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

- Hired a post-doctoral fellow with expertise in cell and molecular biology. This new recruit will play a key role in the research to be undertaken under this contract. All new positions associated with this contract have now been filled.
- Held a day workshop at the Bionic Ear Institute designed to showcase young Australian researches in the field of Auditory Neuroscience. The day was an outstanding success with over 70 participants (see Appendix A).
- Attended and presented at the 24th meeting of the Australian Neuroscience Society, Melbourne, January 2004. The following poster was presented: Hurley, P.A., Serruto, A., Crook J.M. & Shepherd, R.K. TrkB receptor expression in the cochlea following sensorineural hearing loss (Appendix C).

- Manuscript preparation: During the quarter, one manuscript was accepted for publication in *Arch. Otolaryngol. HNS*. Shepherd R.K. and Colreavy M.P. Surface microstructure of the perilymphatic space: Implications for cochlear implants and cell or drug based therapies. Arch. Otolaryngol (in press; Appendix A). A second manuscript was revised in light of reviewer's comments and re-submitted and a third manuscript prepared and submitted based on work performed during the present and our previous contracts. Finally, the following three chapters have been recently published: Shepherd, R.K. The Auditory System. In: Neuroprosthetics: Theory and Practice. K. Horch & G. Dhillon (Eds), World Scientific Publishing, Singapore, pp. 260-280, 2004; Seligman, P.M. & Shepherd, R.K. Cochlear Implants. In: Neuroprosthetics: Theory and Practice. K. Horch & G. Dhillon (Eds), World Scientific Publishing, Singapore, pp. 878-904, 2004; and Shepherd, R.K. Central Auditory Prostheses. In: Neuroprosthetics: Theory and Practice. K. Horch & G. Dhillon (Eds), World Scientific Publishing, Singapore, pp. 1103-1114, 2004.
- Performed *in vitro* functional and *in vivo* biocompatibility tests on our fully implantable small animal stimulator.
- Initiated an anatomical study of the surgical feasibility of cochlear implantation in the rat.
- Continued construction of a rat test chamber for behavioral evaluation of the effects of raising deafened rats with simple patterns of electrical stimulation on their subsequent ability to perceptually distinguish more complex stimulation patterns.
- Obtained experience with AI recordings in rats using the Bionic Technologies and University of Michigan recording electrodes. This work was performed in preparation for the study of plastic change in the AI of rats following behavioral studies performed in the test chamber.
- Completed studies of the trophic effects of chronic neurotrophin delivery in the deaf rat cochlea.
- Completed a second series of chronic stimulation /neurotrophin studies in the adult guinea pig. This work is designed to test the hypothesis that trophic advantage can be maintained by electrical stimulation alone following an initial period of co-delivery of neurotrophin and electrical stimulation (see Final QPR; Contract NIH-NO1-DC-0-2109). Histological analysis of these cochleae is now underway.
- Continued deafening young cats using neomycin in preparation for our first chronic implantation and electrical stimulation study for this new contract.
- Initiated development of a speech processor based stimulator for use in our feline studies. This work will be completed in time to commence our chronic stimulation program next quarter.
- Delivered partially differentiated stem cells into the deaf guinea pig cochlea as the first phase of our SGN regeneration studies.
- Completed histological analysis of cochleae and auditory brainstem structures from our neonatally deafened chronically stimulated cat series (see Final QPR; Contract NIH-NO1-DC-0-2109). This work is designed to test the hypothesis that chronic depolarization provides trophic support to SGNs following the loss of hair cells, and will now be prepared for publication.

- Continued fabricating electrode arrays and leadwire assemblies for our chronic implantation/stimulation studies. Efforts this quarter included the fabrication of electrode assemblies for our feline studies (the electrode arrays are manufactured for us by Cochlear Ltd.; we connect a leadwire assembly to them), and the development of electrode arrays suitable for implantation in the rat.
- Upgraded our website: http://www.bionicear.org/bei/ResProtectiveEffects.html
- Hosted a site visit by Dr. Roger Miller from NIDCD.

3. Plans for Next Quarter

- Continue manuscript preparation and submission.
- Complete the analysis of the cochlear histology (SGN density and soma area measurements) in guinea pigs that have received ES and BDNF for 28 days and then ES alone for periods of up to 10 weeks.
- Complete the analysis of the cochlear histology (SGN density and soma area measurements) in the deafened rat cochlea in order to test the hypothesis that this neurotrophin can rescue SGNs in rat when delivered exogenously.
- Complete the histology and initiate the analysis of cochleae that have received partially differentiated stem cells as the first phase of our SGN regeneration studies.
- Complete the development of an electrode array and surgical protocol for chronic cochlear implantation in the rat.
- Trial the rat behavioral test box using normal hearing subjects in response to acoustic stimulation.
- Implant and commence stimulation of five young cats deafened using multiple application of Neomycin. These animals will be stimulated using a speech processor based stimulation strategy similar to one used clinically.
- Continue to fabricate electrode assemblies for use in our chronic stimulation studies.

4. Personnel

Dr. Lu Wei, an Otologist from 1st Affiliated Hospital, Zheng Zhou University, China is a visiting Research Fellow in our Institute. Dr. Wei will contribute to our research effort in a number of areas, in particular he will play an important role in the development of surgical techniques for cochlear implantation in the rat.

5. Acknowledgements

We gratefully acknowledge the important contributions made by our Histologist, Maria Clarke; Veterinarian Dr Sue Pierce; Elisa Borg for management of our animal house; Helen Feng for electrode manufacture; Frank Nielsen for engineering support; Tracey Wasbutzki for human resources expertise; Dr. Tony Paolini and the Mechanical workshop staff at La Trobe University for advice and assistance in manufacturing the rat test chamber; and Jenny Hardman for expert research assistance.

Appendix A

ANS AUDITORY NEUROSCIENCE WORKSHOP
January 31, 2004
The Bionic Ear Institute
384 Albert Street, East Melbourne 3002

9:15 Welcome: Rob Shepherd
9:20 Jim Pickles (U. Queensland)
Strategic Overview of Auditory Neuroscience in Australia
9:30 Henrik Dahl (Murdoch Research Institute)
Auditory function and hearing impairment: the genetic approach
10:10 Gary Housley (Auckland)
A working hypothesis on electrochemical homeostasis in scala media: an ATP-activated shunt

10:50 BREAK
Moderator: Janine Clarey
11:10 Helmy Mulders (UWA)
Responses of single auditory afferent fibers to electrical stimulation of the guinea pig inferior colliculus
11:40 Sharon Oleskevich (ANU)
Synaptic transmission at two giant terminals in the auditory pathway of deaf mice
12:10 Natalie Rickard (Melbourne)
A new method for imaging and 3D reconstruction of mammalian cochlea using fluorescent confocal microscopy
12:40 Tony Paolini (LaTrobe/BEI)
The role of inhibition in brainstem auditory processing

1.10 LUNCH
Moderator: Don Robertson
1:50 Srdjan Vlakovic (Auckland)
Ectonucleotidase upregulation in noise-exposed cochlea: a role in otoprotection?

2:20 Carl Parsons (Newcastle)
Plasticity in the Adult Auditory System
2:40 Catherine McMahon (Macquarie)
Origin of the P1 peak of the round-window CAP
3:00 Balemir Uragun (Monash)
Classification of Interaural Level Difference Functions using Principal Components Analysis

3:20 Marc Kamke (Monash)
Role of the Cholinergic Basal Forebrain in Cochlear-Lesion-Induced Tonotopic Reorganisation in the Primary Auditory Cortex

3.40 BREAK
Moderator: Dexter Irvine
3:55 Penny Hill (Oxford)
Auditory processing in specific language impairment: a longitudinal study
4:15 Daniel Brown (UWA)
The primary afferent membrane potential and the CAP waveform
4:35 Virginia Best (Sydney)
Spatial hearing with sounds that overlap in both frequency and time
4:55 Richardson Leao (ANU)
Tonotopically organised voltage dependent channels
5:15 Greg O'Beirne (UWA)
Electrophysiological monitoring of outer hair cell homeostasis

5:35 CLOSE & DRINKS

Appendix B

Shepherd, R.K & Colreavy M.P. Surface microstructure of the perilymphatic space: Implications for cochlear implants and cell- or drug-based therapies. *Arch Otolaryngol, HNS* (in press).

Appendix C

Hurley, P.A., Serruto, A., Crook J.M. & Shepherd, R.K. TrkB receptor expression in the cochlea following sensorineural hearing loss. Proc. Aust. Neurosci. Soc. 180, 2004.