Fifth 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 fifth quarter of this contract the following activities were completed:

Publications and conferences

Manuscript preparation: During the quarter two manuscripts were accepted for publication and have been appended to this application (Appendix A and B):

Newbold, C., Richardson, R, Huang, C.Q., Milojevic D., Cowan, R. and Shepherd, R.K. An in vitro model for investigating impedance changes with cell growth and electrical stimulation: implications for neural prostheses. J. Neural Eng. 1: 218-227, 2004.

McGuinness, S.L. and Shepherd, R.K. Exogenous BDNF rescues rat spiral ganglion neurons *in vivo*. Otology & Neurotology (in press).
Three additional manuscripts are currently in the review process.

- Drs. Shepherd and Fallon presented a progress report at the Neural Interfaces Workshop, Bethesda, MD. Dr. Fallon also presented a poster at this meeting: Fallon, J. B., Crook, J. M., Serruto, A., Epp, S. and Shepherd, R. K. Effects of Chronic Stimulation on the Response of Inferior Colliculus Neurons in Neonatally Deafened Cats (Appendix C).

- Dr. Shepherd was an invited speaker and Dr. Fallon attended the 1st Williams Conference on Tissue Engineering the Inner Ear: Re-engineering the Auditory Nerve, Eagle Crest Marriot Resort, Ypsilanti, Michigan, USA, November 2004.

- During the quarter Drs. Fallon and Shepherd visited the Center for Hearing Sciences, Department of Otolaryngology, Johns Hopkins University, Baltimore, MD, and Department of Otolaryngology UCSF, San Francisco, CA. We presented our research at a seminar at each laboratory.

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.

- We implanted a further 10 guinea pigs during the quarter. These profoundly deafened animals received BDNF and chronic electrical stimulation for set periods. This was our major activity for the quarter.

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 present quarter we have continued daily chronic electrical stimulation of six neonatally deafened animals. Animals were monitored daily; current and electrode voltage were recorded from which electrode impedances were calculated. Electrically-evoked auditory brainstem responses were recorded each month. These animals have now been chronically stimulated for periods of 4-5 months using a behaviorally relevant stimulation regime.
- Continued development of a direct interface to the Nucleus® ESPrit 3G processors that will allow us finer control over the Nucleus® CI24 cochlear implants. As part of this process the feasibility of using the Nucleus® processors and implants for performing basic electrophysiological

measurements, particularly EABRs, ECAPs, and behavioral testing will be assessed.
- Repeated behavioral testing of comfort levels for the implanted cats and subsequent adjustment of chronic stimulation levels based on EABR and comfort level measurements.
- We have ordered our new multi-channel Cerebus data acquisition system from Cyberkinetics and additional software from Wavemetrics and Plexon.
- Commenced development of protocols for insertion of the multi-electrode arrays from Cyberkinetics Inc. and the University of Michigan
- Continued analysis of histological and electrophysiological data from the previous series of experiments on the effects of long-term chronic intracochlear electrical stimulation.

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.
- We implanted one rat with a chronic electrode array and stimulated for a period of 1 month.
- A manuscript describing the surgical feasibility of chronic cochlear implantation in the rat was submitted for publication.
- We ordered additional components to allow us to manufacture 20 implantable small animal stimulators over the next three quarters. These stimulators will be used in the rat behavioral experiments currently being designed.
- Following the successful completion of minor modifications to the rat test-box during the quarter, we installed it in our animal house in preparation for initial behavioral studies. The test-box was manufactured at La Trobe University following a design developed by Dr. Tony Paolini and his colleagues.

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.
- Using the plasmids provided by Dr Lessman (Johannes Gutenberg-Universität Mainz, Germany), bacterial work was performed to amplify the quantity of plasmids, such that there are sufficient quantities for use in the Schwann cell transfections.
- Schwann cell cultures were prepared from male rat sciatic nerve; however, problems were encountered in the purification of the cultures from contaminating fibroblasts. We expect to receive the purified cultures from our colleagues at the Howard Florey Institute early in the next quarter.
- All reagents required for the Schwann cell experiments were ordered and received.

Analysis of gene-specific markers altered by deafening in the cochlea

The aim of this study is to investigate how the expression of genes related to neuronal survival and function in the mammalian auditory system are modified by SNHL and by re-activation via a cochlear implant.

- Recombinant DNA procedures have been established in this laboratory aimed at cloning gene-specific plasmids to obtain riboprobes for *in situ* hybridization experiments. We have also performed *in situ* hybridization experiments using riboprobes specific for BDNF, c-Fos and trkB and determined the optimal fixation/decalcification procedures for these riboprobes.
- A protocol has been developed in the cochlea combining *in situ* hybridization and immunohistochemistry on the same section. Such a technique will be useful in answering whether the expression of a specific protein in a cell is related to its endogenous production within the same cell or uptake from neighboring cells.
- We have also examined the cochleae of deafened guinea pigs using immunohistochemistry to look for markers that are altered by deafening. We have observed interesting alterations of both neuronal and trophic markers after chronic deafening. Further experiments will be performed to confirm these changes.

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.

- A manuscript describing our first *in vivo* study was prepared and submitted for publication.

3. Plans for Next Quarter

- Continue manuscript writing and submission, and preparation for attending conferences.
- Install and perform initial studies in the auditory cortex of hearing animals using our multi-channel Cerebus data acquisition system.
- Continue our chronic electrical stimulation studies in the cat.
- Deafen additional cats in preparation for further implant studies.
- Assess a computer-based facility to capture electrode voltage and current waveforms from our chronically implanted cats.
- 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.
- Deafen rats and guinea pigs to investigate short- and long-term effects on neuronal and trophic markers in the cochlea neurons.
- 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

Jennifer Hardman, a part-time Research Assistant on this contract has accepted post-graduate entry to Medical School and completed her activities on this contract at the end of this quarter.

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; and Dr. Tony Paolini from La Trobe University, for advice in using the rat test chamber.

6. Appendix A (attached)

Newbold, C., Richardson, R, Huang, C.Q., Milojevic D., Cowan, R. and Shepherd, R.K. An in vitro model for investigating impedance changes with cell growth and electrical stimulation: implications for neural prostheses. J. Neural Eng. 1: 218-227, 2004.
(This paper was the featured article for the issue and a figure from the paper will be used on the cover of the next issue).

7. Appendix B (attached)

McGuinness, S.L. and Shepherd, R.K. Exogenous BDNF rescues rat spiral ganglion neurons *in vivo*. Otology & Neurotology (in press).

8. Appendix C (attached)

Abstract from the 2004 Neural Interfaces Workshop, Bethesda, MD.

Fallon, J. B., Crook, J. M., Serruto, A., Epp, S. and Shepherd, R. K. Effects of Chronic Stimulation on the Response of Inferior Colliculus Neurons in Neonatally Deafened Cats.