Second 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 second quarter of this contract the following activities were completed:

- Deafened five kittens in preparation for our chronic stimulation study.
- Design and development of an electrode array for our feline experiments.
- Development of stimulators and stimulation strategies for our chronic stimulation studies in kittens and guinea pigs.
- Deafened eight adult guinea pigs and initiated an acute electro-physiological study to examine the temporal resolution of CAS neurons following a SNHL.

- An attempt to develop a new deafening procedure for use in cats.
- Completion and submission of a review paper "Deafness-induced changes in the auditory pathway: Implications for cochlear implants" submitted to Audiology & NeuroOtology.

3. Chronic electrical stimulation studies in neonatally deafened cats

3.1 Deafening

Five kittens from one litter 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. Details of the ABR recording procedure have been described previously (eg Shepherd & Martin, 1995; Hardie & Shepherd, 1999).Three animals exhibited a profound hearing loss while two showed evidence of hearing at low frequencies, therefore modelling a severe hearing loss (Table 1).

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

All five animals will be implanted at approximately seven weeks of age. Their chronic stimulation program will commence 10 days following surgery using temporally challenging stimulus waveforms (see 3.3 below). Localised bipolar stimulation from one or two channels will be used to stimulate discrete sector(s) of the auditory nerve.

The two animals with residual hearing exhibited moderate-low thresholds in the 1-2 kHz region. We would expect that thresholds would approach normal levels below 1 kHz based on the known pathology of aminoglycoside lesions in the mammalian cochlea (eg Hawkins, 1959; Kohonen, 1965), however ABRs are not effective at monitoring the hearing status below approximately 1 kHz (Evans and Elberling, 1982). Both animals showed no response to ABRs evoked by tone pips greater than 8 kHz. NDC_3 and NDC_4 will be used to model the effects of cochlear implantation in a severe hearing loss. This work is designed to study the trophic support of spiral ganglion cells (SGCs) following chronic electrical stimulation in cochleae with residual elements of the organ of Corti, and to examine the plastic response of the CAS to joint acoustic/electrical stimulation of the cochlea. Additional animals with residual hearing will be added to this group.

A second litter of kittens were born late this quarter. They will also be deafened in preparation for the chronic stimulation program. Further details will be presented in our next progress report.

3.2 Electrode arrays

We have received eight feline electrode arrays from Cochlear Limited. This array has six active platinum ring electrodes mounted on a Silastic carrier. 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. In preparation for implant surgery next quarter, we have connected each electrode array to a stainless steel leadwire system and manufactured control electrode arrays for implantation in the contralateral ear.

3.3 Stimulator design and output

Eight portable programmable dual channel stimulators have been assembled, programmed and tested in preparation for our chronic stimulation studies. These stimulators are relatively small and light (Fig. 1), and can be readily worn in a backpack without restricting a kittens' mobility.

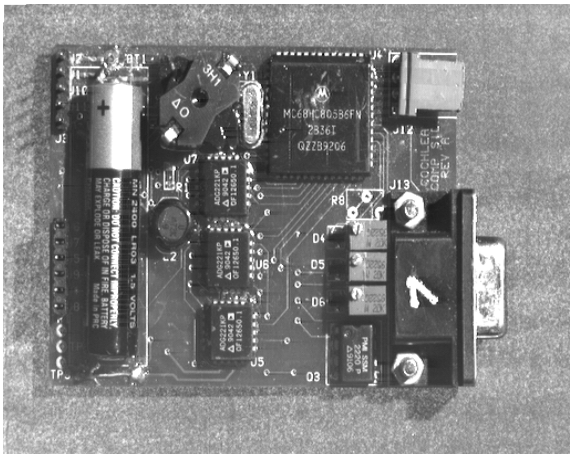

Figure 1. Photograph of the dual channel, programmable stimulator that will be used in this study. The device weighs 54 grams. For scale the battery is an AAA.

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. Each current pulse is 100 μs/phase with a 10 μs interphase gap. This waveform is amplitude-modulated (AM) to a depth of 50% at 30 Hz (Fig. 2). The current amplitude for each channel can be adjusted independently. We anticipate that the minimum current amplitude in the AM waveform will be set at the EABR threshold for a given electrode pair. The stimulator generates alternating leading phase current pulses. 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 before commencing stimulation.

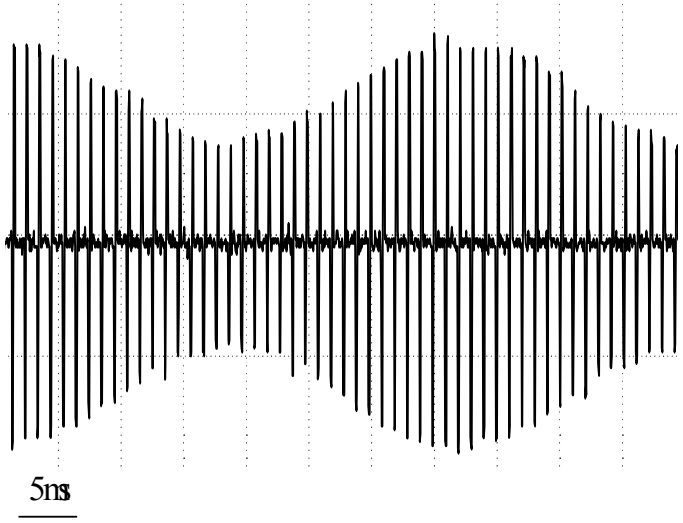

Figure 2. Example of the stimulus waveform. The waveform consists of charge-balanced biphasic current pulses presented at a stimulus rate of 1200 pps. This carrier waveform is amplitude-modulated to a depth of 50% at 30 Hz. This figure shows the output of one channel of the programmable stimulator recorded across a resistive load using a Tektronix TDS710 digital oscilloscope.

4. Electrical stimulation and neurotrophin administration in deafened guinea pigs

4.1 Deafness induced changes in the temporal resolution of neurons in the central auditory pathway

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. In addition, this work is designed to examine the hypothesis that chronic electrical stimulation of the auditory nerve in concert with neurotrophic administration increases the temporal resolution of auditory neurons following a SNHL.

We have previously demonstrated a significant reduction in the temporal resolution of inferior colliculus (IC) neurons following a long-term SNHL in cats (Shepherd et al., 1999). It is important to verify these findings in naive deafened guinea pigs before embarking on chronic studies using electrical stimulation and neurotrophic administration. Our work during this quarter has concentrated on recording the temporal resolution of auditory midbrain neurons in deafened control animals.

Eight otoscopically normal, pigmented guinea pigs (mean weight 546 g; s.e.m. 46 g) were used this quarter. All animals had normal hearing prior to deafening as indicated by ABR thresholds to 100 μs rarefaction clicks of less than 38 dB pe SPL. Each animal was anaesthetised with ketamine and xylazine, and deafened with a single intravenous injection of 100 mg/kg frusemide (FR) followed by 300 mg/kg of kanamycin administered subcutaneously (First Quarterly Progress Report). All animals made an uneventful recovery from the procedure. Hearing was assessed using click-evoked ABRs following a minimum period of seven days after deafening. Both cochleae of all eight animals exhibited a profound hearing loss ie ABRs could not be elicited at the maximum stimulus intensity (98 dB pe SPL).

All animals were used in an acute electrophysiological experiment 1-month following deafening. Briefly, anaesthesia is induced with ketamine and xylazine and maintained for the procedure (typically 10 hours) with pentobarbitone sodium. Atropine sulphate is also administered to minimize mucosal secretions. A tracheal cannulation is performed and the animal's head is secured in a stereotaxic apparatus. The animal's core body temperature is maintained at 38 ^{o}C $\pm$ 1 ^{o}C and respiration and endtidal CO_2 monitored and maintained within normal limits (40-60/min and 3-5% respectively).

The left cochlea is surgically exposed and a banded bipolar electrode array is inserted 5 mm into the scala tympani. Considerable care is taken to ensure a similar insertion depth across experiments. The round window is sealed with muscle and the leadwire system is fixed. Electrically-evoked ABRs are then recorded for subsequent comparison with both normal controls and deafened/treated animals. A craniotomy is performed in the occipital region of the right hemisphere and the dura mater overlying the exposed cerebral cortex carefully removed. In initial experiments an attempt was made to aspirate the cortex overlying the IC however we have abandoned this approach due to excessive bleeding. We now position the recording electrode (a 1-2 MΩ Tungsten microelectrode) above the overlying cortex and advance it in a dorso-ventral direction until contact is made with the underlying IC (Syka et al., 2000). This is determined by the presence of field potentials and background neural activity that is synchronous with the search stimulus (a single 100 μs/phase biphasic current pulse presented at a rate of 2.5/s). The microelectrode is then advanced in 5 μm steps through the external and central nuclei of the IC using a remote-controlled stepping motor.

The signal is amplified by 80 dB and bandpass filtered. Electrical artefact and large field potentials are removed using a sample-and-hold circuit. The output signal is displayed on an oscilloscope and its triggering adjusted to discriminate the leading edge of the action potential. The resultant trigger pulse generated by the oscilloscope is fed to a PC controlled stimulus generation/data acquisition system. An Excel based software system is used to control the stimulus generation and provide real-time peristimulus time, interspike interval and period histograms, while hardware modules (Tucker Davis Technologies) provide the appropriate attenuators, DAC, ADC and event timers. Once isolated, rate intensity functions are recorded in response to stimulus rates of 2.5 - 200 pps to study temporal response properties. Using 100 μs/phase biphasic current pulses, rate-intensity functions are recorded over each unit's dynamic range in response to increasing stimulus rates until the neuron clearly fails to reach saturation.

Of the eight animals used during the quarter, two died under anaesthesia without the collection of any data and a further two animals were used to develop the recording technique. The remaining four animals resulted in successful experiments yielding a total of 85 isolated single IC neurons for analysis. During the next quarter this work will continue using normal hearing guinea pigs, and the data will be analysed statistically.

5. 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 ethacrynic acid (the supply has now been re-established). In our First Quarterly Progress Report we described a new deafening technique for guinea pigs using a single co-administration of kanamycin with the loop diuretic frusemide. Our initial results were based on six guinea pigs. During this quarter a further 12 guinea pigs were deafened for this and other research projects using this technique. In all cases the deafening procedure was successful – ABRs could not be elicited in response to a 98 dB pe SPL acoustic click; there was no clinical evidence of renal impairment; there was no evidence of loss of balance or ataxia. This procedure has now become our laboratory standard for deafening guinea pigs.

Based on this success in guinea pigs, we sought to determine whether this technique could be successfully used to create a profound sensorineural hearing loss in the cat. Two otoscopically normal, adult cats (mean weight 3.47 kg; s.e.m. 0.63 kg) were used. Each animal had normal hearing prior to deafening as indicated by ABR thresholds to 100 μs rarefaction clicks of less than 28 dB pe SPL.

Using ketamine and xylazine anaesthetic, the first animal (KA_FU1) was deafened using a single intravenous injection of 70 mg/kg FR followed by 300 mg/kg of KA administered subcutaneously. Although the animal made a rapid recovery from the procedure, over the following five days it appeared lethargic and was drinking relatively large quantities of water. Given these clinical indications of renal impairment, blood biochemistry was assessed five days following the procedure (Table 2). These data indicated moderate renal impairment and an electrolyte imbalance. The animal's clinical condition improved over the subsequent few days and blood biochemistry results 11 days following the deafening were normal (Table 2). There was no evidence of a loss of balance or ataxia during this period. Subsequent evaluation of this animal's hearing using click-evoked ABRs showed no elevation in threshold (ie threshold < 28 dB pe SPL). We did not use frequency specific stimuli to examine more spatially restrictive regions of the cochlea as our primary objective was to develop a profound hearing loss.

It was decided to increase the dose rate of FU from 70 to 85 mg/kg in an attempt to induce a profound hearing loss in the second animal (KA_FU2). The KA dose rate remained at 300 mg/kg. Again the animal exhibited clinical signs of renal impairment by five days following the procedure (Table 2). In addition, this animal also exhibited a mild balance dysfunction. Blood biochemistry evaluated at five and 13 days following deafening showed evidence of severe renal impairment (Table 2). ABRs recorded at the latter date revealed that the animal was profoundly deaf; ie both ears showed no response to a 98 dB pe SPL acoustic click. The animals were killed using an

overdose of anaesthetic (pentobarbitone sodium) soon after their final blood biochemistry results were obtained.

Table 2: Blood biochemistry following KA/FU deafening in cats (mmol/L)

KA_FU1	*5 days*	*11 days*	*Normal range*
Creatinine	0.16	0.09	0.07-0.16
Urea	**30.2**	8.5	5.4-10.7
Sodium	**138**	151	147-156
Potassium	**3.6**	3.9	3.8-4.6
Chloride	**88**	118	115-123
KA_FU2	*5 days*	*13 days*	*Normal range*
Creatinine	**2.58**	**1.46**	0.07-0.16
Urea	**108.3**	**116.3**	5.4-10.7
Sodium	**138**	**139**	147-156
Potassium	**9.1**	4.5	3.8-4.6
Chloride	**77**	**83**	115-123

Although the co-administration of KA and FU is a safe and effective technique for deafening guinea pigs, its use in cats cannot be recommended. The increased renal and reduced cochlear susceptibility of KA/FU toxicity in cats contrasts with our previous experience using KA and EA (Xu et al., 1993).

6. Plans for Next Quarter

- Complete single unit studies in normal hearing control guinea pigs to study temporal resolution within auditory midbrain neurons prior to commencing chronic stimulation studies in the guinea pig.
- Implant and commence our chronic stimulation study in neonatally deafened kittens.
- Initiate a pilot chronic stimulation program in deafened guinea pigs.
- Continue the manufacture of guinea pig and feline electrode assemblies.

7. Personnel

Dr. Patricia Hurley joined the team this quarter. Dr. Hurley has an MBChB from the University of Edinburgh and is a Fellow of the Royal College of Surgeons of Edinburgh. Dr. Hurley will carry out research directed towards a PhD. Her work will include the study of the pathophysiological response of the cochlea and central auditory system following a SNHL. A novel aspect of Patricia's work will be the study of the acute response of this pathway to "re-afferentation" via electrical stimulation of the auditory nerve. Patricia was recently awarded a University of Melbourne International Fee Exemption Scholarship.

8. 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 Natalie Hardie for general advice and assistance, Helen Feng for electrode manufacture, Maria Clarke for histological support and Frank Nielsen for technical support.

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