First 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) in addition to investigating 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 to the AN and CAS of deafened animals following prolonged 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 deaf 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 first quarter of this contract the following activities were completed:

- Hiring two key members of staff that will be employed in a full-time capacity on this contract.
- Attended and presented at the 31st Neural Prosthesis Workshop in Bethesda, MD.
- The design and development of a prototype electrode array that incorporates a drug delivery system to the scala tympani. This work forms a major section of the present report.

- The design and development of a prototype electrode array for our feline experiments.
- The development of a new deafening procedure for use in guinea pigs.
- Completion of all Animal Ethics approvals for the experiments proposed in this contract with our institutional Animal Research & Ethics Committee.
- Presentation of work associated with this contract at invited talks at the University of Iowa and the Johns Hopkins University.
- Received University of Michigan recording electrodes for use in future studies using guinea pigs.

3. Electrode arrays for chronic stimulation studies

3.1 Feline electrode arrays

An electrode array suitable for chronic implantation in the cat has been designed and a prototype manufactured by Cochlear Limited. This array has six active platinum ring electrodes mounted on a silicon carrier. Three platinum electrodes are located near the tip of the electrode array while the remaining three are located approximately 2 mm more basalward. This arrangement will allow us to stimulate two distinct sectors of the auditory nerve (Shepherd et al., 1999), an important consideration for examining spatial plasticity following chronic stimulation.

University staff have verified the design and quality of manufacture of the prototype electrode array. The manufacture of the feline arrays required for this contract will now commence.

3.2 Electrode arrays for guinea pigs

A major focus of our studies will be to investigate whether electrical stimulation and chronic administration of neurotrophic agents act in synergy to promote AN survival. For studies of this kind we have designed a new scala tympani electrode array suitable for use in guinea pigs. A unique feature of this array is the inclusion of micro tubing that runs longitudinally within the electrode assembly to exit proximal to the platinum electrodes at the tip of the array (Fig. 1). The distal end of this tubing exits the leadwire for attachment to an osmotic pump.

During the quarter, two prototype arrays were manufactured and chronically implanted. Prior to implantation the arrays were ultrasonically cleaned in 100% ethanol followed by distilled water. They were then dried and sterilised using H_2O_2 plasma. Two otoscopically normal adult guinea pigs were unilaterally implanted with these electrode arrays using sterile surgical techniques. In order to assess the patency of the drug delivery system, together with the distribution of the drug within the cochlea, we decided to deliver a solution of neomycin. Neomycin is a highly ototoxic agent and its distribution within the cochlea would be clearly evident through hair cell and AN pathology.

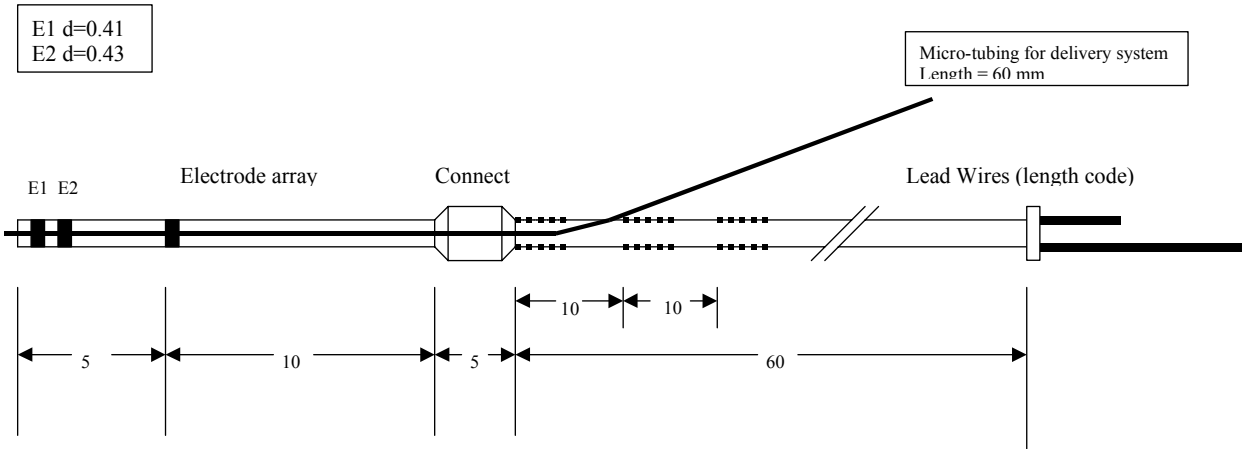


Figure 1. Schematic diagram illustrating the guinea pig electrode array designed to provide delivery of pharmacological agents to the scala tympani via an osmotic pump connected to the micro tube assembly. The array was designed and manufactured by Dr. Jin Xu. All dimensions are in mm.

Both electrode arrays were inserted 5 mm into the scala tympani, the leadwire was fixed in two locations and exited the skin through a small incision in the nape of the neck. Finally, the distal end of the micro tubing was connected to an Alzet® 2004 mini-osmotic pump, which was implanted subcutaneously. Each pump had been primed with ~250 μl of a 22 mM solution of neomycin in sterile phosphate buffered saline 24 hours before surgery. These pumps deliver 0.25 μl per hour of solution over a 28 day period.

On completion of surgery, both electrode voltage waveforms (Fig. 2) and electrically evoked auditory brainstem responses (EABRs; Fig. 3) were recorded to confirm that the electrode array was functional and was stimulating the AN. The recording techniques have been described in detail previously (Hardie & Shepherd, 1999; Huang & Shepherd, 2000). Both animals subsequently made an uneventful recovery from the surgery.

One month following implantation both animals were again anaesthetised to record electrode voltage waveforms and EABRs. The resistive (R_b) and total electrode impedance (V_z) were calculated from the electrode voltage and current waveforms (eg Huang & Shepherd, 2000). Electrode impedances appeared in the normal range, implying normal electrode function (Table 1). EABRs could not be recorded from GP_001, suggesting that the electrode array was no longer within the scala tympani. Microscopic inspection of the auditory bulla confirmed that the array was lying within the bulla surrounded by a thin fibrous tissue capsule. A tissue capsule was evident going into the cochlea suggesting that the array had been located within the cochlea for some time. In contrast EABRs were readily evoked from GP_002 28 days post-operatively (Fig. 3) and microscopic inspection confirmed that the array was entering the cochlea. Moreover, the platinum ring located 5 mm from the tip (Fig. 1) was observed at the round window, indicating that the array had not moved during the implant period.

Table 1: Summary of electrode impedances. Elevated values of R_b and V_z are normally observed following chronic implantation and typically reflect tissue growth in the vicinity of the electrodes.

Animal	Electrode Impedance (kΩ)			
	Post-op		Pre-sacrifice	
	R_b	V_z	R_b	V_z
GP_001	0.72	2.04	3.28	5.20
GP_002	0.84	1.88	1.92	3.96

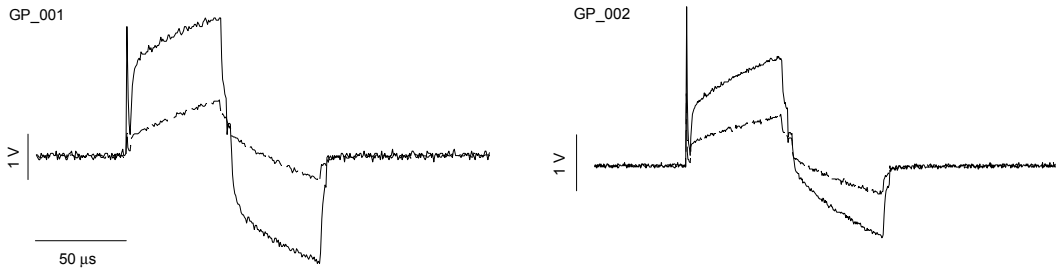


Figure 2. Electrode voltage waveforms recorded from the two guinea pigs used in this study (post-operative responses dashed lines; pre-sacrifice responses solid lines). In both cases the resistive component and the total electrode voltage increased over the implantation period but both electrode arrays were functioning normally at the completion of the implant period. All four voltage waveforms were evoked by a 50 µs biphasic current pulse at an intensity of 0.5 mA .

EABRs not only confirmed the functional status of the prototype electrode arrays but data from GP_002 demonstrated a significant increase in threshold and a reduction in response amplitude (Figs. 3 & 4; Table 2) that is consistent with extensive spiral ganglion cell loss (eg Hardie & Shepherd, 1999).

Table 2: Chronic intracochlear infusion of neomycin: Effects on the EABR (wave III).

GP_002	0 days	28 days
Threshold (mA)	0.5	0.9
Maximum response amplitude (µV)	8.9	6.6
Mean I/O gradient (µV/dB)	0.74	0.81

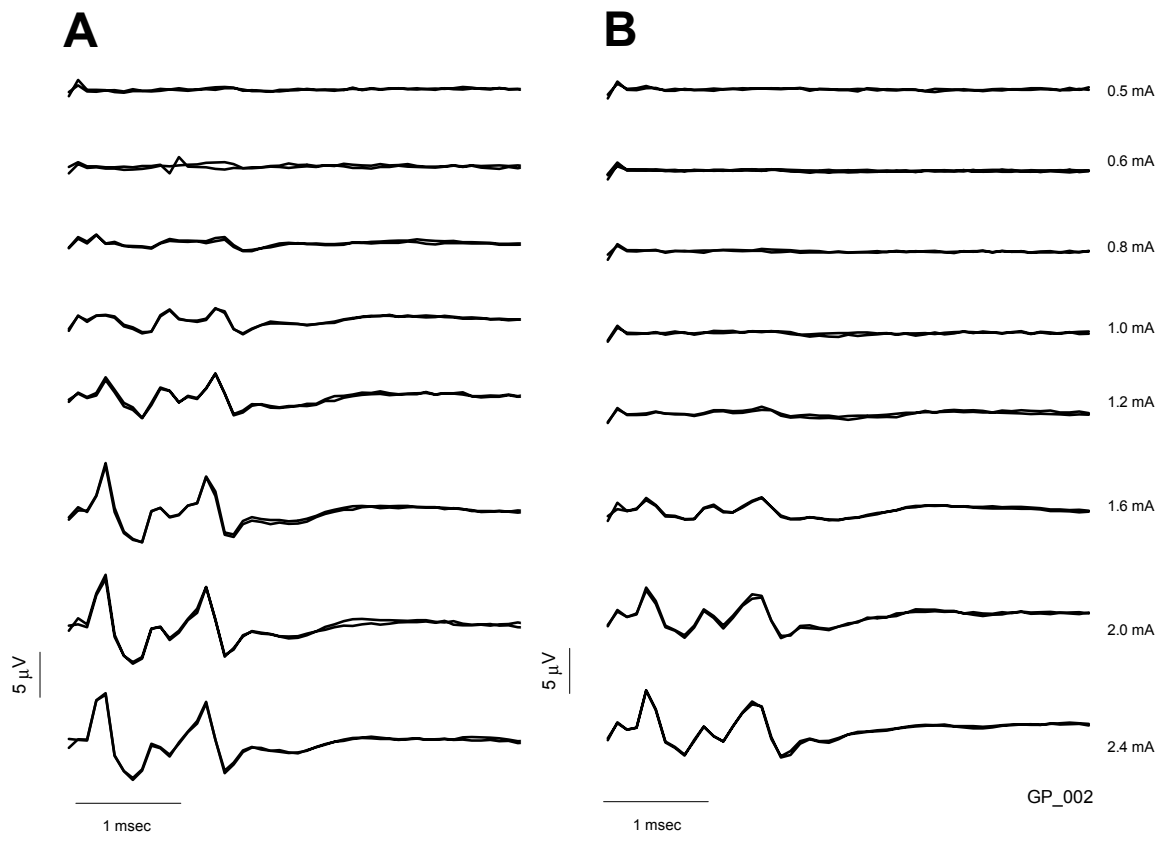
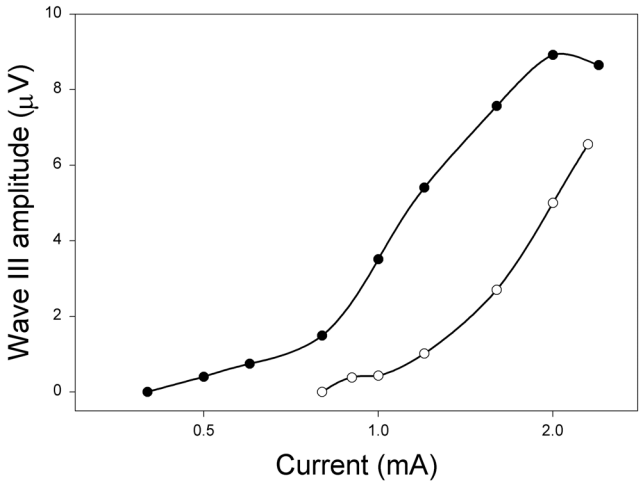


Figure 3. EABRs recorded from GP_002 immediately (A) and 28 days (B) following implantation of a bipolar electrode array that delivered 22 mM of neomycin to the cochlea at a rate of 0.25 µl/hour. EABRs were evoked using 100 µs/phase biphasic current pulses at current amplitudes indicated on the right of this graph. Two responses were recorded at each current level.

Figure 4. EABR input-output functions (wave III) recorded from GP_002 immediately (•) and 28 days (o) following cochlear implantation. Note the elevated threshold and a reduction in the maximum amplitude of the response following chronic neomycin infusion.



Following the acute electrophysiology at completion of the chronic implantation period, both animals were killed with an overdose of anaesthetic, the electrode arrays removed, and the cochleae prepared for light microscope histology (eg Hardie & Shepherd, 1999). The patency of the drug delivery system was confirmed by injecting 1% methyl blue through the micro tubing at a site close to the osmotic pump. Dye was observed at the tip of both electrode arrays.

Figure 5 illustrates representative histology showing the chronic neomycin infused and control cochleae from both animals in this study. Given that this phase of the experiment was designed to evaluate a prototype electrode array we felt it unnecessary to examine these cochleae in any quantitative detail. The following observations are therefore based solely on a qualitative evaluation. While there was evidence of some histological artefact, both control cochleae appeared histologically normal, including near normal organ of Corti throughout all turns, and no evidence of loss of spiral ganglion cells (SGCs) or peripheral processes. In contrast, both implanted cochleae showed no evidence of surviving hair cells, collapsed or completely absent organ of Corti, and a moderate-severe loss of neural elements (30-50% loss of SGCs). Significantly, this pathology was not restricted to the basal turn but extended throughout all turns of both cochleae. It is important to note that such widespread pathology is not observed in cochleae implanted with a standard electrode array. Our previous studies have shown that the cochlea is very tolerant of chronically implanted electrode arrays; any hair cell or neural loss, if present, is typically restricted to regions close to the array (Shepherd et al., 1983; Ni et al., 1992; Xu et al., 1997).

Although limited to two animals, these findings show consistent trends indicative of pathology induced by chronic infusion of neomycin. As the pathology extended throughout all turns it would appear that the infused neomycin spread apicalward from the cochlear base. This finding suggests that drugs delivered via this procedure are capable of spreading throughout the cochlea and are not just restricted to a site proximal to the tip of the array. In addition, both arrays were associated with a minimal tissue response within the cochlea (Fig. 5), indicating that this prototype is biocompatible. Finally, the extent of pathology observed in GP_001L suggests that this array was removed from the cochlea following a relatively long period of implantation.

One interesting feature of the pathology observed in response to chronic neomycin infusion was the relatively large numbers of surviving peripheral processes observed in both cochleae given the extent of both hair cell and SGC loss (Fig. 5B & 5D). This may be related to the low concentration/small volume of neomycin administered resulting in a relatively gradual loss of hair cells (auditory function was not monitored longitudinally so we have no data to support this hypothesis). It is also possible that the neomycin had a direct ototoxic effect on SGCs – many of the surviving cells were pyknotic, indicative of degenerative changes. Any direct toxic action on SGCs would produce a lesion that differs subtly from the normal centrifugal course of degeneration that occurs following loss of hair cells.

In summary, this pilot study has demonstrated that a chronic drug delivery system can be effectively coupled to a scala tympani electrode array. While we clearly need to revise the electrode fixation techniques used during surgery, this work has given us confidence that we can successfully deliver various pharmacological agents to the cochlea while chronically stimulating the auditory nerve.

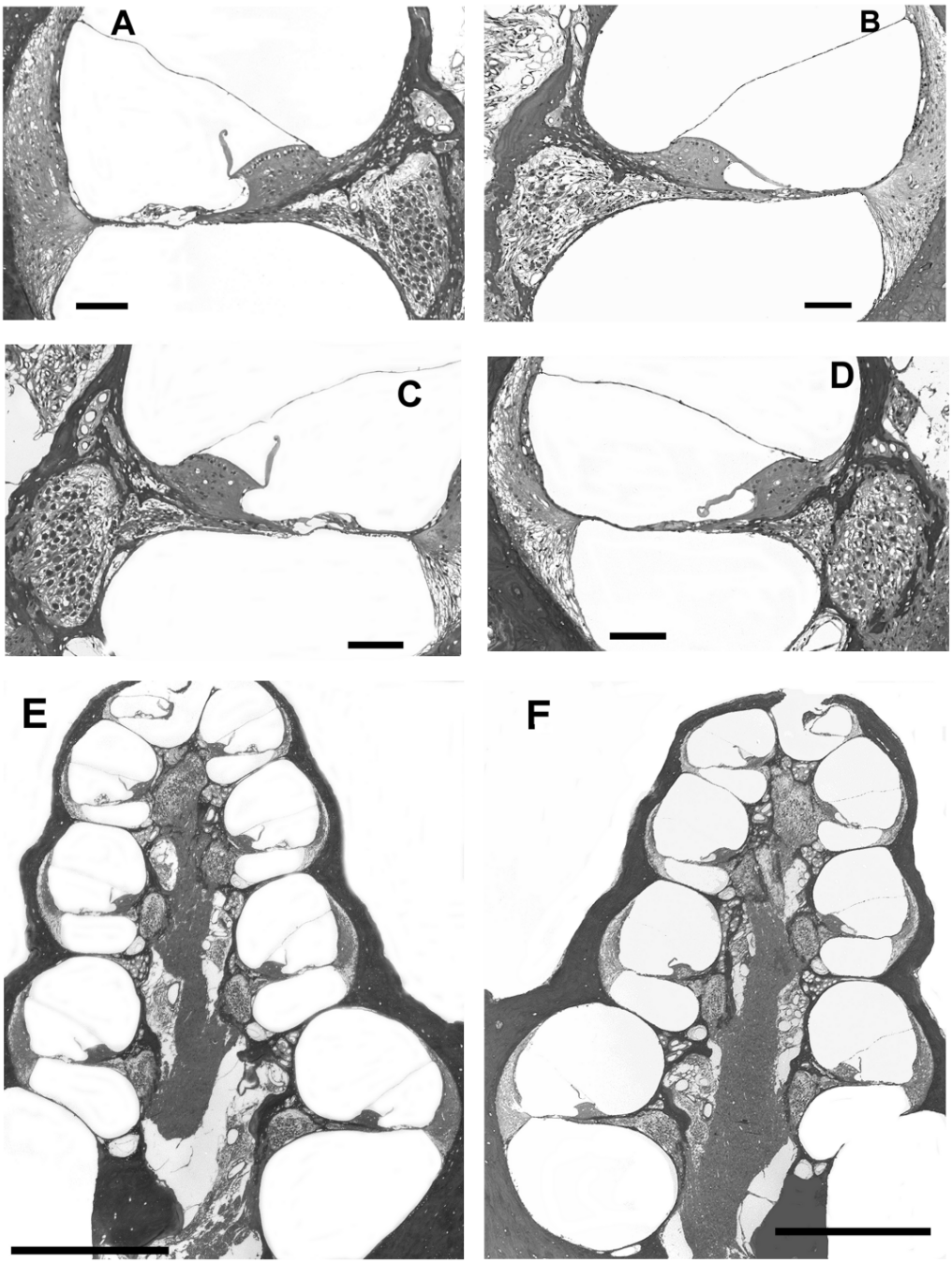


Figure 5. Photomicrographs illustrating the extent of cochlear histopathology associated with the chronic neomycin infusion experiments. A. Upper middle turn (UMT) of control cochlea GP_001R; B. UMT of deafened cochlea GP_001L; C. UMT of control cochlea GP_002R; D. UMT of deafened cochlea GP_002L; E. midmodiolar view of normal cochlea GP_002R; F. midmodiolar view of deafened cochlea GP_002L. Note the loss of the organ of Corti and SGCs throughout the deafened cochleae (right column) compared to the controls (left column). Scale bar = 100 μm A-D and 1 mm E & F.

4. Development of new deafening techniques

Our standard deafening procedure for both cats and guinea pigs has been based on the single co-administration of kanamycin with the loop diuretic ethacrynic acid (West et al., 1973; Xu et al., 1993; Shepherd & Martin, 1995; Hardie & Shepherd, 1999). We recently had difficulties receiving supplies of the loop diuretic (the supply has now been re-established), highlighting our vulnerability to a specific drug regime. In this Quarterly Progress Report we describe a new deafening technique we have developed for use in guinea pigs. This procedure is based on the loop diuretic Frusemide. In a future report we will describe our results using the same technique in cats.

Frusemide was selected as it is a widely available loop diuretic and because of our previous experience with this drug in deafening rat pups (Roberts et al., 2000). Six otoscopically normal, pigmented guinea pigs (mean weight 555.5 g; s.e.m. 57.7g) were used in this study. Each animal had normal hearing prior to deafening as indicated by 100 μs rarefaction click thresholds of lower than 42 dB peak equivalent Sound Pressure Level (dB pe SPL). Details of the ABR recording procedure have been described in detail previously (eg Shepherd & Martin, 1995; Hardie & Shepherd, 1999).

Each animal was anaesthetised with ketamine and xylazine, and deafened with a single intravenous injection of 100 mg/kg Frusemide followed by 300 mg/kg of Kanamycin administered subcutaneously. All six animals made an uneventful recovery from the procedure. No animal showed evidence of a loss of balance or ataxia and there was no clinical evidence of renal impairment (ie there was no evidence of prolonged weight loss or oliguria). Hearing was assessed using click-evoked ABRs following a minimum period of seven days after the deafening procedure. Both cochleae of all six animals exhibited a profound hearing loss (ie ABRs could not be elicited using the maximum stimulus intensity (98 dB pe SPL; Fig. 6).

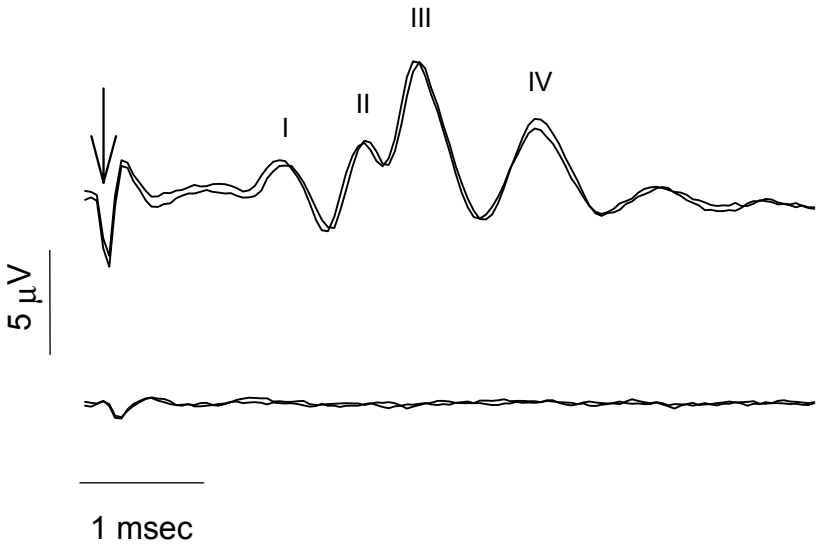


Figure 6. Click-evoked ABRs from a normal guinea pig (top) and an animal deafened using kanamycin and frusemide 20 days previously (bottom). A 100 μs rarefaction click presented at 98 dB pe SPL was used to elicit these responses. The four waves of the guinea pig response are illustrated. Note the artefact associated with the click (arrow). Positive is plotted upward.

While cochlear histopathology from these cochleae will be presented in a future report, this deafening technique appears to be very suitable for use in guinea pigs; we see no evidence of renal dysfunction, the technique appears very reliable with minimal individual variability, and Frusemide is readily available and considerably cheaper than Ethacrynic acid.

5. Plans for Next Quarter

- Commence single unit and evoked potential studies in deafened guinea pigs to study temporal resolution at the level of the auditory nerve and auditory midbrain prior to commencing chronic stimulation studies.
- Deafen our first litter of kittens to be used as part of the feline chronic stimulation study.
- Develop techniques to allow chronic stimulation of adult guinea pigs based on backpack jackets or a leadwire tether system.
- Manufacture guinea pig and feline electrode assemblies.

6. Personnel

During the quarter two key members of staff were recruited to the project. Ms Anne Serruto joined the team in December and is employed as a Research Assistant. Anne has a B.Sc. with Honours in Physiology from the University of Melbourne, and has previous research assistance experience including animal surgery, implantation of Alzet mini-osmotic pumps, general histology and molecular biology, laboratory management and data collection and analysis. Anne will play a key role in all aspects of this project.

Dr Jeremy Crook will join the team in April after completing a two year post-doctoral Research Fellowship at the National Institutes of Mental Health at NIH. Jeremy has a B.Sc. degree with Honours in Neuroanatomy from the University of Tasmania, and a PhD degree in Neurochemistry /Neuropharmacology from the University of Melbourne. He has considerable research experience in neuroanatomy with a particular interest in the localisation and quantification of biological markers underlying neuropsychiatric disorders. Jeremy will play a key role in all aspects of this project, with a particular emphasis on quantitative neuroanatomy and molecular biology.

In addition to the appointment of two key full-time members of staff we are very pleased to have four distinguished scientists act as consultants to this project:

Dr Perry F. Bartlett
Dr Perry Bartlett is the Head of the Neurobiology Laboratory, Walter and Elisa Hall Institute, Melbourne. Dr Bartlett is a distinguished neuroscientist with particular interests in neuronal repair and regeneration and neurotrophin growth factors. A key aspect of this project is to develop an understanding of

the mechanisms underlying trophic support of SGCs via electrical stimulation and the role of neurotrophins in SGC support (Study b). Dr Bartlett's research experience makes him particularly well qualified to contribute to this work. This would form part of a continued collaboration between Dr Bartlett's laboratory and our own.

Dr.Dexter R. F. Irvine
Dr.Dexter Irvine is Professor of Psychology at Monash University, Melbourne. Dr.Irvine is a distinguished neuroscientist with a particular interest in plasticity and neurophysiology of the central auditory system. A central theme of our research is to understand in more detail the mechanisms associated with plastic change following deafness and cochlear implantation (Study a & b). Dr Irvine's research experience makes him an ideal consultant in this area of the contract. Dr.Irvine has collaborated previously with staff from the Department of Otolaryngology.

Dr Edwin W Rubel
Dr Edwin Rubel is the Virginia Merrill Bloedel Professor of Hearing Science, University of Washington, Seattle, WA. Dr Rubel is a distinguished neuroscientist with research interests that include developmental neurobiology, hair cell regeneration, and the fate of denervated neurons. Dr Rubel's expertise will be invaluable when investigating the response of the cochlea and the central auditory pathway to a sensorineural hearing loss and the effects of "reafferentation" via electrical stimulation (Study a, b and c). This would form part of a continued collaboration between Dr Rubel's laboratory and our own.

Dr Peter M. Seligman
Dr Peter Seligman is a Senior Engineer for Cochlear Ltd., Melbourne. Dr Seligman has a distinguished career in cochlear implant research. He was a key member of the team that developed the Cochlear Ltd. multi-channel cochlear implant with a particular interest in the development and improvement of speech processors. His most recent work was the development of a behind-the-ear speech processor. Dr Seligman's engineering expertise makes him an ideal consultant in all engineering aspects of this work with particular emphasis on the development of clinically relevant stimulation strategies (study a & b). Dr Seligman has collaborated with the Principal Investigator on many projects over the last 20 years.

7. Acknowledgments

We gratefully acknowledge the important contributions made by our Veterinarian Dr Peter Reynolds, Elisa Borg and Corina Backhouse for management of our animal house, Dr Christie Huang for the collection of electrode impedance data, Maria Clarke for histological support, Michele Loeliger for digital imaging and Rodney Millard and Frank Nielsen for engineering and technical support.

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