Fourth Quarterly Progress Report

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# Protective Effects of Patterned Electrical Stimulation on the Deafened Auditory System

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**Contents:**

## 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 of spiral ganglion neurons (SGNs) and the central auditory system (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 auditory nerve (AN) survival in both guinea pig and other mammalian models of sensorineural hearing loss (SNHL). This work will also provide insight into the role of neurotrophins in improving synaptic efficiency in the deafened auditory pathway.

(b) The neurophysiological and neuroanatomical response to prolonged electrical stimulation of the auditory nerve following a neonatal SNHL. This work is designed to provide insight into the protective effects of electrical stimulation on SGNs and the plastic response of the CAS to temporally challenging stimuli presented chronically to one or two sectors of the AN. This work will also examine the ultrastructural changes evident at the AN/cochlear nucleus synapse in response to a neonatal SNHL and to chronic electrical stimulation of the AN.

(c) The application of cell based therapies for rescue and regeneration of SGNs following SNHL. These studies are designed to provide insight into the potential clinical application of cell-based therapies in the severe and profoundly deaf prior to cochlear implantation.

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 third quarter of this contract the following activities were completed:

***Publications and conferences***

- Manuscript preparation: During the quarter one manuscript was accepted for publication and has been appended to this application (Appendix A): Shepherd, R.K., Roberts, L.A. and Paolini A.G. Long-term sensorineural hearing loss induces functional changes in the rat auditory nerve. European J. Neuroscience (in press). Two additional manuscripts are currently in the review process.
- Preparation of abstracts and presentations for the 2004 Frontiers in Otorhinolaryngology; the National Institutes of Health National Institute of Neurological Disorders and Stroke Neural Interfaces Workshop; the 2005

Australian Neuroscience Society and the 2005 Association for Research in Otolaryngology conference. Submitted abstracts are located in Appendix B.

- Drs. Gillespie and Tan were invited to present their doctoral research at the 2005 Australasian Auditory Neuroscience Workshop in Perth, WA, while Dr. Fallon was awarded an NIH traveling Scholarship to attend the Neural Interfaces Workshop, Bethesda, MD. Doctoral student Bryony Coleman was awarded most outstanding poster award at the 2004 Frontiers in Otorhinolaryngology, Noosa, QLD. Dr. Shepherd was an invited speaker at the same meeting.

***Chronic electrical stimulation and neurotrophin delivery in the guinea pig***

This work aims at developing techniques for SGN rescue based on the exogenous delivery of neurotrophins in combination with chronic depolarization via a cochlear implant.

- Chronically stimulated five deafened guinea pigs implanted during the previous quarter with electrode arrays incorporating a drug delivery system delivering the neurotrophin brain derived neurotrophic factor (BDNF). At completion of the 28 day implantation period these animals were used in acute experiments recording from single auditory nerve fibers in response to electrical stimulation. This work was performed in collaboration with A/Prof. S. O'Leary and his colleagues D. Sly and L. Heffer.

- We deafened a further 10 guinea pigs during the quarter. These animals will be implanted and receive BDNF and chronic electrical stimulation for set periods. They form the final cohort in a study designed to test the hypothesis that electrical stimulation alone can maintain the trophic advantage on SGNs of an initial application of exogenous BDNF.

***Chronic electrical stimulation in the cat***

As noted in our 3rd QPR, this work uses Nucleus® CI24 cochlear implants in combination with Nucleus® ESPrit 3G behind-the-ear speech processors. The cochlear implants are *not implanted* but are hardwired to connect directly with the animal's percutaneous leadwire system.

- During the quarter we implanted another six animals. All animals were deafened neonatally via daily injections of the aminoglycoside Neomycin. Using revised surgery, we fixed their leadwire to the skull using Titanium screws designed by Dr. David Smith (University of Florida) and Titanium clips (designed in house). Although implant times are to date limited (<2 months) the Ti screws and clips have been effective in fixing the leadwire.
- Programming of the Nucleus® ESPrit 3G behind-the-ear speech processors as part of our feline studies commenced this quarter. The existing clinical programming interface for the Nucleus® ESPrit 3G processors (RSP126®) does not allow us to configure the Nucleus® CI24 cochlear implants to produce the stimulus paradigms required. Therefore, work was begun on our own direct interface to the Nucleus® ESPrit 3G processors that will allow us finer control over the Nucleus® CI24 cochlear implants.
- Chronic intra-cochlear stimulation of 6 animals using the Nucleus® ESPrit 3G behind-the-ear speech processors and the Nucleus® CI24 cochlear implants commenced. As part of the programming of the Nucleus® ESPrit 3G

processors, basic behavioral testing of the animals to confirm their behavioral thresholds and comfort levels was performed

- This quarter work has continued on finalizing the requirements and specifications of our new multi-channel data acquisition system. The system will be used in conjunction with the two acute 25 microelectrode arrays from Cyberkinetics and the 16 electrode Michigan array for electrophysiological studies of the auditory cortex examining plasticity. It is anticipated that this system will be ordered during the following quarter.
- We have obtained several 25x25 recording arrays from Cyberkinetics Inc. in order to practice acute insertion into the auditory cortex prior to these experiments.
- We commenced collaborative studies with Dr. David Ryugo from Johns Hopkins University to study synaptic plasticity in the auditory nerve/cochlear nucleus (end bulb of Held) in our Neomycin deafened/chronically stimulated cats. During this quarter Dr. Ryugo visited the laboratory and tissue specimens taken from our first series of implanted/chronically stimulated cats (see 3rd QPR for more details of these animals) were prepared for light and transmission electron microscopy.

***Chronic electrical stimulation in the rat***

The aim of this project is to develop a small fully implantable stimulator that would provide chronic electrical stimulation of the cochlea in small laboratory animals such as the rat. This work will examine the trophic effects of electrical stimulation in a third animal species. Moreover, it will allow us to perform behavioral studies on animals that have received chronic stimulation and compare their performance at rate and pitch discrimination with implanted, un-stimulated control animals.

- The surgical feasibility of chronic cochlear implantation in the rat continued during the quarter. A manuscript describing the technique is being prepared for publication.
- We have continued work on the development and testing of our fully implantable small animal stimulators as described in our 3rd QPR. An additional animal has been implanted during the present quarter.
- Minor modifications to the rat test-box for behavioral studies are almost complete. This work was performed at La Trobe University in collaboration with Dr. Tony Paolini and colleagues. It is expected that the test-box design will be completed during the following quarter and the box brought to our laboratory for assessment and trials using acoustic stimulation. During this period the box will also be modified to include electrical stimulation of deafened/implanted rats.

***Cellular over-expression of BDNF***

The aim of this study is to use cell transplantation techniques to deliver long-term/ongoing neurotrophic support to SGNs in animal models of deafness. The decision has been made to initiate this study using a non-viral vector system.

- We have established a formal collaboration with Dr. Volkmar Lessman (Johannes Gutenberg-Universität Mainz, Germany), who has kindly donated an expression plasmid containing rat prepro-BDNF fused to the reporter gene enhanced green fluorescent protein (EGFP). Thus, following transfection, secreted BDNF will be identified by the EGFP fluorescence; this will be important in determining the diffusion of the BDNF *in vivo*.

- A collaboration has also been established with a research group at the Howard Florey Institute under the leadership of Dr. Trevor Kilpatrick, for the acquisition of Schwann cells. Due to some concerns over the labeling of these cells for identification *in vivo*, we have decided to implant male cells into female guinea pigs and use Y-chromosome labeling as the identification technique. This requires the establishment of a new Schwann cell culture prepared only from male animals; these cultures will be ready late in the next quarter.

***The application of stem cells for SGN regeneration***

The aim of this study is to develop clinically feasible techniques for the application of stem cell therapy for SGN regeneration in the profoundly deaf.

- While the differentiation of stem cells into neurons is now routine, we are particularly interested in the differentiation of stem cells into SGNs. In an attempt to identify what factors are required for this specific differentiation, we are trialing co-culture models; hair cell explants with partially differentiated stem cells and SGN cultures with partially differentiated stem cells. We hypothesize that stem cells will be encouraged to preferentially differentiate into a SGN under these specific conditions. We are currently optimizing a triple-label for immunohistochemical analysis and completing confocal microscopy on experimental material.

- We have recently completed an *in vivo* study examining the effects of transplanting mouse embryonic stem cells into the deafened guinea pig cochlea via the scala tympani. We observed that although stem cells were detected in the cochlea up to 4 weeks following transplantation, the scala tympani approach used for cell delivery may not be the most efficient for cell-based therapy in this animal model. Future experimentation will be directed towards delivering cells directly into Rosenthal's canal. Importantly, this study found that a proportion of transplanted stem cells were positive for the neuronal marker NF-L (a low molecular weight neurofilament protein specific to neurons). This finding is encouraging for future transplantation studies in the cochlea where we intend to transplant differentiated stem cells. This work will be submitted for publication shortly.

- We continued to examine the osseous spiral lamina of three species of laboratory animals using scanning electron microscopy. This work follows our initial studies in human and cat (Shepherd and Colreavy, 2004) describing small pores in the bony lining of Rosenthal’s canal. These pores may provide passage of drug- or cell-based therapies introduced into the cochlea via the scala tympani to the SGNs. The present research is designed to determine whether there is a suitable animal model for the highly porous human osseous spiral lamina.

## 3. Plans for Next Quarter

- Continue manuscript writing and submission, and preparation for attending conferences.

- Implant deafened guinea pigs and commence BDNF/ES delivery. These animals will complete the cohort designed to test the hypothesis that chronic electrical stimulation can maintain the trophic advantage initially provided by BDNF.
- Continue our chronic electrical stimulation studies in the cat.
- Deafen additional cats in preparation for further implant studies.
- Continue collaborative studies with Dr. David Ryugo
- Complete a computer-based facility to capture electrode voltage and current waveforms from our chronically implanted cats. To date we have recorded these waveforms manually from an electrically isolated oscilloscope. However, now that we are using the CI24M implant and the ESPrit 3G behind-the-ear speech processor, we can significantly increase the number of stimulus channels making a manual recording system redundant. Electrode current and voltage waveforms will be captured using a programmable data acquisition system connected to an electrically isolated PC. In-house software will be developed to provide an accurate calculation of electrode impedance for each electrode.
- Establish recombinant DNA procedures in the laboratory, aimed at cloning specific plasticity-related genes used for the synthesis of riboprobes.
- Establish *in situ* hybridization techniques in the laboratory, taking into consideration how different fixation and decalcification procedures would affect the expression of mRNA transcripts, and to establish semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) as an additional technique that we will use to confirm gene expression changes at the mRNA level.
- Initiate the development of fluorescent *in situ* hybridization (FISH) techniques for Y-chromosome labeling, as well as establishment and maintenance of the male Schwann cell cultures in preparation for transfection and further experimentation.
- Continue *in vitro* and *in vivo* studies directed at further differentiating stem cells towards SGNs.
- Continue to fabricate electrode assemblies for use in our chronic stimulation studies.

## 4. Personnel

Two new postdoctoral fellows commenced during the quarter. In addition, we have appointed another Research Assistant who will contribute to various aspects of our research effort.

Dr. Justin Tan obtained his Dr. rer. Nat. (German equivalent of a Ph D) from the University of Tuebingen, awarded by the Faculty of Biology. He also held a Master degree in Neural and Behavioural Sciences from the University of Tuebingen, awarded jointly by the Faculty of Medicine and Biology. His research activities at the Tuebingen Hearing Research Centre, under the guidance of Professor Marlies Knipper, focus on analyzing how activity-dependent genes in the cochlea and auditory cortex are differentially changed by acoustic over-stimulation and salicylate *in vivo,* using a repertoire of molecular techniques such as *in situ* hybridisation, RT-PCR, immunohistochemistry, Northern and Western blots. Dr. Tan has published 5 papers and presented his work as both oral and

poster contributions in international conferences. His appointment will provide the molecular expertise for our *in vivo* studies.

Dr. James Fallon finished his PhD in Biomedical Engineering at Monash University in 2001, which involved the design and construction of a multi-channel recording system, and the subsequent use of the system in studies of Stochastic Resonance in a variety of biological systems. He then worked for a brief period at the Department of Otolaryngology as part of team for the “Protective Effects of Patterned Electrical Stimulation on the Deafened Auditory System (NIH-N01-DC-0-2109)” project where he was involved in software development and in experiments recording from central auditory centers. In 2002 he moved to the Prince of Wales Medical Research Institute, where he utilized the technique of microneurography in a variety of experiments examining reflexes and motor control stratagems in awake conscious humans. Dr. Fallon provides the team with a valuable combination of both engineering and electrophysiological skills.

During the quarter Jennifer Hardman joined the team and is employed as a research assistant. Jennifer obtained a Bachelor of Science from James Cook University in Townsville awarded through the Faculty of Science and Engineering. Jennifer has previously been working within the Department of Otolaryngology and has obtained extensive experience in tissue culture, animal surgery, molecular biology, general histology, data collection and analysis, and laboratory management which she will be able to bring to the team.

## 5. Acknowledgements

We gratefully acknowledge the important contributions made by our Histologist, Maria Clarke; Veterinarian Dr Sue Peirce; Elisa Borg for management of our animal house; Helen Feng for electrode manufacture; Frank Nielsen for engineering support; Dr. Tony Paolini, his students and the Mechanical workshop staff at La Trobe University for advice and assistance in manufacturing the rat test chamber.

## 6. References

Shepherd, R.K., Colreavy, M.P. 2004. Surface microstructure of the perilymphatic space: implications for cochlear implants and cell- or drug-based therapies. Arch Otolaryngol Head Neck Surg 130, 518-23.

## 7. Appendix A (attached)

Shepherd, R.K., Roberts, L. A. and Paolini A.G. Long-term sensorineural hearing loss induces functional changes in the rat auditory nerve. European J. Neuroscience (in press).

## 8. Appendix B (attached)

Conference Abstracts