Thirteenth 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:

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.

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.

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 thirteenth quarter the following activities were completed:

2.1. Publications

The following papers were accepted for publication:

Coleman, B., Fallon, J.B., Pettingill, L.N., DeSilva, M., & Shepherd, R.K (2007). Auditory hair cell explant co-cultures promote the differentiation of stem cells into bipolar neurons. Exp. Cell Research 313: 232-243.
A copy of this manuscript can be found in Appendix A (attached).

Pettingill, L.N., Richardson, R., Wise, A., O'Leary, S. & Shepherd, R.K. Neurotrophic factors and neural prostheses: potential clinical applications based upon findings in the auditory system. Special issue on sensory neural prostheses. In: IEEE Transactions on Biomedical Engineering. Zeng, F.-G., Dittmar, A. & Miller, R. (Eds). (in press).
A copy of this manuscript can be found in Appendix B (attached).

Wei, B.P.C., Shepherd, R.K., Robbins-Browne, R., Clark, G.M, & O'Leary, S.J. Threshold shift: effects of cochlear implantation on the risk of pneumococcal meningitis. Arch. Otolaryngology (in press).
A copy of this manuscript can be found in Appendix C (attached).

Coco, A., Epp, S.B., Fallon, J. B., Xu, J., Millard, R. E. and Shepherd, R.K. Does cochlear implantation affect residual hair cells and spiral ganglion neurons? Hearing Research (in press).
A copy of this manuscript can be found in Appendix D (attached).

Irvine, D. R. F. Does auditory cortical plasticity provide evidence for cognitive processing in the auditory cortex? Hearing Research (in press).
A copy of this manuscript can be found in Appendix E (attached).

2.2. Conferences

The following papers were presented at conferences during the quarter:

2.2.1 Invited speaker presentations

R. K. Shepherd

"Are Bionic Eye's Possible?" 10th Anniversary Conference, Eye Research Australia, Melbourne, October 2006.

"Sustainable drug delivery to the ear?" Bionics and Regeneration of the Ear, 7th International Academic Conference of Immunobiology in Otolaryngology, Melbourne, 12-14 November 2006.

"Indications for using genes or cells to regenerate the ear". Bionics and Regeneration of the Ear, 7th International Academic Conference of Immunobiology in Otolaryngology, Melbourne, 12-14 November 2006.

"Frontier technologies for brain repair – Bionic prosthetics and neural stem cell approaches". Neural Stem Cells & Frontier technologies for Brain Repair, Adelaide, December 2006.

L.N. Pettingill

"Auditory nerve protection: Neurotrophic factors and cell-based therapies". Bionics and Regeneration of the Ear, 7th International Academic Conference of Immunobiology in Otolaryngology, Melbourne, Australia, 12-14 November 2006.

2.2.2 Abstracts

Andrew J.K., Coco, A., Shepherd, R.K. Schwann cell treatment for sensorineural hearing loss. Bionics and Regeneration of the Ear, 7th International Academic Conference of Immunobiology in Otolaryngology, Melbourne, Australia, 12-14 November 2006.

Backhouse, S.S., Coleman, B., Donley, L., Andrew, J., Shepherd, R.K. Surgical access to the mammalian cochlea for cell based therapies. Bionics and Regeneration of the Ear, 7th International Academic Conference of Immunobiology in Otolaryngology, Melbourne, Australia, 12-14 November 2006.

Coleman, B., Backhouse, S.S., de Silva, M.G., Coco, A., Shepherd, R.K. Hydrogel studies in the deafened mammalian cochlea. Bionics and Regeneration of the Ear, Melbourne, 7th International Academic Conference of Immunobiology in Otolaryngology, Melbourne, Australia, 12-14 November 2006.

Coleman, B., Backhouse, S.S., de Silva, M.G., Coco, A., Shepherd, R.K. Indications for using genes or cells to regenerate the ear. Bionics and Regeneration of the Ear, Melbourne, 7th International Academic Conference of Immunobiology in Otolaryngology, Melbourne, Australia, 12-14 November 2006.

Fallon, J.B., Irvine, D.R.F., Coco, A., Donley, L.M., Millard, R.E., Shepherd, R.K. The effects of chronic implantation and electrical stimulation on residual hair cells and spiral ganglion neurons following a sensorineural hearing loss. Bionics and Regeneration of the Ear, 7th International Academic Conference of Immunobiology in Otolaryngology, Melbourne, Australia, 12-14 November 2006.

Pettingill, L.N., Minter, R.L., Shepherd, R.K. Auditory nerve protection: neurotrophic factors and cell-based therapies. Bionics and Regeneration of the Ear, 7th International Academic Conference of Immunobiology in Otolaryngology, Melbourne, Australia, 12-14 November 2006.

Shepherd, R.K., Coco, A., Epp, S.B. Sustainable drug delivery to the ear? Bionics and Regeneration of the Ear, 7th International Academic Conference of Immunobiology in Otolaryngology, Melbourne, Australia, 12-14 November 2006.

Tan, J., Widjaja, S., Xu, J., Shepherd, R.K. Effects of sensorineural hearing loss and long-tern cochlear implants on activity-dependent gene expression in the rat auditory cortex. Bionics and Regeneration of the Ear, 7th International Academic Conference of Immunobiology in Otolaryngology, Melbourne, Australia, 12-14 November 2006.

Widjaja, S., Tan, J., Xu, J., Shepherd, R.K. Chronic electrical stimulation in deafened rats: effects on spiral ganglion neuron survival. Bionics and Regeneration of the Ear, 7th International Academic Conference of Immunobiology in Otolaryngology, Melbourne, Australia, 12-14 November 2006.

Xu, J., Widjaja, S., Coco, A., Donley, L., Lu, W., Shepherd, R. Highlights of rat cochlear implant surgery with a fully implantable device. Bionics and Regeneration of the Ear, 7th International Academic Conference of Immunobiology in Otolaryngology, Melbourne, Australia, 12-14 November 2006.

A copy of these abstracts can be found in Appendix F (attached).

2.3. Ph. D. theses

The following Ph. D. thesis was passed in this quarter:

B. Wei: “Mechanisms of cochlea infection: strategies to minimize meningitis”.

The summary of this thesis can be found in Appendix G (attached).

2.4. 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.

Histological analysis, in particular, measurements of SGN soma area, was continued during this quarter.

2.5. 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, we commenced neonatal deafening of four animals; these animals will be implanted in the next quarter and will receive low-rate (50 pps/electrode) mono-polar stimulation on all 7 intra-cochlear electrodes and form the basis of our fifth experimental cohort. Seven animals (four deafened un-implanted controls and three chronically stimulated animals (high-rate stimulation cohort)) underwent acute electrophysiological experiments during the quarter. Analysis of the data from the acute electrophysiological experiments from these and our previous cohorts of animals has continued this quarter, and will be presented at the 2007 mid-winter ARO conference.

Following the completion of each acute electrophysiological experiment, the cochleae and CNS from each animal were harvested and prepared for subsequent analysis. SGN density measurements have commenced in the electrically stimulated cohort of animals. These measurements are expected to be concluded in the next quarter.

2.6. 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).

2.6.1 Behavioral model

The ultimate aim of this component of the project is to determine in an animal model 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 will use a rat behavioral model in which rats with implanted stimulators are trained to discriminate different patterns of stimulation in a specially-designed T maze apparatus (described in previous reports).

As reported in the last QPR, the two implanted rats currently being tested had failed to learn a discrimination between pulse trains that were rate modulated in different directions. An attempt was therefore made to introduce the discrimination progressively, by first training on a simpler discrimination (viz., a rate-modulated pulse train against an unmodulated train of shorter duration, and then progressively increasing the duration until the two stimuli were matched in this respect). Both rats learned this discrimination for differences in train duration less than ~400 ms, but discrimination failed for shorter differences. Moreover, discrimination at shorter differences was independent of whether the shorter stimulus was fixed or variable in rate, indicating that the discrimination was based on the difference in duration rather than on differences in pulse train characteristics. These two animals have now been tested for the maximum period permitted by our animal ethics approval, and future investigation of the reasons for the failure of rats to discriminate between pulse trains with different rate modulation characteristics will be carried out on a new cohort of animals.

2.6.2 Chronic stimulation

We have successfully completed our first cohort of five deafened rats chronically stimulated for a period of 7 weeks. We are currently implementing a second cohort involving a longer duration of electrical stimulation. Five deafened rats were successfully implanted with intracochlear electrode arrays connected to a stimulator to undergo a stimulation period of 14 weeks. In one case the implanted device failed immediately after implantation, in a second case it failed after a few weeks. These animals were placed into the dummy implanted cohort. Currently three rats are undergoing chronic electrical stimulation. Cochleae from control animals and one 14 week implanted/stimulated animal are being analyzed.

2.7. Cellular over-expression of neurotrophins

The aim of this study is to use cell transplantation techniques to deliver long-term/ongoing neurotrophic support to spiral ganglion neurons in animal models of deafness.

2.7.1 Long-term BDNF production from BDNF-Schwann cells

Analysis of the longevity of the BDNF production from genetically modified Schwann cells continued this quarter. Further ELISA experiments were performed to quantify the amount of BDNF produced by these cells. In addition, tissue culture experiments, in which the BDNF-Schwann cells were co-cultured with SGNs, were also performed to address the issue of longevity. These data will be presented at the 2007 mid-winter ARO conference.

2.7.2 Encapsulation of BDNF-Schwann cells

We have formed a collaboration with the New Zealand-based company Living Cell Technologies, to utilize their alginate cell encapsulation techniques for this (and other) projects. The encapsulation of our BDNF-Schwann cells will provide immuno-isolation from cochlear tissues while still allowing the transfer of neurotrophic products from the cells, and will also act to secure the cells within the cochlea.

Characterization of the BDNF-Schwann cells following encapsulation is ongoing.

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

In this quarter, we completed our molecular analysis of activity-dependent gene expression changes in the auditory cortex of deafened rats receiving intracochlear electrical stimulation for 7 weeks. Using immunohistochemistry and densitometric analysis, we found that the activity-dependent genes, brain-derived neurotrophic factor (BDNF), phosphorylated cAMP-response element binding protein (pCREB) are re-elevated in the contralateral auditory cortex, in comparison to the ipsilateral auditory cortex.

2.9. 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 the quarter, PhD student Bryony Coleman completed and submitted her thesis. Work also continued on the evaluation of surgical techniques for cell delivery to the cochlea and techniques designed to minimize cell dispersion following cell transplantation. Aspects of these data will be presented at the 2007 mid-winter ARO conference.

2.10. Developing cochlear implantation for the mouse

Compared with other animal models such as the cat, GP and rat, cochlear implantation in the mouse is considered very difficult due to the small size of the cochlear, the associated difficulty in making an electrode array to fit, and the presence of the stapedial artery (SA), located near the round window niche. In this quarter, further investigations on mouse cadavers were performed, and a protocol of the surgical approach and prototype electrode arrays were developed to overcome the insertion difficulty in this species (Fig. 1).

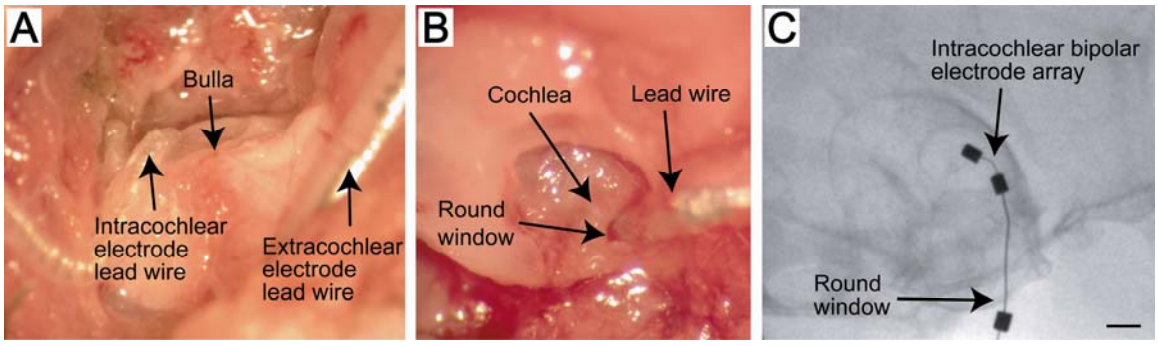

Figure 1. (A) The stapedial artery was cauterized and a round window cochleostomy was made. (B) A scala tympani electrode array was inserted through the round window and into the cochlea. (C) An x-ray of the implanted cochlea shows the bipolar stimulating electrodes in situ. Scale bar = 0.5 mm.

3. Additional activities

Ms Bryony Coleman's Ph.D. thesis was submitted for examination in this quarter.

Ms Coleman was also awarded a Travel Grant from the Australian Stem Cell Centre to attend the mid-winter conference of the Association for Research in Otolaryngology, as well as a Junior Wagstaff Fellowship from the Royal Victorian Eye and Ear Hospital.

4. Plans for next quarter

Plans for the following quarter include:

i. Continued manuscript writing and submission, and preparation for attending conferences.

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

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

iv. Continue training and testing of a group of deafened and implanted rats in the T-maze.

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

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

vii. Continued testing of methods of encapsulating Schwann cells *in vitro*, in preparation for *in vivo* transplantation studies.

viii. Continued investigation of the short- and long-term effects of deafness on neuronal and trophic markers in cochlear neurons.

ix. Completion of histological analysis investigating potential surgical routes for cell based therapies of the inner ear.

x. 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

Catriona Wimberley joined the group as an 'Undergraduate Research Opportunities Program' student via the Bio21 program. She will be working full time during non-instructional periods and approximately one day per week during semester as she continues her undergraduate studies in electrical engineering and biomedical science. Her duties include daily monitoring of our chronically stimulated cats, software development for analysis of our evoked potential recordings and assisting in general laboratory activities.

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, 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.

7. Appendix A

Coleman, B., Fallon, J.B., Pettingill, L.N., DeSilva, M., & Shepherd, R.K (2007). Auditory hair cell explant co-cultures promote the differentiation of stem cells into bipolar neurons. Exp. Cell Research 313: 232-243.

8. Appendix B

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9. Appendix C

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10. Appendix D

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11. Appendix E

Irvine, D. R. F. Does auditory cortical plasticity provide evidence for cognitive processing in the auditory cortex? Hearing Research (in press).

12. Appendix F

Abstracts of presentations at Bionics and Regeneration of the Ear, 7th International Academic Conference of Immunobiology in Otolaryngology, Melbourne, Australia, 12-14 November 2006.

13. Appendix G

Ph. D. Abstract – Dr. Benjamin Wei