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Glial Cell Line-Derived Neurotrophic Factor and antioxidants preserve the electrical responsiveness of the spiral ganglion neurons after experimentally induced deafness

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Abstract

Cochlear implant surgery is currently the therapy of choice for profoundly deaf patients. However, the functionality of cochlear implants depends on the integrity of the auditory spiral ganglion neurons. This study assesses the combined efficacy of two classes of agents found effective in preventing degeneration of the auditory nerve following deafness, neurotrophic factors and antioxidants. Guinea pigs were deafened and treated for 4 weeks with either local administration of GDNF or a combination of GDNF and systemic injections of the antioxidants ascorbic acid and Trolox. The density of surviving spiral ganglion cells was significantly enhanced and the thresholds for eliciting an electrically-evoked brain stem response were significantly reduced in GDNF treated animals compared to deafened-untreated. The addition of antioxidants significantly enhanced the evoked responsiveness over that observed with GDNF alone. The results suggest multiple sites of intervention in the rescue of these cells from deafferentation-induced cell death.

Keywords

Antioxidants; GDNF; spiral ganglion; cochlea; inner ear; neurotrophic factor; intervention; deafferentation; electrical; auditory brainstem response

Introduction

Cochlear implants have become widely established as the treatment of choice for profoundly deaf patients who derive little or no benefit from conventional hearing aids. The principle of the cochlear implant is to by-pass the damaged sensory cells and to directly stimulate neural pathways, thus eliciting a sensation of hearing despite missing sensory receptors. The efficacy of cochlear implants is thought to be related to the number and functional state of the remaining

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spiral ganglion neurons (e.g. Nadol et al., 1989; Incesulu and Nadol, 1998). Thus, interventions to prevent degeneration of auditory sensory neurons would be of therapeutic significance and lead to increased benefits of cochlear implants.

The concept that enhanced survival of spiral ganglion cells provides a better substrate for cochlear implants has been tested in various animal models. The neurotrophic factor hypothesis (see Mattson, 1998 for a review) suggests that deafferentation (in this case, loss of sensory cells) induces a neurotrophic factor deprivation in the afferent nerve fibers, leading to formation of free radicals and up-regulation of cell death pathways. Consistent with that hypothesis, we have demonstrated that a combination of the neurotrophic factors BDNF (brain-derived neurotrophic factor) and CNTF (ciliary neurotrophic factor) applied directly to the inner ear fluids significantly increase both the population of surviving spiral ganglion neurons following deafening, and the efficacy of electrical stimulation as measured by electrically evoked brainstem responses (Shinohara et al., 2002). This is true also if neurotrophic intervention is initiated after delay periods of up to 6 weeks (Yamagata et al., 2004). Ylikoski and co-workers (1998) have previously shown that intracochlear infusion of another neurotrophic factor, GDNF (glial cell-derived neurotrophic factor), enhances the survival of cochlear neurons after noise-induced inner hair cell lesions. They did, however, not investigate the electrical responsiveness of the afferent system. Also the antioxidants ascorbic acid (vitamin C) and Trolox (a water soluble vitamin E analog) reduce deafferentation-induced spiral ganglion cell death (Maruyama et al., 2007), presumably by scavenging neurotrophic factor deprivation-induced free radicals. In these studies, an enhanced survival of spiral ganglion neurons was accompanied by significantly lower thresholds for electrical stimulation.

The purpose of this study was two fold: 1) to test whether GDNF, like BDNF and CNTF, could enhance the electrical responsiveness of the spiral ganglion neurons, and 2) to investigate whether a combination of local treatment with GDNF and systemically administered antioxidants would offer additive protection against auditory nerve degeneration in the deafened ear. Our results demonstrate an enhanced electrically evoked responsiveness in the deafened auditory system treated with GDNF, and an even better effect using the combination of GDNF and antioxidants. These findings are significant both for our understanding of the mechanisms of rescue of the auditory nerve from cell death and for the development of interventions to tissue engineer the auditory nerve to enhance benefits of cochlear implants for the severely hearing impaired.

Materials and methods

Experimental design

An overview of the experimental design is shown in Fig 1. Thirty-two guinea pigs were divided into four groups as follows: (1) Control group; (2) Untreated group; (3) GDNF group; and (4) GDNF+AO group. The Untreated group and the two treatment groups, GDNF and GDNF+AO, were deafened by intracochlear infusion of 10% neomycin for two days. This was achieved by using osmotic pumps (Alzet model 2002, Alza Corp., USA) and priming the cannula (connecting to the inner ear) with 24μl of 10% neomycin (delivered at 0.5 μl/h). For undeafened control animals the cannula was primed with 24μl of artificial perilymph (AP) consisting of 0.1 % guinea pig serum albumin in lactated Ringer's solution. The 2-day treatment (neomycin or AP) was followed by four weeks of delivery of AP for the deafened-untreated group, or GDNF (10μg/ml in AP; Amgen Inc. USA) or GDNF+AO for the two deafened-treatment groups. In the undeafened-control group and untreated group, AP was administered for four weeks via the osmotic pump. The osmotic pump was changed at after two weeks, and the infusion continued through experimental week 4. The GDNF+AO group received daily intraperitoneal injections of the antioxidants (AO) ascorbic acid (20mg/kg/day) and Trolox (1mg/kg/day) for four weeks; while all other animals received an equal volume of saline. Trolox

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(Fluka Chemie, Switzerland) was dissolved in 0.154 N NaOH, neutralized by 0.154 N HCl. The antioxidant solution, prepared immediately before administration, was adjusted to pH 7.2–7.4 with 1N NaOH after addition of ascorbic acid (Sigma-Aldrich Co. Ltd., Germany).

Subjects

A total of 32 pigmented guinea pigs (250 ~ 400 g) with normal Preyer reflexes were used in this study. The animals were anesthetized with xylazine (10 mg/kg i.m.) and ketamine (40 mg/kg i.m.) during the implant surgery and all recording sessions. All animal procedures were performed in accordance with ethical standards of Karolinska Institutet and consistent with national regulations for the care and use of animals (approval no. N113/01).

Implant surgery

All animals underwent aseptic surgery to implant intracochlear stimulating electrodes and an epidural recording electrode as described previously (Maruyama et al., 2007). The left middle ear was exposed by a postauricular approach and a cochleostomy was performed on the basal turn of the cochlea to allow access to scala tympani. A ball electrode was inserted through the round window membrane and placed into scala tympani to elicit electrical auditory brain stem responses (eABRs). The ball electrode was constructed using 75 μm diameter Pt-Ir 90%/10% wire, Teflon-insulated (Advent Research Materials Ltd., England). The return electrode was a Teflon-insulated Pt-Ir wire, 125 μm in diameter (Advent Research Materials Ltd., England). The return electrode was stripped of insulation for 5 mm and placed in the left middle ear. Prior to implantation, each stimulating electrode surface was activated to a stable minimal impedance value (2–3 kΩ) using waveform generator (33120A; Hewlett Packard, USA) for 30 minutes at 2 volts, 3 Hz (triangular wave) in saline. Both electrodes were connected to a percutaneous socket (Scott Electronics, USA), secured with carboxylate dental cement. The epidural recording electrode was a stainless steel screw, implanted at the vertex. The intracochlear cannula was inserted 0.5mm through a carefully made fenestra in the otic capsule 2 mm ventral to the round window, sealed and secured, as described previously (Shinohara et al., 2002). The bulla defect was covered with carboxylate dental cement to fix the electrode and the cannula to the temporal bone. All procedures were performed under aseptic conditions. The tetracycline antibiotic doxycyclin (Nordic drugs, Sweden) was administered after surgery as a prophylactic procedure.

Electrically-evoked auditory brainstem responses (eABRs)

The animals were anesthetized (as described above) and placed in a sound-proof room. First eABRs were recorded on day 3 and then weekly for six weeks. The recording procedures have been described in detail previously (Yamagata et al., 2004; Maruyama et al., 2007). In short, thresholds were defined as the lowest stimulus level that evoked at least a 0.4μV reproducible waveform. Averages of 2048 responses to 50-μs computer-controlled monophasic current pulses, presented at 50pps with alternative polarity were amplified using a preamplifier (HS4, Tucker-Davis Technologies Inc., USA) and recorded between the screw on the vertex (active) and a needle inserted at the left mastoid (reference) by an analogue to digital converter (Tucker-Davis Technologies Inc., USA). Ground was a needle electrode inserted subcutaneously at the neck. High- and low-pass filters were set at 8 Hz and 15 kHz, respectively, and a gain of 1000 was used. Stimulus current was generated by a custom-built isolated constant current stimulator (C. Ellinger, Kresge Hearing Research Institute, USA) and monitored using an oscilloscope (PM3226, Philips, Germany). The intensity of the stimulus current was changed from approximately 30 μA to 800 μA in 50 μA steps (in 10 μA steps near the threshold) in order to determine the stimulus intensity required to obtain a reproducible waveform, and to obtain response amplitudes, here defined as the amplitude of P1, the first positive wave following the stimulus artifact (cf. Fig 1 in Maruyama et al., 2007).

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Histology

To determine the survival of spiral ganglion neurons, the animals were sacrificed by cardiac perfusion (saline followed by 3% glutaraldehyde in 0.1M phosphate buffer) after the final eABR measurement. Following decalcification in 0.1M EDTA and embedding in paraffin, 4-μm sections were cut along the paramodiolar plane. Every third section was mounted on a glass slide and stained with toluidine blue. Six sections were randomly selected from the ten most mid-modiolar sections for each animal and used for quantitative analysis of spiral ganglion neurons. The counting was performed double blind. All neurons meeting size and shape criteria to be considered type I spiral ganglion neurons (i.e., cell diameter 12–25 μm with a nucleus 5–10 μm in diameter) within each profile of Rosenthal's canal from base to apex of the cochlea were counted. The outline of the Rosenthal's canal profile was then traced to generate a spiral ganglion neuron density, expressed as the number of spiral ganglion neurons for an area of 10,000 μm^2.

Statistical analysis

Statistical assessment of differences in eABR thresholds and spiral ganglion neuoron density between the groups was performed using Mann-Whitney's U test.

Results

Electrophysiological findings

Electrically-evoked auditory brainstem responses (eABR) were recorded from day 3 and then weekly throughout the experiments in order to monitor threshold changes related to the different treatment protocols. Thresholds in the three deafened groups (untreated, GDNF and GDNF+AO) were elevated compared with the control group (receiving no neomycin and no treatment) as early as day 3, demonstrating that the 2-day intracochlear infusion of neomycin caused rapid inner ear injury. The eABR thresholds (as well as P1 amplitudes) in the control group were stable (35~50 μA) throughout the experiment. The mean eABR thresholds and standard deviations in each experimental group are shown in Figure 2 as a function of time. Following an initial slight increase in eABR thresholds in the two treatment groups (GDNF and GDNF+AO), the thresholds started to decrease. Already at week 2 the thresholds in both treatment groups were statistically significantly lower than in the control group. The treatment-induced reduction in threshold continued throughout the treatment period and was maintained after the cessation of the 4-week treatment (i.e., at weeks 5–6). The mean eABR threshold in the GDNF treated group was 125 μA at 6 weeks after deafening. For the animals receiving both intracochlear GDNF and intraperitoneal injections of antioxidants, this effect on eABR threshold was significantly greater, with a threshold of 94 μA at 6 weeks ($p < 0.01$). These values should be compared to the mean threshold in the untreated group, which had reached 256 μA at this time point, i.e. more than twice that observed in the treated subjects. From week 2 and onwards, there were statistically significant differences between the GDNF group and the untreated group ($p<0.01$ at weeks 2–6). Animals receiving both GDNF and antioxidants (GDNF+AO) displayed further reduced eABR thresholds; significantly reduced compared to the untreated group as early as one week following the initiation of treatment throughout the experiment ($p<0.01$ at weeks 1–6). Interestingly, there were significant differences between the GDNF and GDNF+AO groups, especially at weeks 3–6 ($p<0.01$).

The response amplitudes, here defined as the amplitude of the first positive wave, P1, varied significantly across the four groups. The P1 amplitudes at the highest level of stimulation (800 μA) are shown in Figure 3. In normal hearing subjects PI amplitudes were 60 – 65μV and stable throughout the study. P1 amplitudes in the deafened-untreated group started at approximately 40 μV and then dropped to below 20 μV. Interestingly, the P1 amplitudes in the treated groups (GDNF and GDNF + AO) started at about the same level as that of the deafened-untreated

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group at day 3 but then increased to above 50 μV by week 2 and was maintained at this level through week 6.

Input-output functions of the P1 amplitude, indicating the dynamic range of the electrically-evoked response, are shown in Figure 4 for each experimental group. In the control group (normal hearing, no treatment; Fig. 4A), P1 amplitudes were stable throughout the experiment whereas in the untreated group (deafened, no treatment; Fig 4B), the input-output function shifted significantly downwards already by day 3 after deafening. This was followed by a gradual further decrease in P1 amplitudes. The amplitudes stabilized at about week 3. Deafened-treated groups (GDNF and GDNF+AO; Fig 4C and D, respectively), demonstrated a marked initial reduction in P1 amplitudes compared to the control subjects. However, at week 2, the input-output functions shifted upwards and remained relatively stably elevated for the remaining study period (also after ending the treatment). The GDNF+AO treated group displayed slightly higher amplitudes compared to the subjects receiving GDNF alone. Thus, the treatment enhanced sensitivity and the dynamic range of evoked responsiveness of the auditory nerve.

Histological findings

Representative sections of the basal cochlear turn at week 6 in each experimental group are shown in Figure 5. In all deafened groups (Figs. 4A–C), the organ of Corti was severely damaged or completely missing. The density of spiral ganglion neurons in Rosenthal's canal differed between the groups. While there were very few neurons remaining following 6 weeks of deafness in the untreated animal (Fig. 4C), the number of spiral ganglion neurons in the treated animals (Figs. 4A and B) seemed comparable to the control ear (Fig. 4D). This was confirmed by estimating the density of surviving spiral ganglion neurons throughout the entire cochlea, calculated as the mean spiral ganglion density in sections of Rosenthal's canal along a midmodiolar plane of the cochlea (Fig. 6). In the control group, the mean spiral ganglion density was 8.0 cells/10,000mm^2 whereas for the untreated group it was nearly 4 times lower (2.1 cells/10,000mm^2). In the GDNF and GDNF+AO treated groups, mean spiral ganglion density was 7.0 and 7.4 cells/10,000mm^2, respectively. These values while close to the cell density of the control group, they are statistically significantly lower. In addition, there was a distinct and statistically significant difference between each of the two treatment groups compared to the untreated group ($p=0.0008$). However, there was no significant difference between the GDNF and GDNF+AO groups ($p>0.05$).

Discussion

Pharmacological treatment of the inner ear is rapidly becoming a realistic possibility. Several experimental studies have demonstrated that certain drugs can prevent and treat noise-induced hearing loss (Le Prell et al, 2007a, b), suggesting that the clinical treatment of hearing impairment will dramatically change in the near future. It has been suggested that when a neuron is deafferented, the subsequent loss of neurotrophic factor support will lead to an altered oxidative state of the cell (e.g. Dugan et al., 1997; Mattson, 1998). The formation of reactive oxygen species (ROS) then injures cellular components and eventually induces apoptosis or necrosis (Davies, 1996; Greenlund, et al., 1995; Deshmukh and Johnson, 1997; 1998; Van De Water et al., 2004). Based on this concept, there are two classes of drugs, which are especially interesting for inner ear treatment, neurotrophic factors and antioxidants.

Neurotrophic factors are macromolecules that affect development and survival of neurons in the central and peripheral nervous systems. In the auditory system, BDNF (brain-derived neurotrophic factor) and NT-3 (neurotrophin-3) are known to contribute to neurogenesis (Staecker et al., 1996a; Sokolowski, 1997). In situ hybridization has shown that BDNF and NT-3 are synthesized in the sensory epithelium of the otic vesicle, and that their high-affinity

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receptors, trkB and trkC, are present in cochlear ganglion neurons of mammals (Pirvola et al., 1992, 1994; Schecterson and Bothwell, 1994, Wheeler et al., 1994). Moreover, BDNF and NT-3 have both been shown to protect auditory neurons from neural degeneration caused by ototoxic drugs in vitro (Zheng and Gao, 1996; Gabaizadeh et al., 1997b), and, more importantly, to prevent spiral ganglion cell degeneration following loss of hair cells in vivo (Staecker et al, 1996b; Ernfors et al., 1996; Miller et al., 1997). GDNF (glial cell line-derived neurotrophic factor) appears to be of less importance during early inner ear development but to be a significant survival factor for the neonatal and adult cochlea (Ylikoski et al., 1998). Ylikoski and colleagues (1998) have shown that GDNF is synthesized by the inner hair cells but GDNF has also been shown present in the spiral ganglion (Stöver et al., 2001). GDNF signaling occurs through a receptor complex consisting of the GDNF family receptor α1 (GFRα1) and Ret (encoded by the *c-ret* protooncogene), although a signaling pathway independent of Ret has been demonstrated (see Trupp et al., 1999 and Sariola and Saarma, 2003 for overviews and references). Ylikoski et al. (1998) reported the expression of GFRα1 in the rat auditory spiral ganglion neurons. This was later confirmed by Stöver et al. (2001), who also showed the presence of Ret in the spiral ganglion cells. A positive effect of GDNF was shown by Ylikoski et al. (1998), demonstrating that intracochlear infusion of GDNF enhanced the survival of the spiral ganglion neurons after noise-induced inner hair cell damage in the guinea pig. The beneficial effect of GDNF on spiral ganglion cell survival was further studied in vitro by Wei et al. (2007), who also showed the presence of GFR α1, GFR α2, GF α3 as well as Ret.

Several studies have thus demonstrated that neurotrophic factors can enhance spiral ganglion neuron survival after deafness. However, from a clinical point of view, e.g. in combination with a cochlear implant device to electrically stimulate auditory neurons, it is crucial to demonstrate that surviving spiral ganglion neurons maintain their electrical excitability. Shinohara et al. (2002) thus reported that administration of BDNF and CNTF not only enhanced spiral ganglion neuron survival, but also improved the sensitivity of the electrically evoked auditory brainstem responses (eABRs). Yamagata et al. (2004) extended this observation, demonstrating that delayed treatment (up to six weeks following deafening) resulted in significantly lower response thresholds and survival of spiral ganglion neurons. The present study demonstrates that GDNF, when administered locally to the cochlea directly after deafening, enhances the electrical responsiveness, as shown by significantly lower eABR thresholds compared to untreated animals (cf. Fig 2). The positive effect may to a large extent be explained by the increased survival of spiral ganglion neurons, the number of which were not very much lower than in control (not deafened) animals (cf. Fig 6). The results confirm the positive effect on spiral ganglion cell survival previously reported (e.g. Ylikoski et al., 1998; Wei et al., 2007) and, more importantly, extend the observations by and Shinohara et al. (2002) in two vital aspects. First, the positive effects on the eABR thresholds were maintained for at least two weeks after the cessation of the GDNF administration. This finding differs from the results by Gillespie et al. (2003), where the positive effects of BDNF on spiral ganglion cell survival disappeared within two weeks. This discrepancy is likely due to differences in the action of BDNF and GDNF. Nevertheless, our findings may suggest that after a critical period of time following inner ear trauma, endogenous survival factors are able to maintain the remaining spiral ganglion cell population. Second, during the GDNF treatment period, the eABR thresholds gradually decreased and at the time of treatment cessation, were clearly lower than at the start of GDNF application (cf. Fig 2). This could reflect a “recovery” of surviving spiral ganglion neurons following exposure to the ototoxic drug and perhaps a change in membrane characteristics (e.g. membrane capacitance) enhancing the electrical excitability of the tissues. It is also possible that a neurotrophic factor-induced growth of peripheral processes towards the stimulating electrode may contribute to reduced response thresholds (Altschuler et al., 1999).

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ROS are highly reactive molecules and thought to be involved in ageing and in virtually all neurodegenerative disease processes. An increased production of ROS has been demonstrated in the cochlea following exposure to intense noise or ototoxic compounds (e.g. Ravi et al., 1995; Rybak et al., 1995; Sha and Schacht, 1999; Ohinata et al., 2000; Le Prell et al., 2007b). However, as ROS formation is a consequence of most normally occurring cellular processes, the organism has several endogenous mechanisms to control the effects of ROS. These mechanisms include free radical scavengers (e.g., vitamins C and E, iron chelators, alpha-lipoic acid) and antioxidant enzymes (e.g., superoxide dismutase). It has been shown experimentally that administering antioxidants can protect the inner ear against noise and drug induced damage (e.g. Seidman et al., 1993; Song and Schacht, 1996; Rybak et al., 1999; Teranishi et al., 2001; Le Prell et al, 2007a). Yamagata and co-workers (2007) recently demonstrated that a combination of the antioxidants vitamin C and E (ascorbic acid and Trolox) significantly enhanced both the survival of spiral ganglion neurons and the electrical responsiveness following experimental deafening. The positive effect was evident both when the antioxidants were applied directly to the cochlea using osmotic pumps and when administered systemically by intraperitoneal injections.

The relation between neurotrophic factor deprivation and increased intracellular ROS levels as proposed by the neurotrophic factor hypothesis (Mattson, 1998) and experimentally demonstrated in the inner ear (Gabaizadeh et al., 1997a), suggests that a combination therapy including both neurotrophic factors and antioxidants would be of interest for inner ear treatment. This hypothesis was tested in the present study, by combining local, intracochlear GDNF treatment with systemic administration of the antioxidants ascorbic acid (vitamin C) and Trolox (a water soluble vitamin E analog). As can be seen in Figure 2, the addition of the antioxidants resulted in a statistically significant improvement of the eABR thresholds, maintained through and beyond the treatment period. The combined treatment caused only a slight difference in maximal P1 amplitude (cf. Fig. 3) while there was a more distinct effect on the P1 input-output functions (cf. Fig. 4) suggesting that the combined treatment enhanced sensitivity and dynamic range). The reason for the lack of a similarly positive effect of the combined treatment on spiral ganglion cell survival can only be speculated on. It cannot be ruled out that the histological evaluation was not sensitive enough to detect a possible difference. However, it is also possible that the antioxidant effect was not only mediated by increased cell survival (which would have been reflected in the spiral ganglion cell density) but possibly by an enhanced electrical responsiveness caused by, for example, better preservation of neural processes or altered membrane properties.

The previous study by Maruyama et al. (2007) showed that the effects on eABR thresholds by the antioxidants alone were in the same range as the present GDNF effects. The fact that the improvement obtained by administering also antioxidants was relatively small may suggests synergism between the two groups of compounds rather than an additive effect. However, the present experimental protocol does not allow us to define the interaction between the treatments. Nonetheless, the present observation is important in that it indicates that the best treatment effect is likely to be provided by a combination of different pharmacological compounds acting at multiple sites in the auditory system.

For an inner ear treatment strategy, antioxidants would clearly offer two main advantages: (i) under normal circumstances they have no deleterious side effects, and (ii) they are easy to administer, either systemically as in the present study or as a pill for oral use. The ease of use and safety make antioxidants very interesting but the qualitative differences in their effects on spiral ganglion neuron function compared to the neurotrophic factors should be noted. As mentioned previously, GDNF has a pronounced effect on electrical responsiveness, causing the eABR thresholds to improve beyond the acute stage when treatment was initiated (cf. Fig 2). A similar effect was reported by Yamagata et al. when administering BDNF and CNTF up

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to six weeks following deafness (cf. Fig. 3 in Yamagata et al. 2004). Although antioxidants also had a significant effect on eABR levels, they failed to improve the response beyond the initial values, irrespectively of applying the compounds directly to the cochlea or systemically (cf. Figs. 3 and 6 in Maruyama et al. 2007). Neurotrophic factors may thus in the long-term be of greater clinical interest, at least for severe inner ear degeneration. In the treatment of deaf patients with cochlear implants, we have an opportunity to offer local neurotrophic factor treatment. There are several studies aiming at investigating their effects on neurodegenerative disorders such as Huntington's disease and Parkinson's disease (e.g. Alberch et al., 2004;Kirik et al., 2004) and the results from such studies will be very valuable for appreciating not only the beneficial effects of neurotrophic factors but, importantly, also their side effects.

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References

Alberch J, Perez-Navarro E, Canals JM. Neurotrophic factors in Huntington's disease. Prog Brain Res 2004;146:195–229. [PubMed: 14699966]

Altschuler RA, Cho Y, Ylikoski J, Pirvola U, Magal E, Miller JM. Rescue and regrowth of sensory nerves following deafferentation by neurotrophic factors. Ann NY Acad Sci 1999;884:305–311. [PubMed: 10842602]

Davies AM. The neurotrophic hypothesis: where does it stand? Philosophical Transactions of the Royal Society of London - Series B Biolog Sci 1996;351:389–394.

Deshmukh M, Johnson EM Jr. Programmed cell death in neurons: focus on the pathway of nerve growth factor deprivation-induced death of sympathetic neurons. Molecular Pharmacol 1997;51:897–906.

Deshmukh M, Johnson EM Jr. Evidence of a novel event during neuronal death: development of competence-to-die in response to cytoplasmic cytochrome c. Neuron 1998;21:695–705. [PubMed: 9808457]

Dugan LL, Creedon DJ, Johnson EM Jr, Holtzman DM. Rapid suppression of free radical formation by nerve growth factor involves the mitogen-activated protein kinase pathway. Proc Natl Acad Sci USA 1997;94:4086–4091. [PubMed: 9108109]

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]

Gabaizadeh R, Staecker H, Liu W, Van De Water TR. BDNF protection of auditory neurons from cisplatin involves changes in intracellular levels of both reactive oxygen species and glutathione. Brain Res Mol Brain Res 1997a;50:71–78. [PubMed: 9406919]

Gabaizadeh R, Staecker H, Liu W, Kopke R, Malgrange B, Lefebvre PP, Van de Water TR. Protection of both auditory hair cells and auditory neurons from cisplatin induced damage. Acta Otolaryngol 1997b;117:232–238. [PubMed: 9105457]

Gillespie LN, Clark GM, Bartlett PF, Marzella PL. BDNF-induced survival of auditory neurons in vivo: Cessation of treatment leads to accelerated loss of survival effects. J Neurosci Res 2003;71:785–790. [PubMed: 12605404]

Greenlund LJ, Deckwerth TL, Johnson EM Jr. Superoxide dismutase delays neuronal apoptosis: a role for reactive oxygen species in programmed neuronal death. Neuron 1995;14:303–315. [PubMed: 7857640]

Incesulu A, Nadol JB Jr. Correlation of acoustic threshold measures and spiral ganglion cell survival in severe to profound sensorineural hearing loss: implications for cochlear implantation. Ann Otol Rhinol Laryngol 1998;107:906–111. [PubMed: 9823838]

Kirik D, Georgievska B, Björklund A. Localized striatal delivery of GDNF as a treatment for Parkinson disease. Nat Neurosci 2004;7:105–110. [PubMed: 14747832]

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Le Prell CG, Hughes LF, Miller JM. Free radical scavengers vitamins A, C, and E, plus magnesium reduces noise trauma. Free Radic Biol Med 2007a;42:1454–1463. [PubMed: 17395018]

Le Prell CG, Yamashita D, Minami S, Yamasoba T, Miller JM. Mechanisms of noise-induced hearing loss indicate multiple methods of prevention. Hear Res 2007b;226:22–43. [PubMed: 17141991]

Maruyama J, Yamagata T, Ulfendahl M, Bredberg G, Altschuler RA, Miller JM. Effects of antioxidants on auditory nerve function and survival in deafened guinea pigs. Neurobiol Dis 2007;25:309–318. [PubMed: 17112730]

Mattson, MP. Neuroprotective strategies based on targeting of postreceptor signaling events. In: Mattson, MP., editor. Neuroprotective Signal Transduction. Humana Press; New Jersey: 1998. p. 301-335.-345.

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]

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]

Ohinata Y, Miller JM, Altschuler RA, Schacht J. Intense noise induces formation of vasoactive lipid peroxidation products in the cochlea. Brain Res 2000;878:163–173. [PubMed: 10996147]

Pirvola U, Arumae U, Moshnyakov M, Palgi J, Saarma M, Ylikoski J. Coordinated expression and function of neurotrophins and their receptors in the rat inner ear during target innervation. Hear Res 1994;75:131–144. [PubMed: 8071140]

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;89:9915–9999. [PubMed: 1409719]

Ravi R, Somani SM, Rybak LP. Mechanism of cisplatin ototoxicity: antioxidant system. Pharmacol Toxicol 1995;76:386–394. [PubMed: 7479581]

Rybak LP, Ravi R, Somani SM. Mechanism of protection by diethyldithiocarbamate against cisplatin ototoxicity: antioxidant system. Fundam Appl Toxicol 1995;26:293–300. [PubMed: 7589918]

Rybak LP, Husain K, Whitworth C, Somani SM. Dose dependent protection by lipoic acid against cisplatin-induced ototoxicity in rats: antioxidant defense system. Toxicol Sci 1999;47:195–202. [PubMed: 10220857]

Sariola H, Saarma M. Novel functions and signaling pathways for GDNF. J Cell Science 2003;116:3855–3862. [PubMed: 12953054]

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

Seidman MD, Shivapuja BG, Quirk WS. The protective effects of allopurinol and superoxide dismutase on noise-induced cochlear damage. Otolaryngol Head Neck Surg 1993;109:1052–1056. [PubMed: 8265189]

Sha SH, Schacht J. Stimulation of free radical formation by aminoglycoside antibiotics. Hear Res 1999;128:112–118. [PubMed: 10082291]

Shinohara T, Bredberg G, Ulfendahl M, Pyykkö I, Olivius NP, Kaksonen R, Lindström B, Altschuler R, Miller JM. Neurotrophic factor intervention restores auditory function in deafened animals. Proc Natl Acad Sci U S A 2002;99:1657–1660. [PubMed: 11818566]

Sokolowski BH. Quantitative analysis of long-term survival and neuritogenesis in vitro: cochleovestibular ganglion of the chick embryo in BDNF, NT-3, NT-4/5, and insulin. Exp Neurol 1997;145:1–15. [PubMed: 9184104]

Song BB, Schacht J. Variable efficacy of radical scavengers and iron chelators to attenuate gentamicin ototoxicity in guinea pig in vivo. Hear Res 1996;94:87–93. [PubMed: 8789814]

Staecker H, Van De Water TR, Lefebvre PP, Liu W, Moghadassi M, Galinovic-Schwartz V, Malgrange B, Moonen G. NGF, BDNF and NT-3 play unique roles in the in vitro development and patterning of innervation of the mammalian inner ear. Brain Res Dev Brain Res 1996a;92:49–60.

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 1996b;7:889–894. [PubMed: 8724667]

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Stöver T, Nam YJ, Gong TWL, Lomax MI, Altschuler RA. Glial cell line-derived neurotrophic factor (GDNF) and its receptor complex are expressed in the auditory nerve of the mature rat cochlea. Hearing Res 2001;155:143–151.

Teranishi M, Nakashima T, Wakabayashi T. Effects of alpha-tocopherol on cisplatin-induced ototoxicity in guinea pigs. Hear Res 2001;151:61–70. [PubMed: 11124452]

Trupp M, Scoss R, Whittemore SR, Ibáñez CF. Ret-dependent and –independent mechanisms of glial cell line-derived neurotrophic factor signaling in neuronal cells. J Biol Chem 1999;274:20885–20894. [PubMed: 10409632]

Van De Water TR, Lallemend F, Eshraghi AA, Ahsan S, He J, Guzman J, Polak M, Malgrange B, Lefebvre PP, Staecker H, Balkany TJ. Caspases, the enemy within, and their role in oxidative stress-induced apoptosis of inner ear sensory cells. Otol Neurotol 2004;25:627–632. [PubMed: 15241246]

Wei D, Jin Z, Järlebark J, Scarfone E, Ulfendahl M. Survival, synaptogenesis and regeneration of adult mouse spiral ganglion neurons in vitro. J Neurobiol 2007;67:108–122.

Wheeler EF, Bothwell M, Schecterson LC, von Bartheld CS. Expression of BDNF and NT-3 mRNA in hair cells of the organ of Corti: quantitative analysis in developing rats. Hear Res 1994;73:46–56. [PubMed: 8157505]

Yamagata T, Miller JM, Ulfendahl M, Olivius NP, Altschuler RA, Pyykkö I, Bredberg G. Delayed neurotrophic treatment preserves nerve survival and electrophysiological responsiveness in neomycin-deafened guinea pigs. J Neurosci Res 2004;78:75–86. [PubMed: 15372491]

Ylikoski J, Pirvola U, Virkkala J, Suvanto P, Liang XQ, Magal E, Altschuler R, Miller JM, Saarma M. Guinea pig auditory neurons are protected by glial cell line-derived growth factor from degeneration after noise trauma. Hear Res 1998;124:17–26. [PubMed: 9822899]

Zheng JL, Gao WQ. Differential damage to auditory neurons and hair cells by ototoxins and neuroprotection by specific neurotrophins in rat cochlear organotypic cultures. Eur J Neurosci 1996;8:1897–1905. [PubMed: 8921280]

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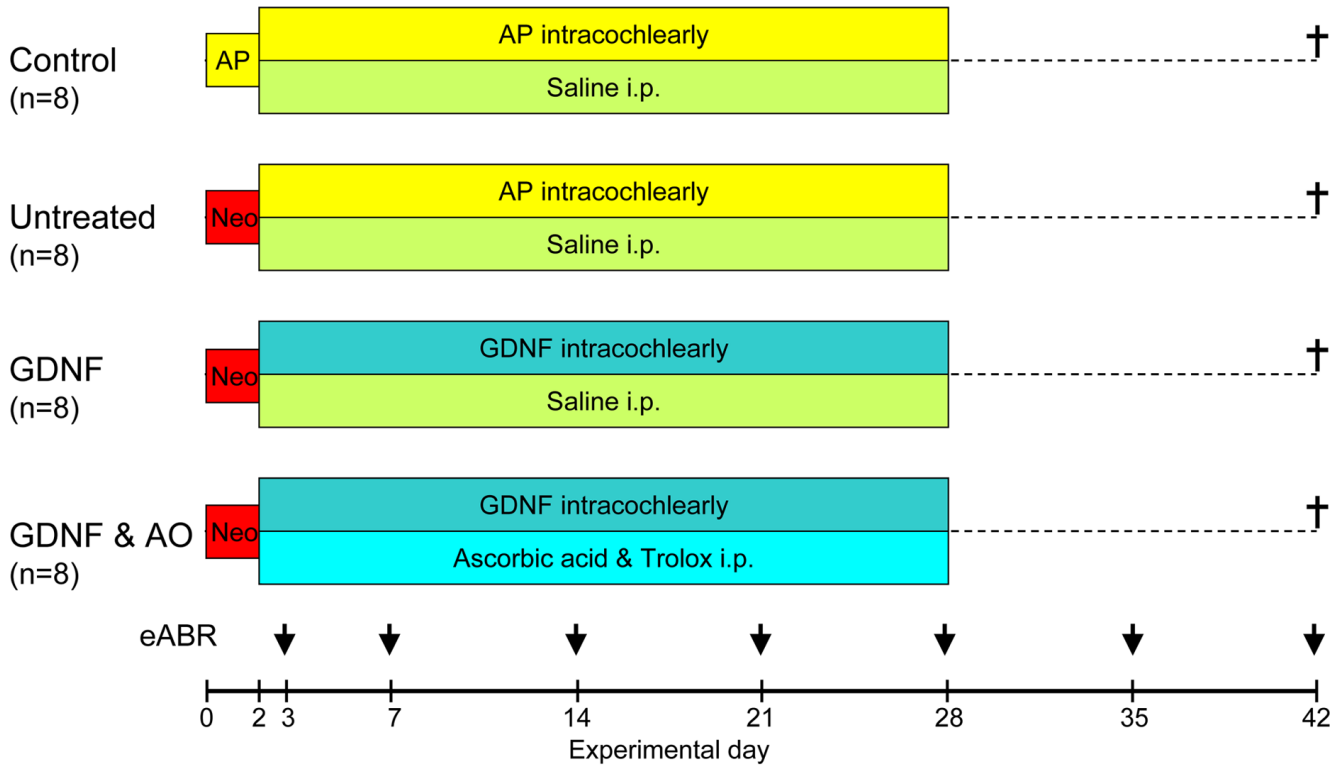

Figure 1.

Graphical presentation of the experimental design. The four groups received identical treatment but were administered different compounds. Immediately after a two-day infusion into the cochlea (artificial perilymph, AP, or neomysin, Neo), the animals received intracochlear infusions of AP or GDNF for almost four weeks (Day 2–28). During the same time period, saline or a mixture of the antioxidants ascorbic acid and Trolox was administered intraperitoneally (i.p.) daily. After a two-week follow-up period, the animals were sacrificed (†) for histological evaluation. Throughout the experimental protocol, electrically-evoked auditory brainstem responses (eABR) were obtained (↓).

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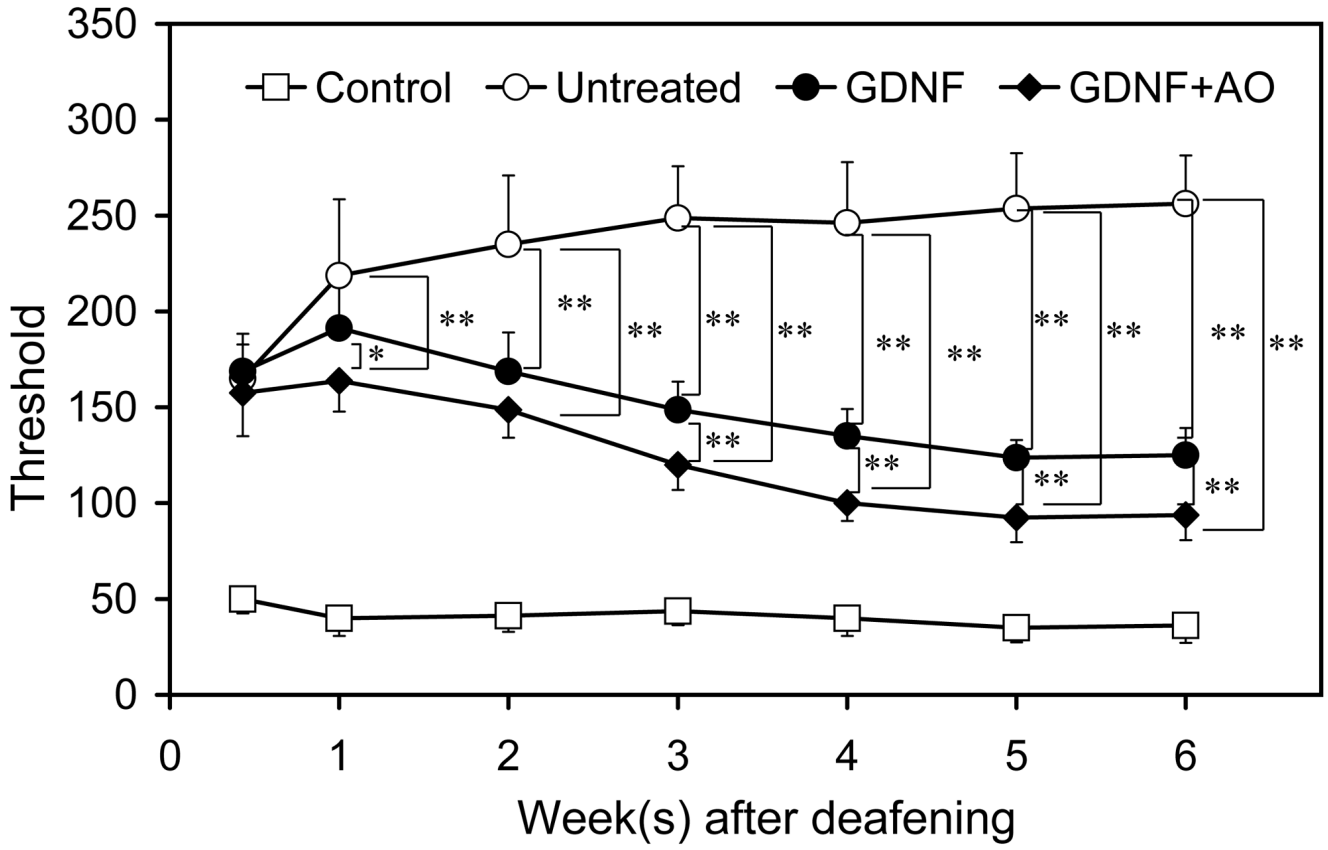

Figure 2.

Mean eABR thresholds in untreated and treated (GDNF and GDNF+AO) guinea pigs compared to control subjects (not deafened). The vertical bars indicate one standard deviation. Already at 2 weeks, all treated animals (unfilled symbols) expressed significantly lower eABR thresholds compared to the untreated animals (filled circles). The animals receiving both GDNF and antioxidants (GDNF+AO) had significantly lower thresholds than animals given only GDNF, at least from week 3 and onwards. Measurements at weeks 5 and 6 were made one and two weeks, respectively, after the cessation of treatment. * $p<0.05$, ** $p<0.01$.

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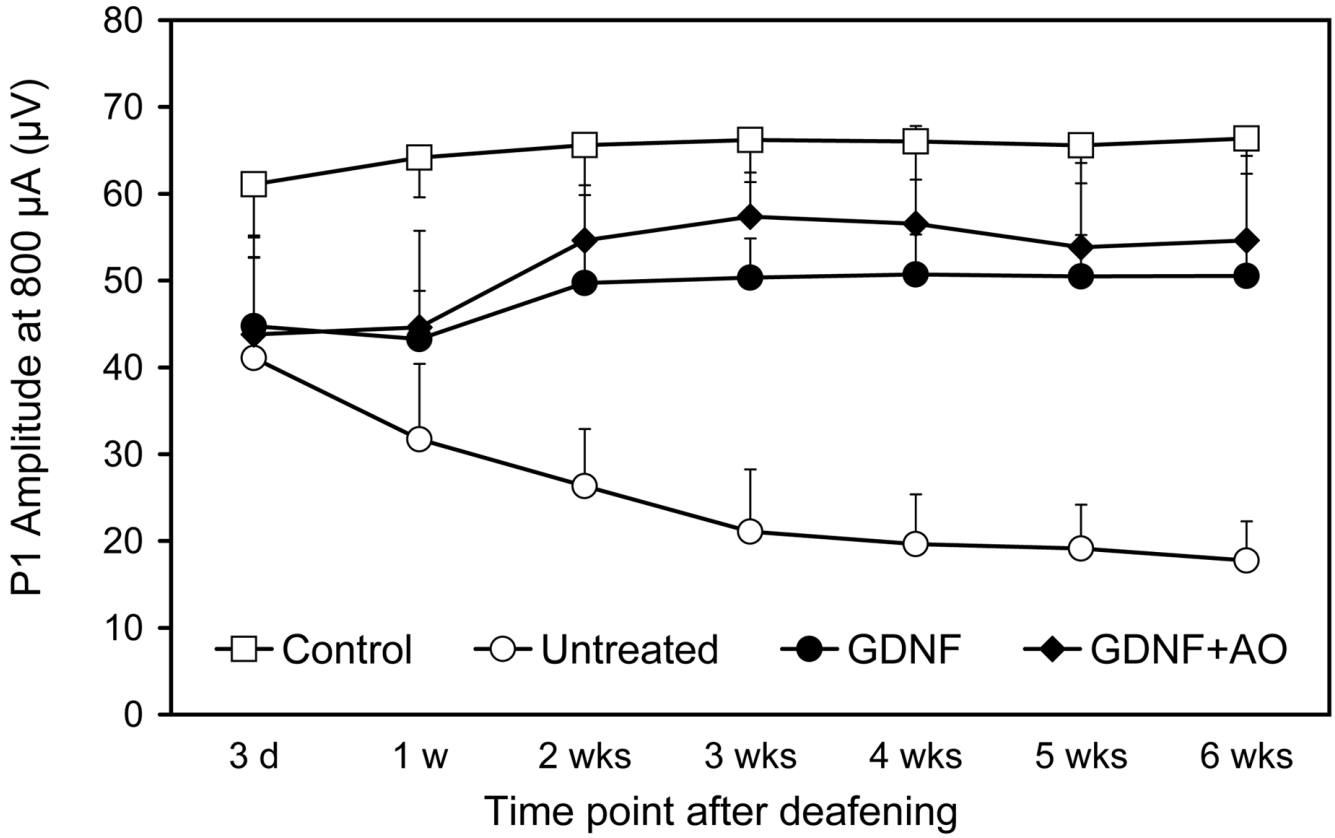

Figure 3.
P1 amplitudes at a stimulus level of 800 μA at different time points after deafening. The P1 amplitudes increased in the treated groups (GDNF and GDNF + AO) during the treatment period.

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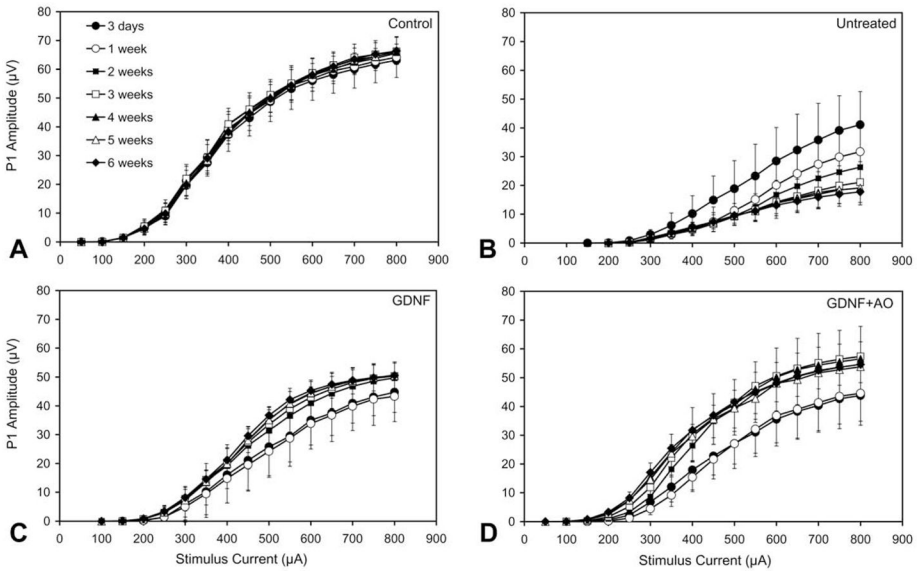

Figure 4.

Input-output functions of the P1 amplitude at different time points for each experimental group (A, control; B, untreated; C, GDNF; D, GDNF + AO). In the untreated group (deafened, no treatment; B), the input-output functions waere shifted downwards throughout the 6-week period, most drastically during the first two weeks. In the two treatment groups (GDNF, C; GDNF+AO, D) the amplitudes were stable for about a week and then increased.

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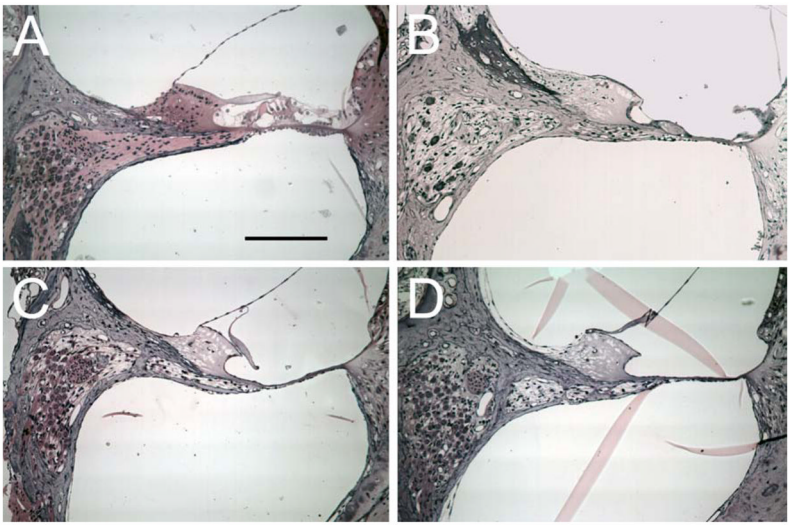

Figure 5.
Light micrographs illustrating representative sections of the hearing organ region and Rosenthal's canal in the basal part of the cochlea from (A) control, (B) untreated, (C) GDNF, and (D) GDNF + AO groups. There was a dramatic loss of spiral ganglion neurons in Rosenthal's canal in the untreated animal (B) whereas a relatively large cell population remained in the treated cochleas (C and D). Scale bar, 200 μm.

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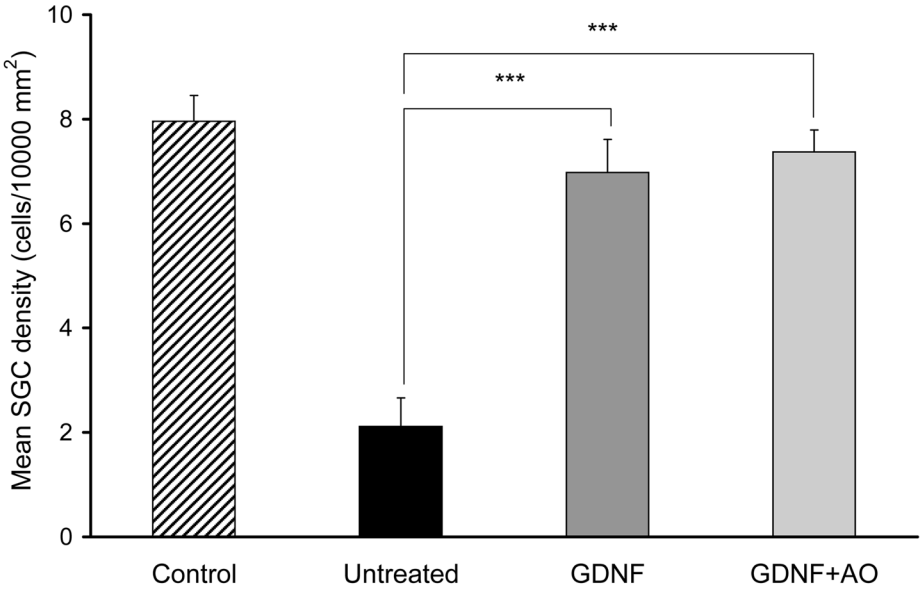

Figure 6.

Mean spiral ganglion cell (SGC) density in the four experimental groups. The vertical bars indicate one standard deviation. Spiral ganglion density was significantly higher in the GDNF and GDNF + AO groups as compared to the untreated group (***p<0.001).