Seventh 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 seventh quarter the following activities were completed:

Publications and conferences

- Four manuscripts are currently in the review process.

- The following paper was presented as a poster by PhD student Bryony Coleman at the 3rd Annual Meeting of the International Society for Stem Cell Research Meeting, San Francisco June 23-25, 2005 (Appendix A):

  Coleman, B., Hardman, J., de Silva, M., Crook, J. & Shepherd R. “Strategies for stem cell-based therapy in the mammalian cochlea.” p217.

In addition Ms. Coleman visited and gave talks at the following laboratories:

- Olivius Laboratory, Karolinska Institute, Stockholm, Sweden.
- Reader Laboratory, Centre for Tissue Engineering and Regenerative Medicine, Imperial College, London, UK.
- Forge Laboratory, Centre for Auditory Research, University College, London, UK.
- Raphael Laboratory, Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI, USA.

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.

- The 10 guinea pigs that completed their BDNF/chronic electrical stimulation program during the 6th quarter had their histology completed during the present quarter. SGN density counts will be performed over the following two quarters.

Chronic electrical stimulation in the cat

We are using Nucleus® CI24 cochlear implants in combination with Nucleus® ESPrit 3G behind-the-ear speech processors. The cochlear implants are *not implanted* but are hardwired to connect directly with the animal's percutaneous leadwire system.

- We continued daily chronic electrical stimulation of five neonatally deafened animals. These animals have now been chronically stimulated for periods of 8-10 months using a behaviorally relevant stimulation regime.
- Performed four acute electrophysiological experiments recording from four of these chronically stimulated animals, the result of which will be discussed in more detail in a later QPR.
- Following the completion of these acute experiments histological preparation of the cochleae and CNS was performed for subsequent histological analysis. The cochlear nuclei from each animal were processed for transmission electron microscopy and sent to Prof. David Ryugo for ultrastructural analysis.
- During the quarter we deafened seven additional animals and chronically implanted five of them with an 8-ring scala tympani electrode array. The remaining two animals will serve as unimplanted deafened controls. The implanted cats have commenced their daily chronic electrical stimulation program.
- Developed a computer-based facility to capture electrode voltage and current waveforms from our chronically implanted cats.
- We continue behavioral testing of comfort stimulus levels for the implanted animals and to make minor adjustment of these levels based on EABR and these behavioral measures.

- Performed two acute electrophysiological experiments recording from the normal hearing rat auditory cortex using our new 96-channel Cerebus data acquisition system and Cyberkinetics Inc. multichannel recording arrays. The result of this and subsequent experiments planned for the next quarter will be discussed in detail in a future QPR.
- Hosted a three day visit by Prof. Patricia Leake from UCSF where we had a fruitful discussion regarding differences in methodology been our groups, the details of which are summarized in Appendix B.
- We completed the analysis of histological and electrophysiological data from the previous series of experiments from contract NO1-DC-0-2109 on the effects of long-term chronic intracochlear electrical stimulation. Manuscript preparation on this research has commenced.

Chronic electrical stimulation in the rat

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).

- The preliminary study to establish procedures for training the animals to perform discrimination tasks in the apparatus using visual and auditory discriminations, prior to deafening and the investigation of electrical discrimination, has continued. A group of three (normal hearing) rats have been trained to perform the task and have subsequently reached criterion on two visual discrimination tasks (one simple and one complex). They were then shifted to an auditory discrimination, and all three reached criterion on an auditory frequency discrimination using pure-tone stimuli. The next stage of this study is to train the rats to discriminate between click trains comparable to the pulse trains to be used in the electrical discrimination studies. The existing software does not permit the presentation of these stimuli with adequate controls for discrimination based on loudness levels, and the software is currently being modified by Rodney Millard. We anticipate the pulse train discrimination trials using acoustic clicks to be completed during the following quarter.

- The second major activity within this project is the development of a chronic cochlear stimulation model using the deaf rat. To this end we have developed a fully implantable stimulator (3rd QPR) and published a surgical technique for cochlear implantation in this species (Lu et al., 2005). During the quarter we revised our electrode leadwire design following failure of a previous design. This design change also resulted in a revision to our electrode fixation techniques.

- Following these revisions, two adult rats were profoundly deafened and the stimulators implanted unilaterally. Magnetically induced EABRs (mEABRs) were successfully evoked from both animals 14 days, one and two months

following surgery (e.g. Fig. 1). The animals continue to undergo daily stimulation via their intracochlear electrode arrays. These results give us confidence that our totally implantable stimulator can be successfully applied chronically as required for our behavioral experiments.

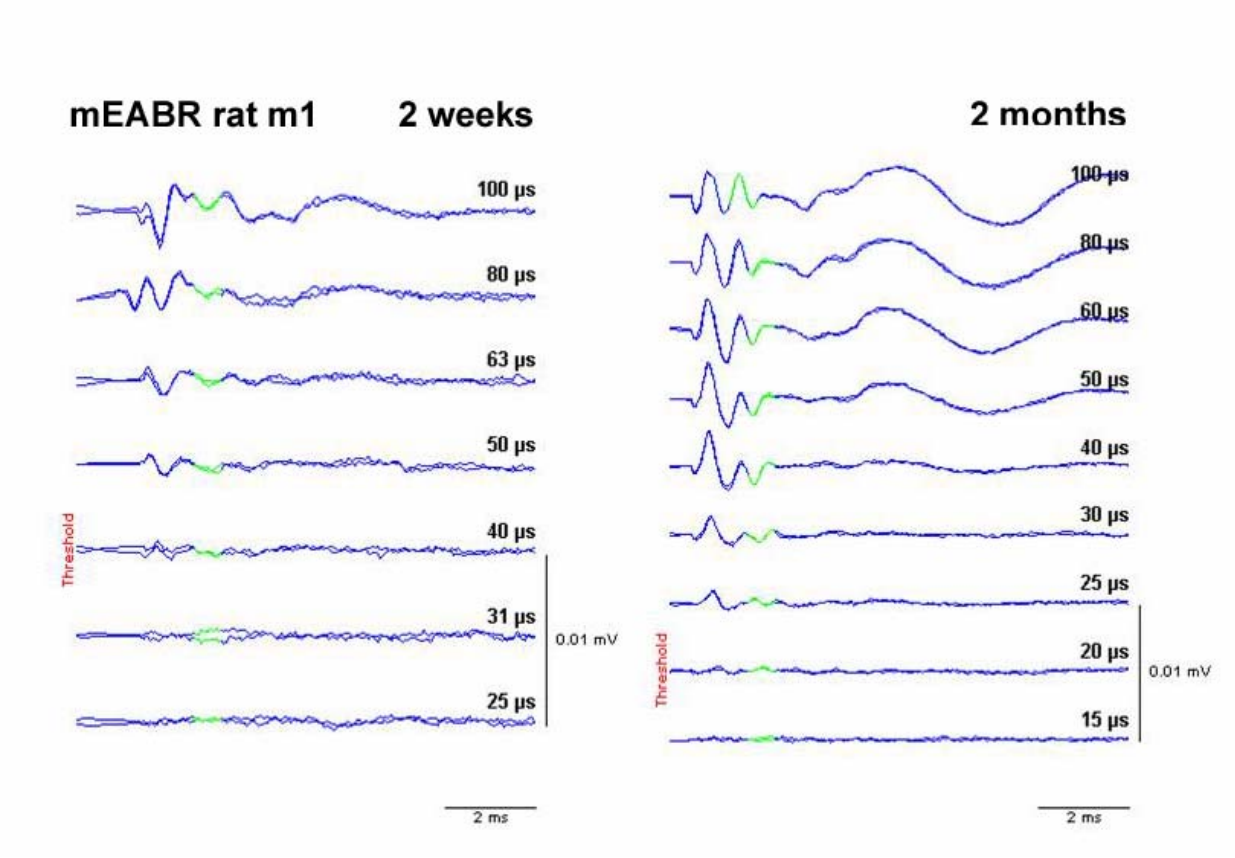

Figure 1. mEABRs evoked from one of our deafened, chronically implanted animals. The implantable stimulator induces a charge balanced biphasic current pulse of fixed current (500 μA peak) in response to a pulsed magnetic field. Charge delivery is controlled by varying pulse width. High quality mEABRs have been recorded during the chronic implantation period. The waveform morphology is similar to conventional EABRs recorded in the same species (Lu et al., 2005).

Cellular over-expression of BDNF

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.

- Molecular biology experiments were performed to produce the quantities of BDNF and NT-3 DNA required for the transfection of Schwann cells. This involved bacterial cell transformation and plasmid isolation.

- Honours student, Ricki Minter, was successfully trained in aspects of immunohistochemistry, cell counting and statistics required for her studies. Ms. Minter was also trained in molecular biology techniques including bacterial cell transformation and plasmid amplification.

- Schwann cell transfections were performed to genetically modify Schwann cells to over-express either BDNF or NT-3. Control transfections using the GFP reporter gene, as well as transfections using GFP-tagged BDNF confirmed successful uptake of the foreign DNA. However, the amount of neurotrophin produced is less than can be detected using Western blot analysis. Enzyme-linked immunosorbent assays (ELISAs) will be performed in the next quarter to quantify the BDNF and NT-3 released from these cells.

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

The initial aim of this project was to develop a mouse model of profound SNHL using ototoxic drugs. Our previous attempts underscore the difficulty of adopting this approach to achieve a deaf mouse model. As an alternative, we have analyzed a genetic mouse model in which a transmembrane protease gene has been deleted. This work is performed in collaboration with the Walter and Eliza Hall Institute of Medical Research in Melbourne. Our aim was to analyze this mouse to determine if it can be an appropriate model of profound SNHL.

From our hearing measurements, we have evidence to show that the knock-out mouse exhibited a profound SNHL while the wild-type control had normal hearing. From our morphological analysis, there was no evidence of hair cell or SGN loss. However, we did observe that the tectorial membrane was abnormally enlarged in the adult mouse cochlea of the profoundly deaf homozygote knock-out mouse compared to the wild-type control. This would suggest that the protease gene mutation is involved in recessive deafness. Examination of cochleae from the neonatal homozygote knock-out mouse demonstrated that this is a developmental defect in the cochlea that fails to correct itself during maturation.

Analysis of myelin gene expression using immunohistochemistry reveals a reduction of myelination of the peripheral myelin (P0) around the SGNs of the adult homozygote knock-out mouse in comparison to wild-type normal hearing control mice. In both peripheral and central processes P0 expression was reduced in the homozygote knock-out mouse. As myelin sheaths the nerve fibers and facilitates rapid nerve impulse conduction, a decline of myelin gene expression would be expected to affect auditory nerve fiber function, thus providing an explanation for the hearing deficit in these gene-deleted mice.

In comparison to rats profoundly deafened with ototoxic drugs, this genetic mouse model does not exhibit a loss of SGNs nor hair cells and as such, the cellular and molecular correlates of their hearing deficits are clearly different from ototoxin-induced hearing loss. This is an *interesting* finding as it supports recent results demonstrating that hearing deficits may not necessarily have to be associated with a loss of SGNs or hair cells, as encountered in the rat and guinea pig model of ototoxin-induced hearing loss.

The application of stem cells for SGN regeneration

The aim of this study is to develop clinically feasible techniques for the application of stem cell therapy for SGN regeneration in the profoundly deaf.

- Final *in vitro* co-culture work is being completed and quantitative results analyzed statistically. Preliminary results appear promising. A manuscript will be prepared for publication in the near future.

- As mentioned briefly in our previous QPR, we have developed a novel procedure to quantify the differentiation of stem cells into neurons *in vitro*. A second manuscript is being prepared, detailing this new research methodology. We intend to submit this to the Journal of Neuroscience Methods.

3. Plans for next quarter

- Continue manuscript writing and submission, and preparation for attending conferences.
- Continue studies in the auditory cortex of hearing animals using our multi-channel Cerebus data acquisition system.
- Continue our chronic electrical stimulation studies in the cat.
- Continue to investigate short- and long-term effects on neuronal and trophic markers in the cochlea neurons.
- Continue *in vitro* and *in vivo* studies directed at further differentiating stem cells towards SGNs.
- Continue to fabricate electrode assemblies for use in our chronic stimulation studies.
- Complete an auditory discrimination study in normal hearing rats where the animals will be required to discriminate between acoustic click trains presented at different rates.
- Perform enzyme-linked immunosorbent assays (ELISAs) to quantify the BDNF and NT-3 released from genetically modified Schwann cells.
- Perform *in vitro* experiments to determine the survival activity of neurotrophin producing Schwann cells on SGN cultures.

4. Personnel

- Lauren Donley joined the group as Research Assistant as noted in our previous QPR. During the quarter she was trained to undertake daily monitoring of our chronically stimulated cats and rats, the recording of ABR and EABRs, preparation of solutions for histology and other laboratory tasks.

- Jacqueline Andrew has rejoined our group after working overseas for two years. Jacqueline is working as a casual Research Assistant; her duties include working with Prof. Dexter Irvine and Rodney Millard to optimize procedures for the behavioral training of rats on an auditory discrimination task and preparing a publication based on her Honours research in our

laboratory. It is anticipated that Ms. Andrew will pursue a PhD in our laboratory in the near future.

5. Collaboration

During the Quarter we initiated a collaborative study with Dr. Douglas Heartly and Prof. Andrew King from the University Laboratory of Physiology, Oxford University. This research will involve the study of bilateral cochlear implants in an animal model. Dr. Heartly visited our laboratory for two months in order to initiate this collaboration. A summary of our initial results are presented in Appendix C.

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, and Dr. Tony Paolini from La Trobe University for advice in using the rat test chamber.

7. References

Lu, W., Xu, J., Shepherd, R.K. 2005. Cochlear implantation in rats: a new surgical approach. Hear Res 205, 115-22.

8. Appendix A (attached)

Coleman, B., Hardman, J., de Silva, M., Crook, J. & Shepherd R. Strategies for stem cell-based therapy in the mammalian cochlea. 3rd Annual Meeting of the International Society for Stem Cell Research Meeting, San Francisco June 23-25, 2005. p 217.

9. Appendix B (attached)

Summary of discussions during the laboratory visit of Prof. Patricia Leake

10. Appendix C (attached)

Summary of the collaboration developed with Dr. Doug Heartly and Prof. Andrew King, University Laboratory of Physiology, Oxford University.