Eleventh Quarterly Progress Report

NIH-N01-DC-3-1005

Protective Effects of Patterned Electrical Stimulation on the Deafened Auditory System

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April 1 – June 30, 2006

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 four papers were accepted for publication during the Quarter:

Tan, J & Shepherd, R.K. (2006) Aminoglycoside-induced degeneration of adult spiral ganglion neurons involves differential modulation of TrkB and p75 neurotrophin receptor signaling. American Journal of Pathology 169: 528-543. (Appendix A)

Irvine, D. R., Fallon, J. B. & Kamke, M. (2006). Plasticity in the adult central audtiory system. Acoustics Australia 34: 13-17. (Appendix B)

Wei, B.P.C., Shepherd, R.K., Clark, G.M, Robbins-Browne, R. & O'Leary, S.J. Pneumococcal meningitis threshold model: a potential tool to assess infectious risk

in new or existing inner ear surgical intervention. Otology & Neurotology (in press). (Appendix C).

Wei, B.P.C., Shepherd, R.K., Clark, G.M, Robbins-Browne, R. & O'Leary, S.J. Pneumococcal meningitis post cochlear implantation: development of an animal model. Otology & Neurotology (in press). (Appendix D).

Invited speaker presentations for the Quarter:

R. K. Shepherd "Can chronic depolarization maintain SGNs following cessation of exogenous neurotrophin delivery?" 2nd Williams Conference on Tissue Engineering the Inner Ear: Re-engineering the Auditory Nerve, Hotel Astoria, Vienna Austria, June 2006.

Published conference abstracts for the Quarter:

Shepherd, R.K., Coco, A., Epp, S., Fallon, J.B., Xu, J. & Millard, R.E. Preservation of hearing following long-term cochlear implantation. 9th International Cochlear Implant Conference, Vienna, Wiener Medizinische Wochenschrift p 4, 2006.

Shepherd, R.K., Coco, A., Epp, S., Xu, J., Donley, L., Hurley, P.A., McGuinness, S. & Fallon, J.B. Rescue of auditory neurons: Implications for cochlear implants. 9th International Cochlear Implant Conference, Vienna, Wiener Medizinische Wochenschrift p 84, 2006.

Heasman, J.M., Prado-Guitierrez, P., Fewster, L.M. McKay, C.L. & Shepherd, R. K. Measurement of the electrically evoked auditory brainstem and cortical responses using the Nucleus® Freedom™ cochlear implant.) 9th International Cochlear Implant Conference, Vienna, Wiener Medizinische Wochenschrift p 39, 2006.

Hartley, D.E., Xu, J., Shial, A., Clarke, M., Ahmed, B., Schnupp, J., Shepherd, R.K. and King, A.J. Bilateral cochlear implantation in the ferret (Mustela putorius). Medical Research Society Annual Meeting for Clinical Scientists in Training, London, 2006

Published conference abstracts are reproduced in Appendix E.

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.

This work is being prepared for publication.

2.3. Chronic electrical stimulation in the cat

This work addresses the question of whether chronic depolarization alone, via a cochlear implant, can (i) prevent SGN degeneration and (ii) produce plastic reorganization within the central auditory pathway.

During this quarter, three animals were neonatally deafened and implanted, with an additional two animals neonatally deafened, but not implanted, to serve as controls. The implanted animals received our new 7-active ring intra-cochlear array and an extra-cochlear ball electrode. These animals are receiving high-rate (500 pps/electrode) mono-polar stimulation on all 7 intra-cochlear electrodes and form the basis of our fourth experimental cohort. At the end of the quarter we had five deafened un-implanted controls and six deafened implanted animals receiving chronic stimulation. 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.

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).

Chronic electrical stimulation in the rat – overview

Over the last two quarters total of 14 deafened Hooded Wistar rats have been successfully implanted and chronically stimulated with a refined version of the rat intracochlear electrode and stimulator (Fig. 1). The surgical techniques have been improved and now includes the use of gaseous anesthesia, revised dissection and cauterization techniques, and improved electrode fixation procedures. Normal morphology of magnetically induced electrically-evoked auditory brainstem responses (mEABRs) in all implanted animals have demonstrated that the implantation of this device in rats is effective and reliable for chronic application.

As noted in our previous QPR, we have developed a novel micro-focus x-ray imaging system for use *in vitro* and in experimental animals. The key feature of this system is the use of a micro-focus x-ray source providing a resolution of less than 10 μm and an appropriate choice of source-object and object-image distance. During the quarter we used this technique to image a live rat implanted with an intracochlear electrode-stimulator package. The animal was sedated, and to reduce movement artifact associated with breathing, exposure time was minimized. The result showed that micro-focus radiography provides improved visualization of anatomical details and definition of the location of the electrode array as illustrated in Fig. 2.

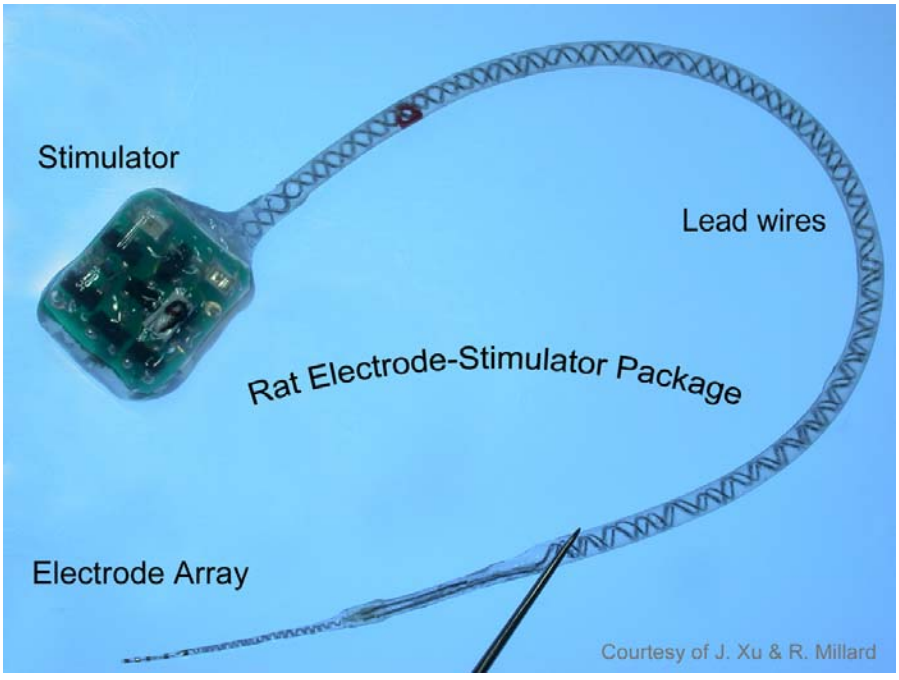

Figure 1.

Photomicrograph of the fully implantable electrode array /stimulator designed for chronic implantation in the rat. The scala tympani electrode array includes 4 Platinum band electrodes and has a diameter of 0.3 mm.

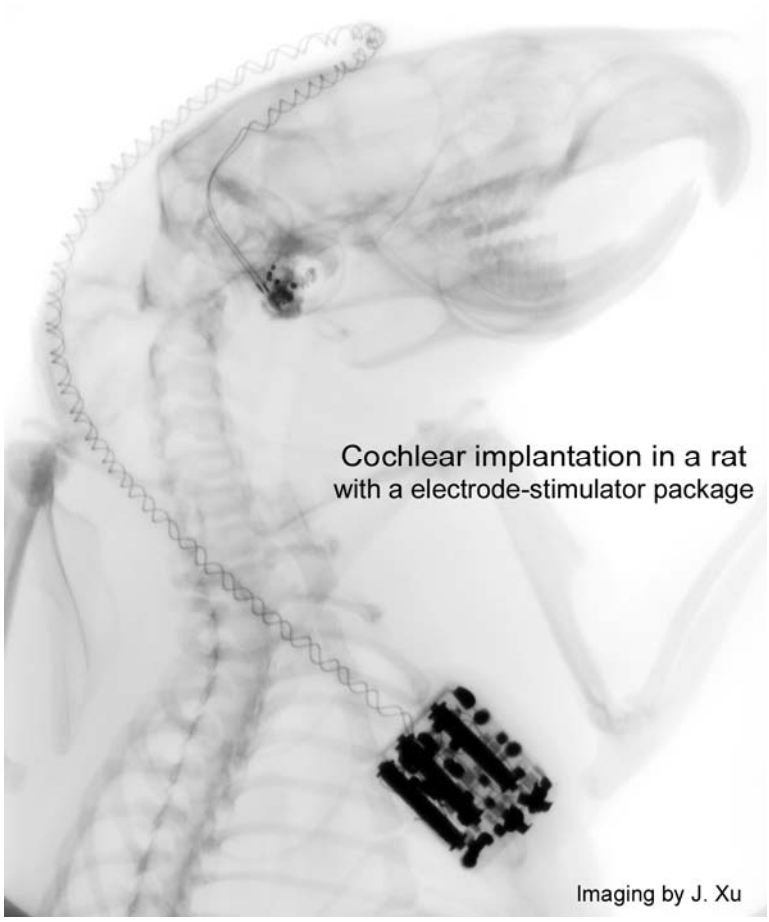

Figure 2. Micro-focus x-ray image of a live rat implanted with the electrode array/stimulator assembly. Three Platinum ring electrodes are located in the scala tympani while the leadwire system courses back to the stimulator located in a subcutaneous pocket on the back of the animal. Charge balanced biphasic current pulses, induced in the implanted stimulator via an external magnetic field, are delivered to a bipolar electrode pair within the scala tympani.

Chronic electrical stimulation in the rat – trophic effects of electrical stimulation

During the quarter four implanted and chronically stimulated rats were sacrificed and the cochleae were harvested. SGNs were counted under light microscopy and additional immunohistochemistry was performed. The data is now being analyzed. Each animal had a two week period to recover from the deafening procedure before cochlear implantation, and a one week recovery period after surgery before commencing the chronic electrical stimulation program. Each animal received 4 hours of stimulation daily at a stimulus level 3 dB above its mEABR threshold. Total implantation time was 8 weeks; the animals received 7 weeks of electrical stimulation (total 140 hours). Four animals in the second cohort were deafened during the quarter and will be implanted and undergo chronic electrical stimulation in the next quarter.

Chronic electrical stimulation in the rat – behavioral studies

The ultimate aim of this component of the project is to determine 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).

Since the last QPR, training on electrical discrimination tasks of the two previously deafened and implanted rats, and of an additional subsequently implanted rat, has

continued. All three rats learned a simple discrimination between a pulse train and no stimulus. Subsequently they were shifted to a discrimination between two pulse trains in which the pulse rate increased and decreased, respectively (upward and downward frequency modulated (FM) pulse trains). One rat receiving monopolar stimulation has learned this discrimination, but two rats receiving bipolar stimulation have so far failed to discriminate significantly above chance. It therefore appears that this discrimination might be too difficult to use in our subsequent experiments, and further efforts will be directed to selecting a more appropriate discrimination task. The mEABR thresholds in these rats have been determined monthly, and have varied relatively little over time, providing evidence of good stability in the stimulating system.

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.

During the quarter, work in this project concentrated on manuscript preparation, as a result two manuscripts have been submitted.

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.

Currently, we are in the process of extending our molecular analysis further along the central auditory system to the auditory cortex. We are examining how plasticity-related genes are altered in expression as a consequence of activity deprivation and whether cochlear implantation has any impact on the expression of these genes. Among the genes that have been analyzed are brain-derived neurotrophic factor, c-Fos and phosphorylated cyclic AMP-response element binding protein (CREB). These genes have been selected because their expression is highly dependent on neuronal activity and they are *bona fide* activity-dependent genes as they contain elements responsive to calcium signaling.

We also concluded our analysis of deaf mutant mice lacking a serine protease in a collaboration with Drs. M. Guipponi and H. Scott from Walter Eliza Hall Institute of Medical Research. A manuscript describing this research is in preparation.

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 the quarter we commenced a pilot study transplanting stem cells via a hydrogel matrix into the deafened guinea pig cochlea in order to study the ability of this matrix to hold and provide a suitable environment for differentiated stem cells. In addition we have implanted microspheres in hydrogel to model potential surgical techniques designed to access the auditory nerve. All 52 cochleae were sectioned and stained during this quarter and tissue analysis is underway. During the next we will complete the statistical analysis evaluating the potential surgical techniques for stem cell delivery to the auditory nerve and complete

immunohistochemistry for the study of differentiated stem cells and hydrogel within the deafened mammalian cochlea.

3. Additional activities

Our electrode fabrication team of Dr. Jin Xu and Helen Feng visited Cochlear Ltd. during the quarter to review improved electrode fabrication techniques and discuss the development of smaller diameter electrode arrays for use in our research program.

4. Plans for next quarter

Plans for the following quarter include:

a) Continued preparation and submission of manuscripts.

b) Analysis of data from the deafened, chronically stimulated cats, including acute multichannel electrophysiological data and cochlear histology.

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

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

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

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

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

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

i) 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. 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 and Dr. Tony Paolini from the Bionic Ear Institute for advice on our behavioral studies.

6. Appendix A (attached)

Tan, J & Shepherd, R.K. Aminoglycoside-induced degeneration of adult spiral ganglion neurons involves differential modulation of TrkB and p75 neurotrophin receptor signaling. American Journal of Pathology 169: 528-543, 2006.

7. Appendix B (attached)

Irvine, D. R., Fallon, J. B. & Kamke, M. (2006). "Plasticity in the adult central auditory system." Acoustics Australia 34: 13-17.

8. Appendix C (attached)

Wei, B.P.C., Shepherd, R.K., Clark, G.M, Robbins-Browne, R. & O'Leary, S.J. Pneumococcal meningitis threshold model: a potential tool to assess infectious risk in new or existing inner ear surgical intervention. Otology & Neurotology (in press).

9. Appendix D (attached)

Wei, B.P.C., Shepherd, R.K., Clark, G.M, Robbins-Browne, R. & O'Leary, S.J. Pneumococcal meningitis post cochlear implantation: development of an animal model. Otology & Neurotology (in press).

10. Appendix E (attached)

Conference abstracts published during the quarter.