Twelfth 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:

Lu, W., Xu, J. (2006) Micro-focus X-ray for cochlear implant research on small animals. Chinese Journal of Otorhinolaryngology - Head and Neck Surgery, 41(9):702-704. A copy of this manuscript is Appendix A (attached).

Wei, B.P.C., Shepherd, R.K., Azzopardi, K., Clark, G.M, Robbins-Browne, R. & O'Leary, S.J. Protective effects of local administration of ciprofloxacin on the risk of pneumococcal meningitis following cochlear implantation. Laryngoscope (in press). A copy of this manuscript is Appendix B (attached).

Wei, B.P.C., Shepherd, R.K., Clark, G.M, Robbins-Browne, R. & O'Leary, S.J. Effects of inner ear trauma on the risk of Pneumococcal meningitis. Arch. Otolaryngology (in press).

A copy of this manuscript is Appendix C (attached).

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.

During preparation of this data for publication it was decided that further histological analysis was required for this project, specifically measurements of SGN soma area. These measurements were commenced this quarter and will continue during the following quarter.

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, eight animals (two normal hearing controls, four deaf hearing controls and two chronically stimulated animals) underwent acute electrophysiological experiments. At the end of the quarter we had four deafened un-implanted controls and three deafened implanted animals receiving chronic electrical stimulation. Analysis of the data from the acute electrophysiological experiments on our previous cohorts of animals has continued this quarter, and has been prepared for presentation at the mid-winter conference of the Association for Research in Otolaryngology.

Following the completion of each acute electrophysiological experiment, the cochlea and CNS from each animal were harvested and prepared for subsequent analysis.

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).

2.4.1 Behavioral model

Since the last QPR, training on electrical discrimination tasks of three previously deafened and implanted rats has continued. As reported previously, one rat receiving monopolar stimulation learned to discriminate between two pulse trains in which the pulse rate increased and decreased, respectively (upward and downward frequency modulated (FM) pulse trains), but two rats receiving bipolar stimulation failed to learn this discrimination. The first rat was subsequently used as a control to test for the possibility that some aspect of the stimulus delivery other than the electrical stimulus itself might have served as the basis of the discrimination (e.g., mechanical stimulation associated with the passage of current through the inducing coil surrounding the stem of the T-maze). When the leads from the implanted stimulator were shorted, however, this rat's performance fell to chance, and the rat failed to relearn the discrimination, establishing that the discrimination was in fact based on the electrical stimulus.

The failure of the other two rats to learn the discrimination indicated that it would be too difficult to use in our subsequent experiments, and we are therefore currently exploring the possibility of introducing the discrimination progressively, by first training on a simpler discrimination (viz., an FM pulse train against an un-modulated train of different duration, and then progressively increasing the duration until the two stimuli are matched in this respect, before introducing modulation in the opposite direction).

2.4.2 Chronic stimulation

Four deafened animals were implanted with electrodes and stimulators and underwent chronic stimulation five days a week for seven weeks. A group of four animals were deafened and implanted with dummy electrodes as a control cohort. Two implanted animals and two dummy implanted animals were sacrificed at the end of the seven week period. Their cochleae were harvested and are to be analyzed during the follow quarter. One implanted animal had to be sacrificed prior to completing the seven week stimulation period due to lead-wire breakage (see below) and one dummy implanted animal died from a respiratory infection. Following on from preliminary analysis of the data from the 7 week cohort, we have begun work on a third cohort with a stimulation period of 14 weeks.

We use micro-focus fluoroscopy in determining intra-cochlea electrode position and in the detection of the breaks in the lead-wires of our experimental animals. Included below is a figure illustrating a breakage that occurred in one of our chronically stimulated animals.

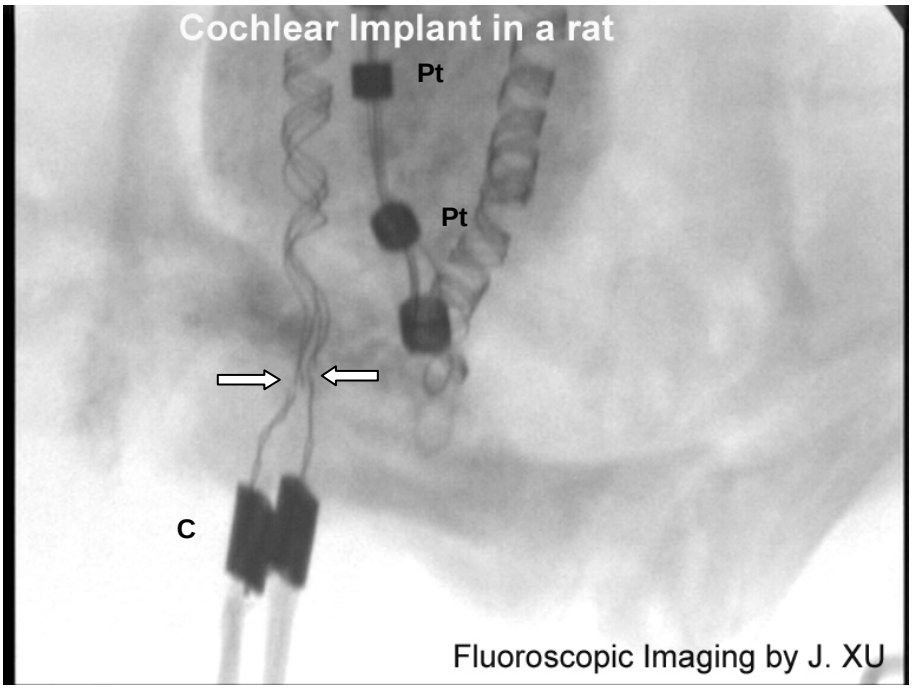


Figure 1 Micro-focus image of Pt lead-wires.

Image of Pt ring electrodes (Pt) and lead-wires in a chronically implanted rat. Arrows indicate a break in the lead-wires just proximal to the connector (C).

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 auditory neurons in animal models of deafness.

2.5.1 Long-term BDNF production from BDNF-Schwann cells

Previously, conditioned media from BDNF-Schwann cells was collected over a time-period of up to two months. During the current quarter, this media was analyzed for BDNF content using enzyme-linked immunosorbent assays (ELISAs). Unfortunately, preliminary data suggests that while the amount of BDNF produced by the BDNF-Schwann cells is strong for the first month, as evidenced by the amount of BDNF detected in the media collected from the cells, this is followed by an apparent decrease in BDNF production (Figure 2). Future experiments, to be conducted in the next quarter, will assess the survival effects of these “older” BDNF-Schwann cells on spiral ganglion neurons *in vitro*.

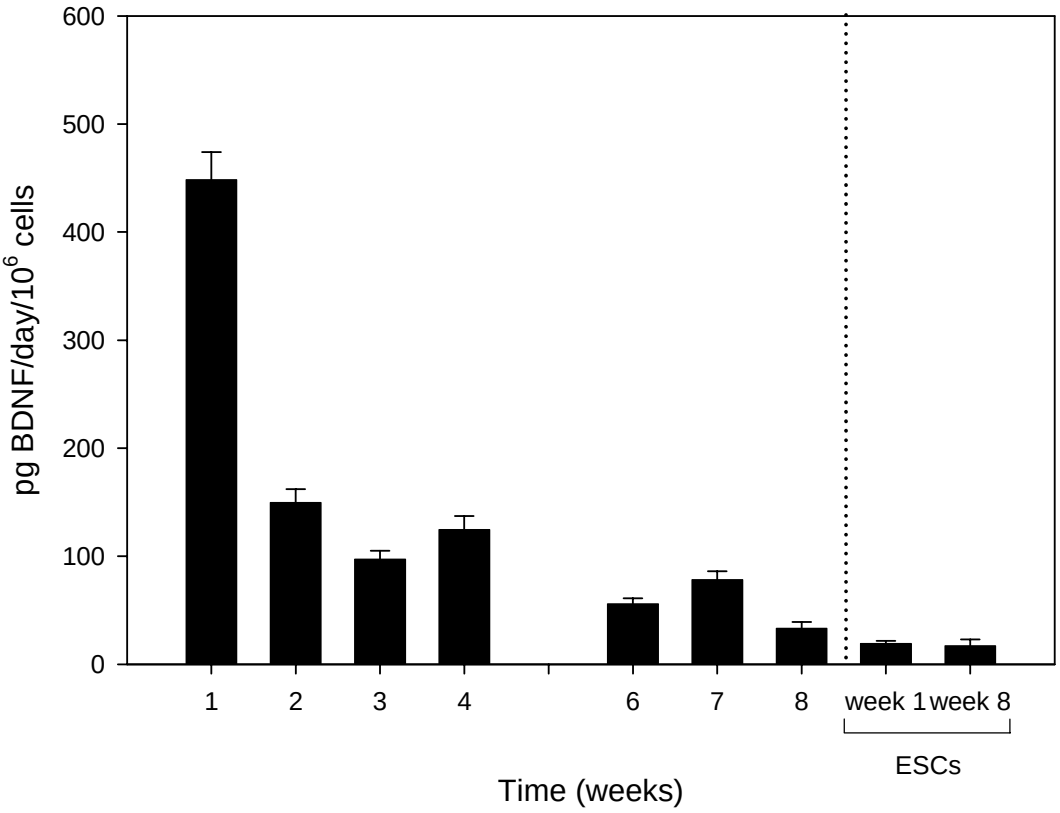


Figure 2 BDNF production over time by BDNF-Schwann cells.

The amount of BDNF produced is greatest during the first week, with a subsequent ongoing drop in production. The amount of BDNF produced by Schwann cells after 6-8 weeks was not significantly different to the BDNF quantities produced by control Schwann cells (ESCs; $p > 0.05$).

2.5.2 Encapsulation of BDNF-Schwann cells

Earlier this year a collaboration was established with a 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 ensuring the cells are located within the cochlea and do not disperse via the cochlear aqueduct to the CNS.

During the current quarter, we had some BDNF-Schwann cells encapsulated, and we are now in the process of analyzing a number of features of the encapsulated cells, such as cell growth, BDNF production and survival effects on spiral ganglion neurons *in vitro*. These studies will continue into the next quarter.

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 this quarter, we completed our molecular analysis of activity-dependent gene expression changes in the auditory cortex of deafened rats. Using a combination of Western blotting and

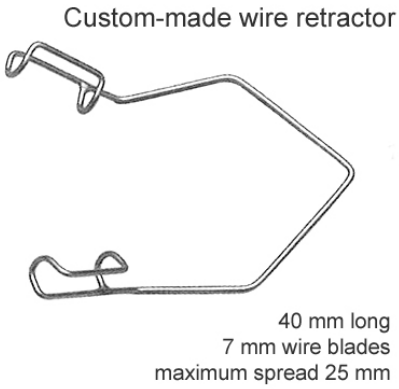
immunohistochemistry, we found the activity-dependent genes, BDNF, c-Fos, phoshorylated cAMP-response element binding protein (pCREB) are reduced in expression levels in the deafened auditory cortex.

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 continued histological analysis of data from our study of transplanting stem cells in a hydrogel matrix into the deafened guinea pig cochlea.

2.8. Developing cochlear implantation for the mouse

Initial development of a surgical method for cochlear implantation in the mouse commenced this quarter. Preliminary investigation showed that the mouse is a challenging surgical subject. Its small size does not allow easy placement of electrodes and may require the development of specialized surgical tools. However, the mouse inner ear is anatomically similar to that of rats, and therefore, some of surgical techniques we have developed for the rat can be adapted, such as cauterizing stapedial artery, cochleostomy etc.



3. Additional activities

Dr Ben Wei's PhD thesis was submitted for examination.

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

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

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

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

k) 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

There were no changes to personnel this quarter.

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 (attached)

Lu, W., Xu, J. (2006) Micro-focus X-ray for cochlear implant research on small animals. Chinese Journal of Otorhinolaryngology - Head and Neck Surgery, 41(9):702-704.

8. Appendix B (attached)

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9. Appendix C (attached)

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