Tenth 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 replacement 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 tenth quarter the following activities were completed:

2.1. Publications and conferences

The following papers were accepted for publication.

Prado-Guitierrez, P., Fewster, L.M., Heasman, J.M., McKay, C.M., and Shepherd, R.K. Effect of interphase-gap and pulse-duration on electrically evoked potentials is correlated with auditory nerve survival. Hearing Research (in press). A copy of this manuscript is attached (Appendix A)

Shepherd, R.K. & McCreery, D. Basis for electrical stimulation of the cochlea and the cochlear nucleus. In: Cochlear and Brainstem Implants, A. Moller (Ed), Karger, Basel. (in press). A copy of this manuscript is attached (Appendix B)

The following papers were presented at conferences during the quarter:

Coleman, B. Hair cell co-culture promotes neural differentiation of embryonic stem cells. *Proceedings of the Australian Neuroscience Society* 17: ORAL-22-3
(Australian Neuroscience Society Meeting, Sydney, 1st-3rd February 2006).

Coleman, B. Hair cell co-culture promotes neural differentiation of embryonic stem cells.
(3rd Australasian Auditory Neuroscience Workshop, Sydney, 31st January 2006, received student travel award for this conference).

Fallon, J.B. Plastic effects of patterned electrical stimulation of the deafened auditory system.
(3rd Australasian Auditory Neuroscience Workshop, Sydney, 31st January 2006).

Gillespie, L.N., Minter, R. and Shepherd, R.K. Neurotrophin over-expressing Schwann cells enhance auditory neuron survival *in vitro*.
Proceedings of the Australian Neuroscience Society 17: POS-THU-50.
(Australian Neuroscience Society Meeting, Sydney, 1st-3rd February 2006).

Gillespie, L.N. Neurotrophin over-expressing Schwann cells enhance auditory neuron survival *in vitro*.
(3rd Australasian Auditory Neuroscience Workshop, Sydney, 31st January 2006).

Hartley, D., Xu, J., Shial, A., Clarke, M., Ahmed, B., Schnupp, J., Shepherd, R.K. and King, A. Bilateral cochlear implantation in the ferret (*Mustela putorius*).
Proceedings of the 29th Annual Mid-winter Meeting of the Association of Research in Otolaryngology: 85.

Tan, J. and Shepherd, R.K. TrkB and p75NTR signaling pathways are differentially altered in degenerating spiral ganglion neurons in vivo
Proceedings of the 29th Annual Mid-winter Meeting of the Association of Research in Otolaryngology: 72.

Tan, J., Guipponi, M., Qingyu, W., Shepherd, R.K. and Scott, H. The type II transmembrane serine protease, TMPRSS1, is required for normal hearing (invited podium presentation).
Proceedings of the 29th Annual Mid-winter Meeting of the Association of Research in Otolaryngology: 3.

Published conference abstracts are reproduced in Appendix C.

2.2. 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.

Data analysis was completed this quarter. The results will be prepared for publication in future quarters.

2.3. Chronic electrical stimulation in the cat

This work continues to address the questions of whether chronic depolarization alone, via a cochlear implant, can prevent SGN degeneration. Additionally, the question of whether patterned chronic electrical stimulation of the auditory nerve can produce plastic reorganization within the central auditory pathway is being addressed.

During this quarter, three animals were neonatally deafened and implanted. One of the animals received our new 8-acitve ring array (previously all arrays had only 6 active rings). These animals will complete our first two experimental cohorts and will begin their chronic stimulation in the next quarter. Additionally, two neonatally deafened, un-implanted control animals underwent acute electrophysiological experiments. At the end of the quarter we had four deafened un-implanted controls and three deafened implanted animals ready to begin their stimulation. Finally, analysis of the data from the acute electrophysiological experiments on our previous cohorts of animals has continued this quarter, detailed results of which will be presented in future reports.

Following the completion of each acute electrophysiological experiment, the cochlea and CNS from each animal were harvested and prepared for subsequent analysis. Additionally, the cochlear nuclei from each animal were processed for both light microscopy and transmission electron microscopy and sent to Prof. David Ryugo for ultrastructural analysis of the end bulb of Held.

2.4. Chronic electrical stimulation in the rat

This work aims to address (i) whether chronic depolarization of the auditory nerve via a cochlear implant can rescue SGNs in the deaf rat cochleae; and (ii) whether early experience with simple forms of electrical stimulation enhances the ability to perceive differences between more complex patterns of electrical stimulation later in life. The experiments to examine this issue use a rat behavioral model in which rats with fully implanted stimulators are trained to discriminate different patterns of stimulation in a specially designed T-maze apparatus (described in previous reports).

Following the preliminary work with hearing rats (reported in previous QPRs), in which training techniques, apparatus, and software were developed, two deafened and implanted rats have been trained on a visual discrimination task in the maze. They have reached asymptotic performance levels on the visual discrimination, and are now to be tested on a simple electrical discrimination task of the sort to be used in later experiments. Magnetically induced EABR (mEABR) thresholds in these two rats have been measured at monthly intervals, and the stimuli to be used in the electrical discrimination task will be presented at a level 3dB above mEABR threshold.

The two chronically implanted rats for which data were presented in QPRs 7 and 8 were sacrificed after an implantation period of four months. At the time of sacrifice, the threshold currents for eliciting a magnetically induced EABR remained close to the levels obtained two weeks and three months after implantation, and examination of the two implantable stimulators indicated that they were in good condition. The cochleae from these animals have already been processed for histological assessment.

Dr Sandra Widjaja, a medically qualified Research Fellow from Switzerland, has joined our group and under the supervision from Dr Jin Xu, she has successfully implanted four deafened animals this quarter. These animals are now undergoing chronic electrical

stimulation using a stimulator designed by our Research Engineer Mr. Rodney Millard. Under Mr. Millard's supervision, four additional stimulator units have been manufactured during the quarter. Details of this novel stimulator and implant assembly, designed for use in small laboratory animals, is currently being prepared for publication.

Dr Jin Xu and colleagues have built a novel micro-focus X-ray imaging system which is adapted for capturing X-ray images of inserted cochlear implants in small mammals such as the rat. Using this system, the position of the electrode array and lead wire can be determined. Details of this system will also be compiled into a manuscript in the future.

2.5. Cellular over-expression of neurotrophins

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. Schwann cells, genetically modified to overexpress neurotrophins, are being grown, passaged, and media collected on a regular basis for analysis of the longevity of neurotrophin production. The neurotrophin content will be quantified using ELISAs in the next quarter.

Collaboration has been established with Dr Giles Plant from the School of Anatomy & Human Biology at The University of Western Australia. Dr Plant will provide us with lentiviral-infected neurotrophin-overproducing Schwann cells, allowing us to compare this viral infection technique with the plasmid-based transfection technique we are currently using. Such comparisons will help us determine which technique is more appropriate for our purposes in terms of duration and quantity of neurotrophin production; as well as allowing us to evaluate safety issues relating to viral techniques. Experiments for this collaboration will be conducted in future quarters.

2.6. 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 is modified by sensorineural hearing loss and by re-activation via a cochlear implant. During the quarter activities associated with this study centered around the preparation and submission of a manuscript examining TrkB and p75 neurotrophin receptor signaling following an aminoglycoside induced SNHL.

We also evaluated a gel documentation system for semi-quantitative documentation of gene expression. We have decided to purchase the system from Amersham Pharmacia for its more superior specifications. Funding for the purchase of this system came from grants from the Marian & E. H. Flack Trust and the H.D.T. Williamson Foundation awarded to Dr. Justin Tan.

2.7. The application of stem cells for SGN replacement

The aim of this study is to develop clinically feasible techniques for the application of stem cell therapy for SGN replacement in the profoundly deaf. During this quarter, we commenced a pilot study transplanting stem cells in a hydrogel matrix into the deafened guinea pig cochlea. All animals were deafened, underwent transplantation surgery, and were sacrificed. The tissue has been prepared for histological and immuno-labeling. Data analysis will occur over the following two quarters.

Bryony Coleman and Dr. Steven Backhouse were awarded a project grant from the Royal Victorian Eye and Ear Hospital to investigate potential surgical routes for cell-based therapy in the guinea pig cochlea. Work on this project commenced during the quarter.

3. Additional activities

Dr Patricia Hurley's thesis, submitted during the previous quarter, was examined and approved for the award of PhD at the University of Melbourne.

4. Plans for next quarter

Plans for the following quarter include:

a) Continued manuscript writing and submission, preparation of book chapter on neurofilament proteins (Dr. Lisa Gillespie) and preparation for attending conferences.

b) Analysis of data from the guinea pig study involving chronic electrical stimulation and neurotrophin delivery.

c) Analysis of data from the deafened, chronically stimulated cats, including acute electrophysiological data.

d) Commence preliminary training and testing of a group of deafened and implanted rats in the T-maze.

e) Continue chronic electrical stimulation programs in deafened/implanted cats and rats.

f) Continued fabrication of electrode assemblies for use in our chronic stimulation studies.

g) Test methods of encapsulating Schwann cells *in vitro*, in preparation for *in vivo* transplantation studies.

h) Refinement of the protocols for the transfection and selection for stable transformants of the male Schwann cells.

i) Continues investigation of the short- and long-term effects of deafness on neuronal and trophic markers in cochlear neurons.

j) Immunohistochemical analyses and cell quantification will be performed on tissues from the stem cell/hydrogel pilot study.

k) Continued investigation of potential surgical routes for cell based therapies of the inner ear.

l) Continued ultrastructural analysis of the end bulb of Held in ototoxically deafened/chronically stimulated cats compared with normal and deafened unstimulated controls (Prof D. Ryugo).

5. Personnel

Dr Sandra Widjaja from Switzerland has joined the group, funded by the "Freiwillige Akademische Gesellschaft, Basel, Switzerland" for a 12-month position as a visiting Research Fellow in our group. Dr Widjaja will play a key role in our chronic electrical stimulation studies, focusing specifically on the rat.

6. 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; Prof. Trevor Kilpatrick and Dr. Simon Murray from the Howard Florey Institute for their collaboration in obtaining Schwann cells, Dr Giles Plant from the School of Anatomy & Human Biology at The University of Western Australia for his collaboration in viral infection techniques, Prof. David Ryugo and colleagues from the Department of Otolaryngology/ Center for Hearing and Balance, Johns Hopkins University for collaboration associated with the ultrastructural examination of the VIIIth nerve/cochlear nucleus synapse and Dr. Tony Paolini from La Trobe University for advice in using the rat test chamber.

7. Appendix A (attached)

Prado-Guitierrez, P., Fewster, L.M., Heasman, J.M., McKay, C.M., and Shepherd, R.K. Effect of interphase-gap and pulse-duration on electrically evoked potentials is correlated with auditory nerve survival. Hearing Research (in press).

8. Appendix B (attached)

Shepherd, R.K. & McCreery, D. Basis for electrical stimulation of the cochlea and the cochlear nucleus. In: Cochlear and Brainstem Implants, A. Moller (Ed), Karger, Basel. (in press).

9. Appendix C (attached)

Conference abstracts published during the quarter.