Third 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 third quarter of this contract the following activities were completed:

- Continued our deafening program in preparation for the feline chronic stimulation study.
- Implanted and commenced chronic electrical stimulation of eight deafened kittens.
- Manufactured five electrode arrays for chronic stimulation studies in guinea pigs. These arrays have the facility to deliver pharmacological agents to the scala tympani via an osmotic pump/micro-tube assembly.

- Deafened, implanted and commenced a chronic stimulation study in an adult guinea pig. This animal will form the first of the control animals, receiving electrical stimulation with the simultaneous delivery of Ringer's solution into the cochlea (this will be reported in detail in our next report).
- Commenced upgrading the Brain Plasticity and Molecular Biology Laboratories in preparation for future neuroanatomical and neurochemical studies.
- Initiated the development of cryoembedding and sectioning of cochleas for future immunohistochemistry studies.
- A review paper "Deafness-induced changes in the auditory pathway: Implications for cochlear implants" by R.K. Shepherd & N. A. Hardie was revised and accepted for publication in Audiology & NeuroOtology.

3. Chronic electrical stimulation studies in neonatally deafened cats

3.1 Deafening

In addition to the five kittens deafened in the previous quarter we deafened a further four animals this quarter (NDC_6-NDC_9, Table 1). The kittens were deafened at 10 days after birth using a single co-administration of kanamycin (KA) and ethacrynic acid (EA; Shepherd & Martin, 1995). All animals made an uneventful recovery from the procedure. Auditory brainstem responses (ABRs) were recorded two weeks following the deafening procedure in order to assess their hearing status using recording procedures outlined in Hardie and Shepherd (1999).

Table 1: Summary of hearing thresholds (in dB SPL). Click thresholds were determined for both ears while frequency specific ABRs were determined unilaterally

Animal	1 kHz	2 kHz	4 kHz	8 kHz	Click (l r)
NDC_1	-	-	-	-	>98 >98
NDC_2	-	-	-	-	>98 >98
NDC_3	63	71	91	>93	83 63
NDC_4	38	41	66	93	58 48
NDC_5	-	-	-	-	>98 >98
NDC_6	58	81.5	86	>93.5	72 -
NDC_7	43	56.5	76	77.5	- 57
NDC_8					>98 >98
NDC_9		66.5	>91	>93	83 83

3.2 Implant surgery

Eight kittens (NDC_1-NDC_8; Table 1) were bilaterally implanted at seven-eight weeks of age. NDC_9 (Table 1) was not implanted, and will be used as an unimplanted control in this study. The left cochlea of each animal was implanted with a stimulating electrode array (Figs. 1 & 2) while the opposite side was implanted with a control array (identical in design to the stimulating electrode array but without a leadwire assembly).

Surgery was performed under sterile conditions. Each animal received a Acepromazine/Atropine sulphate (Anamva) premed. The animals were then intubated and anesthesia was maintained for the duration of the surgery using Halothane in oxygen. A dorso-lateral approach was used to expose the bulla and the underlying cochlea. Two small holes were drilled near the large opening into the bulla as sites for fixation of the leadwire. The bulla cavity was flushed with antibiotics (Amoxycillin) and the round window membrane was incised using a fine needle. The intracochlear electrode array was gently inserted into the scala tympani for a distance of 6-7 mm (all eight Pt ring electrodes typically lay within the scala tympani). The round window was then sealed with muscle, the leadwire proximal to the electrode array was fixed using Dacron mesh ties, and the connector assembly (Figs. 1 & 2) placed into the bulla cavity. The distal leadwire passed subcutaneously from the bulla to the skull, fixed to the parietal bone with an additional Dacron mesh tie, then passed subcutaneously to exit the skin via a small incision in the neck. The wounds were sutured in two layers and the wound sites sprayed with Opsite.

During surgery the animals temperature was maintained at 37^{o}C using a heating pad, and respiration rate and expired CO_2 levels were monitored (Normocap 200) and maintained within normal levels (respiration rate 12-25/min; expired CO_2 2.6-5%). All eight kittens made an uneventful recovery from surgery, and their chronic stimulation program commenced ~2 weeks following surgery.

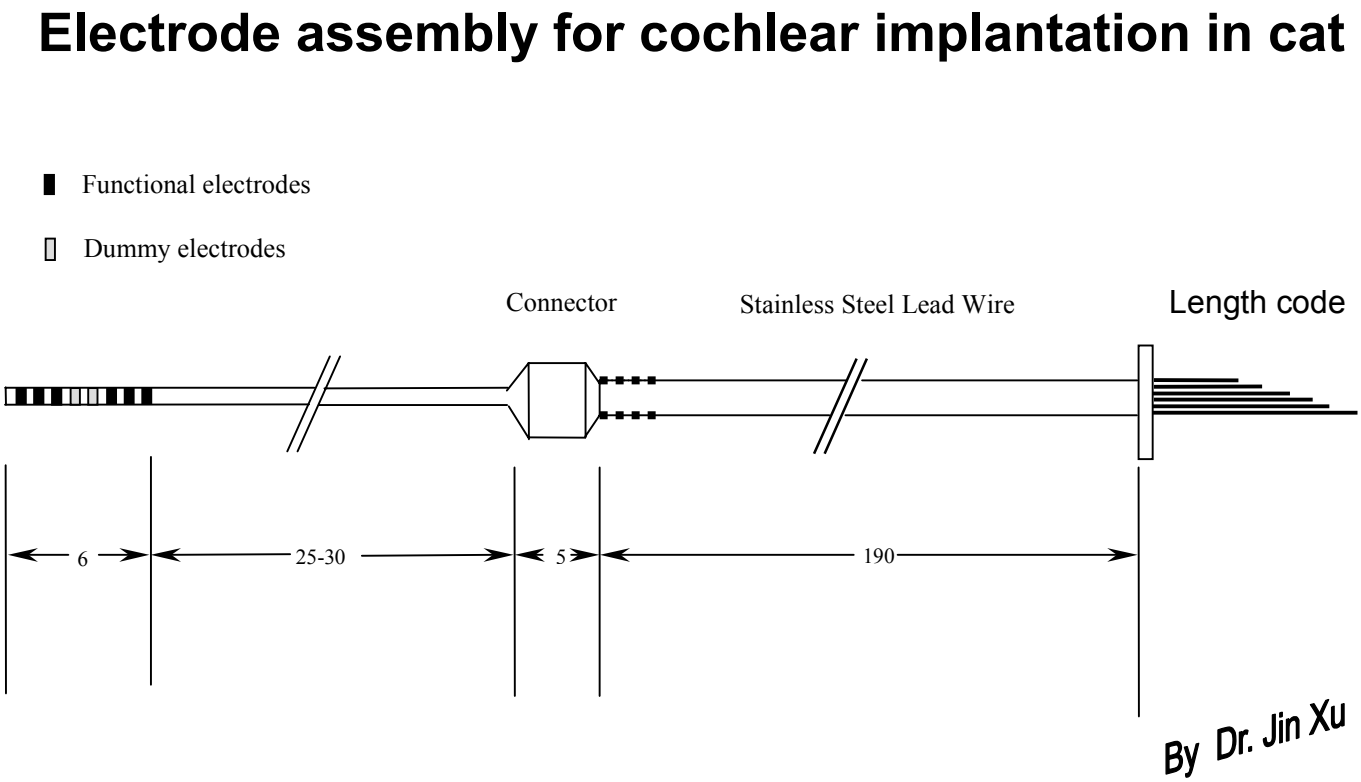

Figure 1. Schematic diagram illustrating the cat electrode array used in the present study. The array, which was designed by members of our staff, is manufactured for us by Cochlear Corp. The stainless steel leadwire assembly is connected to the electrode array in our laboratory. All dimensions are in mm.

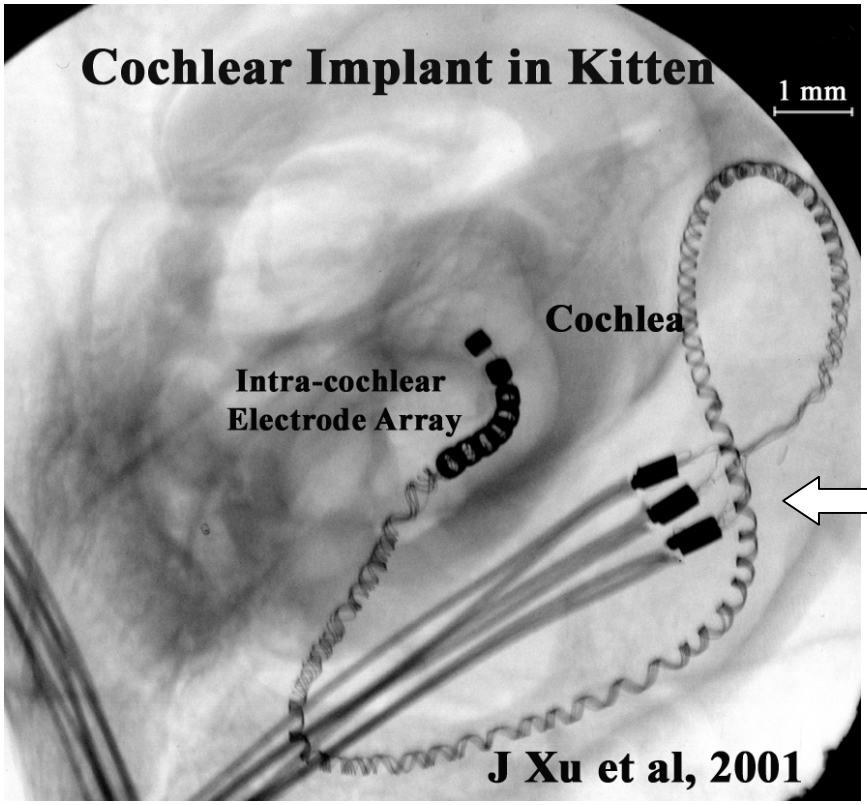

Figure 2. A micro-focus X-ray image of the left cochlea and auditory bulla of one of our implanted kittens. The eight-ring electrode array can be seen within basal turn of the cochlea. The platinum leadwire system is assembled into a helix to maximize stress relief, and welded to the more robust stainless 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 on the nape of the neck.

Each animal was implanted with an eight ring-electrode array manufactured by Cochlear Limited. This array has six active platinum ring electrodes mounted on a silicone carrier (Figs. 1 & 2). Three platinum electrodes are located near the tip of the electrode array while the remaining three electrodes are located approximately 2 mm more basalward to allow stimulation of two distinct sectors of the auditory nerve. Each electrode array has a stainless steel leadwire system assembly to allow direct electrical connection between the scala tympani electrodes and a body worn programmable stimulator (see Fig 1, *Second Quarterly Progress Report*). Control electrode arrays were identical to the stimulated arrays but without a leadwire assembly.

3.3 Chronic stimulation program

The dual channel stimulators have been programmed to provide a temporally challenging stimulus waveform by delivering charge balanced biphasic current pulses non-simultaneously to one or two electrode pairs at a stimulus rate of 1200 pulses per second (pps) per channel. Each current pulse is 100 μs/phase with a 10 μs interphase gap (Fig. 3). This waveform is amplitude-

modulated (AM) to a depth of 50% at 30 Hz (see Fig. 2, *Second Quarterly Progress Report*). The current amplitude for each channel can be adjusted independently and was based on the electrically-evoked auditory brainstem response (EABR) threshold for a given electrode pair (see below). The stimulator generates alternating leading phase current pulses, which has been shown to minimize the production of direct current (Huang et al., in press). Residual charge is removed by electrode shorting between current pulses. In addition, a 1 μF capacitor has been placed in series with one output of each channel. A discharge switch has been added to remove any charge from its output as the stimulator is connected to the stimulating electrodes, and the stimulator has been programmed to gradually apply electrode shorting during a 1-second start up period prior to the start of stimulation.

Chronic electrical stimulation of this group of animals commenced 13-17 days following surgery. All animals were stimulated using localised bipolar stimulation from one or two channels to stimulate discrete sector(s) of the auditory nerve. Over the course of these experiments one group of animals will have a single sector of auditory nerve stimulated; a second group will have two sectors stimulated; while in a third group we shall switch the sector of stimulated nerve from the lower basal to the upper basal cochlear turn.

The amplitude of the AM stimulus waveform for each for each electrode pair was typically set so that the minimum current level equalled the EABR threshold (i.e. the maximum stimulus intensity was 6 dB above EABR threshold). These stimulus levels were subsequently confirmed to be acceptable in the awake animal. A representative example of longitudinally recorded EABRs is illustrated in Fig. 4. The recording procedures have been outlined in Hardie and Shepherd (1999). To date, maximum stimulus current amplitudes used in this study have been in the range 0.3-1.26 mA developing charge densities in the range 6.4-25.6 $\mu C.cm^{-2}$ geom. per phase. These charge densities are within the range previously shown to be safe following chronic electrical stimulation in the cat (Shepherd et al., 1983; Xu et al., 1997).

The stimulators are carried in a harness worn by the cat to enable continuous stimulation without confining the animal's activities. Each animal has been stimulated approximately 6 h per day, five days per week for implant periods, to date, of up to 130 days. Both stimulus current and electrode voltage waveforms are monitored twice daily - just after the stimulator is turned on and just before it is turned off. Electrically isolated monitoring equipment is used for this purpose. Monitoring is used to confirm that the appropriate stimulation levels are set for each animal, and to determine electrode impedance. The peak electrode voltage (V_p; Fig. 3) and the current amplitude (i; Fig. 3) are measured and the electrode impedance calculated ($Z_p = V_p/i$). Two examples of this longitudinal monitoring of Z_p are illustrated in Fig. 5.

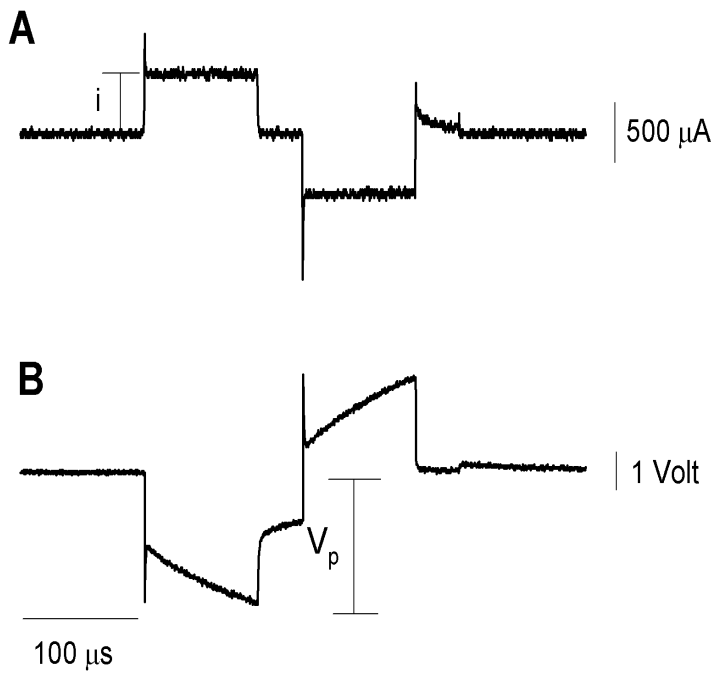

Figure 3. Examples of electrode current (A) and voltage (B) waveforms recorded from a chronically implanted animal. These waveforms are monitored twice daily to ensure the animal is being stimulated at the appropriate setting and to monitor the impedance of the bipolar scala tympani electrodes. The current waveform is obtained by measuring the voltage generated across a 100 Ω resistor placed in series with the output of one side of the portable stimulator.

Monitoring electrode impedance provides an important measure of the electrical stability of the stimulating electrodes. Changes in impedance generally reflect changes occurring at the electrode-tissue interface due, for example, to changes within the tissue environment adjacent to the electrodes (Agnew et al., 1983; Shepherd et al., 1990; Xu et al., 1997). Our previous work using this electrode array indicates that their *in vitro* impedance is ~3 kΩ, which gradually increases over the first 20-30 days of implantation (Shepherd et al., 1990). Although Z_p was not monitored in the immediate post-implant period in the present study, the data suggest a general increase over the first 30 days. Thereafter Z_p appears relatively stable. These results suggest that in both animals, a relatively fine tissue capsule has formed around the electrode array soon after implantation after which the electrode-tissue interface has remained relatively stable. Electrode impedance will be correlated with cochlear tissue response at the completion of these studies.

One observation made during the analysis of these data was evidence of a small but systematic *decrease* in Z_p as a function of electrical stimulation. We consistently observed a 5-8% *reduction* in Z_p at completion of the daily stimulation program. Although a relatively small change, longitudinal comparison of Z_p measured just prior to, and on completion of the daily stimulation program, showed that this stimulus induced change was highly statistically significant (Table 2). Note that this stimulus induced reduction in Z_p is not cumulative i.e. the impedance had returned to pre-stimulation levels by the following morning (e.g. Fig. 5). Moreover, we have only completed a detailed analysis for two of our eight animals to date. A more comprehensive analysis of this phenomenon will be presented in a future report.

Table 2: Median (inter-quartile range) electrode impedance measured after the stimulator is turned on (Z_{pmax}on) and before it is turned off (Z_{pmax}off) after ~6 h of stimulation

Cat	Z_{pmax}on	Z_{pmax}off	n	P*
NDC_1	3.9 (3.6-4.1)	3.5 (3.4-3.9)	81	<0.0001
NDC_3	4.5 (4.3-4.7)	4.0 (4.0-4.2)	73	<0.0001

Note: * Wilcoxon Signed Rank test

Over the next two quarters the chronically stimulated kittens described here will complete their six-month stimulation program. Each animal will be used in an acute electrophysiological experiment prior to euthanasia.

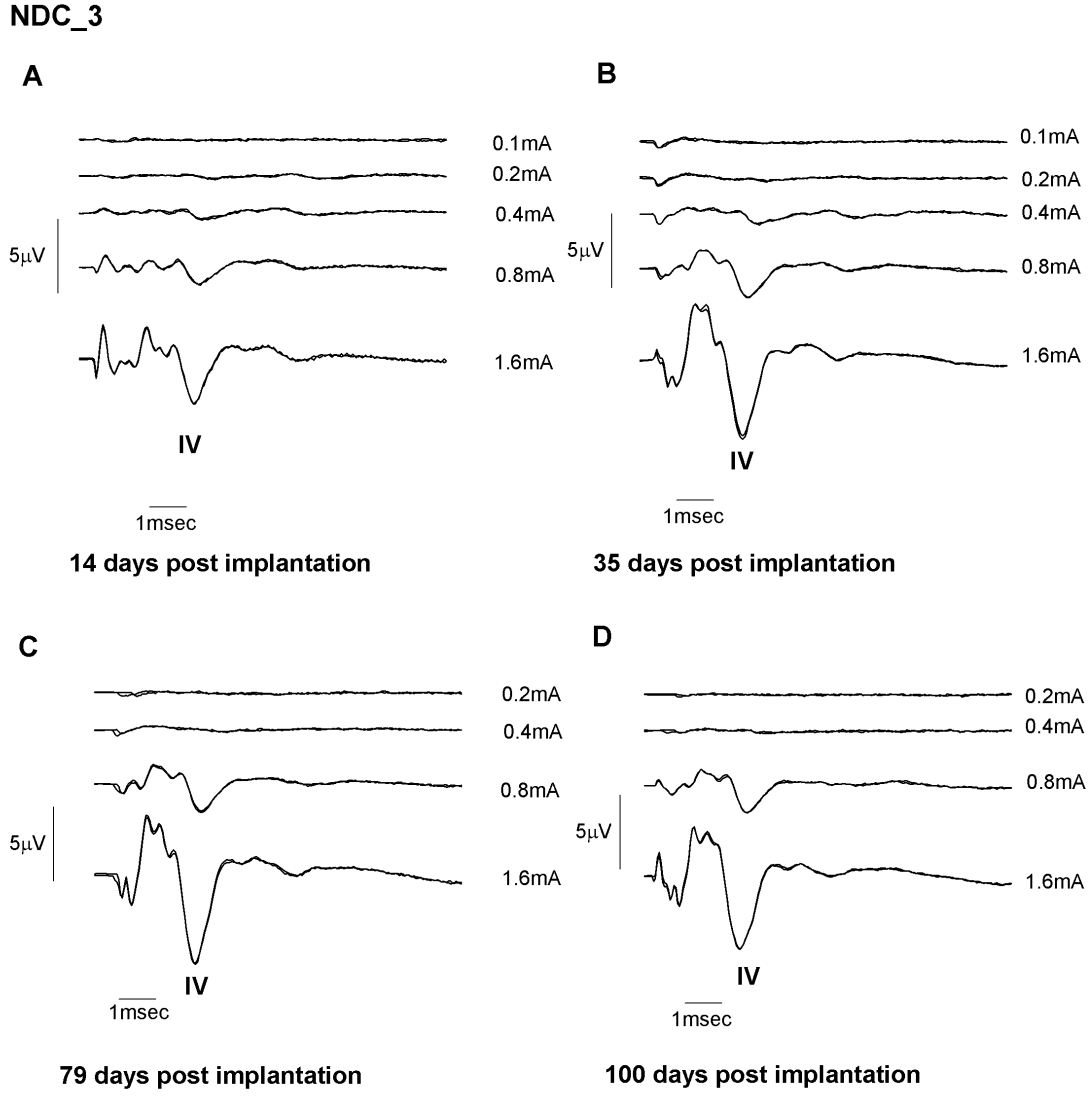

Figure 4. Representative EABRs recorded from NDC_3 periodically during the animal's chronic stimulation program. Although there is a slight increase in threshold, response amplitudes and waveform morphology have remained relatively constant throughout the stimulation program. These responses were evoked by bipolar electrode pair 1/2 (electrode #1 being the most apical on the electrode array). The trough of wave IV is illustrated.

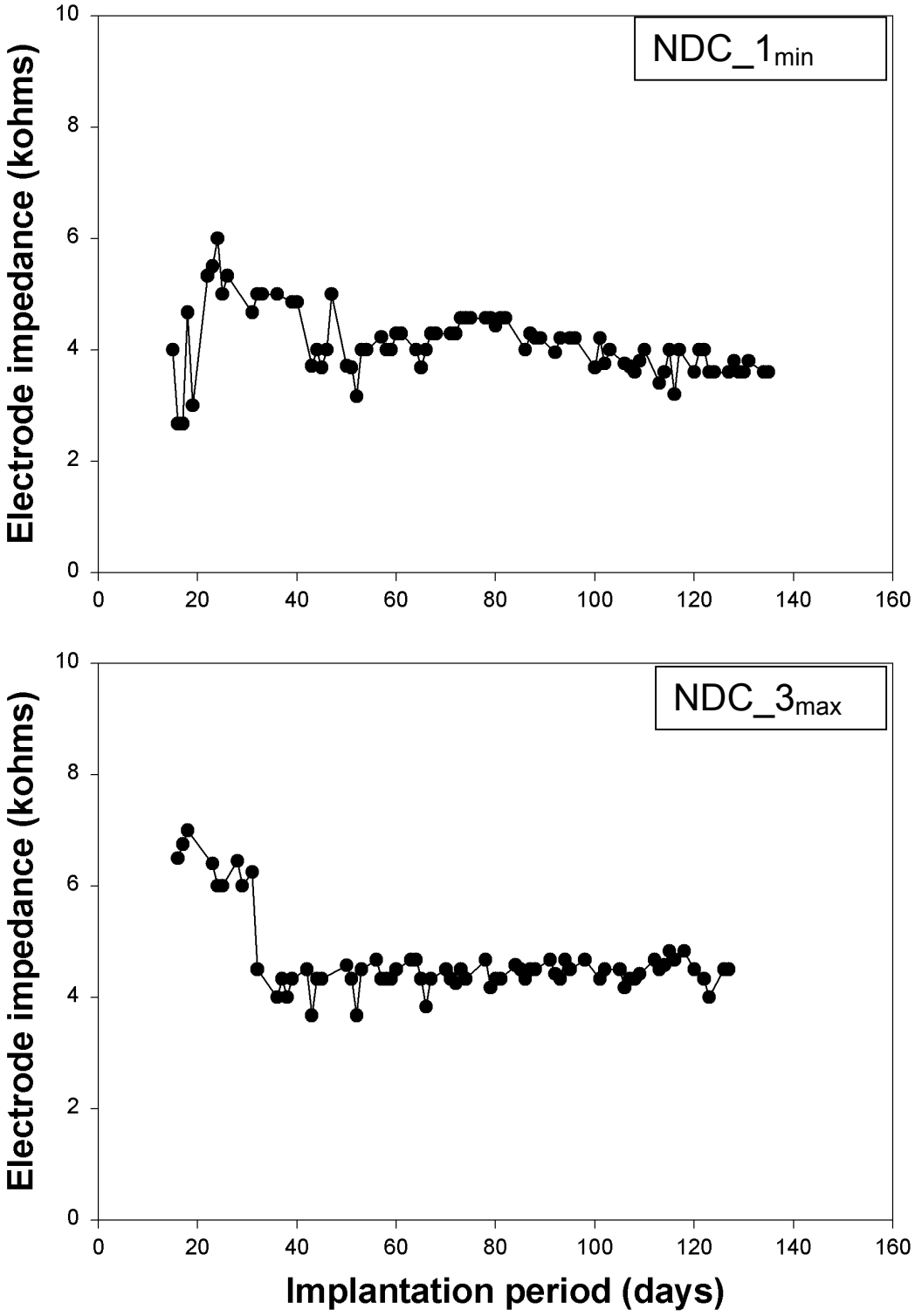

Figure 5. Longitudinal electrode impedance data illustrated for two representative animals from the present study. Impedance measurements commenced with the chronic stimulation program (~2 weeks following implantation of the electrode array). Although there was some variability in Z_p in the period shortly after initiation of the stimulation program, the impedances became quite stable thereafter. “min” refers to the impedance measured using the minimum current in the AM waveform; “max” was measured using the peak current of the AM waveform.

4. Electrical stimulation and neurotrophin administration in deafened guinea pigs

In the *Second Quarterly Progress Report* we noted the initiation of a study designed to examine the temporal resolution of auditory midbrain neurons in deafened control animals. Due to its labor intensive nature and invasive procedures we have decided to postpone this work so that we can concentrate our efforts on initiating the chronic stimulation study in guinea pigs. In our initial experiment, we shall implant and chronically stimulate deafened guinea pigs using an identical stimulator and stimulus waveform as used in the cat study described above. These guinea pigs will have artificial perilymph delivered to the implanted cochlea and hence will form our control animals. During this quarter an additional six portable stimulators and five electrode array/leadwire assemblies were manufactured in preparation for the chronic stimulation study scheduled to commence next quarter. For these experiments the temporal properties of the auditory nerve and CAS will be evaluated by measuring EABR refractory properties following procedures that have been described previously (van den Honert and Stypulkowski, 1986; Abbas and Brown, 1991; Zhou et al., 1995).

5. Neuroanatomical and neurochemical studies of the deafened auditory system

To investigate further the neurobiological effects of electrical stimulation and administration of neurotrophic agents on the deafened auditory system, we will initially perform quantitative and qualitative immunochemical studies of markers for endogenous excitatory and inhibitory neurotransmitter function, neurotrophic activity, and synaptic structure and function. More specifically, immunocytochemistry and Western immunoblot techniques will be used to investigate the localisation and levels of immunoreactivity of marker proteins deemed relevant to auditory system structure and function. Studies will be performed on deafened guinea pigs following completion of their chronic stimulation/neurotrophin administration program (see Section 4, above).

During the present quarter we have undertaken a comprehensive reorganisation and outfitting of the Brain Plasticity and Molecular Biology Laboratories in the Department of Otolaryngology. This effort will soon allow us to perform these basic studies as well as providing a platform for further investigation using pharmacological and molecular biological techniques. Finally, in an attempt to compliment and broaden studies to be performed in the department, we have begun to seek collaborative links with other research organisations, including The Bernard O'Brien Institute of Microsurgery, St Vincent's Hospital, Victoria, and the National Institute of Mental Health, NIH, Maryland. The successful establishment of these collaborations will provide access to equipment deemed useful for future research.

6. Plans for Next Quarter

- Continue our chronic stimulation studies in deafened kittens and guinea pigs.
- Continue the manufacture of guinea pig and feline electrode assemblies.
- Initiate our first immunochemistry protocols using normal hearing animals.
- Prepare and present an invited paper at the 2001 Implantable Auditory Prosthesis Conference, Monterey, CA (Dr. R. Shepherd).

7. Errata

In our *First Quarterly Progress Report*, we described a new deafening technique suitable for use in guinea pigs. The procedure involved a single intravenous injection of 100 mg/kg of Frusemide followed by a subcutaneous injection of Kanamycin. Unfortunately the text read Kanamycin administration at a dose of 300 mg/kg, this was in *error* - it should have read 400 mg/kg of Kanamycin.

8. Acknowledgments

We gratefully acknowledge the important contributions made by our Veterinarian Dr Peter Reynolds, and Elisa Borg for management of our animal house, Dr. Michael Tykocinski for surgical advice, Jacqueline Andrew for research assistance, Helen Feng for electrode manufacture, Maria Clarke for histological support, Dr. Phillip Marzella and Lisa Gillespie for advice on neurotrophin delivery systems and Frank Nielsen for technical support.

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