Ninth Quarterly Progress Report

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Protective Effects of Patterned Electrical Stimulation on the Deafened Auditory System

R. K. Shepherd, S. B. Epp, J. M. Crook and A. Serruto,

Department of Otolaryngology
University of Melbourne
32 Gisborne Street
East Melbourne 3002,
Victoria, AUSTRALIA

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Contents:

1. Introduction

The goal of this contract is to develop methods of protecting the remaining portions of the auditory system from degeneration after loss of hair cells and to improve its effectiveness in extracting information provided by auditory prostheses. We have taken a broad neurobiological approach to this goal in order to study both the short and long-term response of the auditory system to loss of hair cells and the subsequent introduction of afferent input via an auditory prosthesis. Our studies are divided into three major areas of investigation:

(a) The neurophysiological and neuroanatomical response to prolonged electrical stimulation of the auditory nerve following a neonatal sensorineural hearing loss (SNHL). This work is designed to provide insight into the protective effects of electrical stimulation on the auditory nerve (AN) and the plastic response of the central auditory system (CAS) to temporally challenging stimuli presented chronically to one or two sectors of the AN.

(b) The neurophysiological and neuroanatomical response of the AN and CAS following chronic intracochlear electrical stimulation in combination with neurotrophic support of the auditory nerve. This work is designed to investigate whether electrical stimulation and chronic administration of neurotrophins act in synergy to promote AN survival. This work will also provide insight into the role of neurotrophins in improving synaptic efficiency in the deafened auditory pathway.

(c) The neurophysiological and neuroanatomical response to acute electrical stimulation of the auditory nerve following a neonatal SNHL. These studies are designed to provide insight into the acute response of the AN and CAS to intracochlear electrical stimulation in deafened animals with little prior auditory experience.

While these studies are designed to provide insight into the plastic response of the deafened auditory pathway to re-activation via an auditory prosthesis, a major objective of this work is to apply our findings to the clinical environment.

2. Summary of activities for the quarter

During the ninth quarter of this contract the following activities were completed:

- Continued histological analysis of cochleae and auditory brainstem structures in both cat and guinea pig following completion of their chronic electrical stimulation programs. A detailed summary of the cochlear histology of deafened guinea pigs treated with chronic electrical stimulation and neurotrophin delivery is presented in this report.
- Continued developing our immunocytochemical protocols.
- Continued deafening and preparing rat cochleae and CNS tissue for ongoing neuroanatomical and neurochemical studies of the deafened auditory system (see *Fourth Quarterly Progress Report).*
- Attended and presented our results at the 33rd NIH Neural Prosthesis Workshop, Bethesda, MD.
- Continued recording from the inferior colliculus of neonatally deafened unstimulated control cats, and completed experiments recording from the

auditory cortex in severely deafened, chronically stimulated cats. The latter experiments were performed in collaboration with Prof. Dexter Irvine and Dr. Mel Brown of Monash University.

- Completed and submitted three chapters for "Neuroprosthetics: Theory and Practice", edited by K. W. Horch and G. Dhillon, World Scientific Publishing.

3. Electrical stimulation and neurotrophin administration in deafened guinea pigs

A major study associated with this contract is the investigation of the extent of protective effects of patterned electrical stimulation in association with simultaneous neurotrophin delivery to the deafened cochlea. Loss of spiral ganglion neurons (SGNs) following a SNHL is considered, at least in part, a result of the withdrawal of neurotrophic support normally provided by hair cells (Ylikoski et al., 1993; Fig. 1).

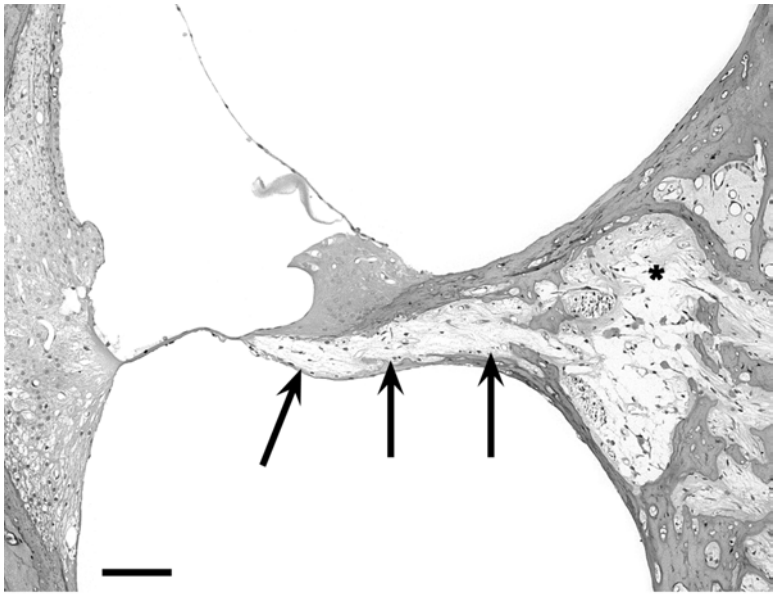

Figure 1. Significant loss of SGNs in Rosenthal's canal occurs following loss of hair cells. Loss of trophic support normally provided by the hair cells is considered a major influence on SGN loss. In this case the animal had been deafened for a period of 12 months using an aminoglycoside antibiotic. Both the organ of Corti and the peripheral processes (pp) normally innervating the organ of Corti, have undergone complete degeneration in this region of the cochlea. The SGN loss was ~90%. Arrows illustrate the OSL devoid of pp; * location of SGNs within Rosenthal's canal. Bar=100 μm (from Shepherd and Hardie, 2001).

We have completed the first phase of our study of chronic electrical stimulation/ neurotrophin administration in guinea pigs. The present report will describe in detail our histological results. These data compliment the electrophysiological data presented from the same animals described in our *Eighth Quarterly Progress Report*.

3.2 Methods

Detailed methods have been presented in our *Eighth Quarterly Progress Report*. Briefly, twenty guinea pigs were used in this study. The animals were divided into four treatment groups (Table 1). Each animal was deafened using a single co-administration of Kanamycin and Frusemide (*Fourth Quarterly Progress Report*). Five days following deafening, Auditory Brainstem Responses (ABRs) were recorded to confirm the hearing loss (only animals with click thresholds >92 dB SPL in both ears were accepted for this study). Deafened animals were then *unilaterally* implanted with a scala tympani electrode array incorporating a drug delivery system (Shepherd and Xu, 2002). The electrode array consists of three platinum ring electrodes located within the guinea pig scala tympani, and a micro-delivery system connected to a 200 μl mini-osmotic pump (Alzet 2004). The contents of the osmotic pump are, therefore, delivered to the scala tympani of the cochlea through the tip of the electrode array. This pump has a flow rate of 0.25 μl/hour, providing a continuous infusion period of 28 days.

We compared the chronic infusion of artificial perilymph (AP) with the neurotrophin Brain Derived Neurotrophic Factor (BDNF). This neurotrophin was selected following encouraging results in previous *in vitro* (Marzella et al., 1999) and *in vivo* application (Staecker et al., 1996; Miller et al., 1997; Gillespie et al., 2003).

Details of the implant surgery were presented in our *Fourth Quarterly Progress Report*. Immediately following implant surgery electrically evoked auditory brainstem responses (EABRs) were recorded from each of the three bipolar scala tympani electrode pairs. Five days following implant surgery, two of the four treatment groups commenced a chronic electrical stimulation program (Table 1). Details of the electrical stimulus waveform and the portable stimulators have been presented previously (*Second Quarterly Progress Report*). Briefly, the output of the stimulator generates charge balanced biphasic current pulses non-simultaneously to two electrode pairs at a stimulus rate of 1200 pulses per second (pps) per channel and is amplitude-modulated (AM) to a depth of 50% at 30 Hz. In the present study only a single bipolar electrode pair is used. The current pulse is 100 μs/phase with a 10 μs interphase gap. Electrode shorting and capacitive coupling are used to ensure complete charge recovery. The amplitude of the AM stimulus waveform was set so that the maximum stimulus intensity was 6 dB above EABR threshold. These stimulus levels were confirmed to be acceptable in the awake animal using basic behavioral indicators.

The stimulators are carried in a harness worn by the guinea pig to enable continuous stimulation without confining the animal's daily activities. Each animal was stimulated approximately 6 h per day, five days per week from day 10 to day 33. Both stimulus current and electrode voltage waveforms are monitored twice daily and the electrode impedance is therefore monitored longitudinally over the implant period (see *Third Quarterly Progress Report* for further details). The impedances are typically within 1.5-6.5 kΩ; the normal range observed for this type of electrode assembly (Xu et al., 1997). Finally, at completion of the 28-day implantation period, EABRs were again recorded in order to assess the functional status of the auditory pathway. On completion of these recordings each animal

was euthanased with an overdose of anesthetic and systemically perfused. The cochleae and brain were harvested for anatomical analysis.

Table 1: Summary of treatment groups

Treatment group	Implant duration (days)	Chronic electrical stimulation	Contents of osmotic pump
BDNF/ES[1]	28	Yes	BDNF[2]
BDNF/C[3]	28	No	BDNF
AP/ES	28	Yes	Artificial perilymph[4]
AP/C	28	No	Artificial perilymph

Notes: [1] ES denotes chronic electrical stimulation; [2] 62.5 μg of BDNF/ml in 0.1% guinea pig albumin in 200 μl of Ringer's solution; [3]C denotes control (i.e. electrode assembly implanted but no chronic electrical stimulation); [4] 200 μl Ringer's solution.

3.3 Results

Representative low power micrographs of cochlear turn 1, 2 & 3 from a deafened animal that had received chronic electrical stimulation and BDNF delivery to the left cochlea (left panel) are shown in Fig. 2. For comparison, photomicrographs from similar regions in the deafened, unimplanted, contralateral control cochlea are also illustrated (right panel). There are a number of histological features of note. First, both cochleae exhibit a complete absence of the organ of Corti in the basal turns 1 and 2, while turn 3 shows evidence of collapsed support cells presumably in the process of degeneration. Second, SGNs within Rosenthal's canal and their peripheral processes, even at this low magnification, show far greater level of survival in the stimulated/BDNF treated cochlea (left), compared with the unimplanted, deafened control (right). Third, there is no evidence of extensive mechanical disruption to the treated cochlea, such as displacement of Reissner's or the basilar membrane, following chronic BDNF infusion at a rate of 0.25 μl/hour. Finally, while a fine fibrous tissue capsule is evident in the basal turn of the treated cochlea, illustrating the location of the electrode array, there is no evidence of any extensive tissue response throughout the cochlea.

Figure 3 illustrates the Rosenthal's canal region of the two cochleae shown in Figure 2. The virtually normal SGN packing density in all three turns of the stimulated/BDNF treated cochlea (left panel), contrasts with the extensive degeneration apparent in the untreated, deafened control cochlea (right panel). Although not clearly illustrated in this figure, the packing density of peripheral processes in the stimulated/BDNF treated cochlea was, at least under visual inspection, far greater than that evident in the untreated control cochlea in all cochlear turns examined.

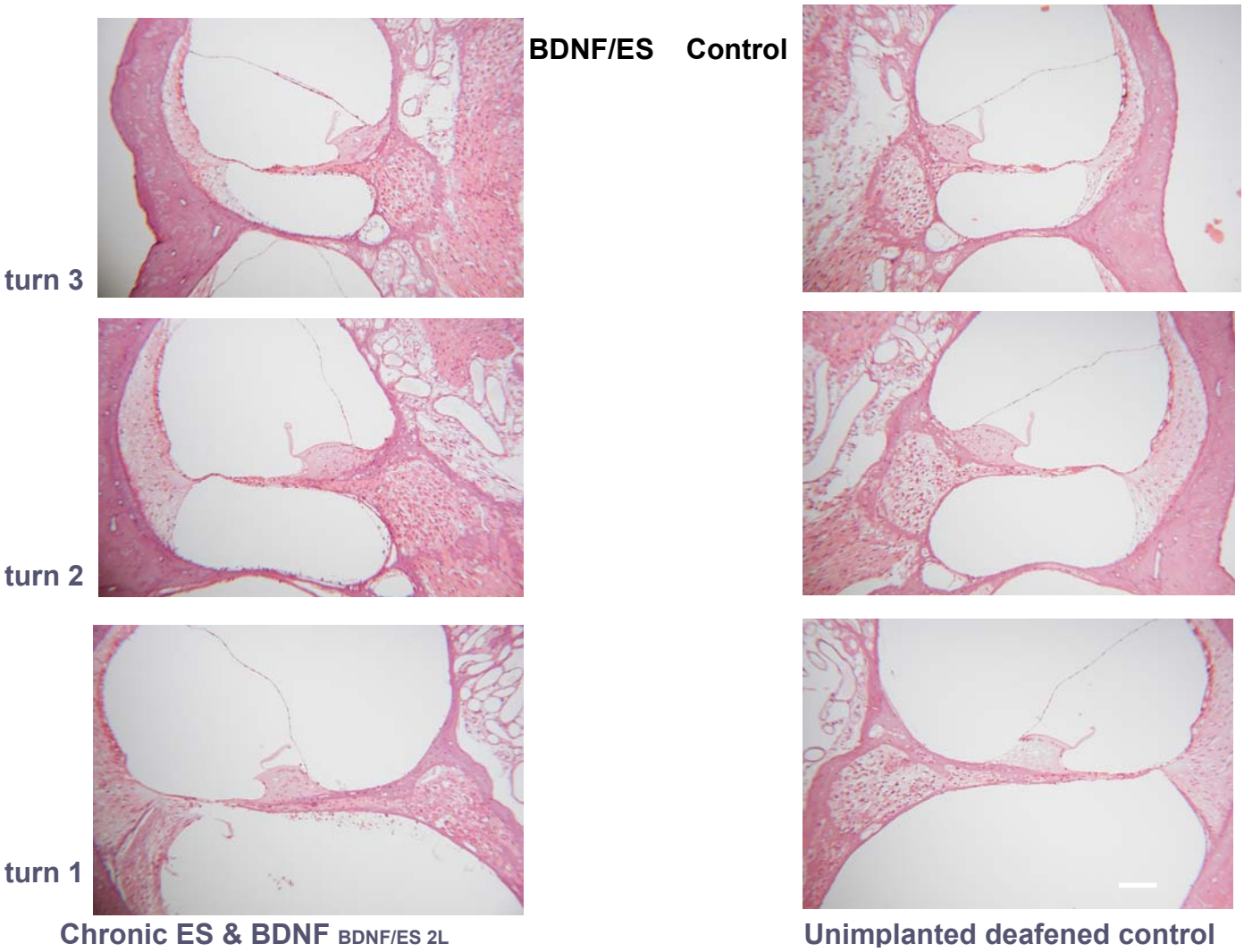

Figure 2. Representative low power micrographs of cochlear turns 1, 2 & 3 . The left panel illustrates a cochlea that received chronic electrical stimulation and neurotrophin (BDNF) delivery over a 28-day period. The contralateral unimplanted deafened control cochlea is illustrated in the right panel. Note the absence of hair cells and organ of Corti in all turns, reflecting the effectiveness of the deafening process. Note also a fine tissue capsule in the basal turn of the treated cochlea (left panel) associated with the electrode array. Bar=100 μm.

Mean spiral ganglion neuron densities across each turn for the electrically stimulated and BDNF treated cochleae are compared with their contralateral unimplanted deafened controls in Figure 4. These data illustrate the significant trophic support electrical stimulation and BDNF provides to SGNs in deafened cochleae. Figure 5, compares mean SGN density across all treatments. Of significance in these data is the clear lack of neurotrophic support provided by electrical stimulation alone (AP/ES) over implanted cochleae treated only with artificial perilymph (AP/C). The other striking feature of these data is the small but significant *increase* in SGN density associated with the BDNF/ES treatment group over BDNF alone (BDNF/C). This finding implies that electrical stimulation potentiates the trophic effects of BDNF on mature auditory neurons.

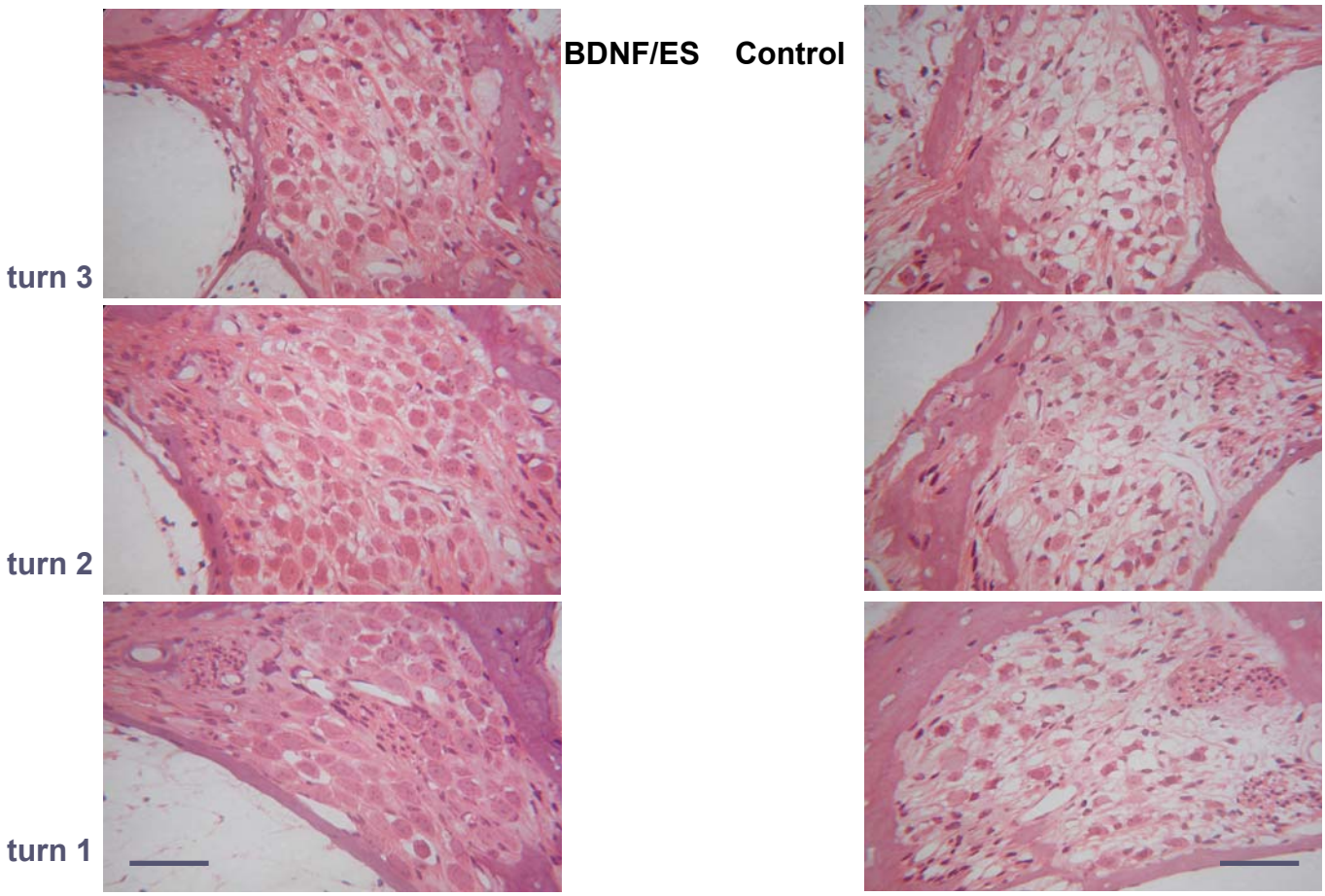

Figure 3. Higher power micrographs of the same cochlear sections illustrated in Fig. 2, showing the degree of SGN survival in Rosenthal's canal of a stimulated/BDNF treated cochlea (left panel), versus an unimplanted deafened control cochlea (right panel). Bar =50 μm.

Finally, we also have measured the soma area of SGNs in some treated cohorts as well as the deafened, untreated control cochleae (data for the remaining treated cohorts is currently being collected). Figure 6 illustrates the mean soma area (± SEM) for each turn of the BDNF/ES and AP/ES treatment groups versus deafened, unimplanted control cochleae. While there was no significant difference in the mean soma area of the AP/ES group compared with the controls, there was a highly significant increase in soma area of the BDNF/ES cohort in the basal turn, which decreased apicalward, until there was no difference in the mean soma area between cohorts in turn 4 (Fig. 6).

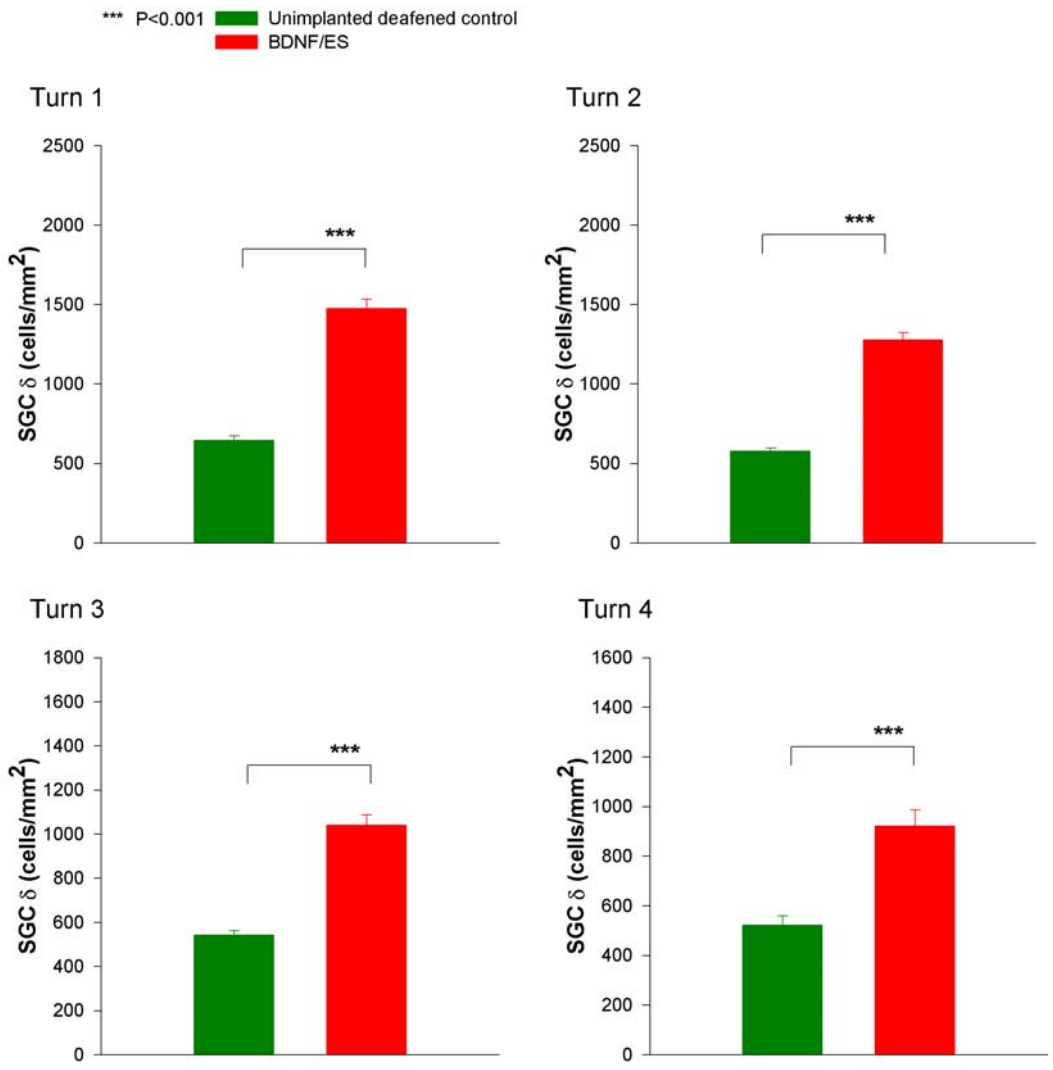

Figure 4. Mean SGN densities (cells/mm^2) from guinea pigs in this study illustrating turn 1 (basal) to turn 4 (apical) from deafened control cochleae 33 days after deafening (green). In contrast, far greater SGN survival is evident in deafened cochleae that received chronic electrical stimulation and BDNF administration for a period of 28 days (red). The trophic influence of this treatment on SGNs was highly significant for all cochlear turns. These data are based on 5 cochleae in each treatment. *** P< 0.001 (t-test). Bar=SEM.

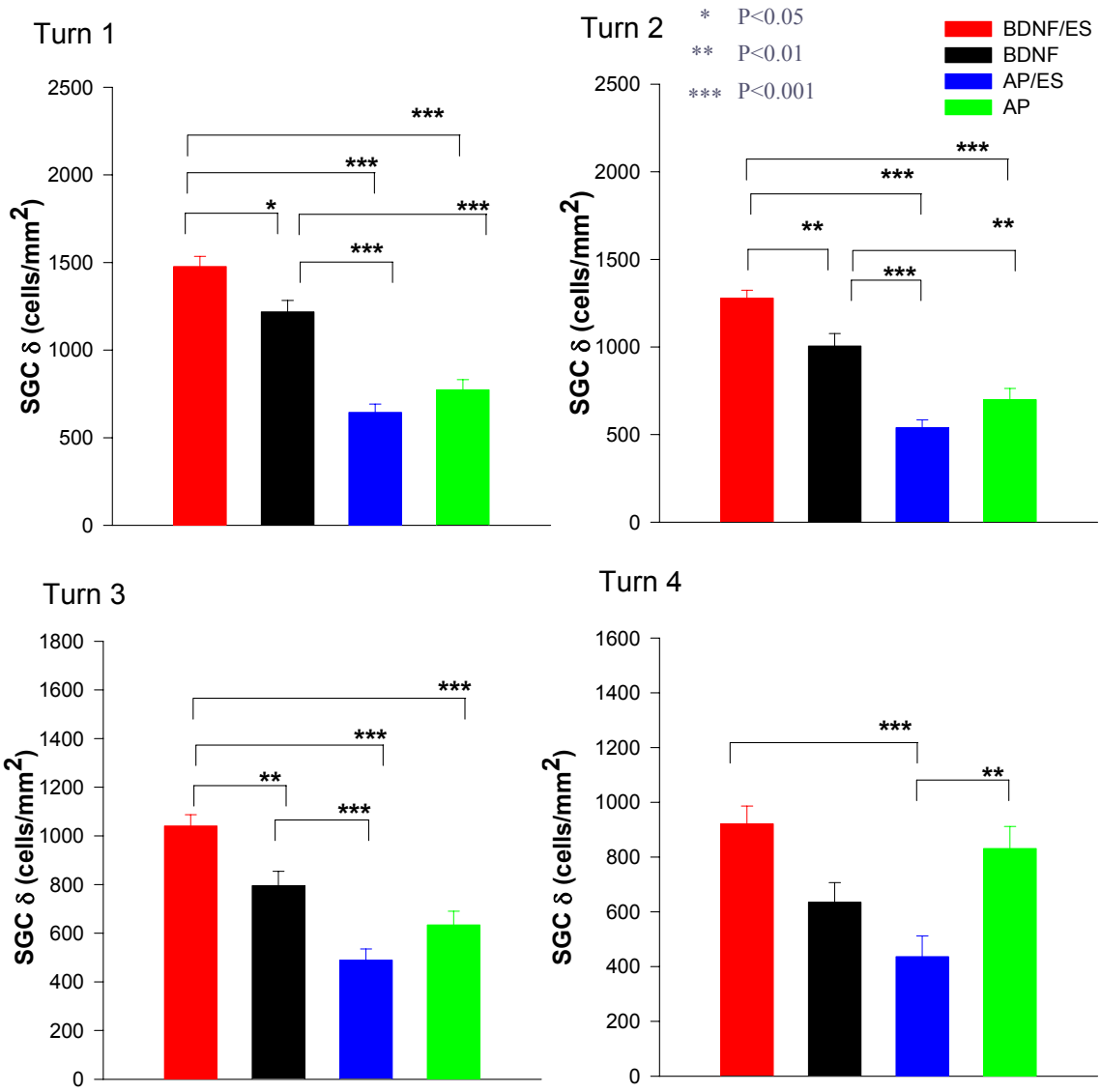

Figure 5. Mean SGN density of implanted side of turn 1, 2, 3 and 4 in BDNF/ES, BDNF alone (BDNF/C), AP/ES and AP alone (AP/C) groups. These data are based on five guinea pig cochleae per treatment group (one cochlea from the BDNF group and one cochlea from the AP group have yet to be analyzed). Note that the trophic effects of BDNF both with and without electrical stimulation appear to be widespread throughout the cochlea. One-way ANOVA. * P<0.05; ** P< 0.01; *** P<0.001. Bar=SEM.

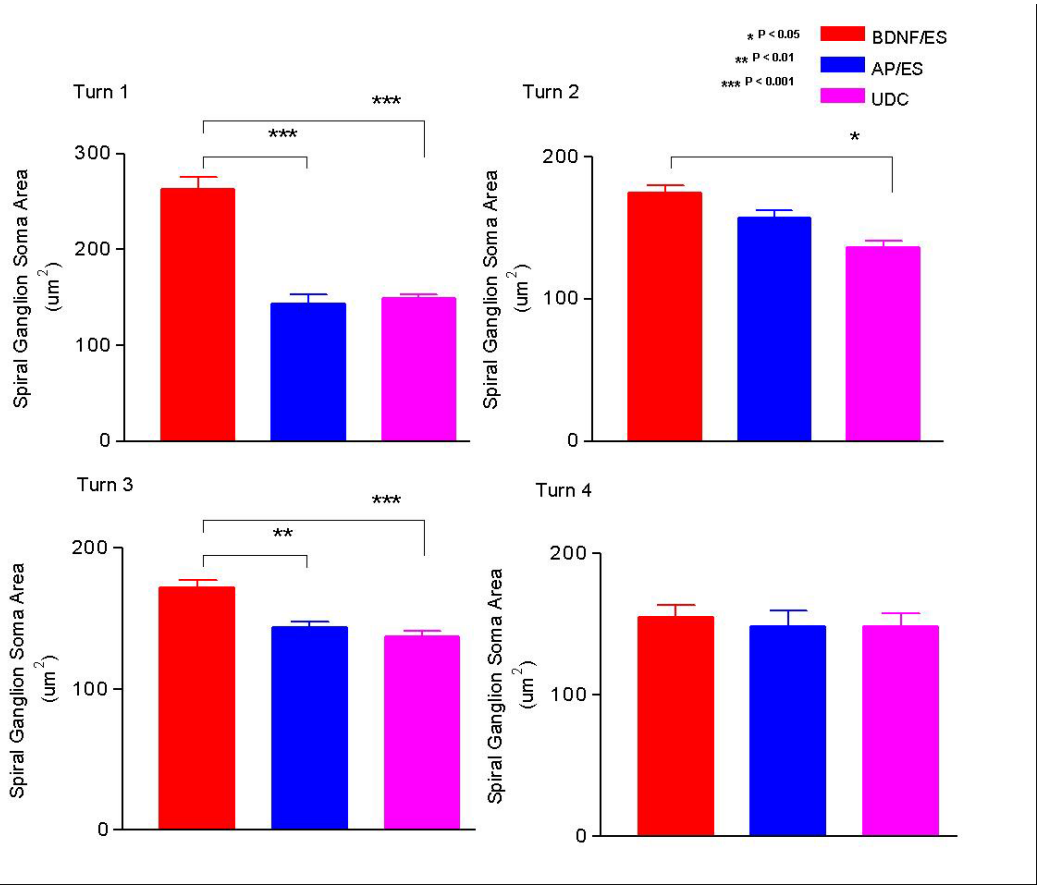

Figure 6. Mean SGN soma area of implanted side of turn 1, 2, 3 and 4 in BDNF/ES, AP/ES and unimplanted deafened control cochleae (UDC). While there was a significantly greater soma area in turn 1 of the BDNF/ES treatment group, this effect tended to reduce apicalward, such that there was no difference in soma area across treatment groups in turn 4. Note that there was no difference in SGN soma area between the AP/ES treated cochleae and the unimplanted deafened control cochlea. One-way ANOVA. Bar=SEM.

3.4 Discussion and Conclusions

Our results show that chronic intracochlear electrical stimulation in the deafened guinea pig does not provide trophic support of SGNs; i.e. the SGN density of AP/ES treated cochleae exhibited no evidence of increased survival compared with the deafened, unstimulated AP treated animals. These results are consistent with our preliminary data obtained from neonatally deafened, chronically stimulated cats (*Seventh Quarterly Progress Report*). Importantly, direct intracochlear delivery of the neurotrophin BDNF resulted in significant increases in SGN survival and this was *enhanced* when the neurotrophin delivery was combined with electrical stimulation.

These anatomical findings using both BDNF and BDNF/ES were also reflected in important functional changes, namely significant reductions in EABR threshold over the 28-day implant period (*Eighth Quarterly Progress Report*). The mechanism underlying this significant reduction in threshold may be associated with the effects BDNF has on firing patterns and ion channel distribution in SGNs

(Adamson et al., 2002). In contrast with the reduced thresholds observed in animals treated with BDNF, both AP/ES and AP/C cohorts exhibited significant increases in EABR threshold over the same period (*Eighth Quarterly Progress Report).* We attribute this threshold increase to the ongoing loss of SGNs evident in these animals.

The shrinkage of SGN soma observed in deafened cochleae presumably reflects a down regulation in biochemical activity at the perikaryon following the SNHL. Interestingly, while treatment with AP/ES showed no advantage over unimplanted, deafened control cochleae, combining BDNF with ES produced significantly larger soma – particularly in the basal cochlear turn. Whether this increase in soma area is a result of BDNF or BDNF with ES, will need to await the completion of our analysis of the soma area of the BDNF/C cohort of animals.

Preservation of SGNs with the use of BDNF has important implications for cochlear implant recipients. For example, a reduction in threshold will lead to a decrease in power consumption of the implant, safe use of smaller electrodes and a potential to increase the number of electrode channels. Future studies will examine whether this advantage is maintained with a single administration of BDNF with ongoing electrical stimulation or whether a continuous supply of BDNF is required. The encouraging results of this and other studies combining neurotrophins with electrical stimulation (Shinohara et al., 2002; Kanzaki et al., 2002), suggest that this technique has potential clinical application following completion of appropriate safety studies.

4. Plans for Next Quarter

- Continue histological preparation and analysis of cochleae and auditory brainstem structures in cats and guinea pigs following completion of the chronic stimulation program.
- Continue developing our immunocytochemistry protocols.
- Continue preparation for manuscript submission and conference presentations.
- Continue terminal acute electrophysiological experiments on neonatally deafened un-stimulated control cats.

5. Acknowledgements

We are grateful to Dr James Fallon for software development, Dr Jin Xu and Ms Helen Feng for electrode manufacture, Ms Maria Clarke for histology and Dr Sue Pierce and Ms Elisa Borg for veterinary advice and animal husbandry. The Animal Research and Ethics Committee of the Royal Victorian Eye and Ear Hospital approved the care and use of the animals involved in this study.

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