Fourteenth Quarterly Progress Report

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Protective Effects of Patterned Electrical Stimulation on the Deafened Auditory System

J. Tan, S. Widjaja, J.B. Fallon, A.K. Wise, L.N. Pettingill *(nee Gillespie)*,
A. Coco, L. Donley, B. Coleman, J. Andrew, D. R. F. Irvine,
J. Xu, R.E. Millard and R.K. Shepherd

The Bionic Ear Institute
384 Albert Street
East Melbourne 3002,
Victoria, AUSTRALIA

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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 fourteenth quarter the following activities were completed:

2.1. Publications

A number of papers were submitted and are under review.

2.2. Conferences

The following papers were presented at conferences during the quarter:

2.2.1 Invited speaker presentations

B. Coleman. "Stem cell based therapy for SGN degeneration". 4th Australasian Auditory Neuroscience Workshop, Mt Eliza, 29-30th January 2007

L.N. Pettingill. "Auditory neuron protection: Neurotrophic factors and Cell-based therapies". 4th Australasian Auditory Neuroscience Workshop, Mt Eliza, 29-30th January 2007

J. Tan. "Activity-dependent plasticity in the auditory cortex after sensorineural hearing loss and cochlear implants: a search for enlightenment". 4th Australasian Auditory Neuroscience Workshop, Mt Eliza, 29-30th January 2007

2.2.2 Abstracts

Coleman B, Backhouse SS, Shepherd RK. A targeted delivery strategy for the transplantation of stem cells into Rosenthal's canal. Mid-winter meeting of the Association for Research in Otolaryngology, Denver CO, 10-15th February 2007.

Fallon J, Irvine D, Coco A, Donley L, Millard R, Shepherd R. Cochlear implantation influences the temporal responsiveness of the primary auditory cortex in the deafened cat. Mid-winter meeting of the Association for Research in Otolaryngology, Denver CO, 10-15th February 2007.

Fallon J, Irvine D, Coco A, Donley L, Millard R, Shepherd R. Cochlear implantation influences the cochleotopic organization of the primary auditory cortex in the deafened cat. Mid-winter meeting of the Association for Research in Otolaryngology, Denver CO, 10-15th February 2007.

Pettingill LN, Minter RL and Shepherd RK. An in vitro study of neurotrophin cell therapy for spiral ganglion neuron survival. Mid-winter meeting of the Association for Research in Otolaryngology, Denver CO, 10-15th February 2007.

Shepherd R, Coco A, Epp S. Chronic electrical stimulation rescues spiral ganglion neurons following removal of exogenous neurotrophins. Mid-winter meeting of the Association for Research in Otolaryngology, Denver CO, 10-15th February 2007.

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

In addition, members who have been involved in this NIH-funded contract were invited to present a poster at the 4th Australasian Auditory Neuroscience Workshop, Mt Eliza, 29-30th January 2007:

J Andrews. Schwann cell transplantation for sensorineural hearing loss.

A Coco. Chronic electrical stimulation rescues spiral ganglion neurons following removal of exogenous neurotrophins.

L Donley. Effects of electrical stimulation on spiral ganglion density in a model of neonatal deafness.

R Minter. Schwann cells over-expressing neurotrophin enhance survival of spiral ganglion neurons in vitro.

A Wise. Intracochlear stimulation in the mouse.

2.3. Ph. D. theses

The following Ph. D. thesis was passed in this quarter:

B. Coleman: “The potential of stem cells for neuronal replacement in the deaf cochlea.”. The summary of this thesis can be found in Appendix B (attached).

2.4. 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.

Histological analysis, in particular, measurements of SGN soma area, was completed during this quarter. These data are now being prepared for publication.

2.5. 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, we neonatally deafened one animal; and implanted two animals that are receiving low-rate (50 pps/electrode) monopolar stimulation on all 7 intracochlear electrodes and form the basis of our fifth experimental cohort. Analysis of the data from the acute electrophysiological experiments of these and our previous cohorts of animals has continued this quarter, with preliminary results presented at the 2007 mid-winter meeting of the Association for Research in Otolaryngology.

Following the 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 in the coming quarters.

2.6. 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. This work is carried out

primarily by Dr Sandra Widjaja who also finished her attachment with us this quarter. In summary, her work showed that chronic unilateral stimulation of deafened rats up to 7 or 14 weeks has no significant effects on spiral ganglion neuron density in the cochlea. However, her studies using immunohistochemistry reveal interesting differences in the expression of neurotrophin receptors between stimulated and unstimulated cochleae. This research used fully implantable stimulators developed in the laboratory. A “Methods” paper describing this stimulator is being prepared for publication.

2.7. 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.

We commenced experiments in which encapsulated BDNF-Schwann cells were unilaterally implanted into the left cochlea of ototoxically deafened guinea pigs. Two guinea pigs have been implanted to date. These animals will be sacrificed and prepared for histological analysis in the next quarter. We have scheduled an additional six implantations to be performed early in the next quarter.

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

In this quarter, we completed our molecular analysis of activity-dependent gene expression changes in the auditory cortex of deafened rats receiving intracochlear electrical stimulation for 7 weeks. Data have been analyzed and figures prepared for a manuscript.

2.9. 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. A review of these techniques have been prepared for submission

3. Additional activities

B Coleman received an award to present her Ph D findings at the *International Society for Stem Cell Research* meeting, to be held in Cairns in June 2007.

Miss Stephanie Misalis commenced the Honours component of her Bachelor of Science degree under the supervision of Dr Pettingill. While this project is not a component of the NIH contract, it is related to studies currently being performed under the contract, and will therefore provide important information for future experiments conducted by Dr Pettingill.

4. 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 training and testing of a group of deafened and implanted rats in the T-maze.

v. Continue chronic electrical stimulation programs in deafened/implanted cats.

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

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

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

ix. Completion of histological analysis investigating potential surgical routes for cell based therapies of the inner ear.

5. Personnel

Lauren Donley, our research assistant, left our group at the end of March to pursue her studies in New Zealand.

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.

7. Appendix A

Abstracts of presentations at the mid-winter meeting of the Association for Research in Otolaryngology, Denver CO, 10-15th February 2007.

8. Appendix B

Ph. D. Abstract – Dr. Bryony Coleman

Appendix A

Chronic electrical stimulation rescues spiral ganglion neurons following removal of exogenous neurotrophins

*R. K. Shepherd, A. Coco, S. B. Epp. Bionic Ear Institute, East Melbourne, Victoria 3002, Australia.

Exogenous neurotrophins (NT) rescue spiral ganglion neurons (SGNs) from degeneration, however, to be effective they must be supplied continuously[1]. We reported a significant rescue advantage when NT administration is combined with chronic electrical stimulation (ES)[2]. Here, we examine whether chronic ES can maintain SGN survival long after cessation of NT delivery. Ten adult guinea pigs were profoundly deafened using ototoxic drugs. Five days later they were unilaterally implanted with a scala tympani electrode array incorporating a drug delivery system. Brain derived neurotrophic factor (BDNF) was continuously delivered to the scala tympani over a 4 week period while the animal simultaneously received ES via a bipolar electrode array. One cohort (n=5) received ES for 6 weeks, including a 2 week period after the cessation of BDNF delivery (ES_6); a second cohort (n=5) received ES for 10 weeks, including a 6 week period following cessation of BDNF delivery (ES_{10}). The cochleae were then harvested for histology and SGN density determined for each cochlear turn for comparison with normal hearing controls (n=4). The withdrawal of BDNF resulted in a rapid loss of SGNs in turns 2-4 of the deafened/BDNF-treated cochleae; this was significant as early as 2 weeks following cessation of the NT when compared with normal controls ($p<0.05$). Importantly, while there was a small reduction in SGNs in turn 1 (i.e. adjacent to the electrode array) after NT removal, this reduction was not significant compared with normal controls. These results demonstrate that chronic ES can at least partially maintain SGNs after initial rescue using exogenous NTs. This finding has implications for the clinical application of NTs and supports earlier work demonstrating a rapid SGN loss after NT removal[1].

[1]Gillespie et al., 2003 J Neurosci Res 71, 785-790. [2] Shepherd et al., 2005 J. Comp. Neurol. 486, 145-158.

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Schwann cells genetically modified to express neurotrophins promote spiral ganglion neuron survival in vitro

Lisa Pettingill, Ricki Minter, Robert Shepherd. Bionic Ear Institute, , East Melbourne, Victoria 3002, Australia.

Spiral ganglion neurons (SGNs) undergo degeneration following sensorineural hearing loss. Their rescue has therapeutic significance as they are the target neurons for cochlear implants. The delivery of exogenous brain-derived neurotrophic factor (BDNF) or neurotrophin-3 (NT-3) via an osmotic pump and cannula can have protective effects on SGNs in animal models of deafness, however, issues associated with infection and the limited delivery period of these devices have resulted in the need to develop alternative delivery strategies. Cell transplantation is now considered a potential avenue for neurotrophin delivery *in vivo*. We genetically modified SCs to over-express either BDNF (BDNF-SCs) or NT-3 (NT3-SCs) to determine if neurotrophic support from a cell-based source elicits survival effects on SGNs. SCs from postnatal day (P) 3 rat sciatic nerve were transfected with expression plasmids encoding enhanced green fluorescent protein (EGFP), BDNF-EGFP or NT3-EGFP using Lipofectamine 2000 (Invitrogen). BDNF- and NT3-SCs produced significantly greater amounts of the respective neurotrophin compared with EGFP-SC controls, as determined by ELISA ($P<0.05$). Genetically modified SCs were then co-cultured with P6 rat SGNs and the survival effects quantified in terms of the number of surviving SGNs after three days *in vitro*. BDNF- and NT3-SCs significantly enhanced SGN survival in comparison to EGFP-SC controls ($P<0.05$). BDNF-SCs also provided significantly greater SGN survival in comparison to recombinant human BDNF ($P<0.05$), while NT3-SCs and recombinant human NT-3 elicited similar survival effects. The transplantation of cells designed to over-express neurotrophins may provide a clinically relevant means of rescuing SGNs in the deaf cochlea.

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Cochlear implantation influences the cochleotopic organisation of the primary auditory cortex in the deafened cat.

James B. Fallon *1,2, Dexter R. F. Irvine 1, Anne Coco 1, Lauren M. Donley 1, Rodney E. Millard 2 and Robert K. Shepherd 1,2,3

1The Bionic Ear Institute, 2Department of Otolaryngology, University of Melbourne, 3The Royal Victorian Eye & Ear Hospital, East Melbourne, Victoria 3002, Australia.

The normal cochleotopic organisation of the primary auditory cortex (AI) can be altered by changes in output from the cochlea. In the extreme case of congenitally- or neonatally-deafened animals, where there is no output from the cochlea, AI is reported to exhibit a 'rudimentary' cochleotopic organisation. Cochlear implants reactivate an otherwise silent cochlea, but the effects of chronic intra-cochlear electrical stimulation (ES) on the cochleotopic organisation of AI in long-term deafened animals are not clear. Therefore, two month after neonatal deafening, four profoundly deaf cats were implanted with a multichannel scala tympani electrode array and received unilateral ES (up to 200 days) to a restricted section of the basal turn from a Nucleus® CI24 cochlear implant and Nucleus® ESPrit 3G speech processor. An additional four animals served as age-matched unstimulated deaf controls. Recordings from a total of 389 multi-unit clusters in AI were made using a combination of single- and multi-electrode arrays. Significant cochleotopic organisation of AI was observed in all but one of the chronically stimulated animals (Pearson correlation; all $p < 0.01$), while only one of the unstimulated control animals exhibited cochleotopic organisation (Pearson correlation; $p < 0.01$). Additionally, electrical stimulation at 3 dB above minimum cortical threshold resulted in a significantly greater area of activation in the chronically stimulated animals than in the unstimulated controls (t-test; $p = 0.04$). These results indicate that chronic ES can result in i) a more defined cochleotopic organisation of AI than is present in the long-term deaf; and ii) an increase in the area of activation produced by supra-threshold stimuli. The re-establishment of a cochleotopically organised AI may partially account for the improved clinical performance observed among implant subjects over time, and reflect the ability of the auditory system to undergo plastic reorganization even following a profound hearing loss.

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Cochlear implantation influences the temporal responsiveness of the primary auditory cortex in the deafened cat.

James B. Fallon *1,2, Dexter R. F. Irvine 1, Anne Coco 1, Lauren M. Donley 1, Rodney E. Millard 2 and Robert K. Shepherd 1,2,3

1The Bionic Ear Institute, 2Department of Otolaryngology, University of Melbourne, 3The Royal Victorian Eye & Ear Hospital, East Melbourne, Victoria 3002, Australia.

Chronic intra-cochlear electrical stimulation (ES) is known to increase the temporal responsiveness of the auditory system in long-term deaf animals. Specifically, chronic ES results in increases in the maximum following frequency and decreases in the latency and temporal jitter of neurons in the inferior colliculus. However, the effect of deafness and chronic ES on the temporal responsiveness of neurons in the primary auditory cortex (AI) is not clear. Therefore, two month after neonatal deafening, four profoundly deaf cats were implanted with a multichannel scala tympani electrode array and received unilateral ES (up to 200 days) to a restricted section of the basal turn from a Nucleus® CI24 cochlear implant and Nucleus® ESPrit 3G speech processor. An additional four animals served as age-matched unstimulated deaf controls. Recordings from a total of 389 multi-unit clusters in AI were made using a combination of single- and multi-electrode arrays. The maximum rate at which units could be driven from chronically stimulated cochlear regions was significantly higher than that at which units could be driven by stimulation of the corresponding regions in deaf control animals (Mann-Whitney; $p = 0.04$). However, chronic ES resulted in no significant change in temporal jitter (Mann-Whitney; $p = 0.2$) and a small but significant *increase* in the latency (Mann-Whitney; $p = 0.04$) of the responses of units in AI. These findings indicate that chronic ES can produce changes in the temporal responsiveness of AI, with increases in the both the maximum following frequency and latency of responses. These changes in the temporal responsiveness of AI have implications for the methods used to encode the fine temporal structure of stimuli used in modern cochlear implants.

Work funded by NIDCD (NO1-DC-3-1005), The Bionic Ear Institute and the RVEEH (Wagstaff Fellowship).

A Targeted Delivery Strategy for the Transplantation of Stem Cells into Rosenthal's Canal

B. Coleman[1,2,] S.S. Backhouse[2] and R.K. Shepherd[1,2]
1The University of Melbourne, 2The Bionic Ear Institute.

The delivery of stem cells (SCs) into the mammalian cochlea is a potential strategy to replace degenerating SGNs following a sensorineural hearing loss. Previous attempts to deliver SCs into the cochlea have demonstrated survival of transplanted cells, however, these cells frequently disperse throughout the cochlea and only low numbers have been reported within the target site, Rosenthal's canal. The purpose of this study was to investigate the efficacy of delivering exogenous cells directly into Rosenthal's canal. For comparison we delivered both coloured microbeads (MBs; 20-45 μm in diameter) and live SCs (5-15 μm in diameter) into normal hearing (NH) and aminoglycoside deafened (AD) adult guinea pigs. MBs or SCs were delivered into the left cochlea via a cochleostomy made in the lower basal turn scala tympani. Rosenthal's canal was opened by fracturing the adjacent osseous spiral lamina wall and both MBs (n=4, NH) and SCs (n=5, AD) were then delivered into Rosenthal's canal within a hydrogel (biocompatible 3D matrix) to minimise their dispersal. These groups were compared to animals that underwent surgery alone (n=4, NH). MBs and SCs were observed in the lower basal turn scala tympani and in Rosenthal's canal, and the hydrogel was effective at retaining both the MBs and SCs at the implant site. An inflammatory tissue response was observed in all treated cochleae, however this was localised to the lower basal turn scala tympani. An observed decrease in the density of SGNs in the lower basal turn of treated cochleae, was again localised to the surgical site. Although further work is required to optimise the delivery of stem cells into Rosenthal's canal, our findings demonstrate the potential of this approach for the targeted delivery of replacement cells. This will be important for future cell replacement therapies incorporating guided neurite outgrowth in a 3D matrix with electrical stimulation.

This work is supported by the University of Melbourne, the NIDCD (N01-DC-3-1005) and the TWJ Foundation (UK).

Thesis summary **Appendix B**

The sensory hair cells in the mammalian cochlea are sensitive to loud noise, ototoxic drugs and ageing. Damage to these hair cells sets in place a number of irreversible changes, which eventually result in the ongoing degeneration of spiral ganglion neurons, the target neurons of the cochlear implant.

Cochlear implants function by directly stimulating spiral ganglion neurons in the absence of hair cells, enabling hearing in severe to profoundly deaf individuals. The efficacy of this electrical device therefore depends on a critical number of surviving spiral ganglion neurons. As such, the preservation of the integrity and density of spiral ganglion neurons in the deafened cochlea is envisaged to provide improved outcomes for cochlear implant recipients.

Previous studies have investigated the potential of neurotrophins to "rescue" spiral ganglion neurons from deafness-induced degeneration. These studies were very successful in terms of enhancing spiral ganglion neuron survival after deafness, however, the survival-promoting effect of these molecules was lost immediately following the cessation of treatment. The identification of longer-term treatments for spiral ganglion neuron preservation is underway, including the engraftment of exogenous cells into the cochlea. This thesis examined the potential of stem cells to provide replacement neurons to the deafened mammalian cochlea, including the *in vitro* differentiation of stem cells toward a spiral ganglion neuron lineage and the engraftment of exogenous stem cells into the deafened auditory system.

Two *in vitro* co-culture models were developed for directing the differentiation of stem cells toward a spiral ganglion neuron lineage. In both cases, co-cultures of early post-natal cochlear tissues with stem cells improved the numbers of bipolar, neuron-like cells produced. Particularly successful was the co-culture of early post-natal organ of Corti explants with stem cells, which resulted in significantly greater numbers of bipolar, neurofilament positive, neuron-like cells. The findings from these models suggest that early post-natal cochlear tissues may contain the combination and concentration of molecular cues required for the directed differentiation of stem cells toward a spiral ganglion neuron lineage. The elucidation of these cues will be essential for the long term replacement of spiral ganglion neurons using stem cells.

The *in vivo* engraftment of stem cells into the deafened cochlea was examined using both a conservative and a more invasive surgical approach. The delivery of stem cells into the scala tympani demonstrated that stem cells were capable of survival in the deafened

mammalian cochlea without causing an inflammatory tissue response. However, their widespread dispersal throughout the cochlea and low numbers detected in the target site, Rosenthal's canal, was not ideal. A second more invasive approach was therefore investigated in an attempt to overcome these difficulties. The direct delivery of stem cells into Rosenthal's canal demonstrated both the survival of exogenous stem cells in the deafened cochlea and the retention of transplanted cells at the implant site. Although this approach was far more invasive than scala tympani delivery, the inflammatory tissue response and decrease in spiral ganglion neuron density observed as a result of surgery, was highly localised to the surgical site. Together these *in vivo* studies illustrated the potential of exogenous stem cells to survive in the deafened cochlea environment. While further investigations will be necessary to improve the numbers of stem cells detected within Rosenthal's canal and to suppress any inflammatory tissue response, stem cell transplantation for spiral ganglion neuron replacement holds promise.

The clinical application of stem cells into the deafened auditory system is likely to be several years away, however, the findings of this thesis have contributed to the development of a spiral ganglion neuron replacement therapy. When combined with electrical stimulation via a cochlear implant, this therapy may bring improved outcomes for cochlear implant recipients, and, if successfully combined with hair cell regeneration therapies may one day result in the regeneration of a fully functional auditory system.