Final Report

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Protective Effects of Patterned Electrical Stimulation on the Deafened Auditory System

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1. Introduction

Cochlear implants have now been implanted in over 50,000 deaf adults and children. There has been continued improvement in clinical performance of cochlear implant patients over the past 20 years; so much so that it is now considered that both severely deaf adults and prelinguistically deaf children are suitable implant candidates (NIH Consensus Statement, 1995). While much of this clinical improvement can be attributed to technical improvements associated with these devices, it is also apparent that the plastic response of the auditory pathway with implant use is an important factor. Indeed, studies investigating significant factors associated with auditory performance in cochlear implant subjects report that auditory experience, both prior to an acquired hearing loss and via the use of a cochlear implant, is strongly positively correlated with clinical performance (Gantz et al. 1993; Blamey et al. 1996; Rubinstein et al. 1999; Sarant et al. 2001; Govaerts et al. 2002; Kirk et al. 2002; Rubinstein 2002).

Adult post-linguistically deafened implant recipients exhibit a wide range of speech perception skills. A number of investigators have evaluated the significant factors associated with auditory performance in cochlear implant subjects (Gantz et al. 1993; Blamey et al. 1996; Rubinstein et al. 1999). Blamey and colleagues (1996) evaluated significant factors affecting clinical performance among 808 implant users. Although the variability was large, this analysis emphasized the importance of auditory experience in speech perception: duration of deafness was strongly negatively correlated with speech perception; experience using a cochlear implant exhibited a strong positive correlation; while age at implantation was negatively correlated. Others have reported similar findings (Gantz et al., 1993; Rubinstein et al., 1999). Collectively these studies have emphasized an important relationship between auditory experience and clinical performance among cochlear implantees.

While post-linguistically deaf subjects exhibit electrode pitch percepts that show consistent variation with tonotopic organization (Cohen et al. 2001; Pfingst et al. 2001; Fu and Shannon 2002), prelinguistically deaf patients implanted as adults do not typically exhibit such organization (Eddington et al. 1978; Tong et al. 1988; Busby et al. 1992; Busby and Clark 2000). Their temporal processing skills are also poor: they are far less successful at perceiving different rates of stimulation and exhibit poorer gap detection abilities. While these studies show evidence of an improvement in performance with increased use of the implant, the level of performance of prelinguistically deaf adults generally remains well below that of post-linguistically deaf adults (Eddington et al. 1978; Busby et al. 1993; Busby and Clark 1999).

Studies such as these emphasize the importance of early auditory experience for developing speech and language skills and provide impetus for the application of cochlear implants in young profoundly deaf children. Such a strategy not only minimizes the duration of deafness prior to implantation, but also maximizes auditory experience during an early, critical period for language acquisition (Svirsky et al. 2000; Kirk et al. 2002). Numerous studies of children with chronic otitis media support the notion of a critical period in language development. Chronic otitis media early in life can have an adverse effect on auditory perceptual skills and language development (Dobie and Berlin 1979; Sak and

Ruben 1981; Hutchings et al. 1992; Ruben et al. 1997; Gravel and Wallace 2000; Klausen et al. 2000), some of which appear to persist at least into early childhood (Ruben et al., 1997).

Consistent with studies of post-linguistically deafened adult implant subjects, clinical experience with cochlear implants in young children has highlighted the importance of auditory experience on speech perception (Osberger et al. 1991; Dawson et al. 1992; Waltzman et al. 1992; NIH Consensus Statement 1995; Fryauf-Bertschy et al. 1997; Blamey et al. 2001). Although children with a congenital hearing loss will initially show poorer speech perception than children with an acquired hearing loss, clinical performance tends to improve with device use. After 2-3 years of implant use, it is reported that a majority of congenitally deaf children obtain open set speech perception (Dowell et al. 1995). It is now clear that the difference in speech perception ability between pre- and post-linguistically deafened children lessens with duration of cochlear implant use (NIH Consensus Report, 1995). While it is difficult to separate the auditory and non-auditory components of language development (Blamey et al. 2001), clinical improvement with device use suggests that the central auditory pathway is capable of undergoing functional reorganization in response to behaviorally relevant auditory cues evoked via a cochlear implant. Finally, evidence is emerging among pediatric implant subjects, that there is a clear sensitive period of ~3-4 years of deafness during which time the central auditory pathway remains maximally plastic (Ponton et al. 1996; Ponton and Eggermont 2001; Govaerts et al. 2002; Kirk et al. 2002; Sharma et al. 2002). Longer periods of delay prior to implantation result in significantly reduced levels of plasticity.

Spiral ganglion neuron (SGN) degeneration and other effects at the level of the cochlea may be associated with the negative correlation between duration of deafness and auditory performance with a cochlear implant. However, to date, there is no histopathological evidence to support such a correlation. To the contrary, two studies have provided evidence that no such association exists. Fayad et al. (1991) showed no significant correlation between auditory performance and SGN numbers in a study of 16 ears from 13 single channel implant patients. While these data are revealing, their interpretation must be made cautiously as single channel devices may not need to stimulate as many neurons as multichannel devices in order to achieve their full clinical potential (Blamey 1997). More recently, Nadol et al. (2001) described a *negative* correlation between total remaining SGNs and word recognition scores in eight post-linguistically deafened adults who had used multichannel cochlear implants. Finally, while there is evidence from the study of human temporal bones that the cause of hearing loss is the most significant determinant of total SGN counts (Nadol et al. 1989; Nadol 1997), the variation in auditory performance with etiology is inconsistent with the expected populations of ganglion neurons (Blamey et al. 1996).

Although limited, these studies suggest that clinical performance with cochlear implants cannot be adequately explained merely in terms of the number of SGNs. Variation in the spatial distribution of surviving SGNs and their functional response to electrical stimulation may contribute to the large range in auditory performance that is observed clinically. Clearly, however, factors associated with

central processing must be considered in order to account for the changes observed in auditory performance over time.

The goal of this contract has been 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 to temporally challenging stimuli presented chronically to one or two sectors of the AN.

(b) The neurophysiological and neuroanatomical response of the AN following chronic intracochlear electrical stimulation in combination with exogenous 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.

(c) Molecular events associated with a SNHL. Using immunochemical techniques, this study was designed to examine, in detail, a number of the important molecular changes that occur longitudinally in response to a neonatal SNHL. Changes in peripheral and central myelin, Schwann cell survival, Trk receptor expression and Na^+ and K^+ ion channel expression are under investigation.

While these and other 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. Chronic electrical stimulation of the deafened cat cochlea

2.1 Trophic effects at the level of the cochlea

Following a SNHL, SGNs in the cochlea degenerate over time (e.g. Leake and Hradek 1988; Shepherd and Hardie 2001). Since SGNs are the target neurons for cochlear implants, their maintenance has important clinical implications. Depolarization has been shown to provide important trophic support to SGNs *in vitro* (Hegarty et al. 1997; Hansen et al. 2001). The aim of the present study was to determine whether depolarization via chronic electrical stimulation provides such trophic support *in vivo*. Twenty-three kittens were deafened using KA (330mg/kg; s.c.) and EA (27.5mg/kg; i.v.), at 14 days of age. Half the population

were profoundly deaf while the remaining animals were severely deaf, in order to test the hypothesis that remaining (apical) hair cells would provide global trophic support of SGNs in response to chronic electrical stimulation. At 2 months of age an Auditory brainstem response (ABR) was used to confirm deafness. Fifteen animals were then bilaterally implanted with an 8-ring electrode array using sterile surgical techniques (Fig. 1; Xu et al., 1997). The remaining eight animals served as unimplanted deafened controls. The implanted animals were electrically stimulated unilaterally at an intensity 6dB above the electrically-evoked ABR (EABR) threshold, for ~6 hours/day over a 6 month period (range 1.3-8.4 months), using charge balanced biphasic current pulses at a rate of 1200 pulses per second and amplitude modulated to a level of 50% at 30 Hz (Fig. 2). Each current pulse was 100μs/phase with a 14 μs interphase gap. EABRs were recorded periodically to confirm that stimulus levels were above threshold of the auditory nerve. At completion of their chronic stimulation program each animal was used in an acute electrophysiological experiment examining the spatial and temporal response properties of neurons in the central nucleus of the inferior colliculus, and – in animals with residual hearing – spatial excitation patterns in the auditory cortex. The animals were then sacrificed with an overdose of anaesthetic and their cochleae were processed for histology.

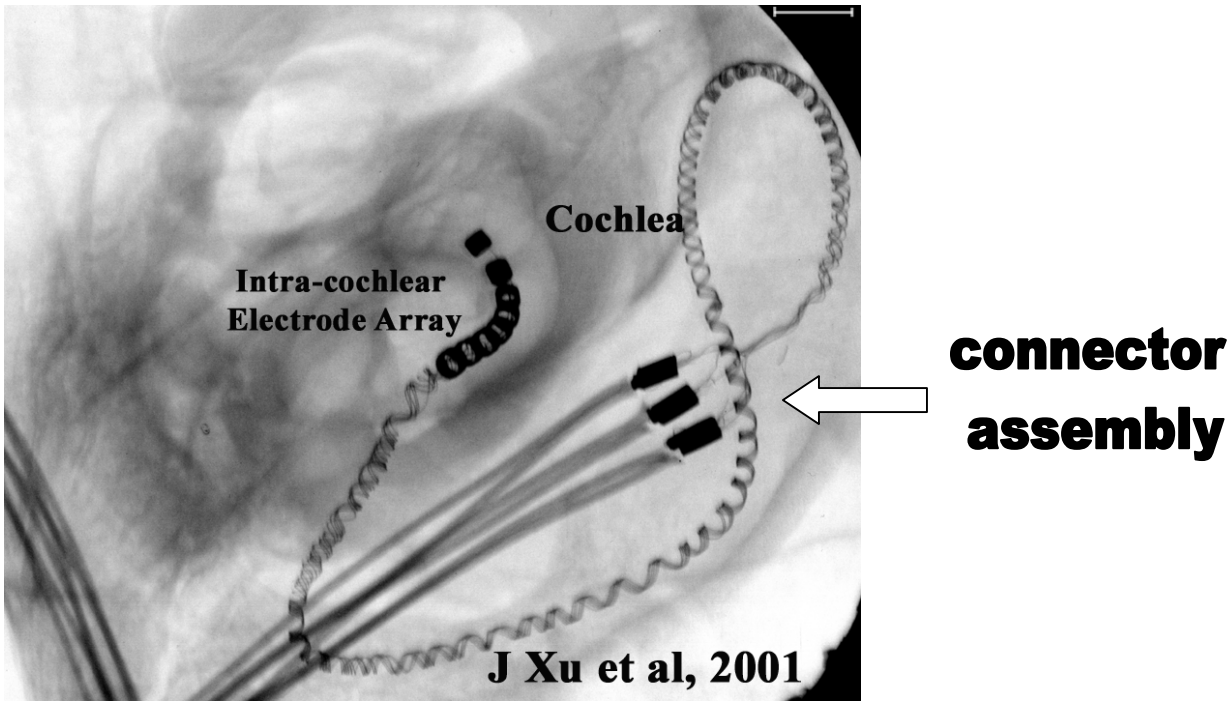


Figure 1. A micro-focus X-ray image of the left cochlea and auditory bulla of one of our implanted kittens. The eight-ring electrode array can be seen within the basal turn of the cochlea. The platinum leadwire system is assembled into a helix to maximize stress relief, and welded to the more robust stainless steel leadwire at the connector assembly. This assembly is placed within the auditory bulla while the stainless steel leadwires project subcutaneously to an exit point at the nape of the neck. Bar=1 mm.

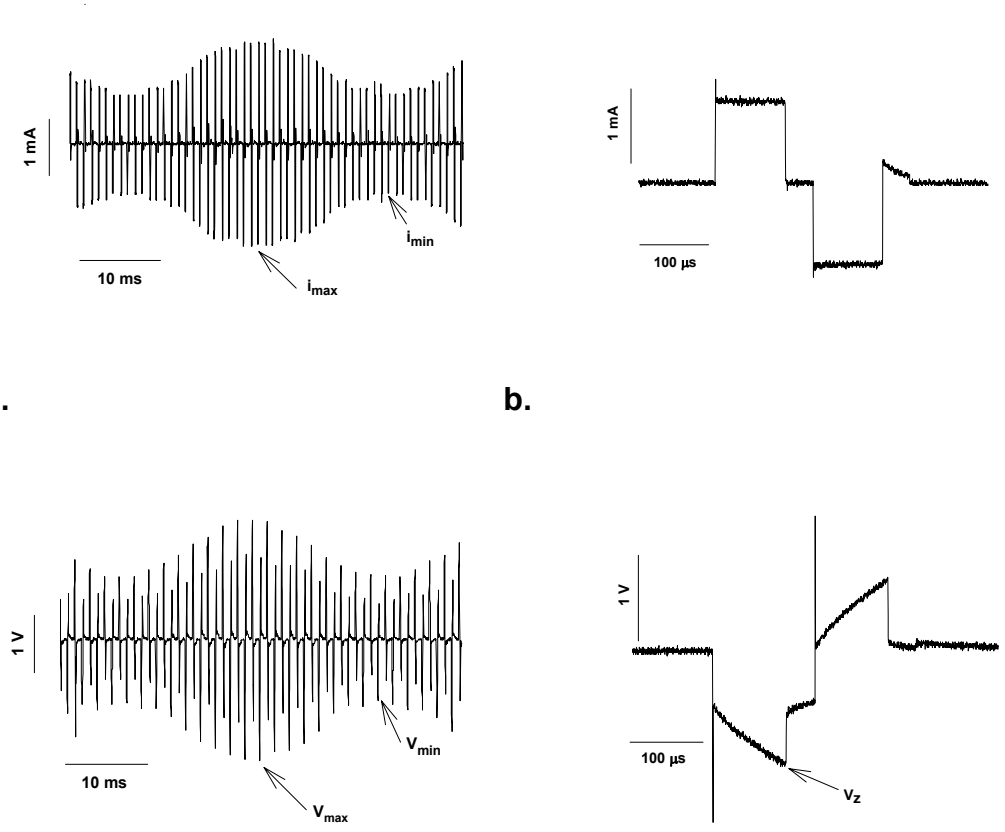


Figure 2. Example of the stimulus waveform used in the present study. Top panels illustrate the current waveform at a wide (a) and narrow (b) time base while the bottom panels illustrate the corresponding electrode voltage waveforms. The stimulus consists of a 1200 pulses per second carrier, amplitude modulated to a depth of 50% at 30 Hz. The current pulses (b) are 100 µs charge balanced biphasic pulses with a 14 µs interphase gap. Additional charge balancing is achieved using capacitive coupling and electrode shorting to ensure minimal DC (Huang et al. 1999). Note that the figure illustrates one of two programmable channels that the portable stimulator can output. The variable height of the voltage waveform in (c) is a result of aliasing.

During the chronic electrical stimulation study the physiological status of the auditory pathway was monitored by periodically recording EABRs. EABR threshold was used as an indicator of functional threshold and the intensity of the chronic stimulus was adjusted on the basis of this measure, in order to ensure our stimulation remained above threshold in each animal. Almost all animals exhibited an increase in EABR threshold as a function of stimulus duration (Figs. 3-6). Typical examples of EABR input-output (IO) functions, are illustrated in Figs. 5 & 6, and show the response amplitude of wave IV and also evidence of increasing thresholds over time.

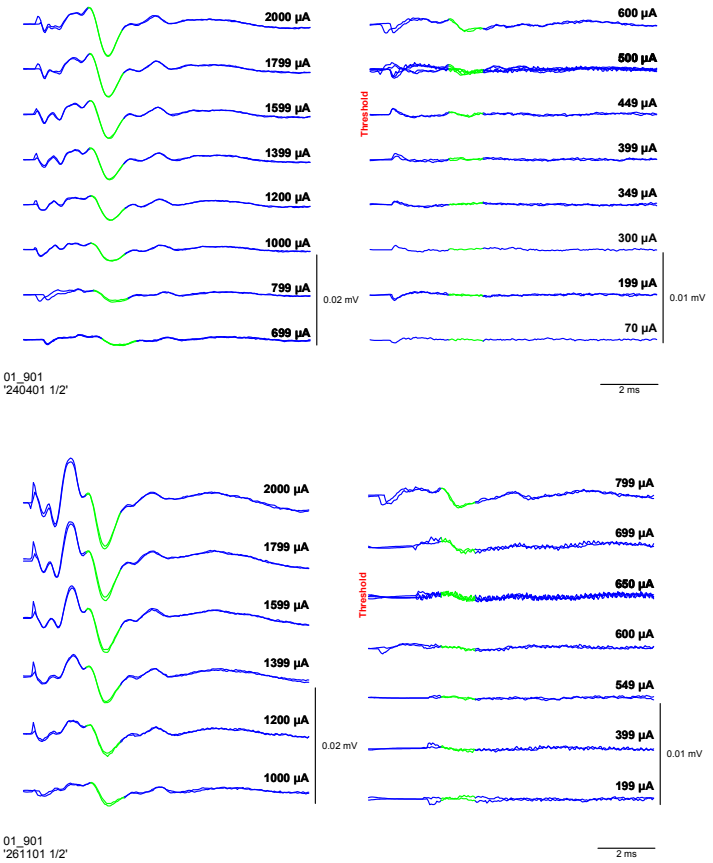


Figure 3. EABRs recorded from NDC_1 immediately following surgery (top panel), and near completion of the stimulation program seven months later (bottom panel). All EABRs were evoked using a 100 µs biphasic current pulse delivered to bipolar electrode pair 1/2. Two responses are typically recorded at each current level. Wave IV, from which response amplitude and latency data are derived, is highlighted in green. Threshold is indicated in each panel.

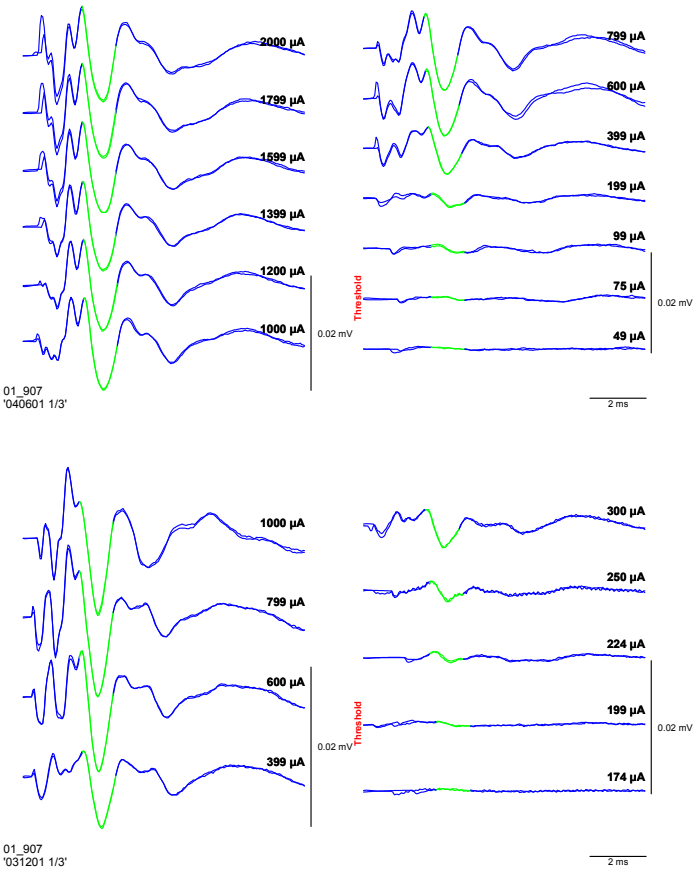


Figure 4. EABRs recorded from NDC_7 immediately following surgery (top panel), and near completion of the stimulation program six months later (bottom panel). These responses were evoked by electrode pair 1/3. The larger inter-electrode spacing resulted in reduced thresholds and a more rapid increase in response amplitude with stimulus intensity compared with electrode pair 1/2 (Fig. 3). There is also an increase in threshold over the implant period in this animal. Note, however, that the normal morphology of the waveform throughout the stimulation period.

Changes in EABR thresholds over time were analysed using linear regression techniques. In 8 out of 12 animals studied, there was a highly significant correlation between increase in EABR threshold and duration of implantation ($P<0.05$; $R^2 \geq 0.8$). These increases in EABR thresholds observed over implantation time support the anatomical evidence of an ongoing loss of SGNs following the deafening procedure (Hardie and Shepherd 1999 and present data).

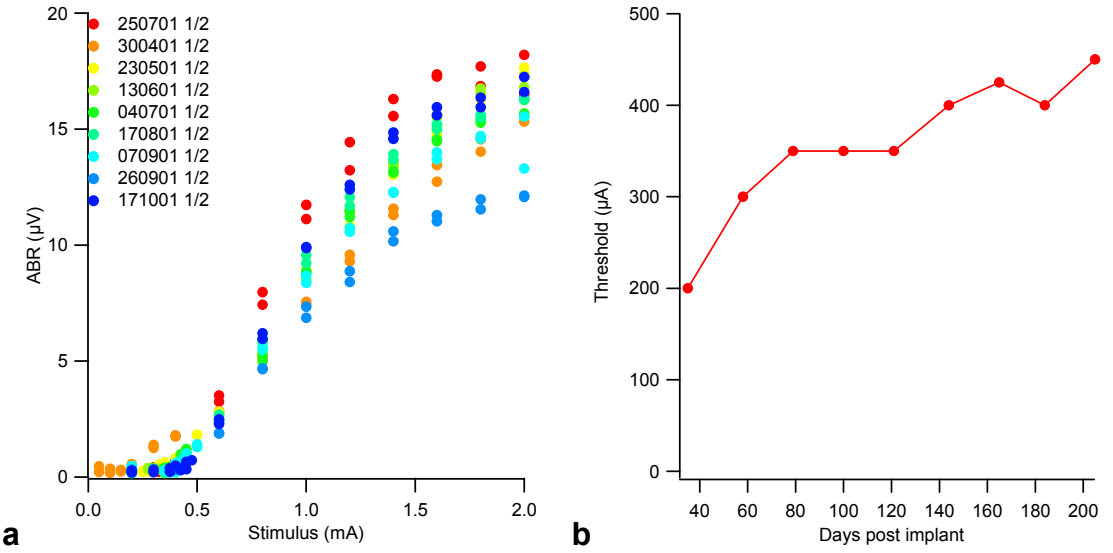


Figure 5. Wave IV EABR I/O functions recorded over the implant period for cat NDC_3 electrode pair 1/2 (a) and threshold changes over implant time (b). While these I/O curves are relatively stable over the course of the stimulation program, we typically observed a small increase in response threshold over time. Two data points are plotted for each recording session; these reflect the response amplitude from each individual recording.

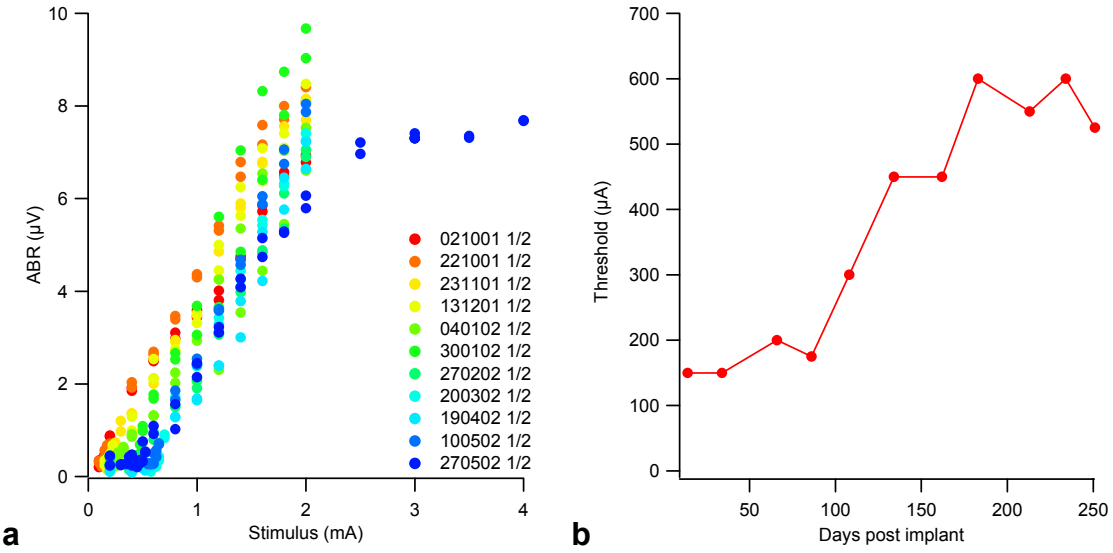


Figure 6. Wave IV EABR I/O functions recorded over the implant period for cat NDC_12 pair 1/2 (a) and threshold changes over implant time (b).

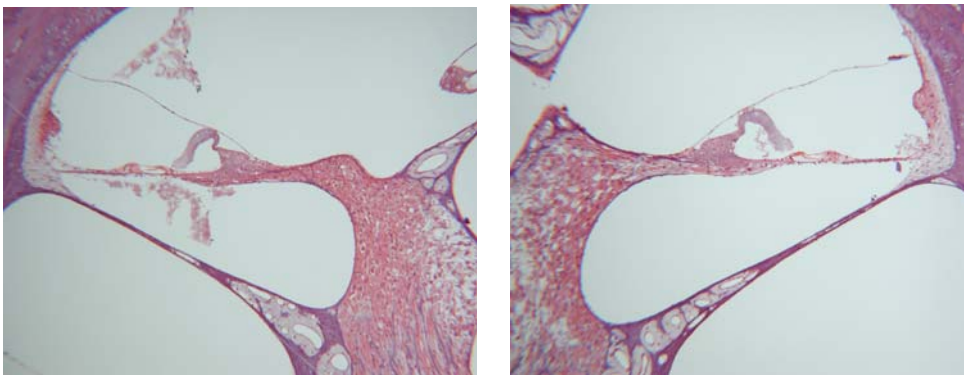
SGN density was measured to quantify the effects of stimulation on neural survival. Each cochlea was divided into the lower basal, upper basal, middle and apical turns. The cochleae were then graphically reconstructed using software developed by contract staff (JBF). All SGNs containing a nucleus were counted. Cells that were in the process of degeneration or those that contained large vacuoles were not included in the count. SGNs were measured in both cochleae and compared statistically using a *t*-test.

Representative examples of the histology, illustrating both stimulated and unstimulated cochleae from the one animal, are shown in Figs. 7-9. Cochleograms, illustrating the extent of soft tissue and new bone within the scala tympani; the extent of inner and outer hair cell survival; the status of the structure of the organ of Corti; and the location of any electrode insertion trauma is illustrated for two animals in Fig. 10. Cochleograms were produced using software developed under this contract as part of a package designed to assist graphic reconstruction of the cochlea (7^{th} Quarterly Progress Report).

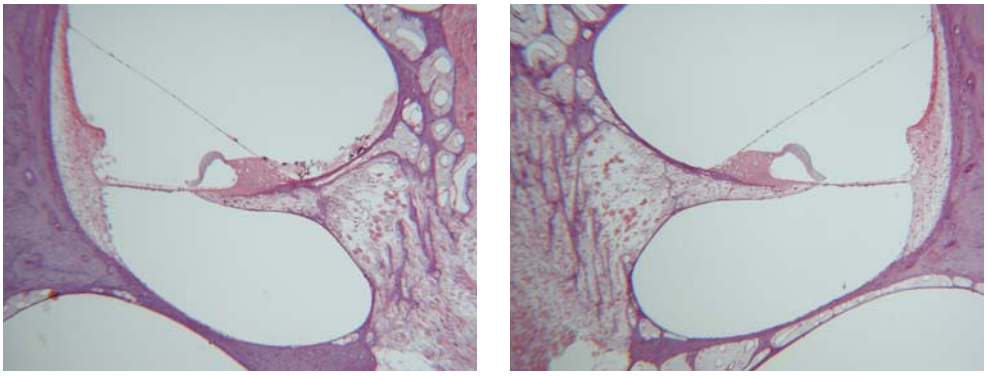
Using our kanamycin/ethacrynic acid (KA/EA) deafening technique in 14 day old kittens we routinely obtain a “U” shaped profile of SGN loss from cochlear base to apex (Fig. 11), with the most extensive loss observed in the upper basal turn adjacent to the stimulating electrode. Examination of SGN density in this region of the chronically stimulated cochleae (i.e. SGNs most subject to electrically induced neural activity) showed no evidence of trophic SGN support compared with the same region in the contralateral, implanted, but unstimulated control cochleae. Furthermore, we observed no evidence of more global trophic support of these neurons in the stimulated cochleae. Indeed, the apical turn was the only cochlear region to exhibit a significant difference, and showed greater SGN survival in the control versus stimulated cochleae (Fig. 11). The mechanisms underlying this difference in the apical turn require further analysis. Finally, there was no evidence of a trophic effect in implanted and chronically stimulated cochleae with residual hearing when compared to their contralateral implanted unstimulated control cochleae.

Although preliminary (additional cochleae are currently undergoing histological processing), the study showed no evidence that chronic electrical stimulation provides trophic support of SGNs in the neonatally deafened cat cochleae, deafened using a single administration of an aminoglycoside and loop diuretic. The increase in EABR thresholds observed over implantation time (e.g. Figs. 5 & 6) supports evidence of an ongoing loss of SGNs. Other studies from our present contract have found that electrical stimulation in conjunction with administration of BDNF directly to the deafened guinea pig cochlea has advantageous effects both on SGN survival (see Fig. 24) and functionally in terms of reduced EABR thresholds (see Fig. 21). Importantly, guinea pigs stimulated without administration of BDNF showed no evidence of trophic SGN survival, a finding consistent with the present feline data.

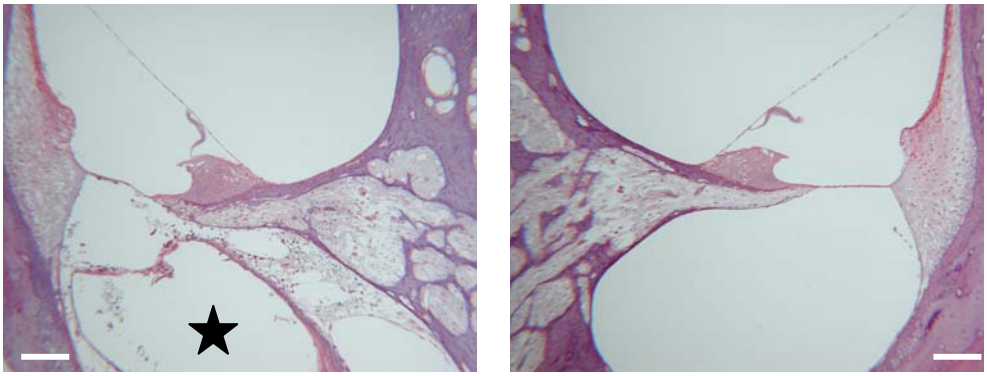
Apical turn



Middle turn



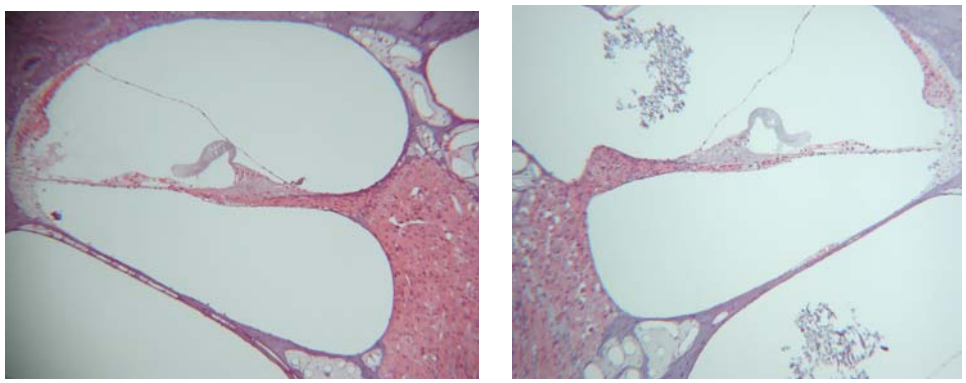
Basal turn



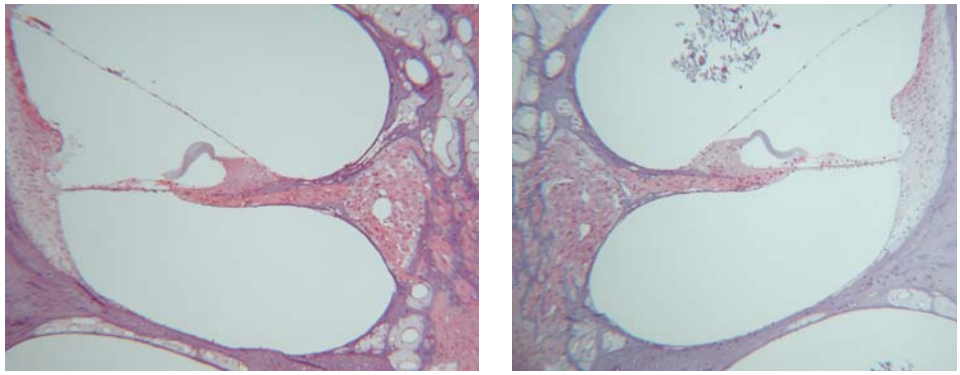
Left (stimulated) Right (control)

Figure 7. Photomicrographs illustrating the apical, middle and basal turns of the stimulated (left) and implanted unstimulated control (right) cochleae from cat NDC_1. Note that a fibrous tissue capsule associated with the electrode array (star) is present in the basal turn of the stimulated side. Although the control cochlea was also implanted, there was no evidence of a tissue response in this example. The electrode array is removed prior to histology. This animal was profoundly deaf. Bar = 100 μm.

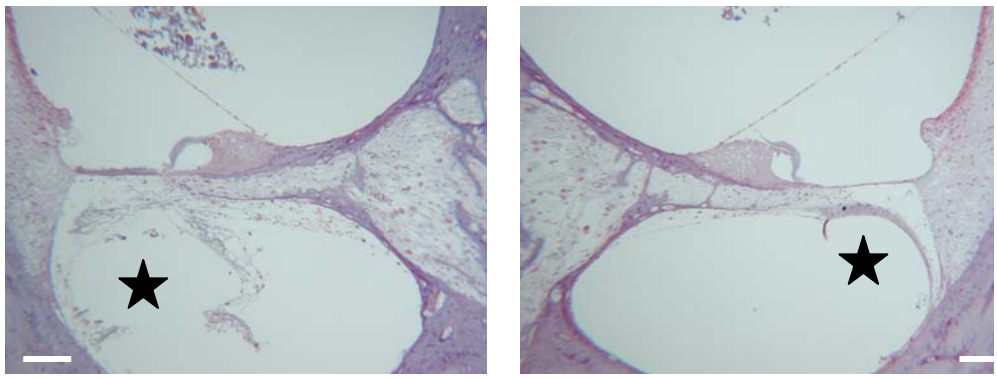
Apical turn



Middle turn



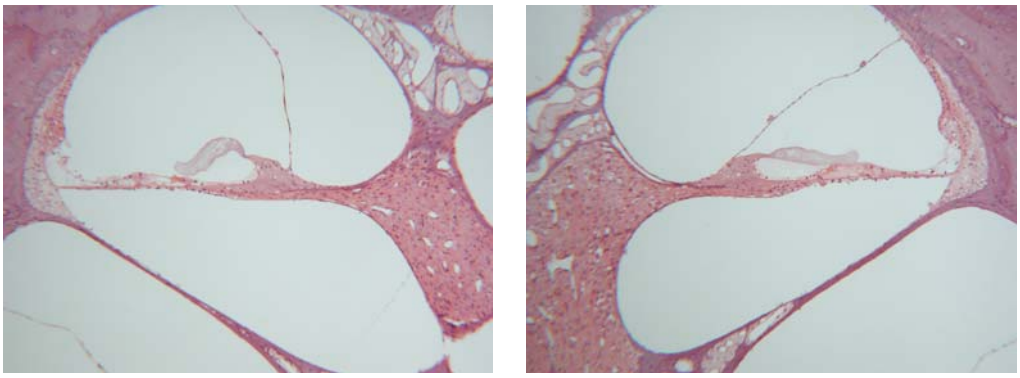
Basal turn



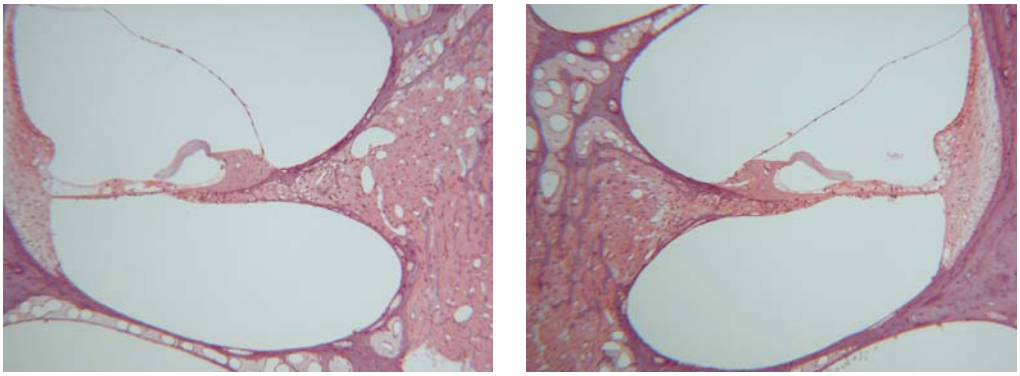
Left (stimulated) Right (control)

Figure 8. Photomicrographs illustrating the apical, middle and basal turns of the stimulated (left) and implanted unstimulated control (right) cochleae from cat NDC_3. Some sections show bone dust in the scala vestibuli; this is a preparation artifact. This animal was severely deaf however there was no evidence of enhanced trophic support of SGNs in the basal turn of the stimulated cochlea. Bar = 100 μm.

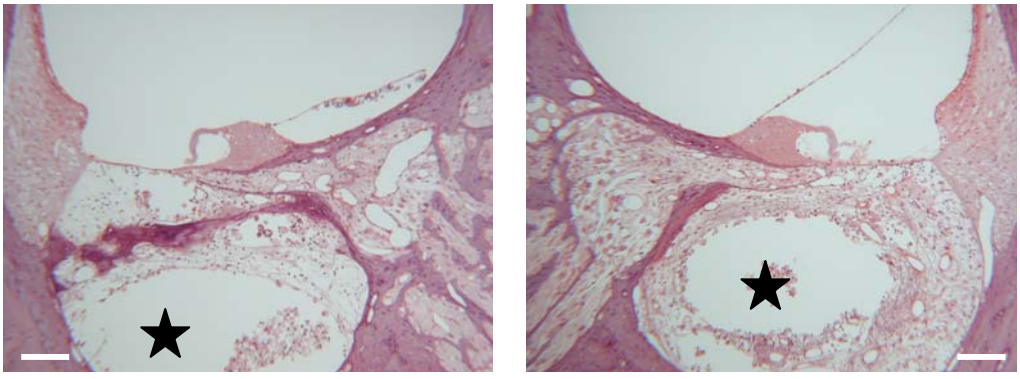
Apical turn



Middle turn

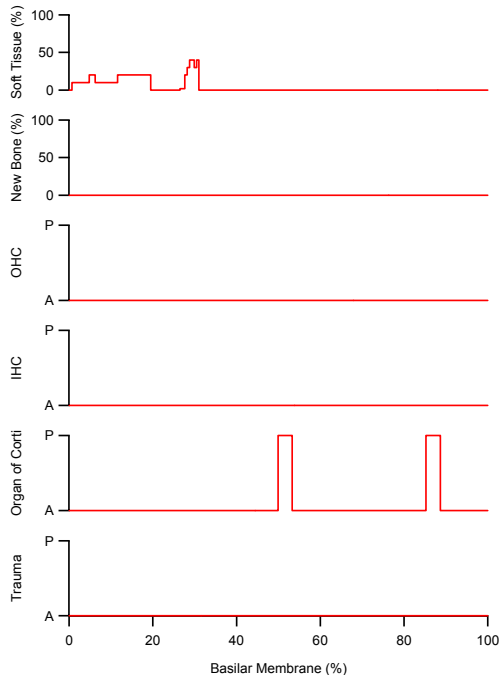


Basal turn

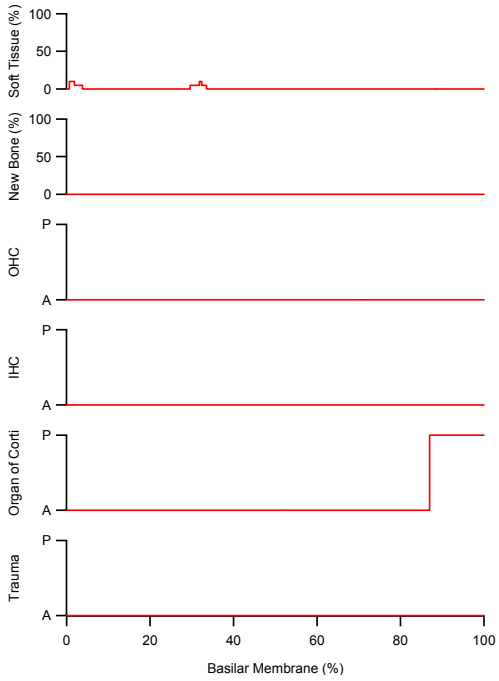


Left (stimulated) Right (control)

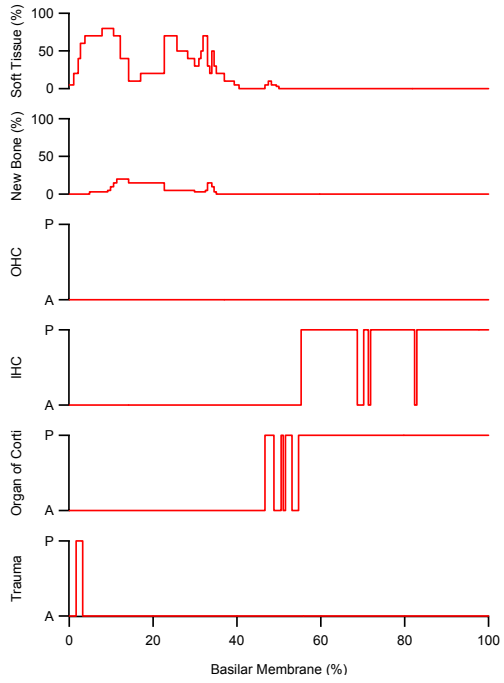
Figure 9. Photomicrographs illustrating the apical, middle and basal turns of the stimulated (left) and implanted unstimulated control (right) cochleae from cat NDC_7. Note that the more extensive fibrous tissue reaction in NDC_7L is associated with a slightly greater electrode impedance measure compared with the other animals illustrated here. This animal was severely deaf. Bar = 100 μm.



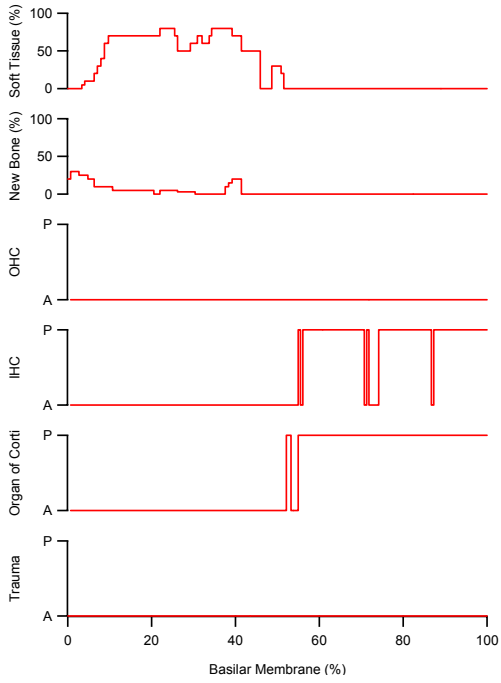
NDC_1L



NDC_1R



NDC_7L



NDC_7R

Figure 10. Cochleograms of the stimulated (left) and control (right) cochleae of NDC_1 (top panels) and NDC_7 (bottom panels), illustrating the extent of soft tissue and new bone within the scala tympani (percent estimate); the extent of inner and outer hair cell survival (absent/present); the presence or absence of the structure of the organ of Corti; and the location of any electrode insertion trauma. These examples are from the cochleae illustrated in Figs. 7 & 9. Note the minimal insertion trauma typically associated with the insertion of this electrode array.

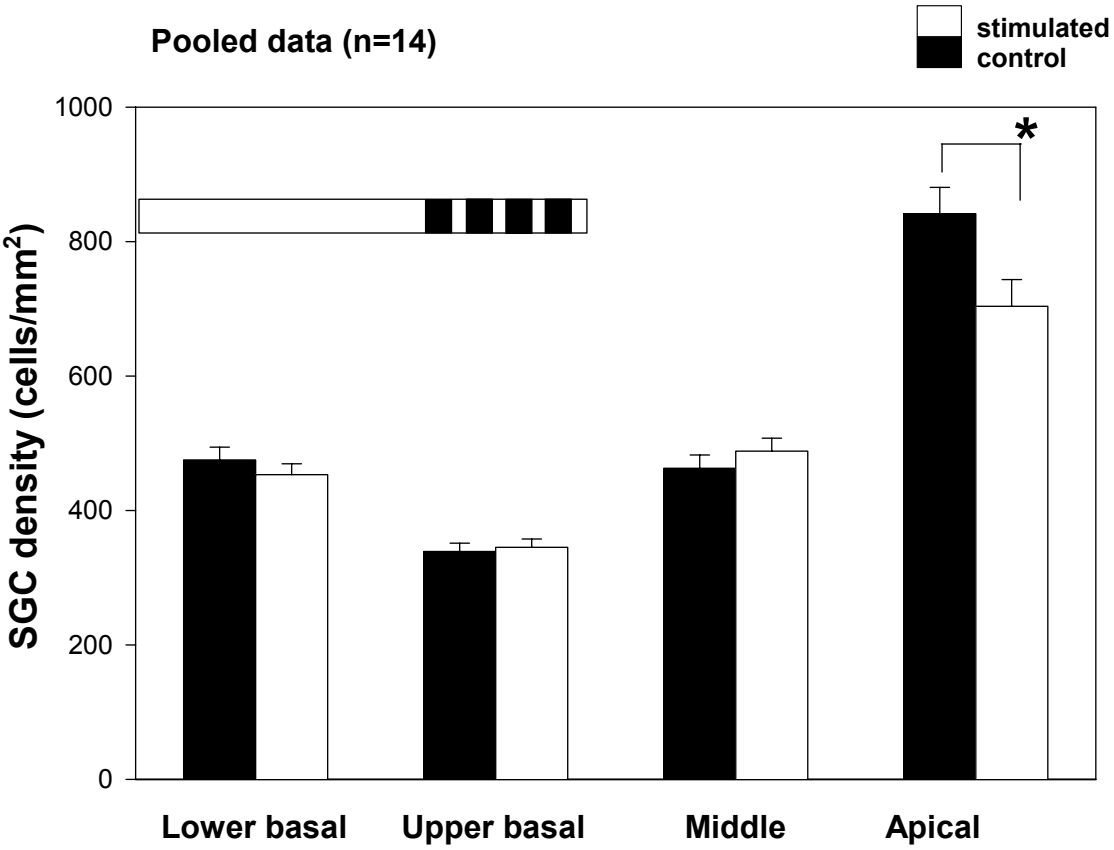


Figure 11. Pooled data from 14 animals (28 cochleae) illustrating the SGN density in the lower basal, upper basal, middle and apical turns. While there is an extensive deafness induced reduction in SGN density in all turns, the most extensive loss is observed in the upper basal turn. There was no evidence of a stimulus induced trophic effect on residual SGNs despite stimulation periods ranging from 1.3-8.4 months. The cell counts used to generate this figure are based on > 50,000 SGNs.

The use of a severely deaf cohort of experimental animals was designed to model severely deaf cochlear implant subjects. The maintenance of low levels of residual hearing following long-term implantation and electrical stimulation, described in our 7th Quarterly Progress Report, is consistent with previous experimental (e.g. Xu et al. 1997) and clinical (von Ilberg et al. 1999) studies.

The inclusion of animals with some intact hair cells also allows us to test the hypothesis that residual elements of the organ of Corti play a key role in trophic support of stimulated SGNs. This follows from the original work of Lousteau (1987) who reported significant preservation of the spiral ganglion *only* in regions of cochleae exhibiting residual elements of the organ of Corti. Although our data must still be regarded as preliminary, these results suggest that residual hair cells do not provide global trophic support to SGNs in implanted, chronically stimulated cochleae.

2.2 Effects of long-term SNHL on neurons in the Inferior Colliculus

Our previous work on the response of central nucleus inferior colliculus (ICC) neurons to electrical stimulation of the auditory nerve following long-term SNHL demonstrated several significant changes in their response properties (Shepherd et al, 1999). The absence of normal afferent input to the central auditory system during development resulted in a significant decrease in temporal resolution, increased latencies and an increase in dynamic range of the ICC neurons. However, the rudimentary cochleotopic organization of the ICC was not affected by the long-term SNHL, suggesting that this organization is laid down during development even in the absence of normal afferent input. The cochleotopic organization of the IC does show some degree of plasticity, as chronic intracochlear electrical stimulation of neonatally (Snyder et al. 1990) and adult (Moore et al. 2002) deafened cats can result in expansion of the ICC represenation of the chronically stimulated cochlear sector.

Therefore, in a second series of experiments we recorded from the ICC of these chronically stimulated, neonatally deafened animals to study spatial and rate plasticity in the auditory midbrain. The present results, summarized below, are being prepared for publication but must be considered preliminary at this stage.

Much of the data analysis has been automated and all analysis is performed within Igor Pro (Wavemetrics) using custom written analysis procedures (JBF; 6th Quarterly Progress Report).

Recordings of ICC single and multi-unit responses are made using tungsten microelectrodes (impedances 0.8 – 2.0 MΩ at 1 kHz). Unit activity is differentially amplified (DAM-5A, WPI) and band-pass filtered (150 Hz – 3 kHz; Khrone-Hite 3750) before being displayed on an oscilloscope (Tektronix 465). The stimulus artifact is eliminated using a sample-and-hold circuit, allowing output trigger pulses from the oscilloscope (adjusted to discriminate the leading edge of the action potentials) to be used to indicate the precise timing of each action potential. The resultant trigger pulse was fed to a PC-controlled in-house stimulus generation and data acquisition system. An Excel based software system was used to control the experiment and provide real-time peristimulus time, interspike interval and period histograms while hardware modules were responsible for synchronizing components and recording event times (Tucker-Davis Technologies).

Stimuli consisted of 100 μs per phase charge balanced biphasic current pulses with a 10 μs interphase gap, and variable stimulus intensities up to 3 mA peak. An in-house, optically isolated, current source stimulator generated the stimuli. Recording electrodes were advanced in 2 μm steps in the presence of an electrical search stimulus.

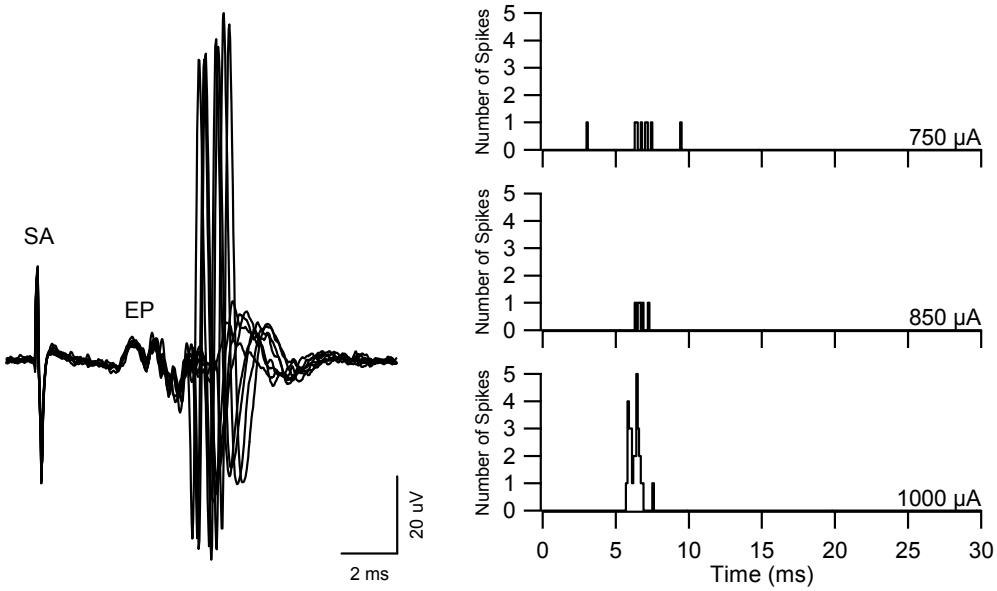


Figure 12. Representative example of an extracellular ICC single-unit response in a chronically stimulated neonatally deafened animal. The stimulus artifact (SA) is typically blanked to avoid contamination of single-unit activity, and adjustment of the oscilloscope trigger level can be used to eliminate the evoked potential (EP). Three poststimulus time histograms (PSTHs) at supra-threshold stimulus currents illustrate the relative levels of spike entrainment with increasing stimulus current.

Single and multi-unit activity in the ICC in response to supra-threshold electrical stimulation of the auditory nerve typically have latencies of between 5 and 20 ms (Fig. 12). As some units exhibited spontaneous activity, all analysis is limited to activity that occurs within an adjustable latency window of the electrical stimulus (typically set 5 to 20 ms). For each single or multi-unit I/O function and Latency curve are determined (Fig. 13). The PSTHs are used to determine the probability of firing and the mean and standard deviation of the latency of firing. In the case of multi-unit activity, or single-units that respond with more than one action potential per stimulus, all measurements are made on the first spike only.

The I/O function is fitted with a saturating Gaussian function (Sachs and Abbas 1974) using a least-squares fitting procedure. The resultant fitted curve is then used to estimate the stimulus current at threshold (probability of firing = 0.1), saturation (probability of firing = 0.9) and mid-dynamic range (probability of firing = 0.5).

Temporal Response Properties

The temporal response properties of each single-unit are examined by recording a series of I/O functions at different stimulus rates (Fig. 14). The new software allows all the I/O functions to be loaded and analyzed together, greatly reducing the time required to analyze each unit. The stimulus current required to reach saturation at each rate is determined and displayed. The maximum stimulus rate at which it is possible to saturate the response of the unit can then be determined.

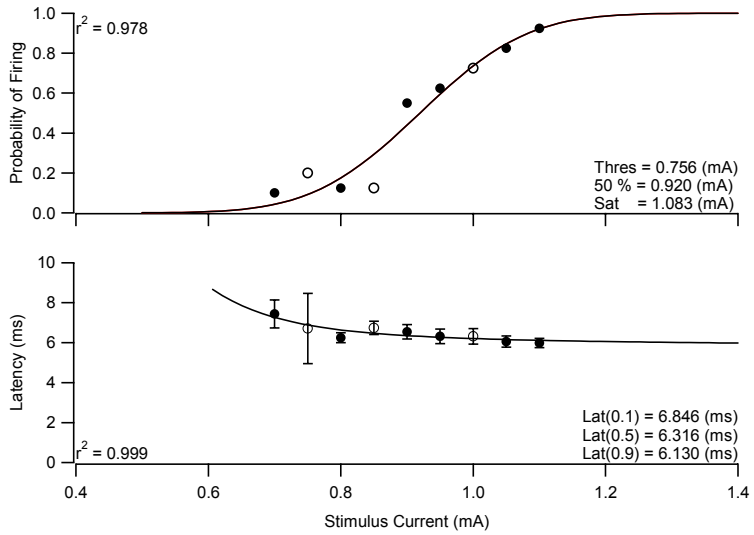


Figure 13. Representative example of an I/O function and latency curve for the same single unit as illustrated in Fig. 12.

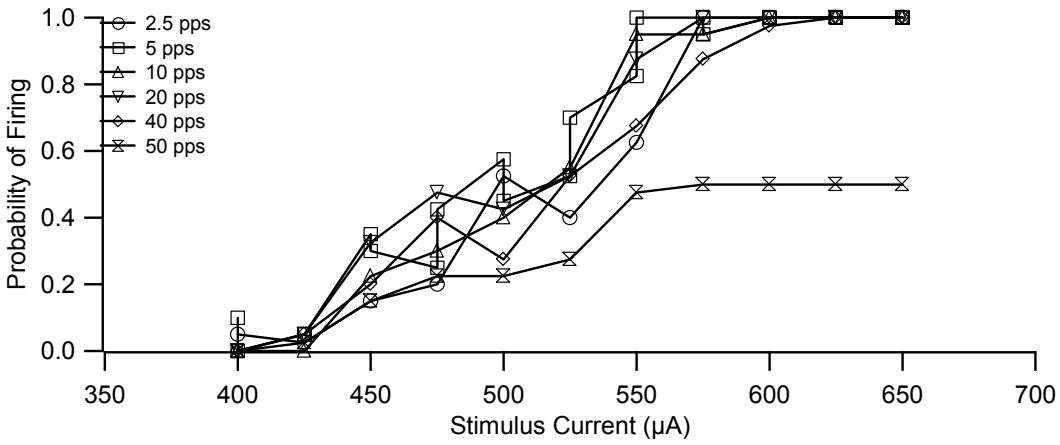


Figure 14. The temporal response properties of an IC single-unit can be determined by measuring several IO functions using different stimulation rates. For this unit, the maximum stimulus rate at which the response saturated was 40 pps.

Spatial Tuning Curves

A spatial tuning curve (STC) can be constructed based on the I/O function of each unit for a given penetration. As the stimulus current for mid-dynamic range is a more robust measure than either threshold or saturation when determined using the least-squares fitting procedures, analysis software allows either threshold, saturation or mid-dynamic range stimulus currents to be plotted against recording depth (Fig. 15). It is also possible to exclude units from the STC based on the r^2-value of their fitted I/O curve, with typical minimal accepted r^2-values being above 0.95.

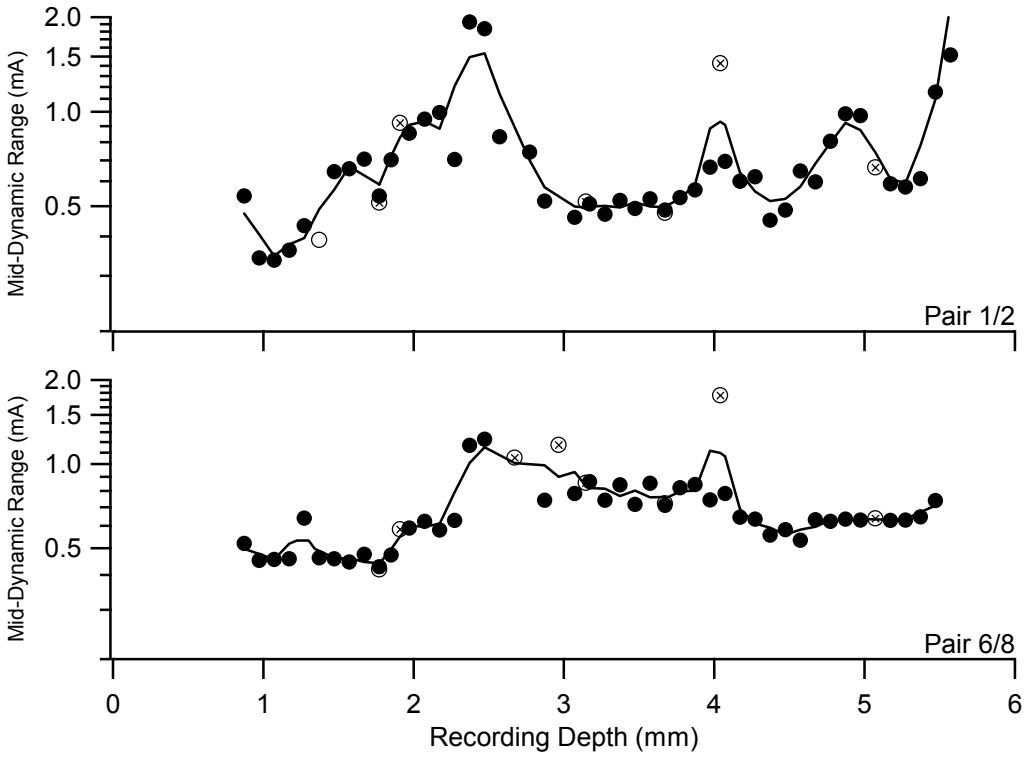


Figure 15. Spatial tuning curves for mid-dynamic range stimulus current for single and multi-units from a single penetration of the IC. Spatial tuning curves for both a basal pair (Pair 6/8) and a more apical pair (Pair 1/2) of stimulating electrodes are shown. Closed symbols represent multi-unit recordings, crossed symbols represent single-unit recordings and open symbols signify units with non-monotonic I/O functions. The solid curve represents a 3-point smoothed spatial tuning curve.

The most striking preliminary result is the alteration of the STCs of the chronically stimulated animals, as illustrated by the example in Fig. 15. The STCs appear to be shallower and wider than previously reported for normal, neonatally deafened and other chronically stimulated animals (see Snyder et al. 1990; Hardie and Shepherd 1999; Moore et al. 2002). This may in part be due to the differences in the stimuli used (charge balanced biphasic current pulses compared to continuous sinusoids) and the recording configurations (single-unit activity compared to single and multi-unit activity) of the various studies. Nevertheless, analysis indicates that this is from an increased representation of the chronically stimulated cochlea sector, rather than a general decrease in the efficacy of the cochlea stimulation.

The maximum stimulus rates at which it is possible to saturate the response of single-units (25 $\pm$ 15 pps, Mean $\pm$ SD) appears to be lower in stimulated neonatally deafened animals than the values for normal animals, but higher than values for neonatally deafened unstimulated animals that have previously been reported. Similarly, the mean first spike latencies appear to be longer in neonatally deafened stimulated animals than for normal animals, but shorter than for neonatally deafened unstimulated animals.

These preliminary results suggest that chronically stimulated, neonatally deafened animals have responses that are intermediate between normal and unstimulated neonatally deafened animals. These data are undergoing further analysis in preparation for publication.

2.3 Status of the primary auditory cortex in severely deafened cats following chronic intracochlear electrical stimulation

Single-unit activity in the primary auditory cortex (AI) in response to both electrical and acoustic stimulation was recorded from five severely deafened cats (ABR threshold 60 – 80 dB SPL), which are a sub-set of the group of cats used in the chronic stimulation experiments (see 2.1 above). Four of the cats received chronic electrical stimulation, while one served as a deafened control. Procedures for the acute experiment were similar to those for the IC experiments (see 2.2 above), including the determination of I/O and latency curves for each neuron. Additionally, the response to acoustic stimulation was also recorded to test the hypothesis that both the acoustic and electrically stimulated sectors of AN will undergo significant expansion in the AI in these animals.

Acoustic Stimulation
For each neuron an acoustic response area (RA) was determined (Fig. 16). Twenty brief (50 ms) tone bursts were given at each acoustic intensity/frequency pairing, which were presented in a pseudo-random order. The number of impulses recorded from the neuron within a 5 – 55 ms window after the tone burst was counted and plotted as shown in Fig. 16. The boundary of the RA defines the frequency tuning curve, and the tip of the tuning curve defines the characteristic frequency (CF; frequency at which threshold is lowest). For the RA illustrated here, the CF and threshold at CF are 744 Hz and 67 dB respectively.

Cortical Maps
The acoustic (CF and threshold at CF) and electrical (mid-dynamic range stimulus current (Pr 50 and latency) responses of 30–50 neurons in each animal were then used to construct cortical maps (see Figs. 17 & 18). Two major types of auditory frequency map were seen. In one, there was a rostral-to-caudal tonotopic gradient from very low to high frequencies within the range of surviving hearing of the most caudal part of AI. However, all points in the more rostral regions of AI, deprived of their normal input by the cochlea lesions, had CF near the lesion-edge frequency (e.g. Fig. 17A). In other cases, all of the mapped area was occupied by an expanded representation of the lesion-edge frequencies (Fig. 17B). These maps are similar to those seen in a previous study of animals with similar lesions using only acoustic stimuli. Preliminary analyses revealed no systematic changes to the acoustic representation as a consequence of chronic electrical stimulation. It must be emphasized, however, that a detailed analysis of these data has not yet been completed.

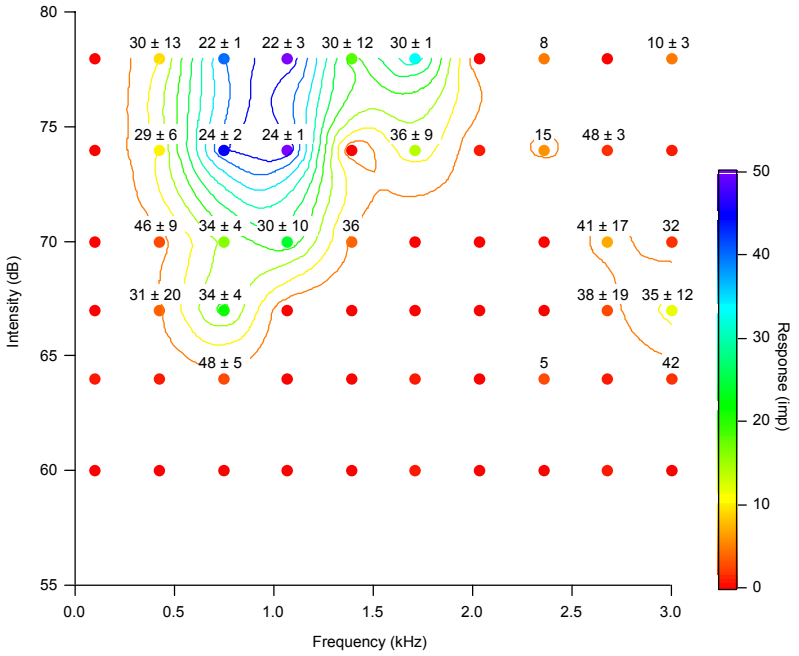


Figure 16. Representative example of an acoustic response area in a partial-hearing cat. Each point represents the number of action potentials evoked by 5 repetitions of a 50 ms tone burst at the frequency and intensity indicated (false color and contour lines). The mean (± SD) latency of the response is also shown.

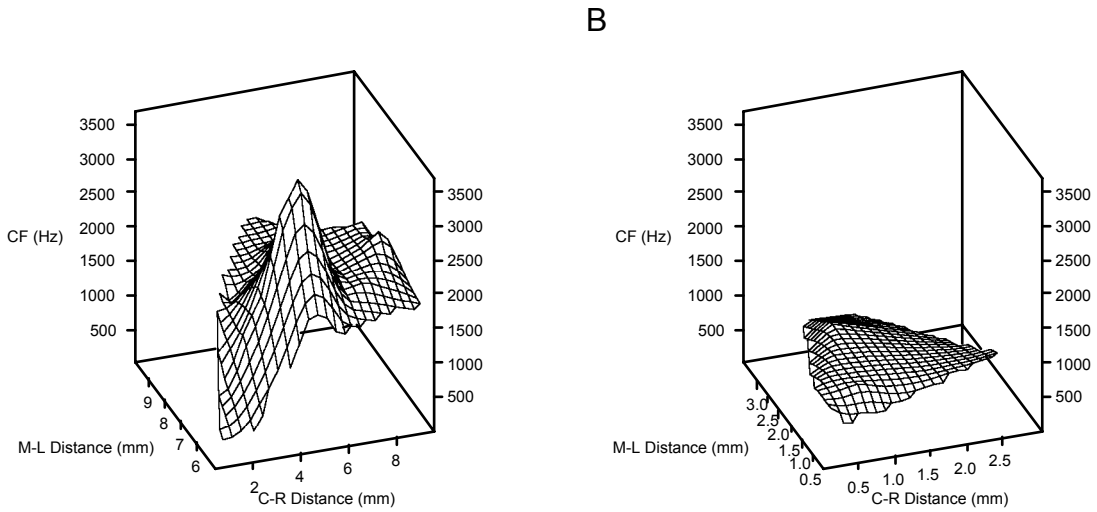


Figure 17. Cortical frequency maps from two different partial-hearing animals. A: Map showing tonotopic gradient up to ~ 3 kHz with a more rostral region containing an expanded representation of frequencies around 2 kHz (same animal as Fig. 16 - 37 weeks of wide sector electrical stimulation). B: Map showing enlarged representation of frequencies in 1 – 1.5 kHz range (43 weeks of localized sector electrical stimulation). M-L, medial-lateral; C-R, caudal-rostral.

Preliminary analysis of the electrical cortical maps suggests that localized cochlear stimulation does not necessarily radically alter the tuning observed with electrical stimulation. A clear 'valley' of lower threshold activation can be seen in the P50 cortical map (Fig. 18). There is an indication that stimulation in a more apical region of the cochlea (grid) results in a more caudal 'valley' than stimulation of a more basal region of the cochlea (false color image). Again, it must be stressed that these results are preliminary and are undergoing further analysis in preparation for publication.

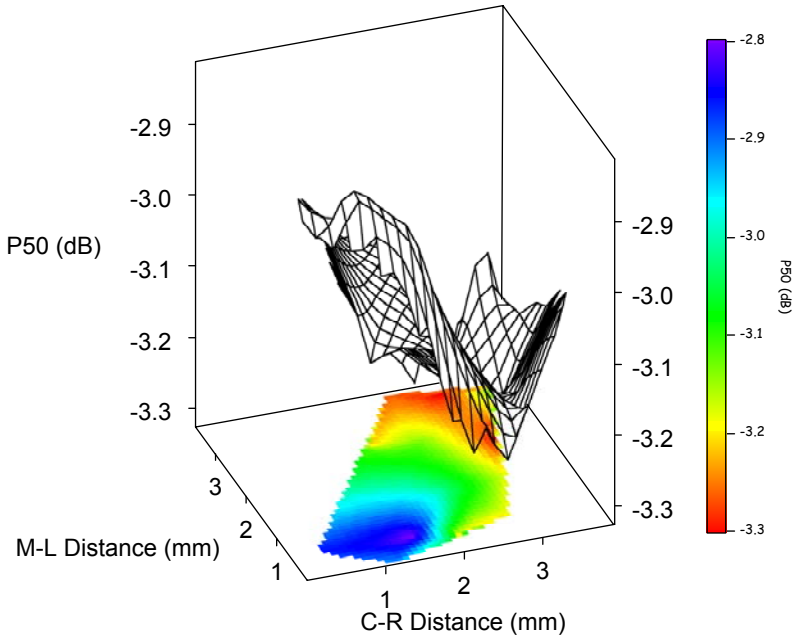


Figure 18. Cortical map for mid-dynamic range current (P50) for an apical pair of electrodes (grid) and a more basal pair of electrodes (false color image).

3. Electrical stimulation and neurotrophin administration in deafened guinea pigs

A major study associated with the present contract is an investigation of the extent of protective effects of chronic intracochlear electrical stimulation combined with simultaneous neurotrophin delivery. Loss of SGNs following a SNHL is considered, at least in part, to be a result of the withdrawal of neurotrophic support normally provided by hair cells (Ylikoski et al. 1993).

We have completed the first phase of this research under our present contract. Twenty-two guinea pigs were used, the animals were divided into four treatment groups (Table 1) with an additional two animals serving as normal hearing controls. Animals were deafened using a single co-administration of kanamycin and frusemide. Five days following deafening, ABRs were recorded to confirm the hearing loss; only animals with click thresholds >93 dB SPL in both ears were accepted. Deafened animals were then unilaterally implanted with a scala tympani electrode array incorporating a drug delivery system (Shepherd and Xu

2002; Fig. 19). The electrode array consists of three platinum (Pt) 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 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. The volume of the guinea pig scala tympani is ~4.76 μl (Dr. A.N. Salt; http://oto.wustl.edu/cochlea/). This pump therefore delivers ~5% of the scala tympani volume per hour. Our initial investigations using this array confirmed its efficacy by chronically stimulating the AN of guinea pigs while delivering the ototoxic agent neomycin (Shepherd & Xu 2002; 1st Quarterly Progress Report).

In the present study we have compared the chronic infusion of artificial perilymph with BDNF. This neurotrophin was selected following encouraging results in previous *in vitro* (Marzella et al. 1999) and *in vivo* applications (Staecker et al. 1996; Miller et al. 1997; Gillespie et al. 2003). Immediately following implant surgery, EABRs were recorded in response to bipolar scala tympani stimulation using 100 μs/phase biphasic current pulses. Five days following implant surgery, two of the four treatment groups commenced a chronic electrical stimulation program (Table 1) using a high rate amplitude modulated (AM) biphasic current waveform (Fig. 2). The amplitude of the AM stimulus waveform was set so that the minimum current level equaled the post-operative EABR threshold (i.e. the maximum stimulus intensity is 6 dB above EABR threshold). These stimulus levels were confirmed to be acceptable in the awake animal using basic behavioral indicators. Charge densities were in the range 8.4-34.3 $\mu C.cm^{-2}$ geom. per phase; well within levels considered electrochemically safe for Pt electrodes (Brummer and Turner 1977; Robblee et al. 1983).

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 ~6 h per day, 5 days per week from day 10 to day 33. Stimulus current and electrode voltage waveforms are monitored twice daily over the implant period to ensure normal electrode function. At completion of the 28-day implantation period, EABRs were again recorded in order to assess the functional status of the auditory pathway following the treatment. Each animal was then killed with an overdose of anesthetic and the cochleae harvested for histological analysis.

Typical EABRs, recorded both immediately post-operatively and at completion of the implantation period 28 days later, are illustrated in Fig. 20. Both AP/ES and AP/- treated animals routinely exhibited a small *increase* in EABR threshold over the implantation period. We have observed similar increases in the EABR threshold as a function of implantation time in our feline studies and have interpreted these changes as reflecting ongoing loss of SGNs due to the deafening process. Importantly, we observed a small but consistent *decrease* in EABR threshold over the implantation period in animals chronically treated with BDNF. These results, which are summarized in Fig. 21, were highly statistically significant.

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/-[3]	28	No	BDNF
AP/ES	28	Yes	Artificial perilymph[4]
AP/-	28	No	Artificial perilymph

Notes: [1] E 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.

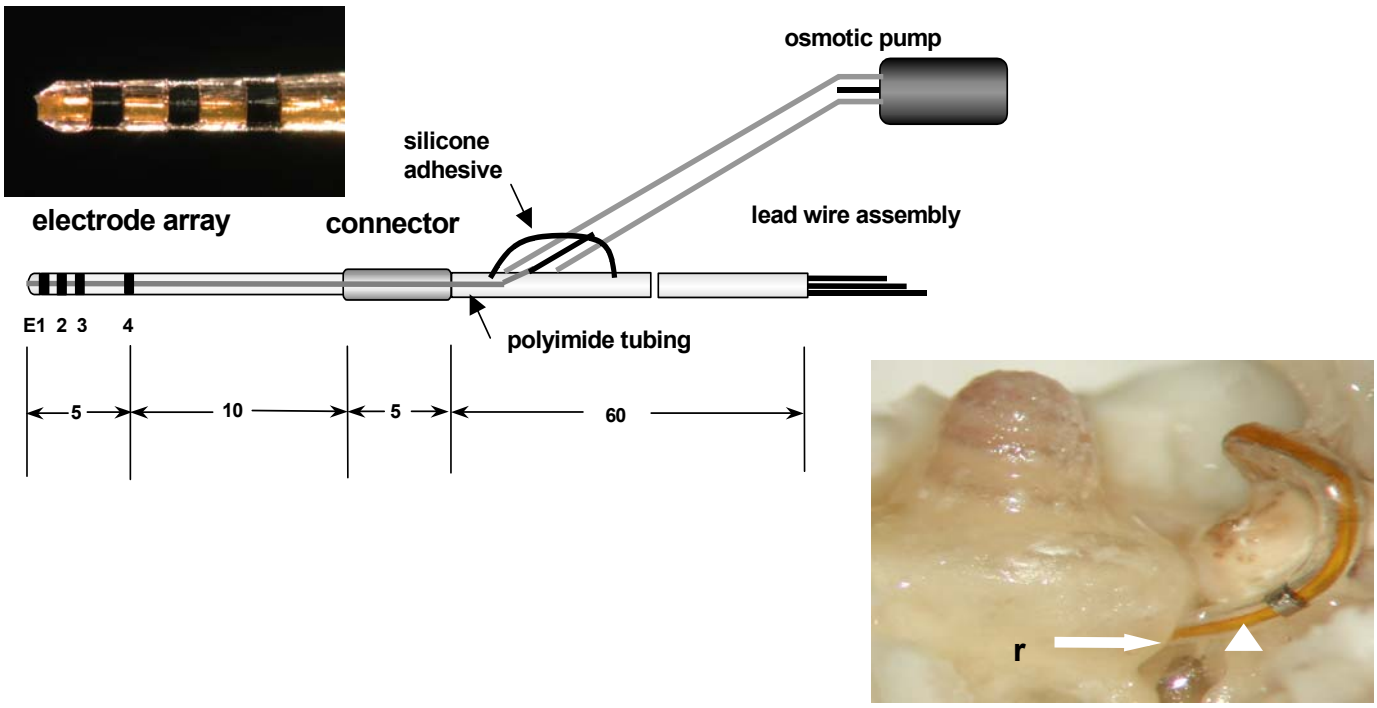


Figure 19. Diagram illustrating the electrode array incorporating a drug delivery system. Three Pt ring electrodes (top inset) are connected to a stimulator allowing controlled depolarization of the AN. A polyimide microtube (brown tube in lower inset) delivers the contents of the osmotic pump directly into the scala tympani. Each Pt electrode is 0.3 mm wide. Adapted from Shepherd & Xu 2002.

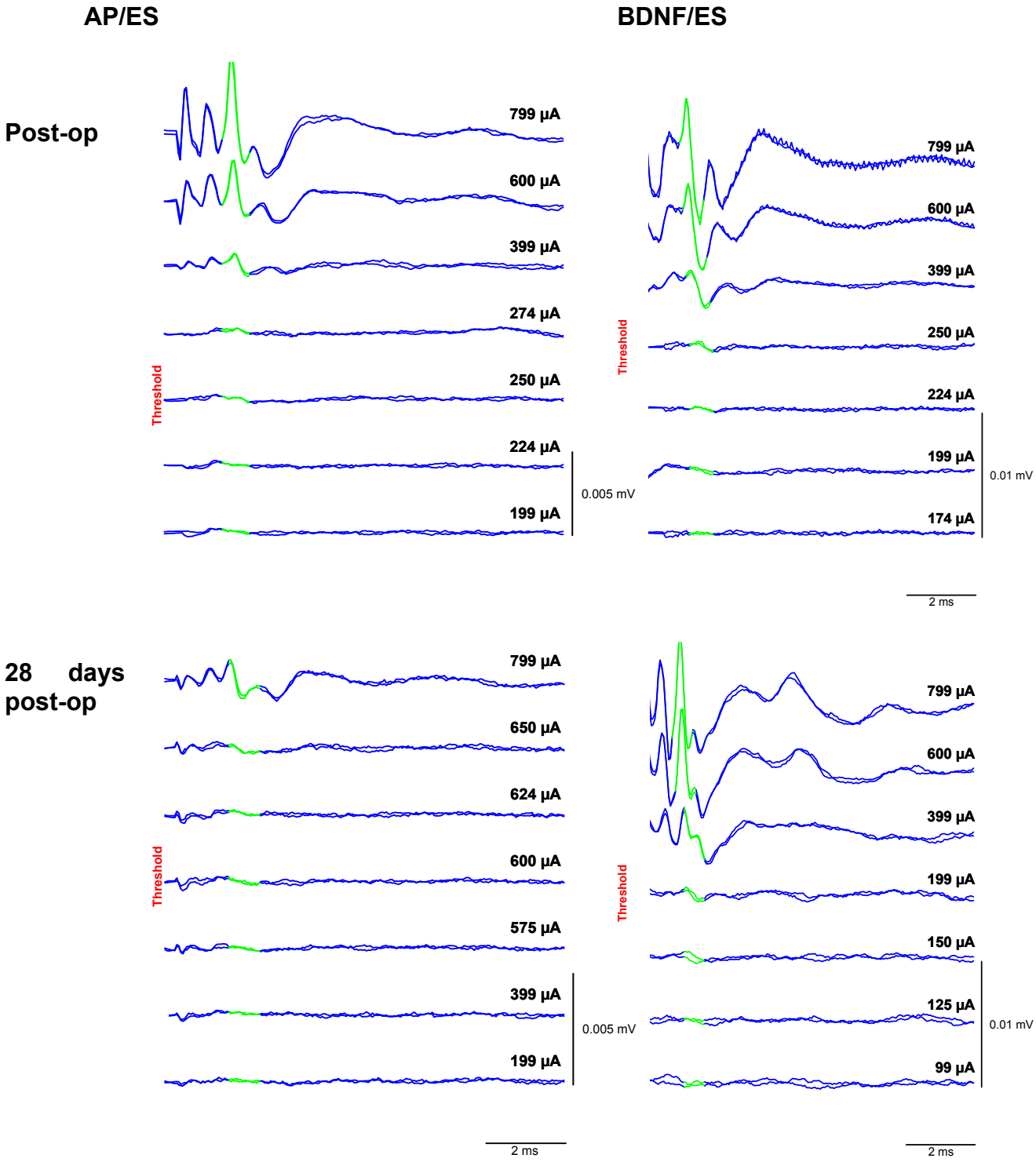


Figure 20. EABRs recorded from a guinea pig treated with AP/ES (left panel) and BDNF/ES (right panel) immediately following surgery (top), and at completion of the 28-day implantation/stimulation program (bottom). All EABRs were evoked using a 100 µs/phase biphasic current pulse delivered to a bipolar electrode pair. Two responses are recorded at each current level; each response is averaged from 500 presentations. Wave III, from which response amplitude, latency and threshold data are obtained, is highlighted in green. Note the reduction in threshold over the implant period in the BDNF/ES treated animal.

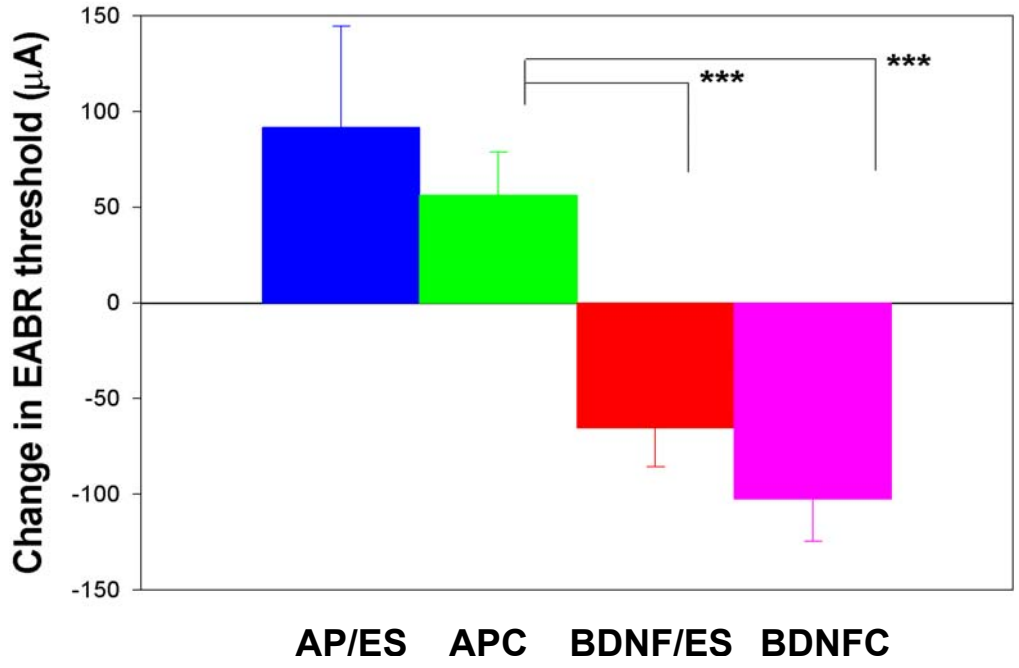


Figure 21. Mean change in EABR threshold over the 28 day implantation period for each of the four treatment groups in this study. The animals treated with artificial perilymph exhibited a systematic increase in EABR threshold over the 28 day implant period of between 55-85 μA. In contrast, both groups of animals treated with BDNF exhibited a systematic decrease in EABR threshold of 60-100 μA. The reductions in threshold associated with the BDNF treatment groups were statistically significant when compared with AP/- animals ($P < 0.001$; t-test). There was no statistical difference between AP/ES and AP/- treated animals. Likewise, there was no statistical difference between BDNF/ES and BDNF/- treated animals. Note that these changes in EABR threshold were independent of whether or not the animals were chronically stimulated. Error bar: SEM

SGN survival

Representative cochlear histology from both a BDNF/ES treated cochlea and its deafened, untreated contralateral control are illustrated in Figs. 22 & 23. The increased SGN survival in the BDNF/ES treated cochlea is evident even at low magnification. Figure 24, compares mean SGN density across 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/-). The other striking feature of these data is the small but significantly greater SGN density associated with the BDNF/ES treatment group over BDNF alone. This finding implies that electrical stimulation enhances the trophic effects BDNF has on mature auditory neurons.

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 cochleae, or deafened untreated control cochleae. These results are consistent with our preliminary data obtained from neonatally deafened, chronically stimulated cats (Fig. 11). Importantly, direct intracochlear delivery of the neurotrophin BDNF resulted in significantly greater SGN survival, which was *enhanced* when the neurotrophin delivery was combined with electrical stimulation. These anatomical findings are also reflected in functional changes (Fig. 21). We also observed significant differences in the soma area of treated versus deafened cochleae (Fig. 25). This work showed significantly larger soma area of SGNs in deafened cochleae treated with chronic electrical stimulation and neurotrophin delivery, compared with BDNF/-, AP/ES and AP/- cochleae. The effect of BDNF/ES on soma area decreased apicalward, and may reflect a reduction in the concentrations of BDNF as one moves more distal from the site of BDNF delivery (i.e. the tip of the electrode array). Of significance was the apparent lack of influence of AP/ES on SGN soma area. We have previously reported a similar deafness induced reduction in SGN soma area in deafened cats that was partially reversed following chronic electrical stimulation (Araki et al. 1998b). The reason for the lack of a stimulus-induced increase in SGN soma area in the present study is unclear, and awaits further analysis. Finally, we interpret the shrinkage of SGNs observed in the deafened cochleae as reflecting a down-regulation in biochemical activity prior to cell loss.

Future studies will examine whether the trophic advantage achieved by the combination of BDNF and ES is maintained with a single administration of BDNF with ongoing electrical stimulation. Importantly, Gillespie et al. (2003) has shown an accelerated loss of SGNs following withdrawal of exogenous neurotrophin in animals that were not subject to ES. Safety studies to investigate the long-term application of trophic agents into the cochlea are also required as are alternative delivery techniques. Finally, if long-term delivery of these peptides is necessary to maintain a trophic advantage, it will be important to examine whether or not the Trk receptors show a plastic response. For example, long-term exogenous application of BDNF may down regulate Trk B receptors on the SGN, leading to a reduction in trophic support of these cells.

The significant reduction in functional sensitivity associated with chronic neurotrophin delivery presented here, has also recently been described by Shinohara et al. (2002). These findings have potentially important clinical implications for cochlear implant recipients. Significant reductions in threshold result in large reductions in power consumption, providing an opportunity to develop smaller, more efficient implants. These results may also mean that increased numbers of smaller electrode contacts can be safely used. Finally, our functional data are consistent with the significant trophic effects on SGNs of BDNF and chronic electrical stimulation.

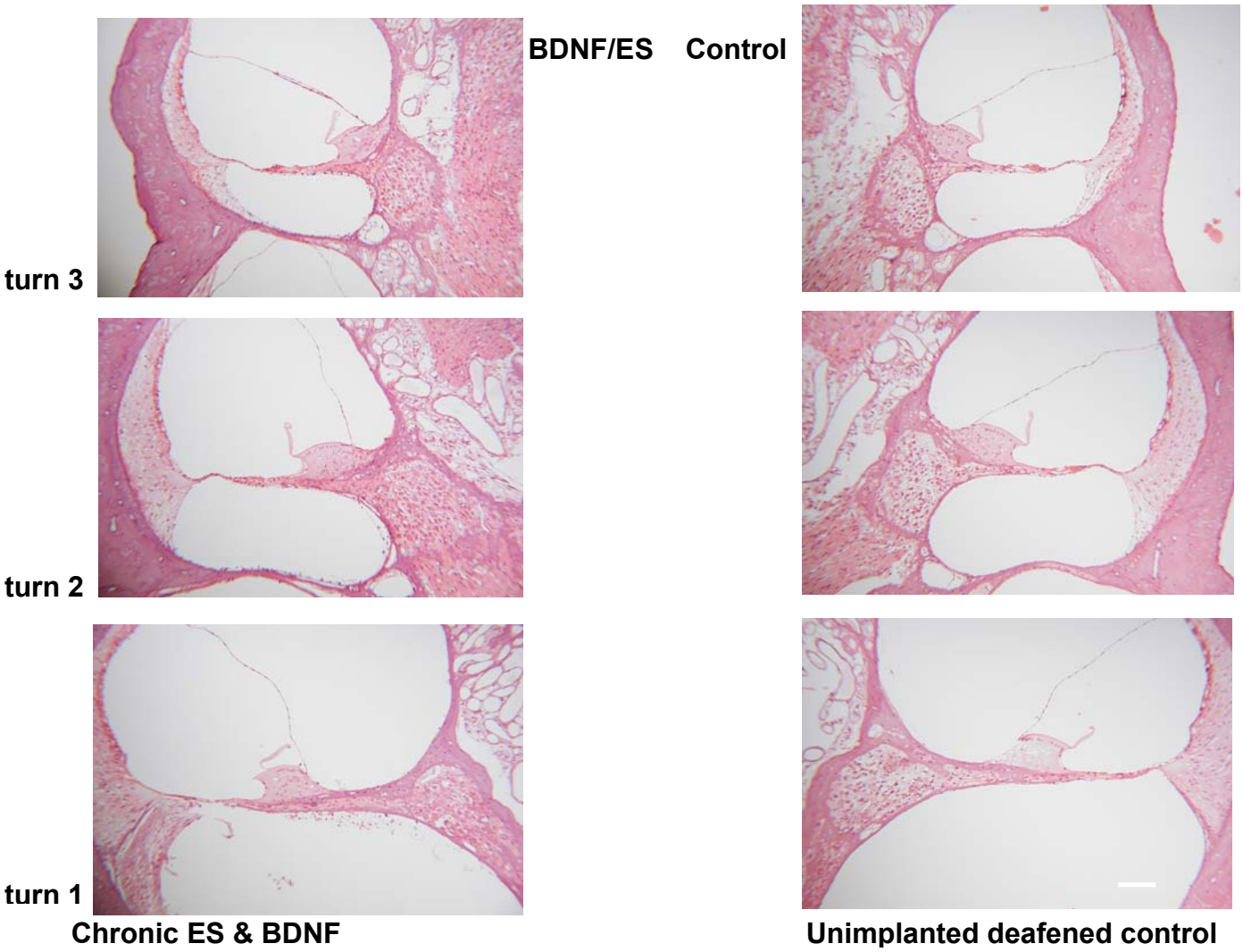


Figure 22. Representative low power micrographs of cochlear turns 1, 2 & 3. The left panel illustrates a cochlea that received chronic electrical stimulation and neurotrophin (BDNF) delivery over a 28 day period. The contralateral unimplanted deafened control cochlea is illustrated in the right panel. 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.

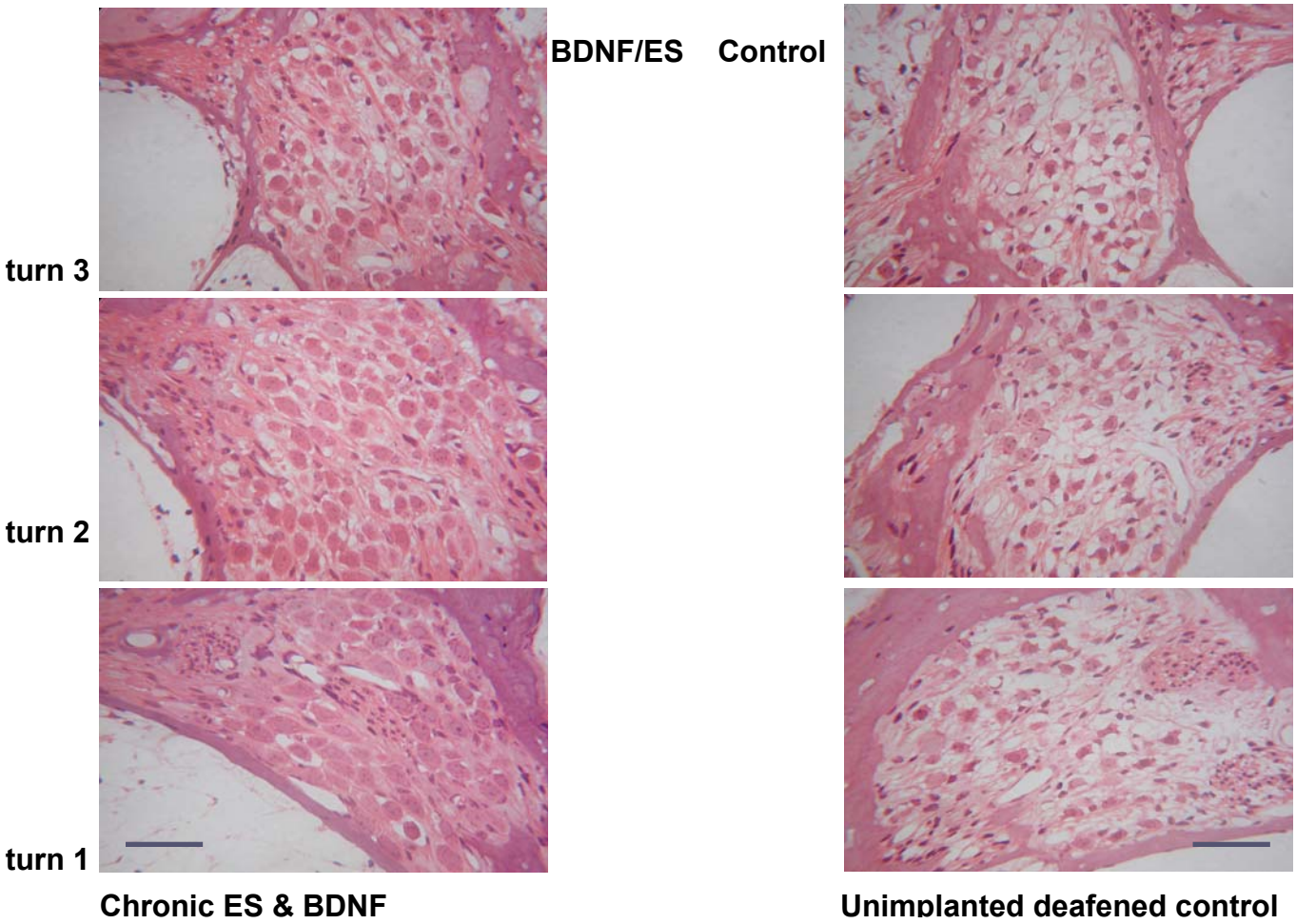


Figure 23. Higher power micrographs of the same cochlear sections illustrated in Fig. 22, showing the degree of SGN survival in Rosenthal's canal of a BDNF/ES treated cochlea (left panel), versus an unimplanted deafened control cochlea (right panel). Bar = 50 μm.

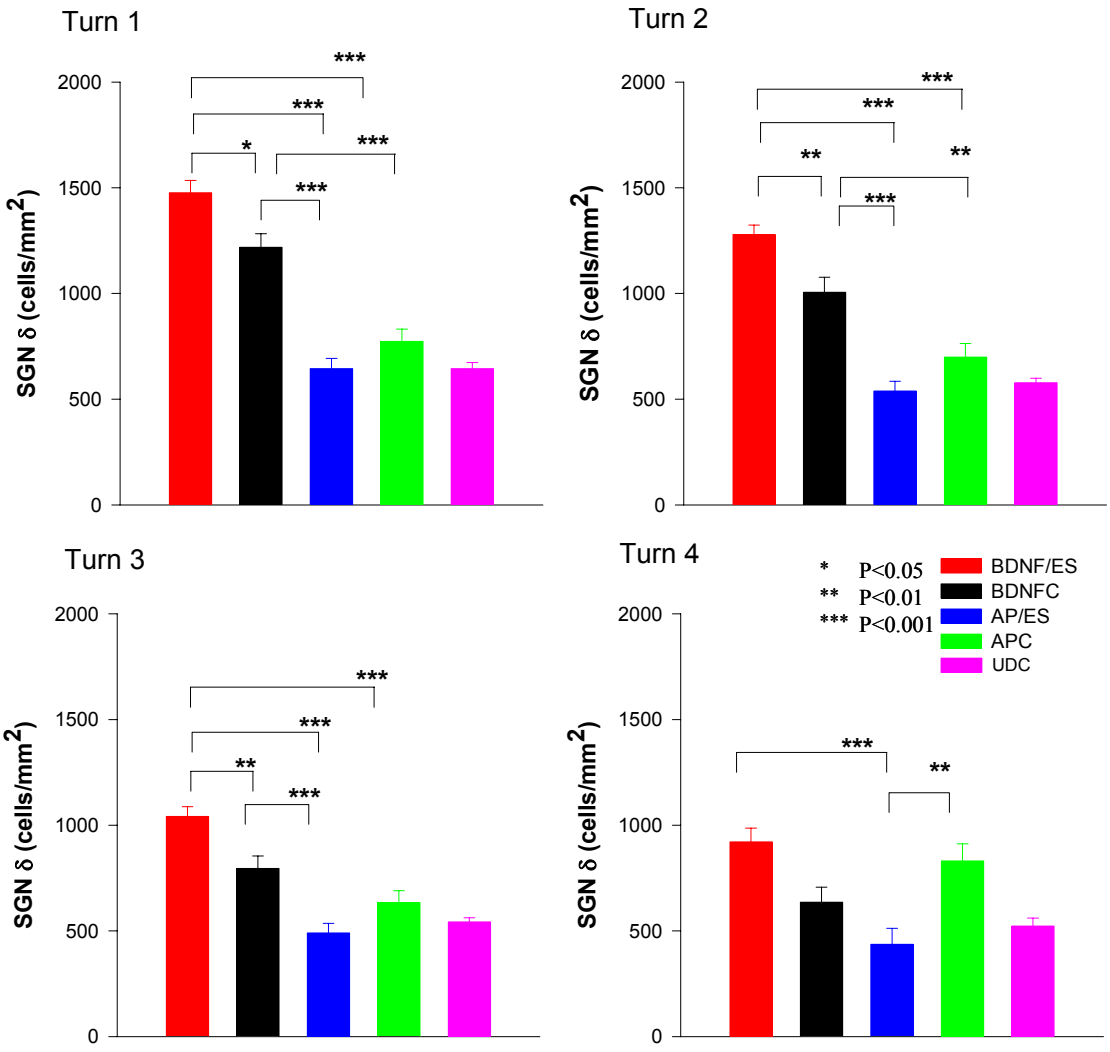


Figure 24. SGN density of implanted side of turn 1, 2, 3 and 4 in BDNF/ES, BDNF/-, AP/ES and AP/- treatment groups. For comparison, SGN density from the deafened, untreated control cochleae (UDC; pink) is also illustrated. These data are typically based on five guinea pigs per treatment group. Note that 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.

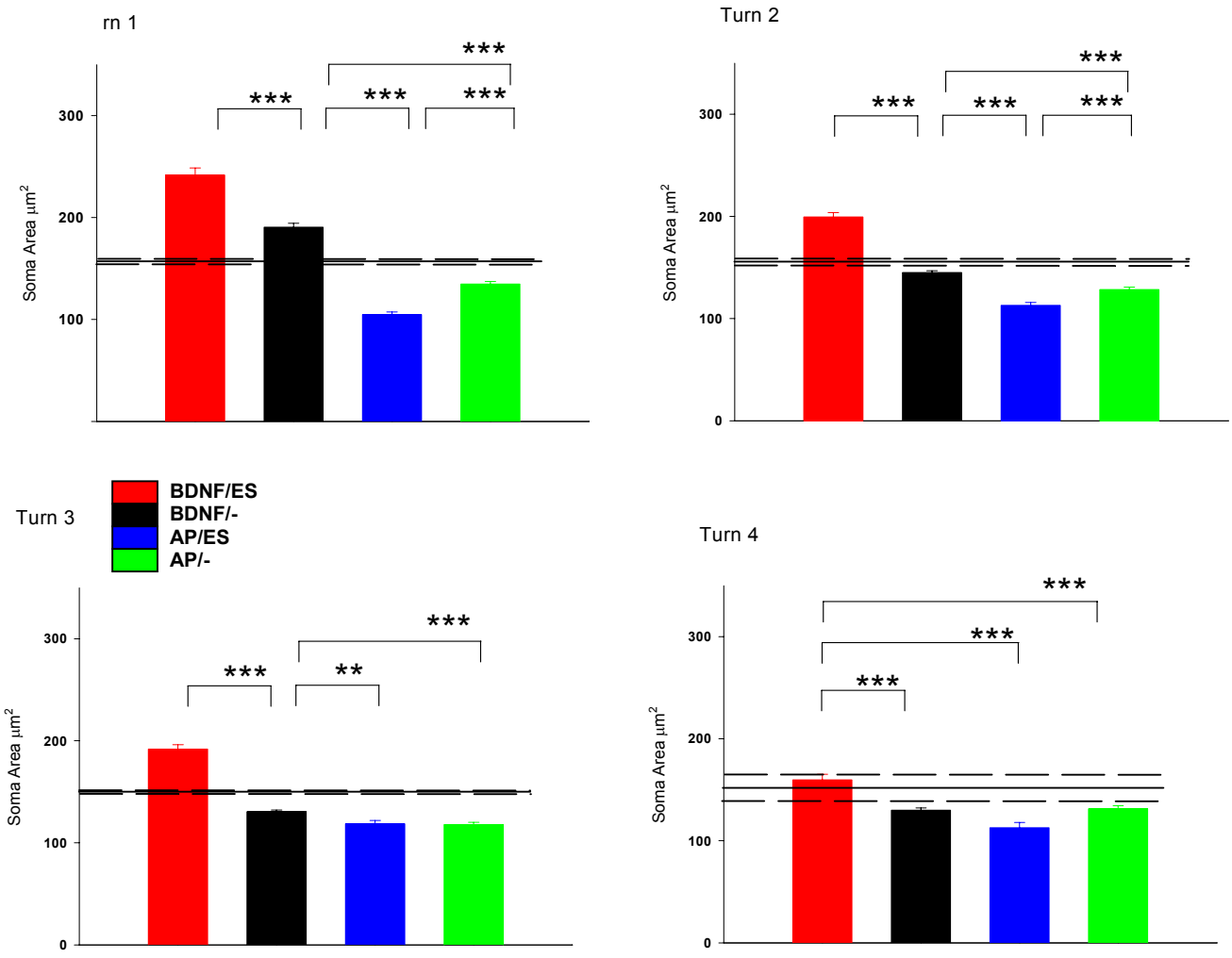


Figure 25. SGN soma area in turns 1, 2, 3 and 4 of BDNF/ES, BDNF/- AP/ES and AP/- treatment groups. ——— represents normal hearing SGN soma area (± SEM) and for clarity, statistics for these data are not illustrated.

4. Cell-based therapies

The survival of normal peripheral nerves relies, in part, on trophic support from surrounding cells including Schwann cells. Following acute trauma to peripheral nerves, the natural secretions and physical support of activated Schwann cells are crucial for neural regeneration (Gestwa et al. 1999). Experimental results show that Schwann cells alone can deliver substantial trophic support (Guenard et al. 1992; Levi and Bunge 1994; Ogden et al. 2000; Fornaro et al. 2001; Mosahebi et al. 2002). The resulting regeneration of axons is robust, precisely directed and concentration dependent, with more Schwann cells leading to greater regeneration (Hadlock et al. 1998). Furthermore, new axons are functionally remyelinated by the Schwann cells (Levi and Bunge 1994).

Given that following a SNHL the peripheral portion of the SGN undergoes degeneration and demyelination (Shepherd & Hardie, 2001) and is known to respond to exogenous neurotrophin treatment (e.g. see *9th Quarterly Progress Report*), we hypothesized that Schwann cell transplantation into the cochlea will provide trophic support to deafened SGNs.

Twelve adult GPs (400-600g) with an ototoxically induced, bilateral SNHL received an infusion of neonatal rat Schwann cells two or four weeks after systemic deafening and were sacrificed two weeks later (Table 2). The round window was exposed via a dorsal approach and a sterile delivery tube, attached to a 5 µL glass Hamilton syringe, was inserted into the scala tympani. Approximately 200,000 cells were gradually (0.5 µL per minute) delivered in a 2 µL suspension into the scala tympani. Two weeks following surgery the animals were euthanased and the cochleae prepared for histological examination. The density of SGNs was quantified by calculating their survival in Rosenthal's canal in Turns 1-3 in mid-modiolar sections. The number of surviving SGNs was determined by counting each SGN with a visible nucleus. The total count divided by the area measured indicates SGN density (cells/mm^2). Neuronal densities for each cochlear turn in the Schwann cell treated groups were statistically compared with the corresponding region in the untreated contralateral control cochlea using the non-parametric Mann-Whitney U test.

Figure 26 illustrates SGN density in upper turn 1 of Rosenthal's canal for two representative 2- and 4-week deafened animals. Figure 27 graphically compares the mean SGN densities for turn 1 between Schwann cell treated and the deafened untreated contralateral control cochleae. All statistical data presented are mean + SEM. Statistical analysis of SGN densities demonstrates a significant difference between the treated (967.7 + 30.5) and control cochleae (835.2 + 28.5) in turn 1 of 2-week deafened animals (Mann-Whitney U test $p<0.05$). No significant difference was detected (Mann-Whitney U test $p>>0.05$) between the density of surviving SGNs in turn 2 or 3 for the 2-week deafened animals or any turns for the 4-week deafened animals.

Table 2: *in vivo* Schwann cell study: summary of experimental animals

Animal Number	n	Deafened	Duration prior to implantation (weeks)	Post-implantation survival times (weeks)
GP 1-6	6	✓	2	2
GP 7-12	6	✓	4	2

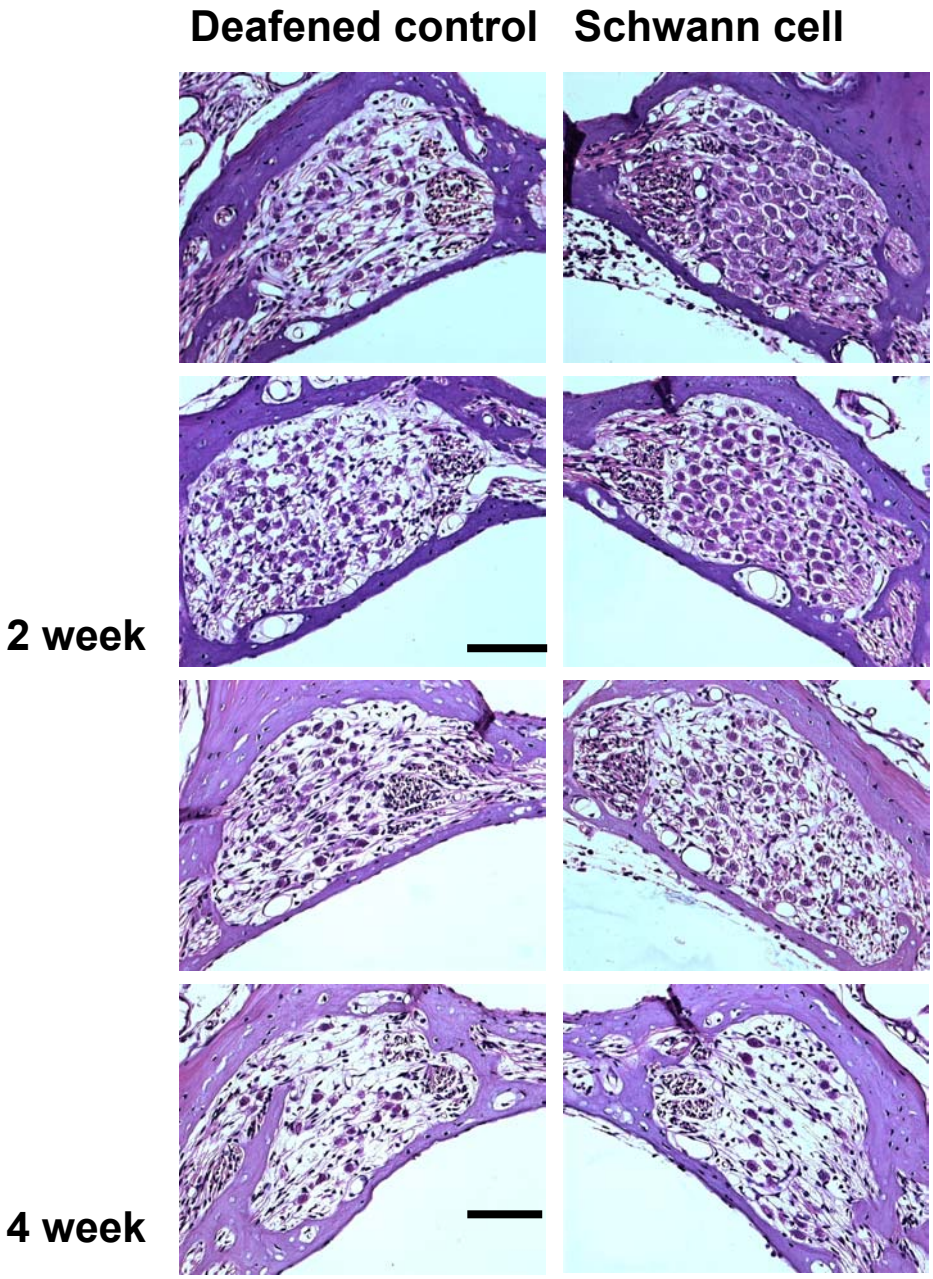


Figure 26. Representative examples of Rosenthal's canal from the upper turn 1 of the contralateral untreated control (left) and Schwann cell treated (right) cochleae from animals in the 2-week (top two panels) and 4-week (bottom two panels) deafened groups. Scale bar = 100μm

This study has shown that a single delivery of Schwann cells can provide trophic support to SGNs following aminoglycoside-induced deafness and is the first *in vivo* report of a trophic influence of transplanted Schwann cells on SGNs. The mechanisms by which transplanted Schwann cells effect these changes on SGN survival are unknown. It is likely to be a result of diffusible trophic factors secreted from the Schwann cell. The characteristics of such secretions are controlled by the interaction of the Schwann cell population with the specific biochemical

environment, and may include various neurotrophins, extracellular matrix molecules and neural cell adhesion molecules.

Although further work is required to refine our knowledge of the mechanisms in play and the exact conditions required for SGN rehabilitation, these results are worthy of continued experimental attention. The study will be expanded to explore the spatial and temporal restrictions of the trophic effects, the effects of vehicle alone (sham control) and the survival and distribution of the Schwann cells within the cochlea.

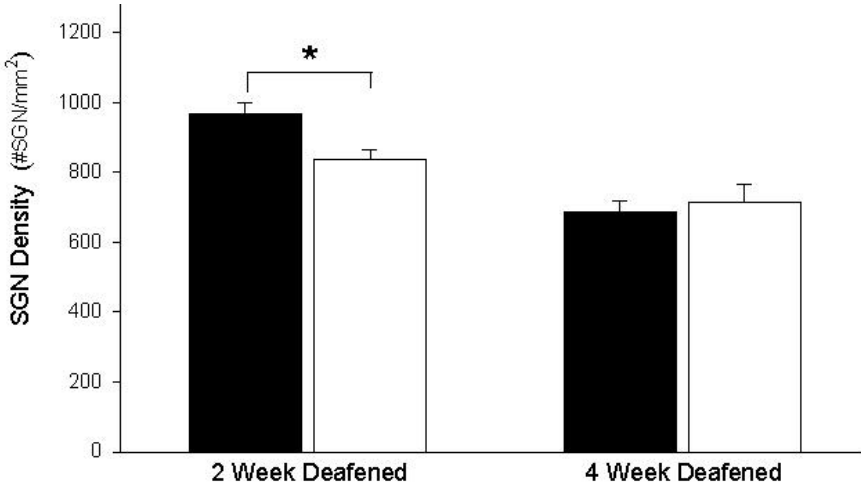


Figure 27. SGN density in the basal turn in Schwann cell treated (black) versus deafened control cochleae (white).

5. Response of the cochlea to a SNHL

Using a neonatal deaf rat model and free-floating immunochemistry protocols (Hurley et al. 2003), we are examining the extent of myelin loss from SGNs as a function of time following a SNHL. Deafening is known to result in loss of myelin surrounding the SGNs (Spoendlin 1984; Shepherd and Hardie 2001). What remains to be determined is how far this loss extends centralward along the central processes of the SGN. The extent of this myelin loss will have important implications for the initiation and conduction of action potentials in the deafened auditory nerve.

Rat pups were profoundly deafened as neonates by co-administration of gentamicin and frusemide. Myelin loss in the peripheral processes, SGN soma, and central processes of the lower two cochlear turns were assessed 2-days, 2-weeks, 1-month, 2-months and >6-months following the onset of SNHL. Cochleae were processed for free-floating immunohistochemistry using antibodies to peripheral myelin (PO) and DAB labeling. The amount of myelin was quantified at each time point using optical densitometry (Figs. 28 & 29).

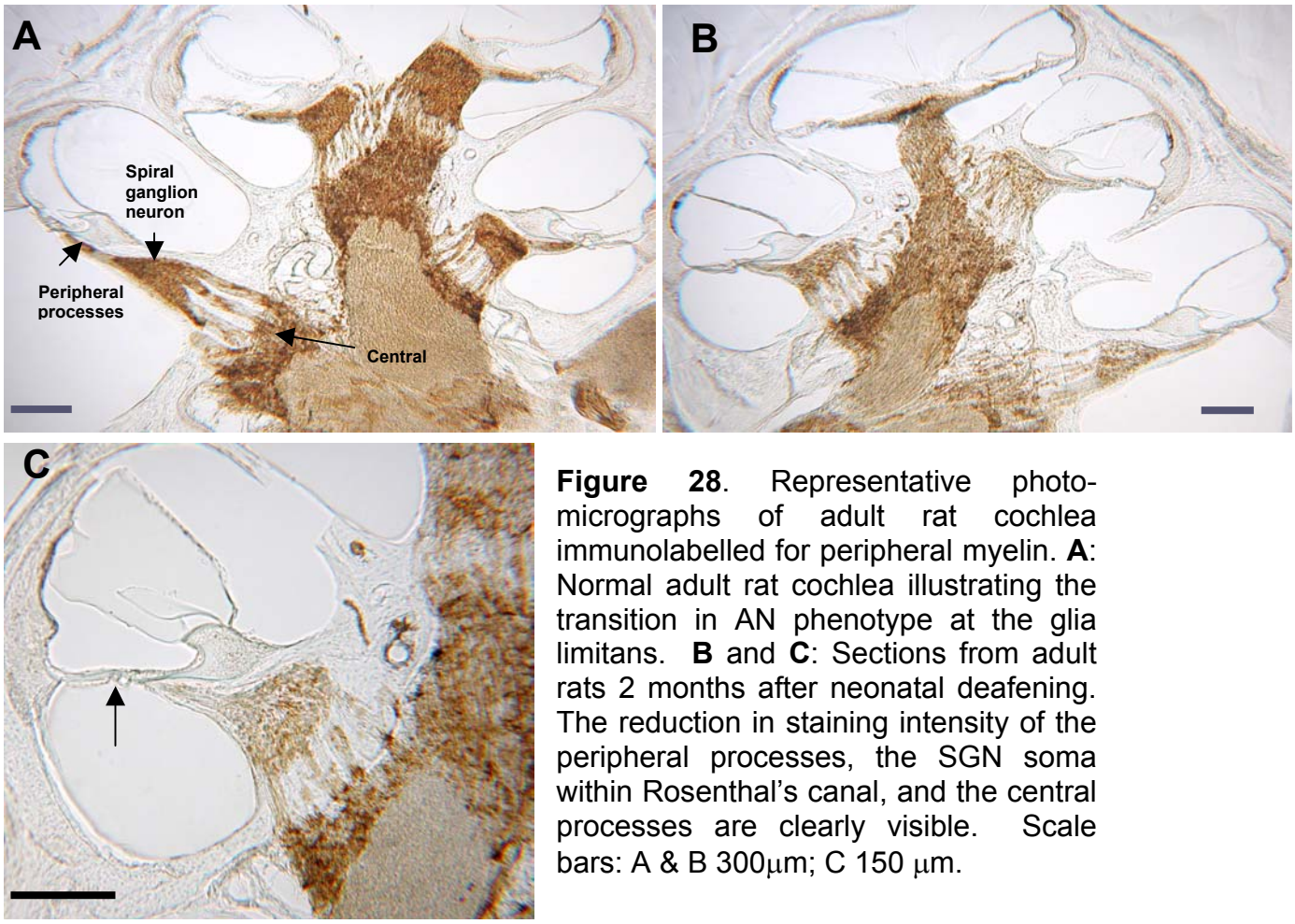


Figure 28. Representative photo-micrographs of adult rat cochlea immunolabelled for peripheral myelin. **A**: Normal adult rat cochlea illustrating the transition in AN phenotype at the glia limitans. **B** and **C**: Sections from adult rats 2 months after neonatal deafening. The reduction in staining intensity of the peripheral processes, the SGN soma within Rosenthal's canal, and the central processes are clearly visible. Scale bars: A & B 300μm; C 150 μm.

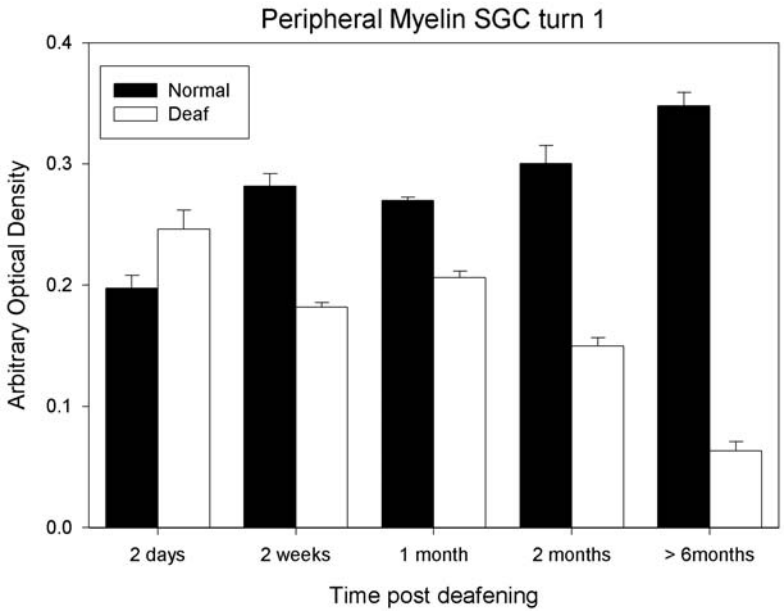


Figure 29. Relative optical density measurement of the extent of peripheral myelin in SGN soma as a function of age post-deafening. Age-matched control data are illustrated in black. These data are based on n=5 animals/group (total n=50) and show an ongoing loss of myelin following deafening, with maximum loss occurring for periods of deafness > 1 month.

This research has demonstrated a gradual centralward loss in PO with duration of deafness. The myelin sheath plays an important role in the initiation and propagation of action potentials (Smith and McDonald 1999). This work will be expanded to study other important changes in the deafened cochlea including Schwann cell survival, expression of neurotrophin receptors, and K^+ and Na^+ ion channel distribution. Efforts will then be made to determine the plastic response of these proteins following exogenous neurotrophin and/or electrical stimulation of the AN.

6. Other research outcomes arising from the present contract

- Development of deaf animal models for rat and guinea pig based on a single co-administration of an aminoglycoside and loop-diuretic.

- Software development base on Igor Pro (Wavemetrics Inc, Lake Oswego, OR), including cochlear graphic reconstruction, ABR, EABR and single/multi-unit analysis.

- A scanning electron microscopy study of the modiolar osseous spiral lamina in human and cat.

7. Conclusions and potential clinical outcomes

Research performed under this contract has shown that electrical stimulation alone does not provide trophic support to SGNs *in vivo* in either a neonatally deafened feline model or a guinea pig model of a post-lingually acquired hearing loss. A number of reports from several laboratories have described significant increases in SGN survival in ototoxically deafened animals following chronic electrical stimulation of the AN (Lousteau 1987; Hartshorn et al. 1991; Leake et al. 1991; Leake et al. 1992, 1995; Miller et al. 1995; Mitchell et al. 1997; Leake et al. 1999; Kanzaki et al. 2002). In contrast to these reports, work from other laboratories, including ours, has reported no such trophic influence on SGNs (Shepherd et al. 1994; Araki et al. 1998a; Li et al. 1999; Shepherd et al. 2002; Andrew et al. 2003). Any attempt to establish key differences between these studies that may indicate underlying mechanism(s) responsible for stimulus-induced trophic support of SGNs, is complicated by the many different variables across these studies (Miller 2001). Factors including species, electrode geometry, age at stimulation (mature versus developing animals), stimulus intensity, stimulus rate, the charge recovery system used (capacitive coupled electrodes), and stimulus duration, don't appear to determine whether or not electrical stimulation provides a trophic influence on SGNs.

One potentially significant factor may be associated with the use of neomycin (NE) as a deafening agent. Studies in which kittens were deafened using multiple injections of neomycin have routinely shown a stimulus-induced trophic effect on SGNs (Leake et al. 1991; 1992; 1995; 1999), while kittens deafened at the onset of hearing using a single co-administration of an aminoglycoside and loop diuretic

show no evidence of electrical stimulation providing a trophic influence on SGNs (e.g. Fig. 11, this report). Multiple injections of NE produces a gradual and delayed SNHL (Shepherd and Clark 1985), unlike the very rapid loss observed using a "single-shot" of kanamycin (KA) and ethacrynic acid (EA) (Xu et al. 1993; Shepherd and Martin 1995). It is likely that when these deafened kittens commence chronic electrical stimulation (~8 weeks of age), there is a far greater SGN population in animals deafened via multiple NE injections versus those deafened via KA/EA. SGNs in KA/EA deafened animals may, therefore, be further down the path towards cell death and have lost their ability to be rescued by chronic electrical stimulation. This effect may be at the individual neuron level or may be a more global effect that also includes the loss of key supporting cells including Schwann cells. Support for this hypothesis comes from our recent data showing a significant trophic effect of electrical stimulation on SGNs when co-treated with BDNF (Fig. 24), where SGNs and possibly support cells were maintained by the neurotrophin. In future studies we will deafen kittens using the multiple NE applications to test this hypothesis.

A major finding associated with the present contract has been the highly significant trophic effects of exogenous neurotrophin delivery combined with chronic electrical stimulation of the AN. This research demonstrated both an anatomical and functional advantage that would have important implications for cochlear implant subjects. There are several significant research questions that must first be carefully addressed prior to the consideration of any clinical application. First, the duration of neurotrophin delivery necessary to maintain a trophic advantage is a key question relating to delivery techniques, safety issues and cost benefits. Recently, Gillespie *et al*. (2003) reported an accelerated loss of SGNs following the withdrawal of exogenous BDNF in deafened animals. We are currently examining the hypothesis that a trophic advantage can be maintained through electrical stimulation alone, following an initial period of co-delivery of neurotrophin and stimulation. Second, the method of neurotrophin delivery must be addressed in more detail. While a drug delivery system incorporated in an electrode array - as developed under the present contract - is a useful experimental procedure, *we do not consider it an adequate solution for clinical application*. Specifically, a major limitation associated with such a design would be the risk of infection spreading from the pump/delivery system directly into the cochlea. This risk would be likely to increase greatly upon the surgical replacement of delivery pumps. Third, it is necessary to demonstrate that the neurotrophic advantage we and others have described in guinea pig, are also evident in other mammalian species. Finally, the effects of long-term delivery of neurotrophins on non-neural cochlear structures, as well as the CNS, must be carefully addressed in safety studies. Aspects of this additional research are currently under investigation.

Our study of the *in vivo* transplantation of Schwann cells into the deafened cochlea demonstrated a small but significant trophic effect localized to the basal turn of the cochlea in animals deafened for a period of 2 weeks. This work again highlights the potential for rescue of SGNs that have not proceeded down the pathway leading to cell death. The work also highlights the important neurotrophic influence support cells - such as Schwann cells - have on SGNs.

We intend to expand our research of cell-based therapies for rehabilitation of the deafened cochlea with an objective of developing potential clinical applications.

Finally, the development of a new histological technique for cochlear immunochemistry, developed under the present contract, provides us with a powerful new tool with which to study the molecular plasticity of the cochlea following a SNHL and in response to SGN rescue via neurotrophin delivery and/or electrical stimulation (Hurley et al. 2003). It is anticipated that this procedure can be adapted to studies examining mRNA expression via *in situ* hybridization.

8. Publications arising from this contract

Peer reviewed journal publications:

1. Shepherd, R.K. and Hardie, N.A. Deafness induced changes in the auditory pathway: Implications for cochlear implants. Audiol & NeuroOtol, 6: 305-318, 2001.

2. Hellier, W.P.L., Wagstaff, S.A., O'Leary, S.J. & Shepherd, R.K. Functional and morphological response of the stria vascularis following a sensorineural hearing loss. Hear. Res. 172: 127-136, 2002.

3. Shepherd R.K., & Xu, J.. A multichannel scala tympani electrode array incorporating a drug delivery system for chronic intracochlear infusion. Hear. Res. 172: 92-98, 2002.

4. Hurley P.A., Clarke, M., Crook, J.M., Wise A., and Shepherd R.K. Cochlear immunochemistry – A new technique based on gelatin embedding. J. Neurosci. Methods 129: 81-86, 2003.

5. Shepherd R.K. and Colreavy M.P. Surface microstructure of the perilymphatic space: Implications for cochlear implants and cell or drug based therapies. (submitted).

6. Shepherd, R.K., Roberts, L. A. and Paolini A.G. A long-term sensorineural hearing loss affects auditory nerve fiber response properties evoked by electrical stimulation. (submitted).

Book Chapters:

1. Shepherd, R.K. The Auditory System. In: Neuroprosthetics: Theory and Practice. K. Horch & G. Dhillon (Eds), World Scientific Publishing, (in press).

2. Seligman, P.M. & Shepherd, R.K. Cochlear Implants. In: Neuroprosthetics: Theory and Practice. K. Horch & G. Dhillon (Eds), World Scientific Publishing, (in press).

3. Shepherd, R. Central Auditory Prostheses. In:Neuroprosthetics: Theory Practice. K. Horch G. Dhillon (Ed), World Scientific Publishing, (in press).

Conference Abstracts:

1. Huang, C.Q., Shepherd, R.K. & Carter, P.M. "Electrical stimulation of the auditory nerve: pH changes in vivo and in vitro. 31st Annual Neural Prosthesis Workshop, National Institutes of Health, Bethesda, MD, U.S.A., October 25-27, 2000.

2. Hardie, N.A. & Shepherd, R.K. Expression of TRK B in the cochlear nucleus of neonatally deafened cats. Proc. Aust. Neurosci. Soc. 12, p. 167, 2001.

3. Rees, S., Rhen A.E., Loeliger M., Hardie, N., Dieni, S. & Shepherd R.K. Chronic placental insufficiency has long-term effects on auditory function. Proc. Aust. Neurosci. Soc. 12, p. 185, 2001.

4. Hellier, W., O'Leary, S.J. & Shepherd, R.K. Cochlear endocochlear potentials following prolonged deafness. 51st Australian Soc. Otolaryngology Head & Neck Surgery Scientific Meeting, Adelaide, March 2001, p 18.

5. Shepherd, R.K., Colreavy, M. P., Hellier, W.P.L. & O'Leary, S.J. Morphological and functional studies of the mammalian cochlea: Implications for cochlear implants. 2001 Conference on Implantable Auditory Prostheses, Monterey CA, p. 9.

6. Hellier, W., O'Leary S.J. & Shepherd R.K. Endocochlear potentials and cochlear strial morphology following sensorineural hearing loss in the guinea pig. IUPS Satellite Symposium, Auckland, New Zealand, August 2001. p. 348.

7. O'Leary, S.J. & Shepherd, R.K. Auditory nerve stochasticity and deafness. Inner Ear Biology meeting, Liège, Belgium, September 7 –10, 2002.

8. Shepherd, R.K., Serruto, A., Crook J.M., Fallon, J.B., Epp, S. & Xu, J. Protective effects of cochlear implantation on the deafened auditory system" Frontiers in Otorhinolaryngology 2002, Noosa Heads, August 2002, pp.27-28.

9. Epp, S.B., Serruto, A., Xu, J., Crook, J.M., & Shepherd, R.K. Does chronic electrical stimulation provide trophic support of spiral ganglion neurones in vivo? Proc. Aust. Neurosci. Soc. 262, 2003.

10. Hurley, P.A., Clarke, M.M., Crook, J.M., Wise, A.K., & Shepherd, R.K. A new technique for optimal cochlear immunochemistry. Proc. Aust. Neurosci. Soc. 263, 2003.

11. Serruto, A., Crook, J.M., Epp, S.B. & Shepherd, R.K. Maintenance of auditory neurones following deafness. Proc. Aust. Neurosci. Soc. 264, 2003.

12. Shepherd, R.K., Serruto, A., Epp, S.B. & Crook, J.M. Protective effects of

electrical stimulation and neurotrophin delivery on auditory neurons *in vivo*: Implications for cochlear implants. Assoc. Res. Otolaryngol. , Abs.: 770 , 2003.

13. Xu, J., Feng, H.N. & Shepherd, R.K. Design of electrode assemblies for research on cochlear implant in live animals. 4th International Symposium on Electronic Implants in Otology & Conventional Hearing Aids, Toulouse, France, June 5-7, 2003.

14. Shepherd, R.K., Serruto, A., Epp, S.B. & Crook, J.M. Protective effects of electrical stimulation and neurotrophin delivery on auditory neurons *in vivo*: Implications for cochlear implants. 2003 Conference on Implantable Auditory Prostheses, Pacific Grove, CA, p 56.

15. Hurley, P.A., Crook, J.M. & Shepherd, R.K. Schwann cells are lost before myelin protein in sensorineural hearing loss. 2003 Conference on Implantable Auditory Prostheses, Pacific Grove, CA, p. 53.

16. O'Leary, S., Sly, D., Heffer, L., White, M., Wise, A., Shepherd, R. & Birch, M. Independence of auditory neuronal spiking at low discharge rate probabilities. 2003 Conference on Implantable Auditory Prostheses, Pacific Grove, CA, p. 60.

17. Hurley, P.A., Serruto, A., Crook, J.M. & Shepherd R.K. TrkB receptor expression in the cochlea following sensorineural hearing loss. Proc. Aust. Neurosci. Soc. (in press).

9. Post-graduate research performed under this contract

Completed Dissertation:

BSc. Honours:

J. Andrew "Rehabilitation of the deafened auditory nerve with Schwann cell transplantation" (H1A).

Submitted Dissertations:

MD

M. P. Colreavy "Cochlear ultrastructural morphology with regard to cochlear implantation". Submitted October 2003.

MAud.

L. C-C. Chen "The effect of chronic neurotrophin delivery on non-sensory tissue within the cochlea" Submitted November 2003.

Research in progress:

PhD.

P.A. Hurley "The response of the auditory system to deafness and reafferentation via a cochlear implant"

B. Coleman "Tissue regeneration in the deaf cochlea using stem cells"

Advanced Medical Science Degree.

S. McGuiness "Trophic effects in the deafened rat cochlea: combining electrical stimulation with neurotrophin delivery *in vivo*".

10. Academic activities of research staff as part of this contract

R. K. Shepherd:

Chair, 2003 Conference on Implantable Auditory Prostheses, Pacific Grove, CA, USA.

Invited Speaker presentations:

1. "Protective Effects of Patterned Electrical Stimulation on the Deaf Auditory System", 31st Neural Prosthesis Workshop, National Institutes of Health, Bethesda, Maryland, USA October 2000.

2. "Morphological and functional studies of the mammalian cochlea: Implications for cochlear implants", 2001 Conference on Implantable Auditory Prostheses, Asilomar CA, USA August 2001.

3. "Protective Effects of Patterned Electrical Stimulation on the Deaf Auditory System", 32nd Neural Prosthesis Workshop, National Institutes of Health, Bethesda, Maryland, USA October 2001.

4. "Protective effects of cochlear implantation on the deafened auditory system" Invited Speaker "Frontiers in Otorhinolaryngology 2002" Noosa Heads, August 2002.

5. "Protective Effects of Patterned Electrical Stimulation on the Deaf Auditory System", 33rd Neural Prosthesis Workshop, National Institutes of Health, Bethesda, Maryland, USA October 2002.

6. "Rescuing auditory neurones following sensorineural hearing loss: implications for cochlear implants", 23rd Annual meeting of the Australian Neuroscience Society, Adelaide, January 2003.

7. "Protective Effects of Patterned Electrical Stimulation on the Deaf Auditory System", 34th Neural Prosthesis Workshop, National Institutes of Health, Bethesda, Maryland, USA October 2003.

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