Eighth Quarterly Progress Report

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Protective Effects of Patterned Electrical Stimulation on the Deafened Auditory System

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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 eighth quarter of this contract the following activities were completed:

- Continued to manufacture guinea pig electrode arrays with chronic delivery systems.
- Completed deafening and chronic stimulation of adult guinea pigs, including terminal acute electrophysiological experiments. Aspects of this work will be presented in the present report.
- Continued histological analysis of cochleae and auditory brainstem structures in both cats and guinea pigs following completion of their chronic electrical stimulation programs.
- 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).*

- Preparation and presentation of a summary of our work at the 2002 Frontiers in Otorhinolaryngology conference held at Noosa Heads, Queensland, Australia July 31-August 2.

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).

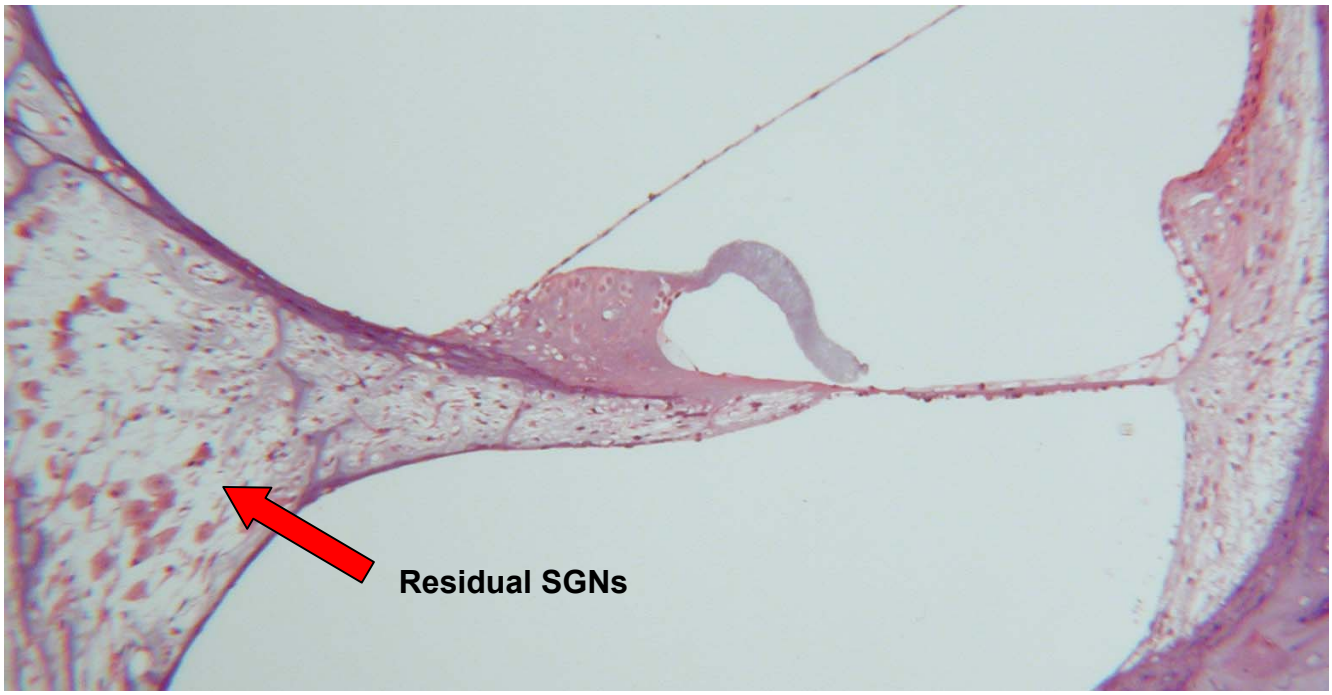


Figure 1. Significant loss of SGNs in Rosenthal's canal occurs following loss of hair cells. Loss of trophic support normally provided by the hair cells is considered a major influence on SGN loss.

We have completed the first phase of our study of chronic electrical stimulation/ neurotrophin administration in guinea pigs. The present report will describe in some detail our electrophysiological results while the next report will present our cochlear histology.

3.1 Methods

Twenty guinea pigs were used in the present 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; Fig. 2). 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. Note that the volume of the guinea pig scala tympani is estimated to be 4.76 μl (Dr. A.N. Salt; http://oto.wustl.edu/cochlea/), therefore this pump will deliver ~5% of the scala tympani volume per hour. We consider this to be an acceptable proportion of fluid replacement in a chronic preparation.

In the present study we have compared the chronic infusion of artificial perilymph 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).

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 in response to 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). 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 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. The maximum stimulus current amplitudes used in this study were in the range 0.39-1.6 mA at 100 μs/phase, developing charge densities in the range 8.4-34.3 $\mu C.cm^{-2}$ geom. per phase. These stimulus intensities are within levels considered safe for cochlear implant applications using platinum electrodes (Shepherd et al., 1983; Xu et al., 1997).

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 is 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 (e.g. 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. In addition to the standard input-output functions, refractory properties of the EABR were also recorded following procedures that have been described previously (Abbas et al., 1991; van den Honert et al., 1986; Zhou et al., 1995; *Fourth Quarterly Progress Report*). On completion of these recordings each animal was killed with an overdose of anesthetic and systemically perfused. The cochleae and brain were harvested for anatomical analysis (cochlear histopathology will be presented in the next progress report).

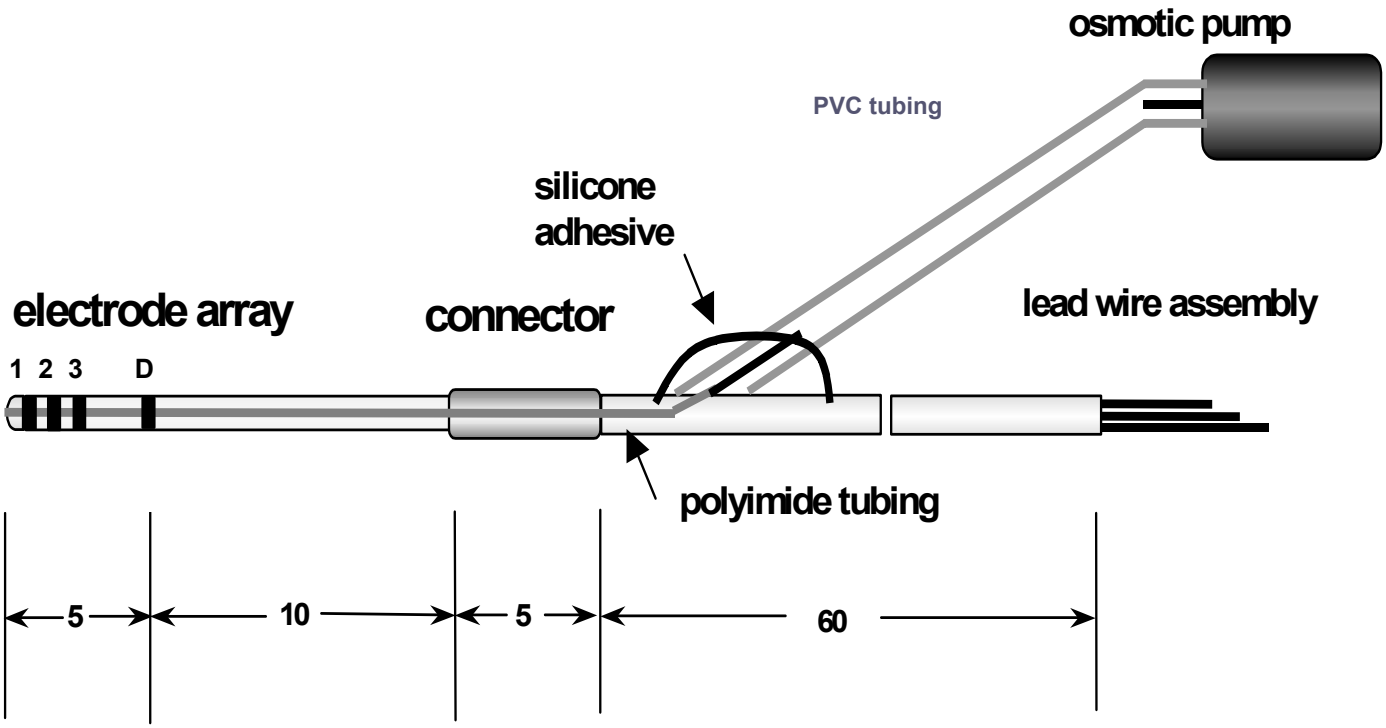
Table 1: Summary of treatment groups

Treatment group	Implant duration (days)	Chronic electrical stimulation	Contents of osmotic pump
BDNFE[1]	28	Yes	BDNF[2]
BDNFC[3]	28	No	BDNF
APE	28	Yes	Artificial perilymph[4]
APC	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.

3.2 Results

Typical EABRs, recorded both immediately post-operatively and at completion of the implantation period 28 days later, are illustrated in Figs. 3 & 4. Both APE and APC 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 previous studies (Shepherd et al., 1983; Xu et al., 1997; *Seventh Quarterly Progress Report*), and have interpreted these changes as reflecting the ongoing loss of spiral ganglion cells 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. 5, were highly statistically significant.



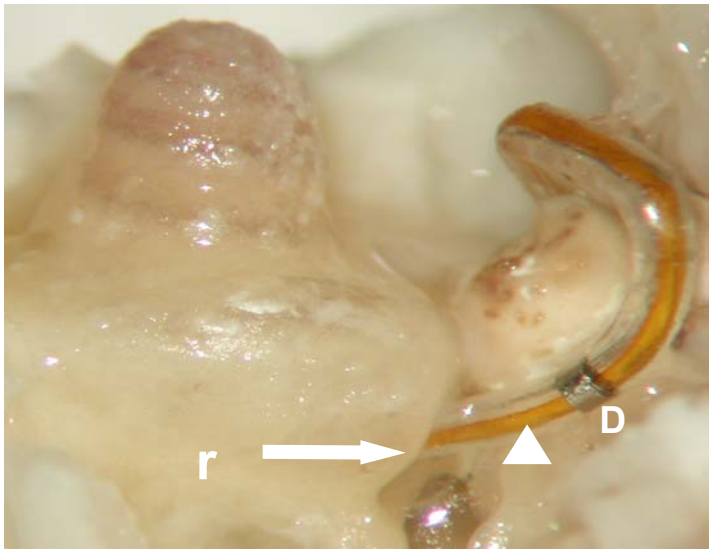


Figure 2. Diagram of the electrode assembly incorporating a drug delivery system. A polyimide microtube provides a path to deliver artificial perilymph or BDNF into the scala tympani via the tip of the electrode array. All dimensions are in mm. (from Shepherd & Xu, 2002). The inset (right) is a micrograph of the electrode assembly entering the round window (r). This micrograph was taken 28 days following cochlear implantation. D, dummy ring; ▲, polyimide microtube.

While we have observed clear functional evidence of a reduction in EABR thresholds in animals chronically administered with BDNF, this reduction was independent of whether or not the animal was subject to chronic electrical stimulation. Potential mechanism(s) underlying a reduction in EABR threshold with chronic BDNF delivery will be discussed following a detailed analysis of SGN survival across treatment groups.

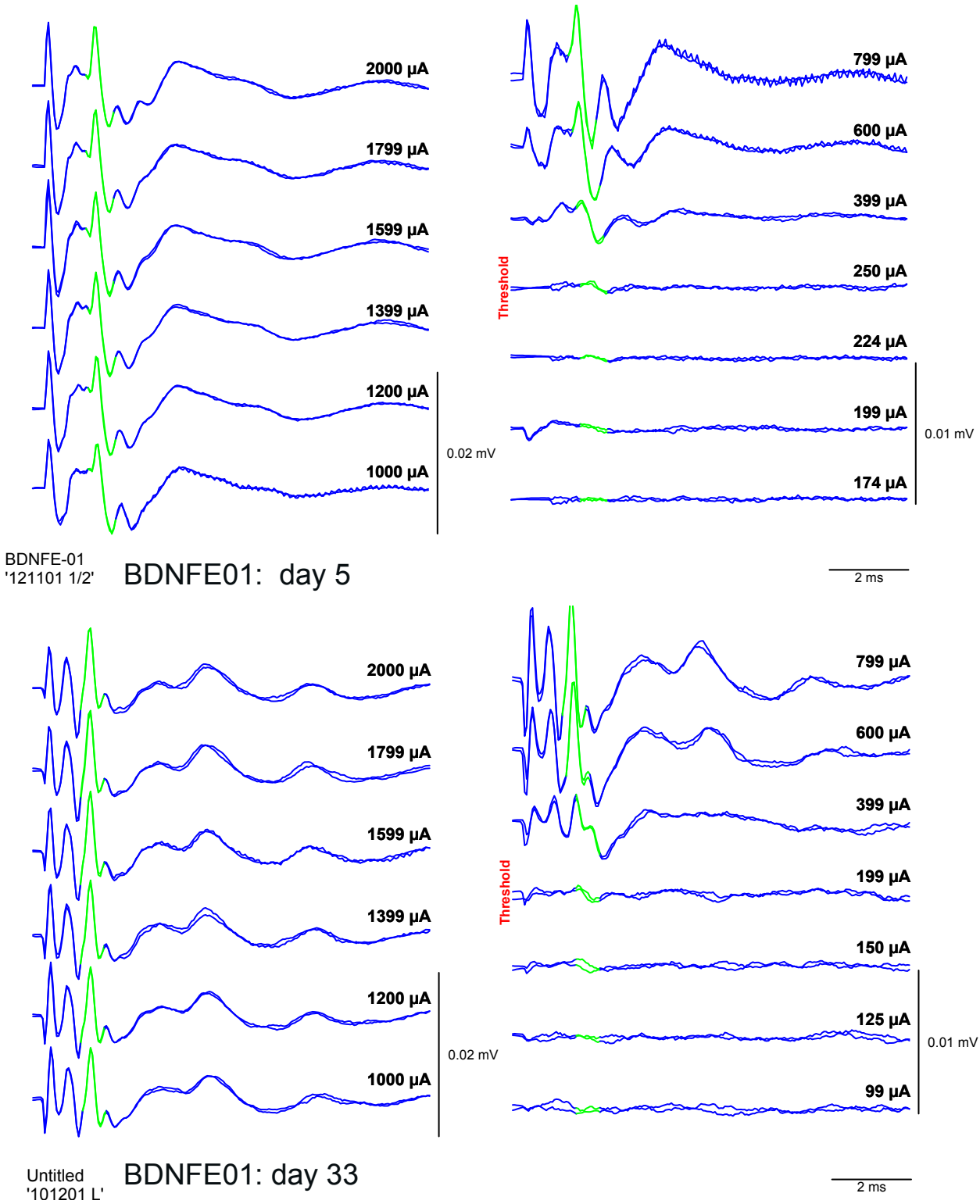


Figure 3. EABRs recorded from guinea pig BDNFE01 immediately following surgery (top panel), and at completion of the 28-day implantation program (bottom panel). The left cochlea of this animal was both electrically stimulated and received BDNF during this implant period. All EABRs were evoked using a 100 µs/phase biphasic current pulse delivered to bipolar electrode pair 1/2. 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 this animal.

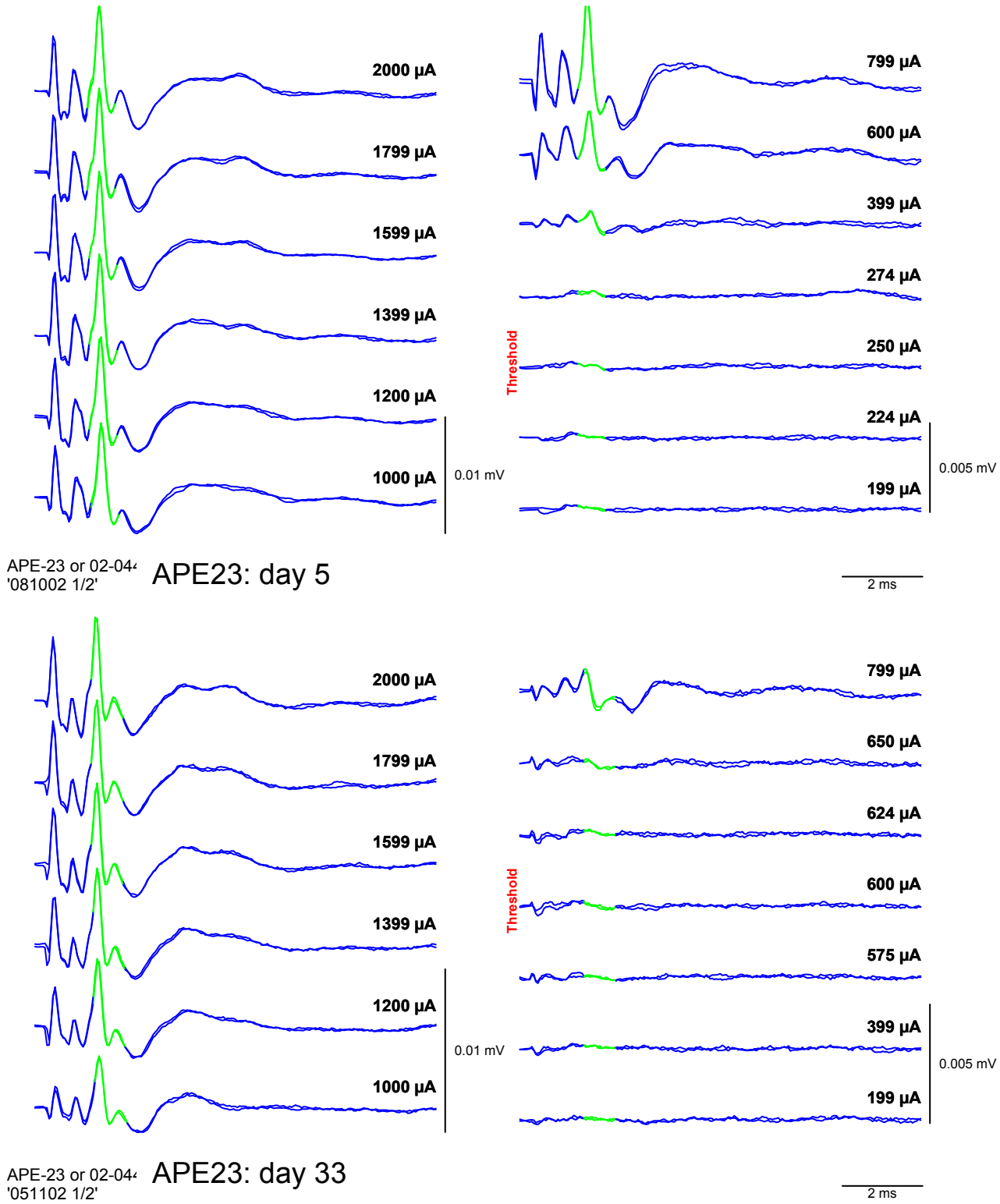


Figure 4. EABRs recorded from guinea pig APE23 immediately following surgery (top panel), and at completion of the 28 day implantation program (bottom panel). The left cochlea of this animal was both electrically stimulated and received artificial perilymph during the implant period. All EABRs were evoked using a 100 µs/phase biphasic current pulse delivered to bipolar electrode pair 1/2. Note the *increase* in threshold over the implant period in this animal. An increase in threshold was observed in all animals not treated with BDNF.

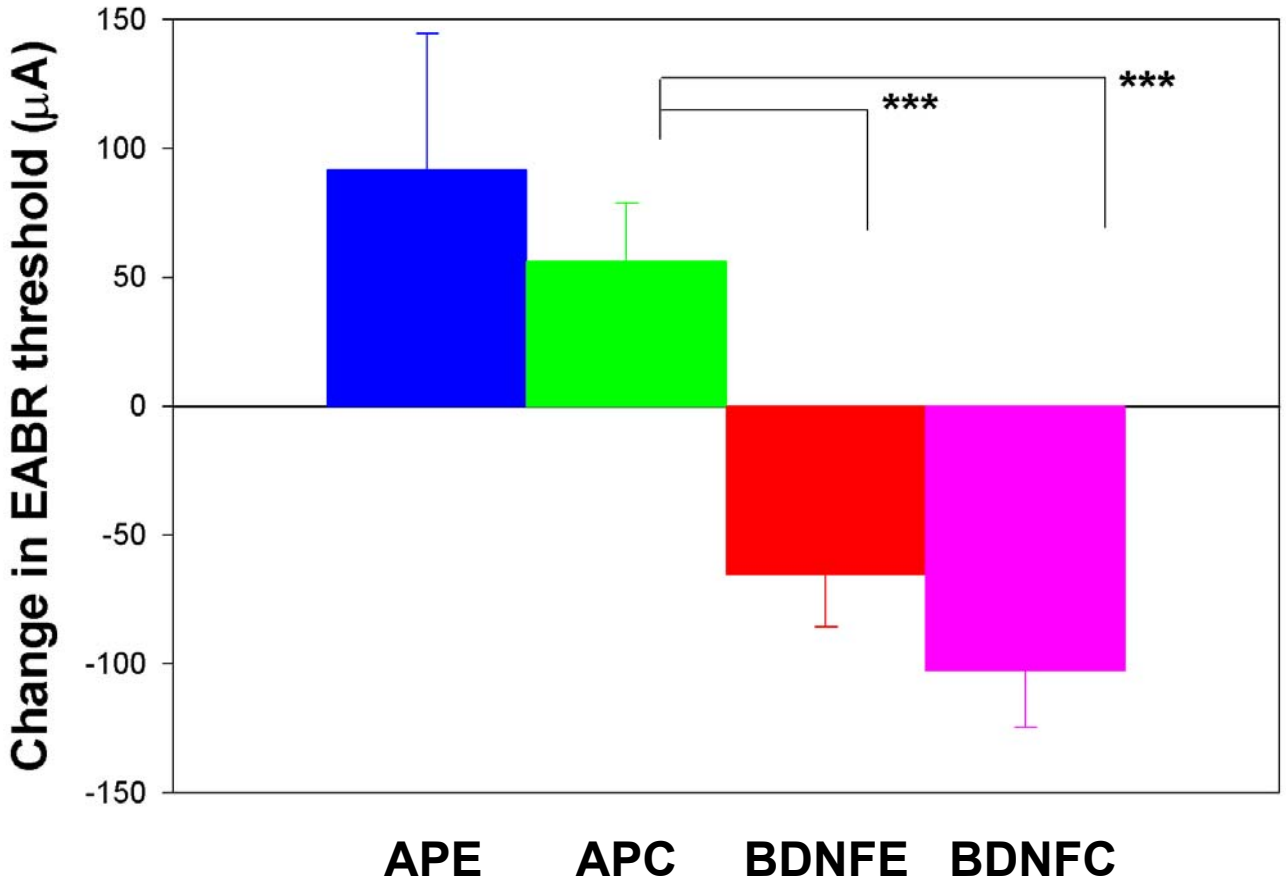


Figure 5. Mean change in EABR threshold over the 28 day implantation period for each of the four treatment groups in this study. The animals treated with artificial perilymph exhibited a systematic increase in EABR threshold over the 28 day implant period of between 55-85 μA. In contrast, both groups 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 APC animals ($p < 0.001$; t-test). There was no statistical difference between APE and APC treated animals. Note that these changes in EABR threshold were independent of whether or not the animals were chronically stimulated. Error bar: SEM.

Refractory properties of the EABR were also measured at completion of the chronic implantation period. We hypothesized that any trophic support of SGNs via chronic electrical stimulation and/or neurotrophin delivery would be reflected in improved refractory properties compared with deafened controls. Typical examples of these derived responses, recorded at 6 and 12 dB above EABR threshold, are illustrated in Fig. 6. In these examples, the response to the masker has been removed, leaving only the response to the probe stimulus.

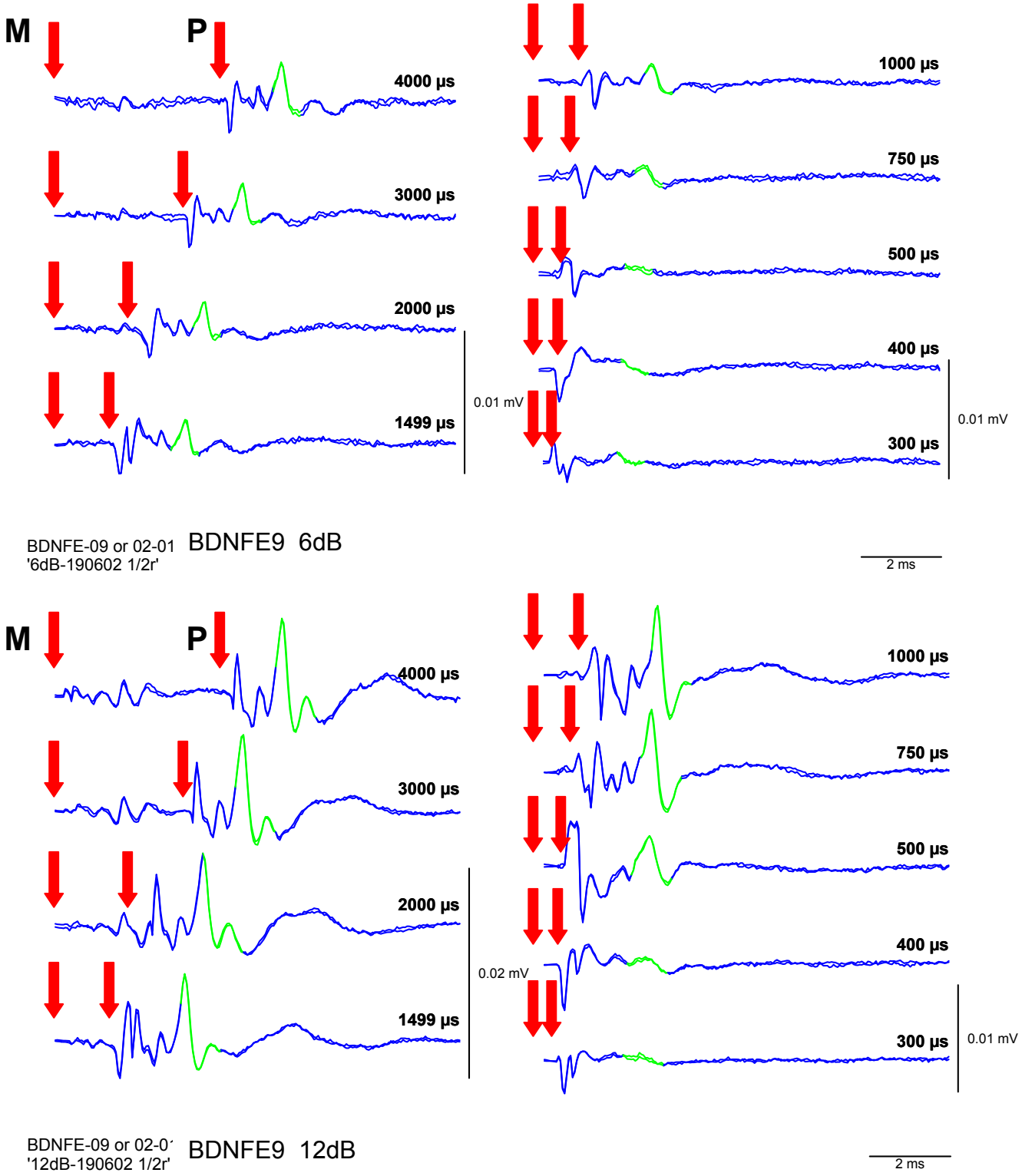


Figure 6. Typical derived EABRs illustrating the response to the probe stimulus (P). In these experiments, the inter-pulse period (illustrated to the right of each set of traces) is reduced from 4000 to 300 µs. When the IPI is < 1000 µs the refractory state of neurons excited by the masker (M) results in a large reduction in the probe-evoked response. Examples here illustrate recordings made at 6 (top panel) and 12 dB (bottom panel) above the EABR threshold for this electrode pair. Wave III of the EABR (highlighted in green) is used to monitor the EABR refractory properties. Masker and probe current pulses are indicated (arrows).

Both sets of derived EABRs illustrated in Fig. 6, show a systematic reduction in the amplitude of wave III as the IPI is reduced below 1000 μs. This reflects a reduction in the neural population contributing to the probe-evoked response, as neurons remain in a refractory state following the masker. Note that the IPI at which the wave III amplitude begins to show a reduction is dependent on the stimulus intensity; the reduction in wave III amplitude occurs at shorter IPIs for probe currents of higher intensity.

By normalizing the amplitude of wave III of the probe-evoked response, it is possible to plot amplitude as a function of the IPI for all three stimulus intensities tested (6, 12 and 18 dB above threshold). In these examples, the amplitude is normalized to the amplitude of wave III at an IPI of 4000 μs.

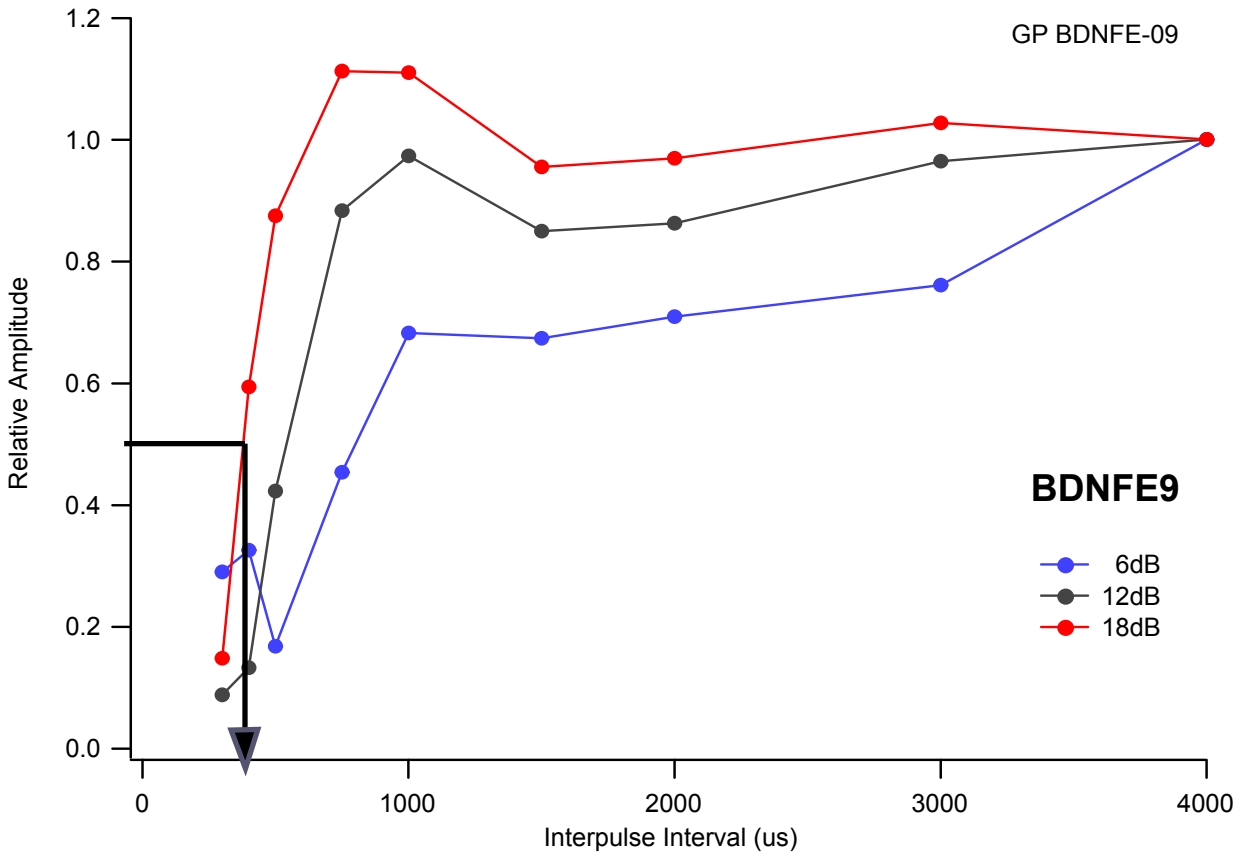


Figure 7. Probe-evoked wave III EABR amplitude versus interpulse interval for three masker/probe intensities (6, 12 and 18 dB above threshold). These data were normalized to the probe-evoked wave III amplitude at an IPI of 4000 μs. In an attempt to quantify EABR refractory properties across treatment groups, we calculated the IPI at which the relative amplitude of the response was half that at 4000 μs ($RP_{0.5}$) using a linear regression procedure (arrow). An example of an $RP_{0.5}$ estimate for the 18 dB response is illustrated. While this example is from an animal co-treated with BDNF and electrical stimulation, the general profile of these normalized data was similar across treatment groups.

$RP_{0.5}$ were pooled for each stimulus intensity (6, 12 and 18 dB) within a treatment group (Table 2). $RP_{0.5}$ generally reduced with increasing stimulus intensity, however we saw no statistically significant differences between treatment groups at a given stimulus intensity (Table 2). This preliminary analysis suggests that there is no significant difference in the refractory properties of EABRs as a function of cochlear treatment. We will present a more detailed analysis of these data in a future progress report.

Table 2. Mean (SEM) $RP_{0.5}$ in μs for each treatment group at 6, 12 and 18 dB above EABR threshold. n=5 per group.

Treatment	6 dB	12 dB	18 dB
APC	524.6 (46.9)	556.5 (39.4)	475.6 (71.1)
APE	614.1 (75.0)	621.3 (39.9)	556.1 (80.8)
BDNFC	731.6 (62.5)	588.5 (55.6)	403.3 (35.8)
BDNFE	1160.8 (397.9)	570.1 (55.3)	444.2 (35.8)
1 Way ANOVA	F=2.79; p=0.086	F=0.37; p=0.775	F=1.25; p=0.334

3.3 Discussion and Conclusions

This report examines the functional response of the deafened cochlea following chronic electrical stimulation with and without co-administration of BDNF. The results show a clear and highly significant reduction in EABR threshold in animals that were chronically administered with BDNF compared with control animals administered with artificial perilymph. Importantly, chronic electrical stimulation did not appear to contribute to a reduction in EABR threshold.

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.

In contrast with the significant reductions observed in EABR threshold associated with chronic BDNF treatment, there was no evidence of the effect of treatment on $RP_{0.5}$ – a simple first order measure of the refractory properties of the EABR. We intend to analyze these refractory EABR data in more detail to determine whether or not these treatments can influence the refractory properties of the deafened auditory pathway.

Finally, our next objective is to complete the remaining cochlear histology and SGN density measurements in order to compliment these functional data with cochlear histopathology. This work will be completed during the next quarter and presented in the following report. Longer-term studies will be directed towards important clinical questions associated with neurotrophin delivery to the cochlea. These include (i) studies to investigate the duration of BDNF delivery (is continuous delivery of BDNF the only suitable technique available to maintain SGNs?); (ii) studies to investigate the advantage associated with other neurotrophins (or combination of neurotrophins); (iii) safety implications of delivery of these drugs directly to the cochlea both on cochlear tissue and the CNS – issues including the neoplastic response and infection must be considered prior to consideration for clinical application.

4. Publications

During the quarter the following papers, funded in part or fully by this contract, were published:

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

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. Hearing Research 172: 127-136, 2002.

In addition, an invited paper was presented at the "Frontiers in Otorhinolaryngology 2002" conference organized by the Garnett Passe & Rodney Williams Memorial Foundation, Noosa Heads, Australia, August 2002. (see Appendix A).

5. Plans for Next Quarter

- Continue our chronic stimulation studies in guinea pigs and long-term deafening studies in the rat.
- Continue the manufacture of guinea pig electrode assemblies.
- 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.
- Commence terminal acute electrophysiological experiments on neonatally deafened un-stimulated control cats.

6. Acknowledgements

We gratefully acknowledge the important contributions made by our Histologist, Maria Clarke; Veterinarian Dr Sue Pierce; Elisa Borg for management of our animal house; Helen Feng for electrode manufacture; and Rodney Millard and Frank Nielsen for engineering support.

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Xu, J., Shepherd, R.K., Millard, R.E., Clark, G.M. 1997. Chronic electrical stimulation of the auditory nerve at high stimulus rates: a physiological and histopathological study. Hear. Res. 105, 1-29.

Ylikoski, J., Pirvola, U., Moshnyakov, M., Palgi, J., Arumae, U., Saarma, M. 1993. Expression patterns of neurotrophin and their receptor mRNAs in the rat inner ear. Hear Res 65, 69-78.

Zhou, R., Abbas, P.J., Assouline, J.G. 1995. Electrically evoked auditory brainstem response in peripherally myelin-deficient mice. Hear. Res. 88, 98-106.

8. Appendix A

Protective effects of cochlear implantation on the deafened auditory system

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Cochlear implants electrically stimulate discrete populations of residual auditory nerve fibres in profound and severely deaf patients in order to provide important temporal and pitch cues for speech perception. In this paper, we will review the effects of a neonatal hearing loss on the cochlea, and discuss the potential protective effects of chronic electrical stimulation of the auditory nerve. The implications of these effect for cochlear implants will also be discussed.

A profound sensorineural hearing loss initiates a gradual, ongoing degeneration of spiral ganglion neurons (SGNs) - the target neural population for stimulation via a cochlear implant. The loss of these cells is due, at least in part, to the withdrawal of neurotrophins normally expressed by hair cells [1, 2]. The degenerative changes observed in SGNs are associated with a loss of peripheral processes and demyelination of the soma of surviving auditory neurons [3]. These changes have been shown to affect neural response properties to electrical stimulation, including elevated thresholds, a reduction in the security of action potential propagation and altered refractory properties [3]. Deafness induced degeneration of SGNs may adversely affect the clinical performance of implant subjects, as evidenced by the strong negative correlation between duration of deafness and speech perception [4]. Moreover, the SGN degeneration may place limitations on the development of more advanced cochlear implants.

Recent *in vitro* studies have shown that neural depolarization provides a strong trophic influence on SGN survival [5]. The possibility of reducing or preventing SGN degeneration via depolarization, using electrical stimulation, has important implications for cochlear implant research. We have examined the extent of this trophic effect on SGNs *in vivo,* by chronically stimulating neonatally deafened cats, via a cochlear implant, for periods of up to eight months. Preliminary results, illustrating the long-term physiological response of the auditory pathway to electrical stimulation, and the extent of survival of SGNs, will be compared with results from unstimulated deafened controls. In a second study, we have developed an electrode array capable of simultaneously delivering neurotrophic agents into the cochlea and electrically stimulating the SGNs. Initial studies examining both the functional and anatomical efficacy of combining depolarization and neurotrophic delivery to SGNs will be described.

These studies are designed to develop techniques that will enhance the survival of auditory neurons, using procedures that can be applied to the clinical setting. Our work also examines the plastic response of the central auditory pathway to both deafness and reactivation via a cochlear implant. Clinical experience consistently shows improved performance with implant use, and suggests that such improvement can be attributed to a reorganization of the central auditory pathway [6].

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