Published in final edited form as:

Hear Res. 2013 August ; 302: 9–16. doi:10.1016/j.heares.2013.04.007.

Effect of monopolar and bipolar electric stimulation on survival and size of human spiral ganglion cells as studied by postmortem histopathology

Mohammad Seyyedi^{a,b}, Donald K. Eddington^{a,b,c}, and Joseph B. Nadol Jr.^{a,b,c,*}

^aDepartment of Otology and Laryngology, Harvard Medical School, Boston, 02114 MA, USA

^bDepartment of Otolaryngology and Cochlear Implant Research Laboratory, Massachusetts Eye and Ear Infirmary, Boston, 02114 MA, USA

^cSpeech and Hearing Bioscience and Technology Program, Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA, USA

Abstract

The spiral ganglion cell (SGC) is the target of electrical stimulation in cochlear implants. This study is designed to test the hypothesis that chronic electrical stimulation tends to preserve SGCs in implanted hearing-impaired ears. A total of 26 pairs of temporal bones were studied from 26 individuals who in life suffered bilateral profound hearing impairment that was symmetric (in degree of impairment and etiology) across ears and then underwent unilateral cochlear implantation. The subjects were divided in two groups by stimulus configuration: bipolar ($n = 16$) or monopolar ($n = 10$). The temporal bones were prepared for histological review by standard methods and two measures of SGC status were made by cochlear segment: count and maximal cross-sectional area. Within-subject comparison of the measures between the implanted-stimulated and the unimplanted ears showed: (1) for both stimulus configurations, the mean (across subjects and segments) of the count difference (implanted ear – unimplanted ear) was significantly less than zero; (2) the mean (across subject) count difference for cochlear segments I, II and III (segments with electrode contacts in the implanted ear) was significantly less negative than the mean difference for cochlear segment IV (no electrode in implanted ear) for bipolar but not for monopolar stimulation; (3) neither implantation-stimulation nor stimulus configuration significantly influenced the measures of maximum cross-sectional cell area. The SGC count results are consistent with the hypothesis that implantation results in a propensity across the whole cochlea for SGCs to degenerate and with chronic bipolar stimulation ameliorating this propensity in those cochlear segments with electrodes present.

1. Introduction

Intracochlear stimulation by cochlear implants (CIs) provides partial hearing restoration for profoundly hearing impaired patients. Because the presumed site of neural excitation is the spiral ganglion cell (SGC), the success of cochlear implantation should be dependent at least in part on the status of the surviving cells. SGCs gradually degenerate following inner hair cell (IHC) loss due to noise, trauma, ototoxicity or other causes of irreversible damage (Spoendlin, 1975; Webster et al., 1981; Xu et al., 1993). Inner hair cells provide the neural activity and neurotrophic support for SGCs, and reduction in this supportive role results in

SGC degeneration (Fritzsche et al., 1999; Hartmann et al., 1984; Shepherd et al., 1997; Ylikoski et al., 1993). Therefore, if this supportive role is provided, it would be expected to preserve SGCs or at least slow the rate of degeneration. Reintroducing neurotrophic agents like the neurotrophins (e.g., brain derived neurotrophic factor (BDNF), and NT3) and glial derived neurotrophic factor (GDNF) in profoundly deafened cochleae has been shown to prevent or slow the loss of SGCs (Ernfors et al., 1996; Kanzaki et al., 2002; McGuinness et al., 2005; Miller et al., 1997; Wise et al., 2005). On the other hand, the efficacy of chronic electrical stimulation (CES) via cochlear implant by itself or in combination with neurotrophics in preserving SGCs in animal and human studies is less clear.

Several animal studies have reported that CES reduces the rate of degeneration of SGCs following aminoglycoside induced hair cell loss (Hultcrantz et al., 1991; Leake et al., 1999, 1991, 1995; Lousteau, 1987; Mitchell et al., 1997). However, in other animal studies no evidence for a CES trophic effect has been shown (Agterberg et al., 2010; Araki et al., 1998; Chatterjee, 1999; Coco et al., 2007; Shepherd et al., 1994; Xu et al., 1997). There are several methodological differences between these studies that could account for the difference in outcome, including differences in species, deafening method, electrode configuration, age at stimulation, stimulus intensity, rate and duration of stimulation, and stimulation current level.

The reported effect of CES on SGC survival is also mixed in studies of humans with cochlear implants. Fayad and Linthicum (2006) found no significant difference between counts in the implanted and non-implanted ears of eight subjects except in the most basal segment where significantly fewer cells were counted in the implanted ear. Khan et al. (2005) and Xu et al. (2012) also counted SGCs in implanted and non-implanted ears of eleven and three subjects respectively. The three more basal cochlear segments (in which electric stimuli were delivered) did not show significant count differences between the two ears; but in the apical segment, SGC counts in the implanted ears were significantly fewer than in the unimplanted ears. As pointed out by Khan et al. (2005), this result is consistent with the electrode insertion initiating a degeneration of SGCs that is minimized by CES in the more basal segments but not in the unstimulated apical segment of the implanted ear.

In this study we revisit the question of whether CES effects the survival of SGCs in humans with CIs using methods that control two variables not always controlled in previous human studies: the likelihood that within-subject SGC survival was similar across ears and stimulus configuration. Stimulus configuration (monopolar vs. bipolar) was controlled because Leake et al. (1999, 1995) suggest that bipolar stimulation preserves SGCs more effectively than monopolar stimulation. SGC status was evaluated using two measures: SGC count and maximum cross-sectional cell area. Temporal bones of profoundly hearing-impaired patients with symmetric hearing loss before implantation and, consequently, a similar number of surviving SGCs across ears (Seyyedi et al., 2011), were selected and segregated into two groups by the CI stimulus configuration: monopolar (MP) or bipolar (BP). Within each configuration group, comparisons of SGC status (count and cell size) were made between implanted and unimplanted ears.

2. Materials and methods

All temporal bones from the collections at the Massachusetts Eye and Ear infirmary (MEEI), House Research Institute (HRI) and University of Minnesota (UM) which met the following criteria were included in the study: (1) both right and left temporal bones of each subject available, (2) the etiology of hearing loss symmetric across ears in each subject, (3) bilaterally symmetric profound hearing impairment before implantation documented by

audiometric test results (all subjects had bilateral pure tone average 90 dB with maximum 10 dB difference between ears at each test frequency before implantation) and (4) unilateral implantation with a multichannel CI. A total of 26 subjects were identified: 10 with devices configured for MP stimulation using Ineraid® or Nucleus®24 cochlear implants and 16 subjects with devices configured for BP stimulation all using Nucleus® 22 CIs.

The temporal bones were removed after death, fixed in Heidenhain Susa solution or 10% buffered formalin and decalcified in ethylene diamine tetra acetic acid (EDTA), and embedded in celloidin (Schuknecht, 1968). The temporal bones were sectioned at a thickness of 20 μ m in the horizontal plane and every tenth section was stained with hematoxylin and eosin and mounted on a glass slide. Rosenthal's canal and the cochlear duct were reconstructed in two dimensions (Fig. 1) by a method described by Schuknecht (1953) and Otte et al. (1978), and the length of Rosenthal's canal and cochlear duct were calculated. All SGCs with visible nuclei were counted on every tenth section. SGC counts for the four cochlear segments identified in Fig. 1 were computed by adding all counts across slides from the same segment. Each segmental count was multiplied by ten to account for unmounted sections, and again multiplied by a correction factor of 0.68 to account for doubly counted SGCs (Konigsmark, 1970; Nadol, 1988; Seyyedi et al., 2011). The total spiral ganglion cell count was calculated as the sum of the four segmental counts (Nadol, 1988; Nadol et al., 1989).

Because the maximum cross-sectional area of SGCs differ by location (segment) in Rosenthal's canal with larger cells at the base and smaller cells at the apex in adults (Nadol et al., 1990), we targeted four positions (one in each segment) for SGC area measurements: 15, 50, 75 and 95% of total Rosenthal's canal length. The maximum cross-sectional area of each SGC with a visible nucleolus in the segment of the mounted/stained section closest to each targeted position was measured (Chiong et al., 1993). In the case illustrated in Fig. 1 for example, the length of Rosenthal's canal is 13.2 mm and 75% of that length is equal to 9.9 mm which corresponds to section 207 in the 2-D reconstruction. The closest mounted/stained section to 207 is section 211. Thus, the cross-sectional area of each SGC with a clear nucleolus and cell boundary in segment 3 of slide 211 was measured.

3. Results

Of the 26 subjects studied, the CI of 10 used MP stimulation (Table 1) and 16 used BP stimulation (Table 2). As shown in these tables, the subjects included 17 males and 9 females ranging in age from 42 to 92 years at the time of death. There was a wide range of duration of CI use (age at death minus age at implantation) which varied between 1 year in case 1 of the BP group to more than 21 years in case 1 of the MP group. Subjects were impaired postlingually by a variety of etiologies, but in 14 of 26 cases, the etiology was unknown. The progression of hearing loss in both ears of each subject was similar. All BP-group cases were implanted with the Nucleus® 22 device and the MP-group cases received either the Ineraid® or Nucleus® 24 devices.

Pre-implantation audiometric test results are presented in Tables 3 and 4. One of the case selection criteria was a symmetric hearing loss: a maximum 10 dB difference in hearing threshold between ears at each frequency eliciting a response. This criterion was easily applied to cases 14 and 15 of the BP group where response thresholds were measured at each frequency tested. Subjects who did not respond at the maximum acoustic intensity are identified by NR with a subscript indicating the maximum intensity delivered. In these cases both the response threshold and no-response entries were symmetric across ears, and the hearing loss was considered symmetric. For example, the thresholds measured at 250 Hz in

case 11 of the BP group were within 10 dB across ears and at frequencies between 500 and 8000 Hz, no response was elicited in each ear for acoustic intensities up to 110 dB (NR₁₁₀).

3.1. Spiral ganglion cell counts

Table 5 (MP) and 6 (BP) represent corrected segmental and total SGC counts for the implanted and unimplanted ears of each subject. In case 10 of the BP group (Table 6) and case 3 of the MP group (Table 5), portions of segments 2 and 4 in the unimplanted ears were lost at bone extraction. Therefore, these segmental counts and consequently total counts were not available for these two cases. There is a wide range in the total SGC count for the unimplanted ears (1068 to 15,334) and implanted ears (626–14,464). It should be mentioned that the counts represent the number of SGCs at the time of death (sometimes long after the preimplantation audiological measurements were made).

Table 7 (MP group) and 8 (BP group) show the differences between the implanted (Im) and unimplanted (Ui) SGC segmental counts for each subject by cochlear segment. The count differences (Im – Ui) ranged from –4284 (negative: implanted count < unimplanted count) to 2442 (positive: implanted count > unimplanted count) for MP stimulation and –1891 to 1231 for BP. We tested whether the across-subject and across-segment variation in age at death and duration of cochlear implant (*durCI*) usage explained any of the variation in count difference using a least-squares fitting procedure to estimate the coefficients of the following linear model: $cDiff = A * age + B * durCI + C$, where *cDiff* is count difference, *A* is the coefficient associated with the age variable, *B* is the coefficient associated with the duration of cochlear implant usage and *C* is a constant. For both MP and BP stimulation, neither *A* (MP: 4.2, $p = 0.82$; BP: 12.2, $p = 0.08$) nor *B* (MP: 59.0, $p = 0.11$; BP: –12.9, $p = 0.46$) were significantly different than zero indicating that neither age nor *durCI* significantly influenced the count difference. Thus, additional analyses proceeded assuming no effect of age at death or duration of cochlear implant use.

The mean (across subjects) difference for each cochlear segment is negative for both MP and BP stimulation showing a tendency for counts to be lower in the implanted than in the unimplanted ear. Using the *t*-test, we found that the overall mean (across subjects and segments) of the count differences of both Table 7 (MP: –573) and Table 8 (BP: –322) were each significantly less than zero (MP: $t = 2.87$, $df = 37$, $p = 0.003$; BP: $t = -3.96$, $df = 61$, $p < 0.0001$) indicating fewer in the implanted/stimulated ears than in the unimplanted ears.

Because the contacts of an electrode array typically extend from segment I through segment III in the implanted ears, the SGCs in segments I–III are likely to experience stronger stimulus excitation than SGCs in segment IV. We reasoned that if CES provides a trophic influence on SGCs, then the magnitude of the differences between the counts made in the implanted ear and those made in the unimplanted ear in segments I–III should be smaller than the magnitude differences of segment IV. This is consistent with the segment means shown in Table 7 (MP) and 8 (BP) where the magnitude of the individual segment mean differences for segments I–III are smaller (less negative) than those for segment IV. To test this hypothesis statistically, we considered the count differences for segments I–III as a stimulated group and tested whether the magnitudes of the means of these differences (MP: –460; BP: –244) were significantly smaller than the magnitudes of the mean segment-IV count differences (MP: –938; BP: –569) using a *t*-test. The magnitude of the mean difference was significantly lower in segments I–III than segment IV for BP stimulation ($t = 1.75$; $df = 24$; $p = 0.046$) but not for MP stimulation ($t = 0.97$; $df = 12.45$; $p = 0.18$). While the *t*-test results for MP stimulation did not reject the null hypothesis that the mean count difference for segments I–III was greater than or equal to the mean difference for segment IV, this result does not prove that the difference is zero. In fact, given the relatively low statistical power associated with these MP data, the 95% confidence limit of the mean

difference between the count differences (Im-Ui) of the segments I-III and of the segment IV data is within the very broad range of ± 1577 of zero.

3.2. Maximum cross-sectional cell area

The maximum cross-sectional area of 1422 cells was measured in the subject group experiencing MP stimulation: 647 in implanted and 775 in unimplanted ears. In the BP group, the cross-sectional area of 2276 cells was measured: 1095 in implanted and 1181 in unimplanted ears. The cell area ranges from $97 \mu\text{m}^2$ to $1296 \mu\text{m}^2$ in the MP group and from $76 \mu\text{m}^2$ to $1460 \mu\text{m}^2$ in the BP group. These ranges are roughly consistent with the results presented by Nadol (1990) for a normal-hearing temporal bone. The mean cell area was computed for each subject by segment and the results are listed in Table 9 (MP) and 10 (BP). Case 10 of the BP group was not included in the analysis because part of the unimplanted specimen was lost during bone removal. The mean cell area ranges from $241 \mu\text{m}^2$ to $812 \mu\text{m}^2$ in the MP group and from $340 \mu\text{m}^2$ to $864 \mu\text{m}^2$ in the BP group. These ranges are roughly consistent with the results presented by Nadol (1990) for a normal-hearing temporal bone.

The difference (Im-Ui) in mean cell area by segment for each subject is shown in Table 11 (MP) and Table 12 (BP). We tested whether the across-subject and across-segment variation in age at death and duration of cochlear implant usage explained any of the variation in the mean cell area difference using a least-squares fitting procedure to estimate the coefficients of the following linear model: $aDiff = A*age + B*durCI + C$, where $aDiff$ is the difference (Im-Ui) in mean cell area, A is the coefficient associated with the age variable, B is the coefficient associated with the duration of cochlear implant usage and C is a constant. For both MP and BP stimulation, neither A (MP: -0.7 , $p = 0.72$; BP: 0.4 , $p = 0.75$) nor B (MP: 3.1 , $p = 0.0039$; BP: 2.4 , $p = 0.48$) were significantly different than zero indicating that neither age nor $durCI$ significantly influenced the difference in mean cell area. Thus, additional analyses proceeded assuming no effect of age at death or duration of cochlear implant use.

Because the range of mean (across subjects and segments) cell area differences (Im-Ui) is quite large for both the MP ($-323 \mu\text{m}^2$ – $197 \mu\text{m}^2$) and BP ($-348 \mu\text{m}^2$ – $258 \mu\text{m}^2$) groups while the segment mean differences are relatively small ($-9 \mu\text{m}^2$ – $64 \mu\text{m}^2$), it is not surprising that a t -test shows that for both MP and BP stimulation, one is not able to reject the hypothesis that mean difference (Im-Ui) in cell area is zero (i.e. the same in the implanted ears as in the unimplanted ears; MP: $t = 0.62$; $df = 37$; $p = 0.54$; BP: $t = 1.14$; $df = 59$; $p = 0.26$). While this result not prove that the difference is zero, given the statistical power associated with the number of samples and the variance associated with these data, we are able to say with a likelihood of 95% that the mean difference is within $\pm 57 \mu\text{m}^2$ of zero for BP stimulation and within $\pm 70 \mu\text{m}^2$ of zero for MP stimulation.

Unlike the cell count results, one is also unable to reject the hypothesis by t -test that the mean difference (Im-Ui) in cell area for the segments receiving direct stimulation (segments I-II) (MP: $29 \mu\text{m}^2$; BP: $45 \mu\text{m}^2$) is equal to the mean cell area difference of unstimulated segment IV (MP: $9 \mu\text{m}^2$; BP: $15 \mu\text{m}^2$) for both MP ($t = 0.22$; $df = 11$; $p = 0.83$) and BP ($t = 1.18$; $df = 31$; $p = 0.25$) stimulation. The 95% confidence limits for the difference = between = the mean cell area differences (Im-Ui) of the stimulated segments (I-III) and the unstimulated segment (IV) are relatively broad: within $\pm 133 \mu\text{m}^2$ of zero for BP stimulation and within $\pm 165 \mu\text{m}^2$ of zero for MP stimulation.

4. Discussion

4.1. Spiral ganglion cell counts

The total SGC counts for the unimplanted subjects group reported here tend to be lower than the counts by other investigators for other adult, profoundly hearing-impaired populations. For example, the total SGC count mean (8268 across all unimplanted subjects) of this study is significantly lower than those reported by Hinojosa and Marion ((1983); mean: 14,600, t = -3.69, df: 24, p : 0.0009) and Linthicum et al. ((1991); mean: 11,716, t = -2.54, p : 0.009) and lower (but not significantly) than Khan et al. ((2005), mean: 10,286, t = -1.69, df: 31, p : 0.051), Fayad and Linthicum ((2006); mean: 11,762, t = -1.48, df: 14, p : 0.080) and Xu et al. ((2012), mean: 9,906, t = -1.26, df: 9, p : 0.12). While it is possible that a methodological difference between the present study and the previous studies accounts for our lower counts, we are not able to identify a credible possibility and adopt the working hypothesis that our population's SGC counts are lower than many other subject populations studied.

Analysis of the difference between the implanted and unimplanted SGC counts for each cochlear segment in each subject showed that the counts made in the implanted bones are significantly lower than the counts in the unimplanted bones. This finding is in agreement with the results reported by other studies where SGCs were counted in the implanted and unimplanted bones of each subject (e.g., Clark et al., 1988; Fayad et al., 2006; Khan et al., 2005; Marsh et al., 1992; Xu et al., 2012; Zappia et al., 1991). Taken together, these results are consistent with one or more factors associated with implantation and/or stimulation having a deleterious impact on SGC survival.

By separating our count differences into two groups (cochlear segments I-III and segment IV) we showed that the amount by which the implanted ear count is lower than the unimplanted ear in a subject is significantly smaller in the segments I-III group (mean: -249) than in the segment-IV group (mean: -569) for BP stimulation. This result, together with the result described in the previous paragraph leads us to a two part interpretation for profoundly-impaired adult ears: (1) implantation typically leads to degeneration of SGCs resulting in the mean SGC count in an implanted ear being lower than the count in an unimplanted ear and (2) BP stimulation promotes SGC survival relative to no stimulation and leads to less SGC degeneration in stimulated cochlear segments than in the unstimulated segment of the implanted ear. This interpretation is consistent with results reported by Khan et al. (2005) and Xu et al. (2012) where the SGC counts in the implanted ears tended to be less than the unimplanted ears in all cochlear segments but the only segment in which this difference was significant was the most apical segment that did not receive direct electric stimulation.

Our result suggesting that BP stimulation promotes survival of SGCs is consistent with data from a number of animal studies. Leake et al. (1999), in their experiment on 8 kittens with BP stimulation, showed 21% higher SGC density in implanted ears in comparison with unimplanted ears. The increase of SGC happened throughout the whole cochlea except the apical sector which showed no significant difference. Leake et al. (1991) in their earlier study on 4 neonate kittens with a shorter time of stimulation (12 vs. 32–56 weeks) and lower stimulation frequency (30 vs. 80–300 pps) also reported higher SGC density in implanted stimulated ears which was statistically significant and generally restricted to the segments adjacent to the stimulating electrode (basal segments) while the apical sector showed fewer cells but did not reach statistical significance. It should also be noted that a number of animal studies did not find a protective effect for CES (e.g., Araki et al., 1998; Coco et al., 2007; Xu et al., 1997).

MP stimulation did not show a significant protective effect on SGCs in the subject sample of this study. We suggest two possible explanations. First, the protective effect of MP stimulation may be weaker than for BP stimulation in human as has been observed by Leake et al. (1999, 1995) in neonatally-deafened cat. Second, the protective effect of MP stimulation is substantial but the statistical power associated with our MP-stimulation subject sample was not sufficient to find the effect of stimulation significant. For example, post-hoc power analyses showed a power of 0.26 for MP stimulation; about half the power (0.53) associated with our BP-stimulation sample. In order to distinguish between the two explanations, the power associated with the comparison of means would need to be increased (e.g., by increasing the number of subjects and/or decreasing the variance) to increase the likelihood that a significant effect would be detected if actually present.

4.2. Maximum cross-sectional cell area

There are two types of SGCs in Rosenthal's canal: (1) larger type-I cells that innervate inner hair cells and constitute 90–95% of all SGCs and (2) smaller type-II cells that innervate outer hair cells and constitute 5–10% of all SGCs (Nadol et al., 1990). Nadol (1990) and Zimmerman et al. (1995) reported that the maximum cross-sectional area of SGCs from normal-hearing and hearing-impaired ears are distributed bimodally with distribution peaks at approximated $200 \mu\text{m}^2$ and $600 \mu\text{m}^2$ on either side of a minimum at $300 \mu\text{m}^2$. The cell area distributions of the current study did not fit this picture. The distributions (by subject or segment or stimulus configuration) were unimodal with a peak at approximately $520 \mu\text{m}^2$ and range from approximately 75 – $1460 \mu\text{m}^2$. Because of the unimodal distribution of cell area, we did not attempt to divide the population of cells measured into type I or type II categories.

Unlike the cell count data, the maximum cross-sectional cell area measures did not differ significantly between implanted/stimulated and unimplanted ears (see results section) for both MP and BP stimulus configurations. Also unlike the cell count data, the mean cell-area difference between stimulated cochlear segments (segments I-III) was not different than that for the unstimulated segment IV. Taken together, this means we see no evidence of implantation or CES influencing maximum cross-sectional cell area. This result is not consistent with animal studies where differences in cell size between implanted and unimplanted ears have been observed. For instance, Coco et al. (2007), Araki et al. (1998) and Leake et al. (1999) reported larger SGCs associated with BP stimulation. Agterberg et al. (2010) also reported larger SGCs in ears with chronic or brief MP stimulation.

5. Conclusion

The SGC counts made in implanted ears were significantly lower than counts made in unimplanted ears for subjects who experienced either MP or BP stimulation. When the SGC count differences (implanted – unimplanted) in each subject were grouped by segments with stimulating electrodes (segments I-III) and segments without (segment IV), the mean difference for the stimulated segments were smaller than the unstimulated segment for BP stimulation but the effect of stimulation was not found to be significant for MP stimulation. These results are consistent with (1) implantation typically leading to degeneration of SGCs in adult humans and (2) BP stimulation (but not MP) promoting SGC survival relative to no implantation/stimulation. The measurements of maximum cross-sectional cell area did not show a significant influence of implantation or stimulation.

Acknowledgments

This work was supported by grant R01-DC000152 from the National Institute of Deafness and Other Communication Disorders.

References

- Agterberg MJ, Versnel H, de Groot JC, van den Broek M, Klis SF. Chronic electrical stimulation does not prevent spiral ganglion cell degeneration in deafened guinea pigs. *Hear. Res.* 2010; 269:169–179. [PubMed: 20600740]
- Araki S, Kawano A, Seldon L, Shepherd RK, Funasaka S, Clark GM. Effects of chronic electrical stimulation on spiral ganglion neuron survival and size in deafened kittens. *Laryngoscope.* 1998; 108:687–695. [PubMed: 9591547]
- Chatterjee M. Effects of stimulation mode on threshold and loudness growth in multielectrode cochlear implants. *The J. Acoust. Soc. America.* 1999; 105:850–860.
- Chiong CM, Burgess BJ, Nadol JB Jr. Postnatal maturation of human spiral ganglion cells: light and electron microscopic observations. *Hear. Res.* 1993; 67:211–219. [PubMed: 8340274]
- Clark GM, Shepherd RK, Franz BK, Dowell RC, Tong YC, Blamey PJ, Webb RL, Pyman BC, McNaughtan J, Bloom DM, et al. The histopathology of the human temporal bone and auditory central nervous system following cochlear implantation in a patient. Correlation with psychophysics and speech perception results. *Acta Otolaryngol. Suppl.* 1988; 448:1–65. [PubMed: 3176974]
- Coco A, Epp SB, Fallon JB, Xu J, Millard RE, Shepherd RK. Does cochlear implantation and electrical stimulation affect residual hair cells and spiral ganglion neurons? *Hear. Res.* 2007; 225:60–70. [PubMed: 17258411]
- Ernfors P, Duan ML, ElShamy WM, Canlon B. Protection of auditory neurons from aminoglycoside toxicity by neurotrophin-3. *Nat. Med.* 1996; 2:463–467. [PubMed: 8597959]
- Fayad JN, Linthicum FH Jr. Multichannel cochlear implants: relation of histopathology to performance. *Laryngoscope.* 2006; 116:1310–1320. [PubMed: 16885730]
- 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; 295:369–382. [PubMed: 10022958]
- Hartmann R, Topp G, Klinke R. Discharge patterns of cat primary auditory fibers with electrical stimulation of the cochlea. *Hear. Res.* 1984; 13:47–62. [PubMed: 6546751]
- Hinojosa R, Marion M. Histopathology of profound sensorineural deafness. *Ann. N. Y. Acad. Sci.* 1983; 405:459–484. [PubMed: 6575668]
- Hulterantz M, Snyder R, Rebscher S, Leake P. Effects of neonatal deafening and chronic intracochlear electrical stimulation on the cochlear nucleus in cats. *Hear. Res.* 1991; 54:272–280. [PubMed: 1938629]
- Kanzaki S, Stover T, Kawamoto K, Prieskorn DM, Altschuler RA, Miller JM, Raphael Y. Glial cell line-derived neurotrophic factor and chronic electrical stimulation prevent VIII cranial nerve degeneration following denervation. *J. Comp. Neurol.* 2002; 454:350–360. [PubMed: 12442325]
- Khan AM, Handzel O, Damian D, Eddington DK, Nadol JB Jr. Effect of cochlear implantation on residual spiral ganglion cell count as determined by comparison with the contralateral nonimplanted inner ear in humans. *Ann. Otol. Rhinol. Laryngol.* 2005; 114:381–385. [PubMed: 15966525]
- Konigsmark, BW. Methods for the counting of neurons. In: Nauta, WJH.; Ebbsen, SOE., editors. *Contemporary Research Methods in Neuroanatomy.* Springer-Verlag; Berlin: 1970. p. 315–338.
- Leake PA, Hradek GT, Snyder RL. Chronic electrical stimulation by a cochlear implant promotes survival of spiral ganglion neurons after neonatal deafness. *J. Comp. Neurol.* 1999; 412:543–562. [PubMed: 10464355]
- Leake PA, Hradek GT, Rebscher SJ, Snyder RL. Chronic intracochlear electrical stimulation induces selective survival of spiral ganglion neurons in neonatally deafened cats. *Hear. Res.* 1991; 54:251–271. [PubMed: 1938628]
- Leake PA, Snyder RL, Hradek GT, Rebscher SJ. Consequences of chronic extracochlear electrical stimulation in neonatally deafened cats. *Hear. Res.* 1995; 82:65–80. [PubMed: 7744715]
- Linthicum FH Jr. Anderson W. Cochlear implantation of totally deaf ears. Histologic evaluation of candidacy. *Acta Oto-laryngologica.* 1991; 111:327–331. [PubMed: 2068918]
- Lousteau RJ. Increased spiral ganglion cell survival in electrically stimulated, deafened guinea pig cochleae. *Laryngoscope.* 1987; 97:836–842. [PubMed: 3600136]

- Marsh MA, Coker NJ, Jenkins HA. Temporal bone histopathology of a patient with a nucleus 22-channel cochlear implant. *Am. J. Otol.* 1992; 13:241–248. [PubMed: 1609853]
- McGuinness SL, Shepherd RK. Exogenous BDNF rescues rat spiral ganglion neurons in vivo. *Otol Neurotol.* 2005; 26:1064–1072. [PubMed: 16151360]
- Miller JM, Chi DH, O’Keeffe LJ, Kruszka P, Raphael Y, Altschuler RA. Neurotrophins can enhance spiral ganglion cell survival after inner hair cell loss. *Int. J. Dev. Neurosci.* 1997; 15:631–643. [PubMed: 9263039]
- Mitchell A, Miller JM, Finger PA, Heller JW, Raphael Y, Altschuler RA. Effects of chronic high-rate electrical stimulation on the cochlea and eighth nerve in the deafened guinea pig. *Hear. Res.* 1997; 105:30–43. [PubMed: 9083802]
- Nadol JB Jr. Quantification of human spiral ganglion cells by serial section reconstruction and segmental density estimates. *Am. J. Otol.* 1988; 9:47–51. [PubMed: 3059814]
- Nadol JB Jr, Young YS, Glynn RJ. Survival of spiral ganglion cells in profound sensorineural hearing loss: implications for cochlear implantation. *Ann. Otol Rhinol Laryngol.* 1989; 98:411–416. [PubMed: 2729822]
- Nadol JB Jr, Burgess BJ, Reisser C. Morphometric analysis of normal human spiral ganglion cells. *Ann. Otol. Rhinol. Laryngol.* 1990; 99:340–348. [PubMed: 2337313]
- Otte J, Schunknecht HF, Kerr AG. Ganglion cell populations in normal and pathological human cochleae. Implications for cochlear implantation. *Laryngoscope.* 1978; 88:1231–1246. [PubMed: 672357]
- Schuknecht H. Temporal bone removal at autopsy. Preparation and uses. *Arch. Otolaryngol.* 1968; 87:129–137.
- Schuknecht HF. Techniques for study of cochlear function and pathology in experimental animals; development of the anatomical frequency scale for the cat. *AMA Arch. Otolaryngol.* 1953; 58:377–397. [PubMed: 13091542]
- Seyyedi M, Eddington DK, Nadol JB Jr. Interaural comparison of spiral ganglion cell counts in profound deafness. *Hear. Res.* 2011; 282:56–62. [PubMed: 22008826]
- Shepherd RK, Javel E. Electrical stimulation of the auditory nerve. I. Correlation of physiological responses with cochlear status. *Hear. Res.* 1997; 108:112–144. [PubMed: 9213127]
- Shepherd RK, Matsushima J, Martin RL, Clark GM. Cochlear pathology following chronic electrical stimulation of the auditory nerve: II. Deafened kittens. *Hear. Res.* 1994; 81:150–166.
- Spoendlin H. Retrograde degeneration of the cochlear nerve. *Acta Otolaryngol.* 1975; 79:266–275. [PubMed: 1094788]
- Webster M, Webster DB. Spiral ganglion neuron loss following organ of corti loss: a quantitative study. *Brain Res.* 1981; 212:17–30. [PubMed: 7225854]
- 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; 487:147–165. [PubMed: 15880560]
- Xu HX, Kim GH, Snissarenko EP, Cureoglu S, Paparella MM. Multi-channel cochlear implant histopathology: are fewer spiral ganglion cells really related to better clinical performance? *Acta Oto-laryngologica.* 2012; 132:482–490. [PubMed: 22264101]
- Xu J, Shepherd RK, Millard RE, Clark GM. Chronic electrical stimulation of the auditory nerve at high stimulus rates: a physiological and histopathological study. *Hear. Res.* 1997; 105:1–29. [PubMed: 9083801]
- Xu SA, Shepherd RK, Chen Y, Clark GM. Profound hearing loss in the cat following the single co-administration of kanamycin and ethacrynic acid. *Hear. Res.* 1993; 70:205–215. [PubMed: 8294265]
- Ylikoski J, Pirvola U, Moshnyakov M, Palgi J, Arumae U, Saarman M. Expression patterns of neurotrophin and their receptor mRNAs in the rat inner ear. *Hear. Res.* 1993; 65:69–78. [PubMed: 8080462]
- Zappia JJ, Niparko JK, Oviatt DL, Kemink JL, Altschuler RA. Evaluation of the temporal bones of a multichannel cochlear implant patient. *Ann. Otol Rhinol Laryngol.* 1991; 100:914–921. [PubMed: 1746827]

Zimmermann CE, Burgess BJ, Nadol JB Jr. Patterns of degeneration in the human cochlear nerve. *Hear. Res.* 1995; 90:192–201. [PubMed: 8974997]

Fig. 1. Two dimensional reconstruction of left cochlear duct, Rosenthal's canal and path of electrode array. The vertical line divides Rosenthal's canal to 4 segments. The black circles on Rosenthal's canal and on the cochlear duct are 1 mm apart.

Table 1

MP subjects

Case	Diagnosis	Gender	Age at death (years)	CI use (years)	Ear implanted	Device	Stimulus configuration
1	Idiopathic	F	87	21	AD	Ineraid	MP
2	Idiopathic	M	55	15	AD	Ineraid	MP
3	Otosclerosis	M	83	12	AS	Ineraid	MP
4	Idiopathic	M	89	6	AD	Nucleus 24	MP
5	Meniere's	M	70	3	AD	Nucleus 24	MP
6	Otosclerosis	M	84	10	AS	Ineraid	MP
7	Idiopathic	F	80	9	AD	Nucleus 24	MP
8	Otosclerosis	F	69	4	AS	Nucleus 24	MP
9	Idiopathic	F	64	4	AS	Nucleus 24	MP
10	Idiopathic	M	72	5	AS	Nucleus 24	MP

CI: Cochlear implant; AD: Right; AS: Left; MP: Monopolar.

Table 2

BP subjects

Case	Diagnosis	Gender	Age at death (years)	CI use (years)	Ear implanted	Device	Stimulus configuration
1	Idiopathic	M	42	1	AD	Nucleus 22	BP
2	Idiopathic	M	78	4	AS	Nucleus 22	BP
3	Otosclerosis	M	87	3	AD	Nucleus 22	BP
4	Idiopathic	F	73	6	AS	Nucleus 22	BP
5	Idiopathic	F	53	6	AS	Nucleus 22	BP
6	Meniere's	M	67	7	AS	Nucleus 22	BP
7	Noise Trauma	M	66	15	AD	Nucleus 22	BP
8	Viral Labyrinthitis	M	67	2	AD	Nucleus 22	BP
9	Coch.Sac.Deg	F	64	9	AS	Nucleus 22	BP
10	Idiopathic	M	80	6	AS	Nucleus 22	BP
11	NFII	M	70	11	AD	Nucleus 22	BP
12	Idiopathic	M	75	12	AD	Nucleus 22	BP
13	Idiopathic	F	77	18	AS	Nucleus 22	BP
14	Idiopathic	M	92	12	AD	Nucleus 22	BP
15	Ototoxicity	F	74	15	AD	Nucleus 22	BP
16	Meniere's	M	92	11	AS	Nucleus 22	BP

CI: Cochlear implant; AD: Right; AS: Left; BP: Bipolar.

Table 3

MP group preimplantation pure-tone audiometry

Case	Left						Right					
	250	500	1000	2000	4000	8000	250	500	1000	2000	4000	8000
1	–	NR ₉₀	85	95	NR ₁₀₀	–	–	NR ₉₀	85	95	NR ₁₀₀	–
2	NR ₁₀₅	NR ₁₂₀	NR ₁₂₀	NR ₁₂₀	NR ₁₂₀	NR ₁₁₀	NR ₁₀₅	NR ₁₂₀	NR ₁₂₀	NR ₁₂₀	NR ₁₂₀	NR ₁₁₀
3	90	95	110	NR ₁₁₅	–	–	90	95	110	NR ₁₁₅	–	–
4	–	65	80	NR ₁₁₀	NR ₁₁₀	NR ₁₀₀	–	65	80	NR ₁₁₀	NR ₁₁₀	NR ₁₀₀
5	–	110	105	110	115	NR ₁₁₀	–	120	115	NR ₁₂₀	NR ₁₂₀	NR ₁₁₀
6	–	NR ₁₁₀	NR ₁₁₀	NR ₁₂₀	NR ₁₂₀	NR ₁₁₀	–	NR ₁₁₀	NR ₁₁₀	NR ₁₂₀	NR ₁₂₀	NR ₁₁₀
7	–	105	110	NR ₁₂₀	NR ₁₂₀	NR ₁₁₀	–	NR ₁₁₅	120	NR ₁₂₀	NR ₁₂₀	NR ₁₁₀
8	–	NR ₁₀₀	NR ₁₀₀	NR ₁₀₀	NR ₁₀₀	NR ₁₀₀	–	NR ₁₀₀	NR ₁₀₀	NR ₁₀₀	NR ₁₀₀	NR ₁₀₀
9	–	90	90	95	NR ₉₀	–	–	90	90	95	NR ₉₀	–
10	80	90	105	115	NR ₁₁₅	NR ₁₁₅	90	95	110	105	NR ₁₁₅	NR ₁₁₅

NR: No response; “–”: no information available.

Table 4

BP group preimplantation pure-tone audiometry

Case	Left					Right						
	250	500	1000	2000	4000	8000	250	500	1000	2000	4000	8000
1	NR ₁₀₀	NR ₁₁₀	NR ₁₁₀	NR ₁₁₀	NR ₁₁₀	NR ₁₀₀	NR ₁₀₀	NR ₁₁₀	NR ₁₁₀	NR ₁₁₀	NR ₁₁₀	NR ₁₀₀
2	75	90	105	NR ₁₁₅	NR ₁₁₅	NR ₁₀₀	85	100	110	NR ₁₁₅	NR ₁₁₅	NR ₁₀₀
3	NR ₁₀₀	NR ₁₀₀	NR ₁₂₀	110	115	NR ₁₀₀	NR ₁₀₀	NR ₁₀₀	NR ₁₂₀	110	NR ₁₂₀	NR ₁₀₀
4	105	115	NR ₁₂₀	NR ₁₂₀	NR ₁₂₀	NR ₁₁₀	105	110	NR ₁₂₀	NR ₁₂₀	NR ₁₂₀	NR ₁₁₀
5	NR ₁₀₀	NR ₁₁₅	NR ₁₁₅	NR ₁₁₅	NR ₁₁₅	NR ₁₀₀	NR ₁₀₀	NR ₁₁₅	NR ₁₁₅	NR ₁₁₅	NR ₁₁₅	NR ₁₀₀
6	NR ₁₀₀	NR ₁₁₅	NR ₁₁₅	NR ₁₁₅	NR ₁₁₅	NR ₁₀₀	NR ₁₀₀	NR ₁₁₅	NR ₁₁₅	NR ₁₁₅	NR ₁₁₅	NR ₁₀₀
7	NR ₁₀₀	NR ₁₁₅	NR ₁₁₅	NR ₁₁₅	NR ₁₁₅	NR ₁₀₀	90	NR ₁₁₅	NR ₁₁₅	NR ₁₁₅	NR ₁₁₅	NR ₁₀₀
8	–	NR ₁₂₀	NR ₁₂₅	NR ₁₂₅	NR ₁₂₀	NR ₁₁₀	–	NR ₁₂₀	NR ₁₂₅	NR ₁₂₅	NR ₁₂₀	NR ₁₁₀
9	90	NR ₁₁₀	NR ₁₁₀	NR ₁₁₀	NR ₁₁₀	NR ₁₀₀	90	100	NR ₁₁₀	NR ₁₁₀	NR ₁₁₀	NR ₁₀₀
10	NR ₁₀₅	NR ₁₂₀	NR ₁₂₀	NR ₁₂₀	NR ₁₂₀	NR ₁₁₀	NR ₁₀₅	NR ₁₂₀	NR ₁₂₀	NR ₁₂₀	NR ₁₂₀	NR ₁₁₀
11	90	NR ₁₁₀	NR ₁₁₀	NR ₁₁₀	NR ₁₁₀	NR ₁₁₀	90	NR ₁₁₀	NR ₁₁₀	NR ₁₁₀	NR ₁₁₀	NR ₁₁₀
12	–	85	85	115	NR ₁₁₅	–	–	80	95	110	NR ₁₁₅	–
13	–	110	110	NR ₁₁₅	NR ₁₁₅	NR ₁₁₅	–	110	110	NR ₁₁₅	NR ₁₁₅	NR ₁₁₅
14	80	85	90	90	80	90	70	90	95	95	90	95
15	–	90	90	90	90	90	–	90	90	90	90	90
16	NR ₁₀₀	NR ₁₀₅	NR ₁₀₅	NR ₁₀₅	NR ₁₁₀	NR ₁₁₀	NR ₁₀₀	NR ₁₀₅	NR ₁₀₅	NR ₁₀₅	NR ₁₁₀	NR ₁₁₀

NR: No response; “–”: no information available.

Table 5

MP group corrected segmental and total SGC counts

Case	Segment I		Segment II		Segment III		Segment IV		Total	
	Im	Un	Im	Un	Im	Un	Im	Un	Im	Un
1	1958	1466	5896	3454	2978	2720	2781	2645	13613	10285
2	728	1897	5270	5392	3563	3679	2591	3543	12152	14511
3	1680	1136	4216	–	2264	3169	1825	–	9985	–
4	170	619	687	938	1455	2421	741	3516	3053	7494
5	517	1258	2700	2938	2216	2203	1075	1496	6508	7895
6	428	449	1442	3835	1374	2428	1448	2944	4692	9656
7	2258	1557	5229	5025	3271	2925	3706	3665	14464	13172
8	68	571	320	4604	163	3094	75	3346	626	11615
9	768	1099	3726	4447	1347	2312	1129	1142	6970	9000
10	333	428	2407	2468	2278	2298	2237	1932	7255	7126

Im: Implanted ears; Un: Unimplanted ears.

Table 6

BP group corrected segmental and total SGC counts

Case	Segment I		Segment II		Segment III		Segment IV		Total	
	Im	Un	Im	Un	Im	Un	Im	Un	Im	Un
1	1251	3142	4903	5970	3318	3182	2863	3040	12335	15334
2	1754	1346	1714	2332	748	1285	1027	613	5243	5576
3	1040	1822	4257	4379	1319	2305	2897	2740	9513	11246
4	748	1047	1856	2754	700	1870	674	2142	3978	7813
5	619	415	4617	5243	3325	3631	2849	3427	11410	12716
6	1496	1564	5481	5392	3577	4379	3230	3815	13784	15150
7	88	292	2849	2693	2271	3237	1516	3387	6724	9608
8	1204	558	2876	2516	2074	1890	1618	2713	7772	7677
9	415	1000	2115	3570	1442	2550	1734	1952	5706	9072
10	721	326	3284	–	3176	1945	3046	–	10227	–
11	258	48	1060	422	544	265	197	333	2059	1067
12	843	734	898	748	1000	1061	1489	1904	4230	4447
13	1163	1197	1353	2482	680	1210	1136	1421	4332	6310
14	666	1027	1931	2434	1979	2094	2230	2496	6806	8051
15	1190	1285	2434	2624	1047	1401	1285	2060	5956	7371
16	524	462	2591	1604	1713	1557	1054	2285	5882	5909

Im: Implanted ears; Un: Unimplanted ears.

Table 7

MP group segmental count differences

Case	SGC count difference (implanted-unimplanted)			
	Segment I	Segment II	Segment III	Segment IV
1	492	2442	258	136
2	-1169	-122	-116	-952
3	544	-	-905	-
4	-449	-251	-966	-2775
5	-741	-238	13	-421
6	-21	-2393	-1054	-1496
7	701	204	346	41
8	-503	-4284	-2931	-3271
9	-331	-721	-965	-13
10	-95	-61	-20	305
Segment mean	-157	-603	-634	-938

Table 8

BP group segmental count differences

Case	SGC count difference (implanted-unimplanted)			
	Segment I	Segment II	Segment III	Segment IV
1	-1891	-1067	136	-177
2	408	-618	-537	414
3	-782	-122	-986	157
4	-299	-898	-1170	-1468
5	204	-626	-306	-578
6	-68	89	-802	-585
7	-204	156	-966	-1871
8	646	360	184	-1095
9	-585	-1455	-1108	-218
10	395	-	1231	-
11	210	638	279	-136
12	109	150	-61	-415
13	-34	-1129	-530	-285
14	-361	-503	-115	-266
15	-95	-190	-354	-775
16	62	987	156	-1231
Segment mean	-143	-282	-309	-569

Table 9MP group mean cell areas (μm^2)

Case	<u>Segment I</u>		<u>Segment II</u>		<u>Segment III</u>		<u>Segment IV</u>	
	Im	Un	Im	Un	Im	Un	Im	Un
1	466	569	467	618	569	434	524	503
2	497	505	621	424	491	372	522	382
3	500	556	514	–	512	601	603	–
4	812	693	595	544	679	639	718	693
5	443	345	282	249	241	290	355	248
6	731	642	556	578	710	649	530	568
7	453	415	432	318	507	316	476	329
8	452	618	418	583	510	701	390	713
9	624	658	510	569	617	472	573	568
10	464	445	504	583	470	483	517	415

Im: Implanted ears; Un: Unimplanted ears.

Table 10BP group mean cell areas (μm^2)

Case	<u>Segment I</u>		<u>Segment II</u>		<u>Segment III</u>		<u>Segment IV</u>	
	Im	Un	Im	Un	Im	Un	Im	Un
1	575	599	680	569	692	616	576	676
2	724	656	570	557	567	569	460	587
3	495	551	546	550	440	611	498	404
4	471	452	444	415	531	341	443	523
5	808	755	597	660	510	528	614	700
6	611	499	669	611	497	487	556	628
7	604	857	498	845	474	575	429	545
8	516	747	630	584	674	613	728	599
9	526	444	567	548	723	567	663	644
11	694	436	663	773	712	469	521	597
12	864	642	591	707	657	658	654	569
13	489	486	536	361	593	363	565	460
14	488	376	444	431	495	366	467	396
15	539	492	530	468	595	448	603	495
16	400	411	464	480	431	427	448	556

Im: Implanted ears; Un: Unimplanted ears.

Table 11MP group cell area (μm^2) differences

Case	Cell area difference (μm^2 , implanted-unimplanted)			
	Segment I	Segment II	Segment III	Segment IV
1	-104	-151	135	21
2	-8	197	118	141
3	-57	-	-89	-
4	119	52	40	25
5	98	33	-49	107
6	89	-21	61	-38
7	38	113	191	147
8	-166	-166	-191	-323
9	-35	-59	145	5
10	19	-79	-13	101
Segment mean	-1	-9	35	21

Table 12BP group cell area (μm^2) differences

Case	Cell area difference (μm^2 , implanted-unimplanted)			
	Segment I	Segment II	Segment III	Segment IV
1	-24	111	77	-100
2	68	13	-2	-127
3	-56	-5	-171	94
4	18	30	190	-80
5	53	-63	-18	-87
6	112	58	10	-72
7	-253	-348	-101	-116
8	-230	46	61	130
9	82	19	156	19
11	258	-110	243	-76
12	222	-116	-1	85
13	3	175	230	104
14	111	13	130	71
15	47	61	146	108
16	-12	-15	4	-108
Segment mean	27	-9	64	-10