Fifteenth 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 fifteenth quarter the following activities were completed:

2.1. Publications

The following papers were accepted for publication.

Guipponi, M., Tan, J., Cannon, P.Z.F., Donley, L., Crewther, P., Clarke, M., Wu, Q., Shepherd, R.K., Scott, H. Mice deficient for the type II transmembrane serine protease, TMPRSS1/hepsin, exhibit profound hearing loss. American Journal of Pathology (in press). A copy of this manuscript is attached (Appendix A).

Guipponi M, Toh MY, Tan J, Park D, Hanson K, Ballana E, Dwong D, Cannon P, Wu Q, Gout A, Smith RJH, Dahl H, Petersen M, Teasdale R, Estivill X, Park WJ, Scott H (2007). An integrated genetic and functional analysis of the role of type II transmembrane serine protease in hearing loss. Hum Mutat (in press). A copy of this manuscript is attached (Appendix B).

Millard, R.E., Shepherd, R.K. A fully implantable stimulator for use in small laboratory animals. J. Neurosci. Methods (in press). A copy of this manuscript is attached (Appendix C).

Sly, D.L., Heffer, L.F., White, M.W., Shepherd, R.K., Birch, M.J., Minter, R.L., Nelson, N.E., Wise, A.K. & O'Leary, S.J. (2007) Deafness alters auditory nerve fiber responses to cochlear implant stimulation. European J. Neurosci. **26**: 510-522. A copy of this publication is attached (Appendix D).

Wei, B.P.C., Shepherd, R.K., Robbins-Browne, R., Clark, G.M, & O'Leary, S.J. Cochlear implantation: Current biological safety debate. British Medical Journal. (in press). A copy of this manuscript is attached (Appendix E).

2.2. Conferences

The following paper was presented during the quarter:

Coleman, B., de Silva, M.G. and Shepherd, R.K. The potential of stem cells for auditory neuron replacement in the deaf cochlea. Proceedings of the International Society for Stem Cell Research, Cairns, June (2007). – This poster received the Australian Stem Cell Centre poster award.

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

2.3. 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.

Analysis of the histological data was completed this quarter and the preparation of a manuscript for submission substantially completed.

2.4. 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 chronic electrical stimulation of the auditory nerve via a clinically available cochlear implant and speech processor can produce plastic reorganization within the central auditory pathway is being addressed.

During this quarter, we neonatally deafened one animal; and implanted one additional animal with a scala tympani electrode array in preparation for chronic electrical stimulation. Currently, three animals are receiving low-rate (50 pps/electrode) monopolar stimulation on all 7 intracochlear electrodes. One acute electrophysiological experiment was performed on a normal hearing animal.

An advantage of the recording techniques we have used in our acute electrophysiological experiments is that we are able to simultaneously record single- and multi-unit data together with evoked potentials. To date we have concentrated our analysis on single- and multi-unit data recorded from the cat primary auditory cortex (AI). Here we present initial analysis of electrically-evoked responses recorded from Cyberkinetics Inc. electrodes acutely implanted in the cat AI. An example of an evoked potential from the auditory cortex of a chronically stimulated cat is illustrated in Figure 1 and an amplitude response curve in Figure 2. Using these recordings it is possible to determine a threshold current for each stimulating electrode, recording electrode pair. These thresholds can subsequently be used to determine cortical maps as illustrated Figure 3. Preliminary analysis of such maps in normal hearing animals demonstrate the well studied cochleotopic organization of the auditory pathway. Consistent with our preliminary analysis of the unit data from the same experiments, the cortical maps of chronically deaf animals appear to show a complete lack of organization, while the maps from chronically stimulated animals appear to show a similar level of organization as that observed in the normal hearing animals (Fig. 3).

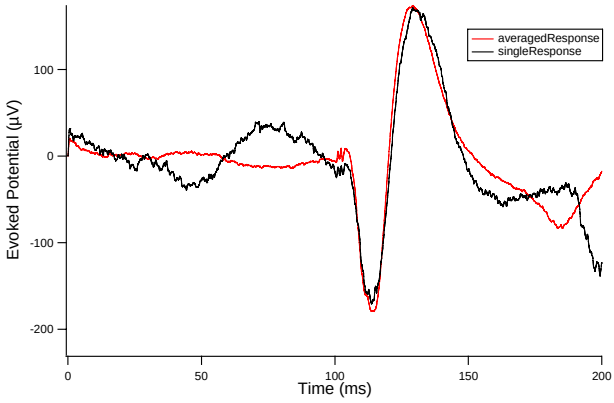

Figure 1 Electrically-evoked response recorded from the AI of a chronically stimulated animal. This recording is taken from electrode number 17 in the electrode array and shows the response to stimulation of electrode 1 (the most apical electrode) stimulated at 1 mA. The red line shows the averaged response of 10 trails, while the black line is the response recorded from a single trial.

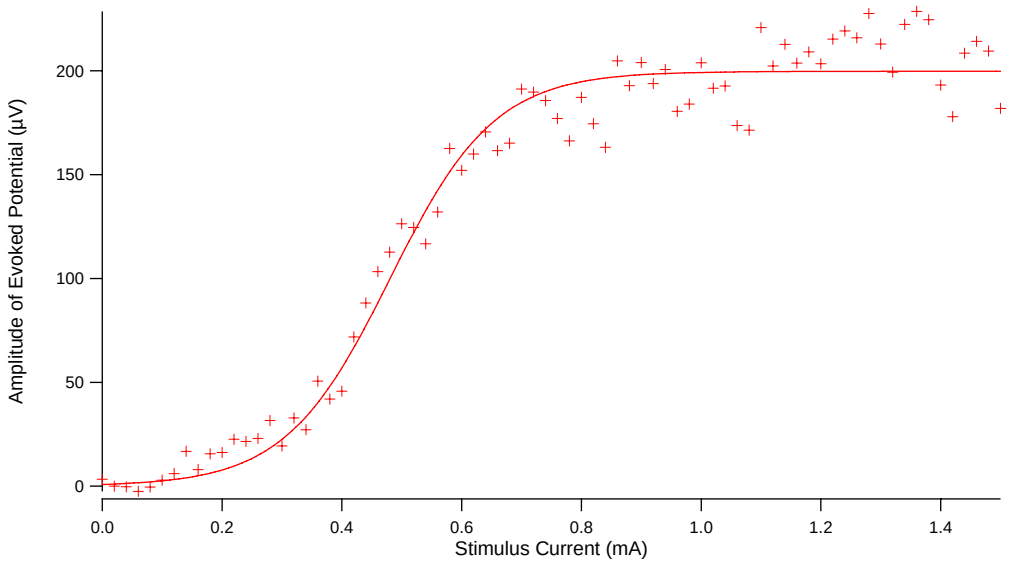

Figure 2 The amplitude response curve of the evoked potential is monotonically increasing. This example is the recording from electrode #17 on a Cyberkinetics Inc electrode array and illustrates the amplitude response recorded to the stimulation of electrode 1 of a scala tympani electrode array. The vertical axis shows p1-n1 amplitude (in µV) from the evoked responses, an example of which is shown in Figure 1, and the horizontal axis shows the current level that the electrode within the cochlear has been stimulated to elicit the evoked potential.

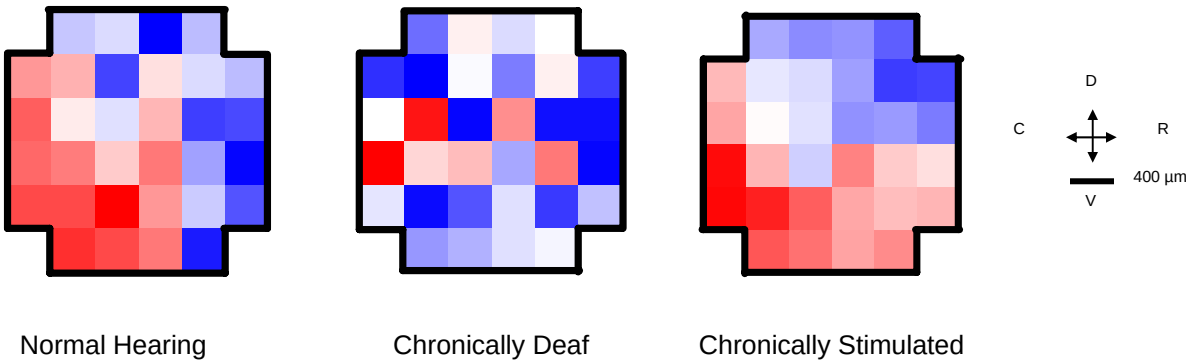

Figure 3 Maps represent part of the primary auditory cortex. Blue coloring represents a lower threshold for more basally placed intra-cochlear electrodes, while red coloring represents a lower threshold for more apically placed intra-cochlear electrodes. The length of each arrow in the directional key is 400 µm. This is also the spacing between each electrode of the electrode array. D, dorsal; R, rostral; V, ventral, C, caudal.

Analysis of the evoked potential data and single- and multi-unit data from these experiments is ongoing. Preliminary results will be presented at upcoming meetings while final preparation for publication is anticipated in the following quarter.

On completion of each acute electrophysiological experiment, the cochleae and CNS from each animal were harvested and prepared for subsequent analysis. The preliminary histological analysis of our cohorts of animals that received chronic high-rate common-ground stimulation has been completed this quarter. These data will be statistically analyzed and prepared for publication.

2.5. Chronic electrical stimulation in the rat

This work aims to address whether chronic depolarization of the auditory nerve via a cochlear implant can rescue SGNs in the deaf rat cochleae.

Although the results from this study have indicated that chronic unilateral electrical stimulation did not results in the preservation of spiral ganglion neurons (Widijaja et al. 2006), there were interesting differences in the expression of neurotrophin receptors between stimulated and unstimulated cochleae and changes in the auditory cortex of these animals. However, the current fully implantable stimulator is only capable of delivering a fixed stimulation (Millard and Shepherd, in press). Therefore we have begun work on improving the fully implantable rat stimulator presently used in this study. The priority thus far as been the capacity for remote adjustment of current level post-implantation.

A design capable of adjustable current level has been successfully verified in bench prototype form. The implant utilizes a digital potentiometer to adjust the level of the current regulator used in the existing implant. This in is turn controlled by an integrated microcontroller and radio transceiver, powered by a small button cell. Testing thus far has indicated implant lifespan of six months or more, depending upon the frequency of current updates.

An implantable prototype is currently under construction (Fig. 4), which will be used for testing of radio performance and battery life prior to *in vivo* trials. A more advanced implant featuring two channels of stimulation is also under development.

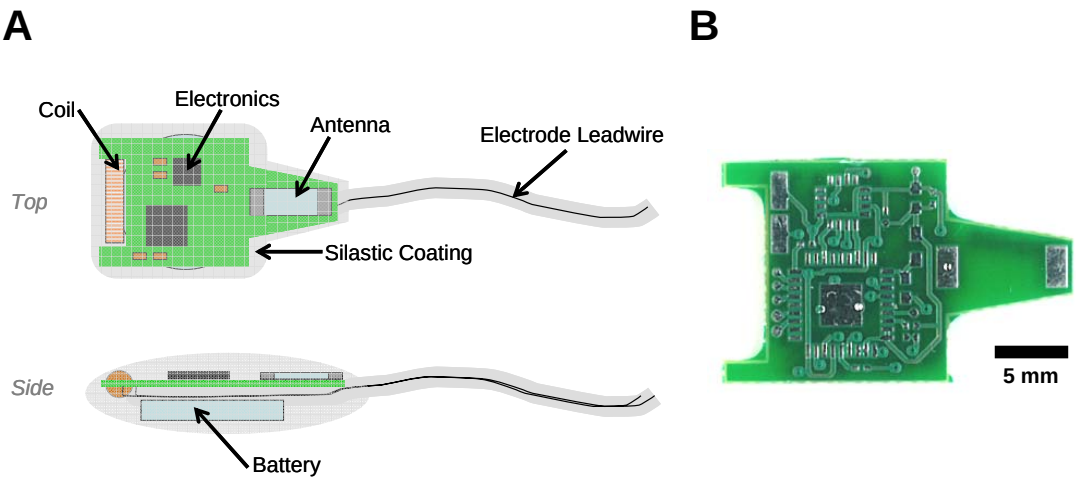

Figure 4. (A) Diagram of improved implant, and (B) photo of unassembled circuit board.

2.6. 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.

In vivo transplantation of encapsulated BDNF-Schwann cells into the deaf guinea pig cochlea continued this quarter, with four animals successfully implanted. These animals, as well as the two animals implanted in the previous quarter, completed their experimental

courses, and tissue specimens from all animals are currently undergoing histological processing.

Additional implantations to complete the experimental cohorts are scheduled to be performed in the coming quarters.

2.7. Analysis of gene-specific markers altered by chronic stimulation

This work aims to identify molecular pathways stimulated by cochlear implants in the auditory cortex after a long-term period of intracochlear stimulation. We observed that in unilaterally implanted rats, the expression and phosphorylation of distinct activity-dependent genes were altered in the contralateral auditory cortex, versus the ipsilateral. The key question is whether these alterations are due to the electrical stimulation *per se* or attributed to long-lasting changes in neurotrophins or neurotransmitter release in the brain. To answer these questions, we performed acute unilateral electrical stimulation of deafened rats and examined the same genes in the contralateral auditory cortex. The analysis is likely to be completed by the next quarter.

3. 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 chronic electrical stimulation programs in deafened/implanted cats.

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

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

vii. In conjunction with Prof. David Moore (MRC Institute of Hearing Research, UK), hosting an international conference “The Auditory brain – A tribute to Dexter Irvine” as a satellite meeting to IBRO 2007. This meeting has attracted 85 participants from the US, Europe, Asia and Australia.

4. Personnel

Tom Landry joined the group as a PhD student and part-time research assistant this quarter. His research assistant duties include monitoring chronically stimulated cats, and measuring acoustically- and electrically-evoked auditory brainstem responses. His PhD project will focus on the role of neurotrophins and electrical stimulation in the re-sprouting of the peripheral processes of spiral ganglion neurons.

Justin Tan was selected by the European Molecular Biology Laboratory in Heidelberg, Germany to attend the course “Practical Course on Microinjection and Detection of Probes in Living Cells” from 17 – 23 Jun 2007.

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.

6. References

Millard RE and Shepherd RK. A fully implantable stimulator for use in small laboratory animals. *J Neurosci Methods*, in press.

Widijaja S, Tan J, Xu J, and Shepherd RK. Chronic electrical stimulation in deafened rats: effects on spiral ganglion neuron survival. *Bionics and Regeneration of the Ear*, Melbourne, Victoria, Australia, 2006.

7. Appendix A

Guipponi, M., Tan, J., Cannon, P.Z.F., Donley, L., Crewther, P., Clarke, M., Wu, Q., Shepherd, R.K., Scott, H. Mice deficient for the type II transmembrane serine protease, TMPRSS1/hepsin, exhibit profound hearing loss. American Journal of Pathology (in press).

8. Appendix B

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9. Appendix C

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10. Appendix D

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11. Appendix E

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12. Appendix F

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