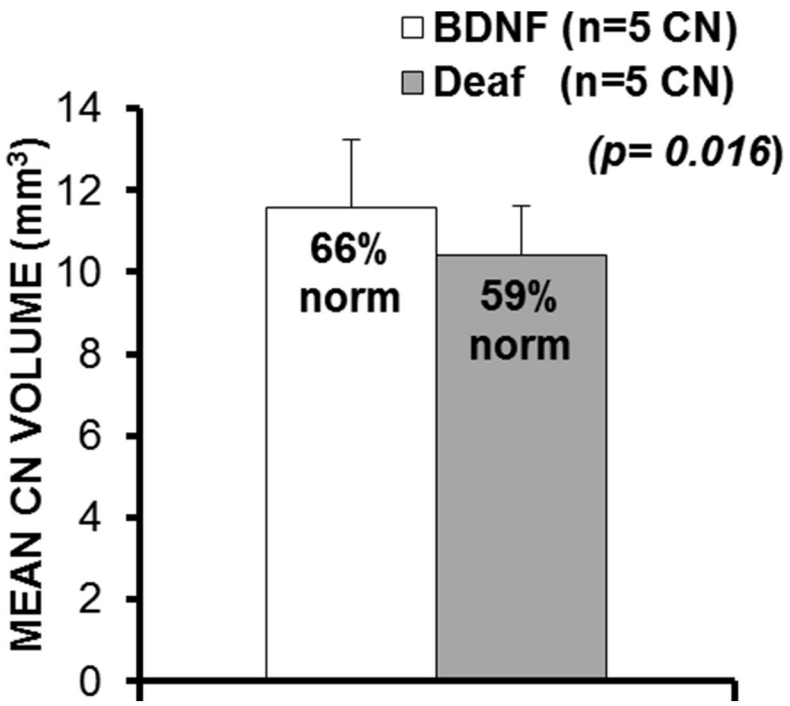

Figure 4. Total CN Volume

Total CN volume was calculated by summing the areas of the 3 major subdivisions and multiplying by the section thickness and sampling step. The CN on the BDNF-treated side was significantly larger than on the deaf side ($t(4) = 3.99$, $p = 0.016$; student's t-test, paired). Expressed as percentage of values obtained in normal adult cats, the CN volumes were 66% of normal on the BDNF-treated side and 59% on the deaf side in these young animals studied at 14–23 weeks of age. Error bars represent +1 standard deviation.

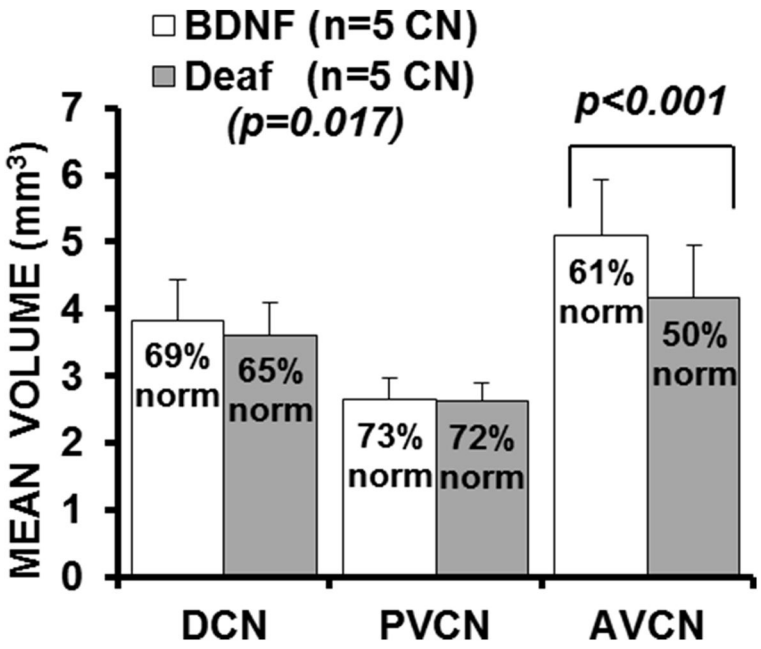

Figure 5. CN Subdivision Volumes

The volumes of the 3 individual subdivisions are shown for the BDNF-treated and control sides. Two-way ANOVA indicated a significant main effect of BDNF treatment ($F_{(1,4)}$ =15.6, p= 0.017) and a significant interaction between BDNF treatment and CN subdivision ($F_{(2,8)}$ 12.45, p= 0.003). Post hoc comparisons showed that the difference elicited by BDNF was significant only for the AVCN (p<0.001; Tukey test). Error bars represent + standard deviation.

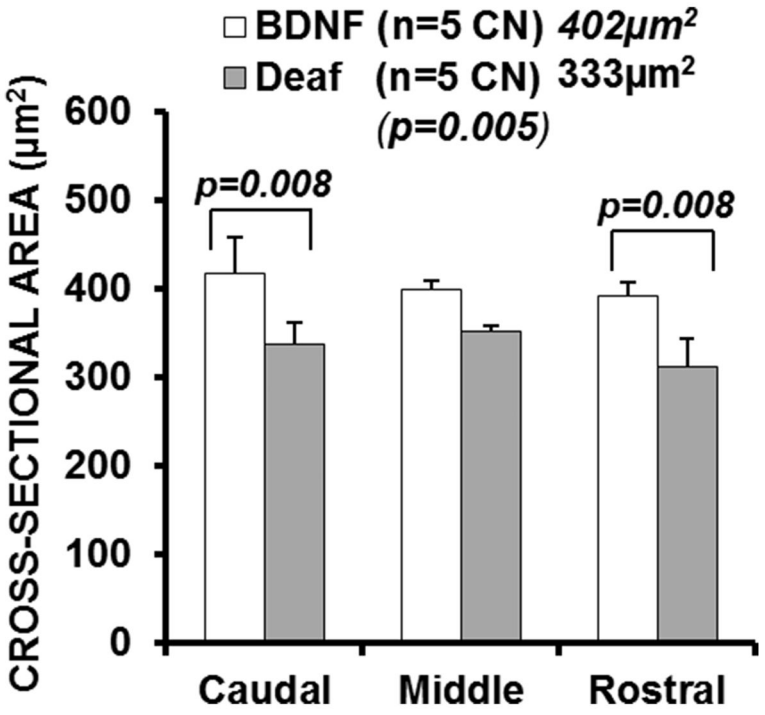

Figure 6. AVCN Spherical Cell Size

Spherical cell areas (in osmium-treated sections) were significantly larger in the AVCN ipsilateral to the BDNF-treated ears as compared to the contralateral AVCN. ($F_{(1,4)}$=31.61, p= 0.005, 2-way ANOVA) with no significant effect of the location within the AVCN. Post-hoc comparisons (Tukey test) showed a significant difference in cell size between the BDNF-treated and the deaf side in the rostral (p=0.008) and caudal (p=0.008) parts of the AVCN. Error bars represent + 1 standard deviation.

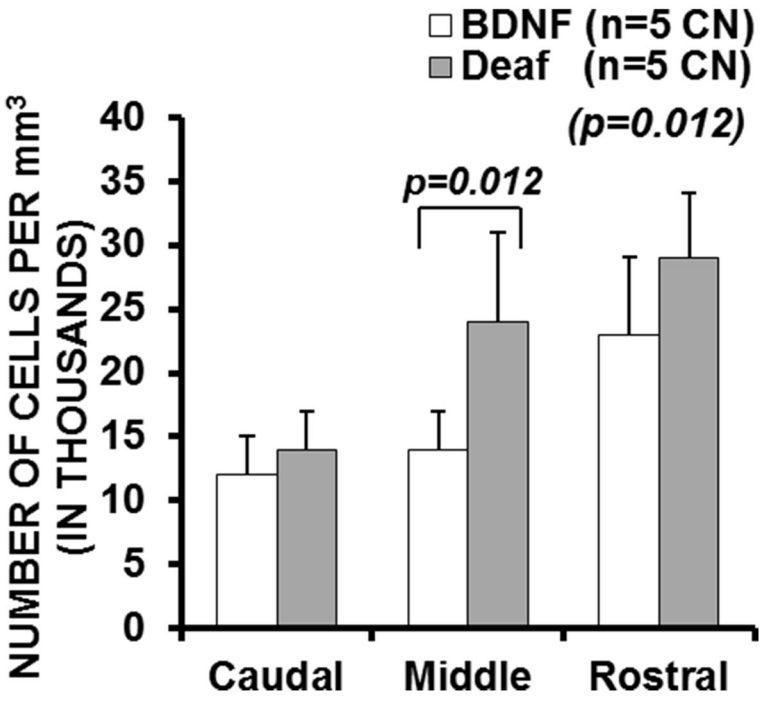

Figure 7. AVCN Spherical Cell Density

The numerical density of spherical cells in the AVCN was significantly lower on the BDNF-treated side ($F_{(1,4)}$=19.15, p=0.012, 2-way repeated measures ANOVA), with a significant main effect of the location within the AVCN ($F_{(2,8)}$=13.17, p=0.003). Post-hoc comparisons (Tukey test) showed a significantly higher numerical density in the middle part of the AVCN (p=0.012). These findings can be explained by a significantly larger AVCN volume on the BDNF-treated side compared to deafened untreated side as shown in Table 3, which would result in the distribution of the spherical cells over a larger area, thereby resulting in a lower density. Error bars represent one standard deviation.

Table 1

Summary of the experimental histories of the 5 neonatally deafened, BDNF-treated animals included in the study.

Experimental Histories for Individual Neonatally Deafened Cats			
Subject	**No. of days (neomycin)**	**Age at implant (weeks)**	**Age at study (weeks)**
#1-K210	18	5	15
#2-K214	16	5	14
#3-K215	16	5	23
#4-K217	16	4	14
#5-K310	18	5	15
Mean (n=5)	17.4	5	16

Table 2

Summary of SG density data for the 5 individual BDNF-treated animals.

Spiral Ganglion Density in Neonatally Deafened Cats		
Subject	**SG Density (% normal)**	
	Implant	**Contra**
#1-K210*	85%	68%
#2-K214*	92%	75%
#3-K215	79%	62%
#4-K217*	66%	49%
#5-K310*	70%	55%
Mean (n=5)	78%	62%

SG density $F_{(1,3)}=150.582$, $p<0.001$ with significant interaction between BDNF treatment and SG sector ($F_{(8,32)}=2.647$, $p=0.024$).

*SG density data were previously reported for 4 of these animals in Leake et al 2011(J. Comp. Neurol)

Table 3

Summary of the subdivision volumes and the total CN volumes for individual neonatally deafened, BDNF treated animals included in this study (ipsilateral and contralateral to the implanted ear).

CN Volumes (mm^3) in Neonatally Deafened Cats (n=5)

	Implant (BDNF)				Contra			
Subject	**DCN**	**PVCN**	**AVCN**	**TOTAL**	**DCN**	**PVCN**	**AVCN**	**TOTAL**
#1-K210	3.52	2.59	4.67	10.78	3.29	2.94	3.89	10.11
#2-K214	4.87	3.06	6.12	14.05	4.21	2.38	5.17	11.76
#3-K215	3.31	2.48	3.98	9.77	3.16	2.44	3.02	8.62
#4-K217	3.64	2.28	4.99	10.92	3.37	2.44	4.41	10.22
#5-K310	3.84	2.85	5.67	12.36	4.05	2.88	4.37	11.30
Mean ±SD	3.84±0.6	2.65±0.3	5.09±0.8 *	11.57±1.6 *	3.62±0.5	2.62±0.3	4.17±0.8	10.40±1.2

* Indicates statistical significance. [AVCN volume ($p<0.001$); Total Mean CN volume ($p=0.017$).]

SD indicates standard deviation.

Table 4

Summary of total and CN subdivision volumes for normal adult cats (comparison group).

Normal Adult Cats				
Subject	**CN volume (mm^3)**			
	DCN	**PVCN**	**AVCN**	**TOTAL**
N3	5.24	3.13	7.69	16.06
N6	5.48	4.37	9.78	19.63
N124	6.44	3.36	8.42	18.22
N001	5.07	3.73	7.38	16.18
Mean (n=4)±SD	5.56±0.6	3.65±0.5	8.32±1.1	17.52±1.6

SD indicates standard deviation