Fifth Quarterly Progress Report

HHS-N-263-2007-00053-C

# The Effects of Intracochlear Electrical Stimulation on Neural Survival and Connectivity

The Bionic Ear Institute

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October 1 – December 31, 2008

**Contents:**

## 1. Introduction

The overall objectives of this contract are to develop techniques that employ intracochlear electrical stimulation (ICES) and drug administration which can support neural survival and function in order to improve the quality of auditory perception from a multichannel cochlear implant. Our goals are threefold; to study the effects of ICES on the developing auditory system for subjects implanted at a young age in order to minimize any delay in auditory stimulation; to examine the effects of ICES on the auditory system over a lifetime of use; and to evaluate the response of the auditory system in adult onset deafness to ICES, and the effect of duration of deafness, using functional, anatomical and behavioral measures.

To achieve these goals we will use a systems approach across a number of sub-disciplines of neurobiology including electrophysiological, behavioral and neuroanatomical / molecular biological techniques in order to maximize data collection from each animal. We have divided our approach into two broad areas of research:

a) Chronic stimulation studies investigating the trophic and plastic response of the deafened auditory pathway to chronic ICES. Studies in this area focus on the role of ICES in shaping both the developing and the mature auditory system. Key outcomes will be a deeper understanding of the effects of ICES on both the spatial and temporal processing ability of the auditory system, and the interaction of these effects with the preceding state of the auditory pathway (i.e. the duration of deafness and developmental state of the auditory pathway).

b) Neurotrophin (NT) studies investigating the trophic and plastic response of the deafened auditory pathway to spiral ganglion neuron (SGN) rescue via ICES and exogenous neurotrophin delivery. The role of exogenous NTs in the rescue of SGN has been well established; therefore, studies in this area focus on developing and using delivery techniques we consider to have potential clinical application. Additionally, we will determine the effects of NT delivery and SGN rescue on the spatial and temporal processing ability of the central auditory system.

A major objective of this work is to apply our findings to the clinical environment. Therefore, while these studies are designed to provide insight into the effects of ICES on neural survival and connectivity across a range of etiologies and animal species, we will be using techniques that are clinically relevant whenever possible.

## 2. Summary of activities for the quarter

During the quarter the following activities were completed:

### *2.1. Publications and conferences*

The following papers were presented at the Medical Bionics conference held in Lorne, Australia. Abstracts are attached as appendix A.

Landry, T.G., Fallon, J.B., Wise, A.K. and Shepherd, R.K. Effects of exogenous neurotrophins in the deaf stimulated cochlea. *Proceedings of the Medical Bionics - a new paradigm for human health.* Lorne, Victoria, Australia, 16-19 November 2008. Awarded MiniFab Best Poster Prize.

Perry, D.W.J., Fallon, J.B., Grayden, D.B., Millard, R.E. and Shepherd, R.K. A fully implantable two-channel cochlear stimulator for rats. *Proceedings of the Medical Bionics - a new paradigm for human health.* Lorne, Victoria, Australia, 16-19 November 2008.

Glynn, F., Tan, J., Wang, Y., Caruso, F. and Shepherd, R.K. A novel therapeutic approach encapsulating brain-derived neurotrophic factor in nanoporous particles for treating sensorineural hearing loss. *Proceedings of the Medical Bionics - a new paradigm for human health.* Lorne, Victoria, Australia, 16-19 November 2008.

Wise, A.K., Fallon, J.B., O'Leary, S.J., Sly, D.J. and Shepherd, R.K. Spatial and temporal characteristic of auditory neurons in response to deafness and chronic electrical stimulation. *Proceedings of the Medical Bionics - a new paradigm for human health.* Lorne, Victoria, Australia, 16-19 November 2008.

Millard, R.E., Fallon, J.B., Coco, A. and Shepherd, R.K. Use of commercial cochlear implants for chronic stimulation of laboratory animals. *Proceedings of the Medical Bionics - a new paradigm for human health.* Lorne, Victoria, Australia, 16-19 November 2008.

Fallon, J.B., Irvine, D.R.F. and Shepherd, R.K. Sensory neural prostheses and brain plasticity. *Proceedings of the Medical Bionics - a new paradigm for human health.* Lorne, Victoria, Australia, 16-19 November 2008.

## *2.2. Chronic intracochlear electrical stimulation*

### 2.2.1. Mouse

Mutations in specific genes account for approximately 50% of childhood deafness. In the past decade, deafness genes in mouse mutants have been identified, providing a platform to study the mechanisms of genetically based deafness in humans. We are seeking to determine whether the auditory systems of these mice have a common cellular and molecular mechanism underlying their deafness and how these compare to the pathologies seen clinically. We are also developing the procedures and techniques to provide chronic ICES in these models to determine if ICES can reverse the deafness-associated pathologies seen in these animals.

The tissue from the animals that successfully completed their chronic stimulation program along with the other implanted but non-stimulated animals has been processed and is ready for histological analysis. This analysis will continue in the coming quarters and the outcomes from this analysis will determine if further refinements to both the electrode assembly and fixation of the leadwire and stimulator assembly are required before any additional animals are implanted in the coming quarters.

### 2.2.2. Rat

As well as providing an additional species to study the effect of ICES on neural survival, the rat provides a useful model to study the effects of temporally challenging ICES on the adult deafened auditory pathway. The small size of the rat cochlea limits the number of intra-cochlea electrodes that can be inserted atraumatically, therefore focusing these studies on the effects of temporally challenging ICES on the temporal processing throughout the central auditory pathway, assessed using both electrophysiological and behavioral measures.

The conditioned avoidance procedures for testing psychophysical thresholds in chronically implanted adult rats were finalized and two normal hearing animals were tested on an acoustic AM detection task. We are continuing with our work on a means to provide behaviorally relevant ICES to chronically implanted rats using a simple operant conditioning task. We have also begun to set up for our chronic and acute experiments that are planned to commence in the next quarter.

### 2.2.3. Cat

This work continues to address the question of whether chronic ICES alone, via a cochlear implant, can prevent SGN degeneration. Additionally, the question of the effects of chronic ICES on the developing nervous system; the effects of early vs late intervention for subjects deafened at a young age; and the effects of early intervention for subjects deafened as adults will be addressed.

During this quarter, we continued our to run the five animals in our late intervention cohort, two have begun their stimulation regime (500 pps/electrode, monopolar stimulation on all 7 intracochlear electrodes using the SPEAK® speech processing strategy) at approximately 8 months of age, the remaining animals will begin in the following quarter. One acute electrophysiological experiment was performed this quarter on the last animal in our low-rate (50 pps/electrode) cohort. Analysis of the data from this cohort is underway and will be reported in a following quarter. Following the completion of the acute electrophysiological experiment, the cochleae and CNS were harvested and prepared for subsequent analysis. Neurotrophins

## *2.3. Neurotrophins*

### 2.3.1. Nanoparticles

While exogenous NT delivery has been shown to promote SGN survival in the deafened cochlea, the clinical application of NTs awaits an acceptable delivery system. The development of a layered nanofilm, sequestering the desired NT, physically designed for implantation in to the cochlea, and engineered for optimal NT release kinetics would offer a potentially elegant solution.

During this quarter, Dr Fergal Glynn, an ENT registrar from Ireland, concluded his research at the Bionic Ear Institute. In this quarter, we performed *in vitro* assays determining if the BDNF-encapsulated nanoparticles were biologically active. Differentiated SHSY-5Y blastoma cells undergo apoptosis when serum is deprived from the culture. This can be reversed by adding defined concentrations of neurotrophins. In this *in vitro* assay, we found that BDNF released from the nanoparticles can rescue these differentiated cells from death in a serum-deprived medium. The extent of rescue does not differ significantly from recombinant BDNF added to positive control cultures.

To examine if these nanoparticles are toxic, we delivered fluorescently-labeled nanoparticles into the round window of rats. No immediate death was observed indicating thus far that these nanoparticles are well-tolerated. To determine the ability of BDNF-encapsulated particles to rescue degenerating spiral ganglion neurons, we have delivered these particles into the round window of deafened rats and hope to determine any biological effects in a long-term study.

### 2.3.2. Guinea Pig

The pro-survival effects of NT delivery (with or without ICES) following aminoglycoside-induced deafening are well established. What is less clear are the effects of NT delivery with different deafness pathologies and the effects of NT delivery and ICES on the spatial and temporal processing ability of the central auditory system.

The previous quarter consisted primarily of the maintenance of chronic treatments and the collection of data from acute experiments performed on six guinea pigs receiving chronic infusion of neurotrophins or artificial perilymph into the scala tympani. Multi-unit excitation patterns were recorded from the inferior colliculus to intracochlear electrical stimulation (ICES) in the experiments, as well as the injection of the neural tracer biotinylated dextran amine (BDA) into the auditory nerve.

The tracer signal was initially weak within the peripheral fibres of SGNs located in the basal turn of midmodiolar sections. However, it was noted that labeling was stronger in the more basal portion of basal turn surface preparations, and the signal was apparent in central axons and somata in the majority of the basal turn. Therefore, we have altered the orientation of the cochlea so that midmodiolar sections capture more basal regions of the Rosenthals canal (Figure.1). This has the added benefit of providing surface views of the majority of peripheral fibres that were likely to have been stimulated by chronic or acute ICES at low amplitude (e.g. 4dB above threshold). The tissue analyzed using the new orientation has only received artificial perilymph chronically. One neurotrophin treated cochlea will be immunolabeled and imaged in the near future.

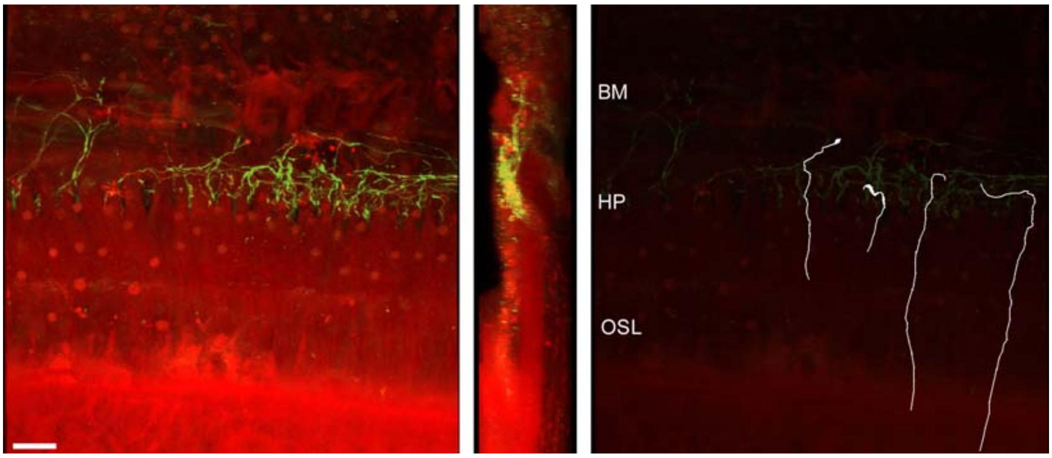


**Figure 1.** Collapsed confocal microscope images of immunolabeled neurofilament (green) and BDA (red – includes much autofluorescence from surrounding tissue) in the basal turn region of a guinea pig cochlea surface preparation following chronic artificial perilymph infusion. The SGN peripheral fibres appear to be growing in a disorganized manner, although the extent of disorganization decreases apically through the cochlea in this condition with the peripheral fibres resembling normal organization by turn 3, including tunnel-crossing fibres (data not shown). The middle image shows a 3D reconstruction of the same tissue rotated 90° about the vertical axis. The right image shows reliable tracings (white) of fibres co-labeled with anti-neurofilament and BDA. BM = basilar membrane, HP = habenula perforata, OSL = osseous spiral lamina, ST = scala tympani; scale bar = 20µm.

Analysis of the preliminary electrophysiological data from the multi unit recordings in the inferior colliculus indicate that the spatial tuning curve widths at 4dB above threshold (an indirect measure of the spatial selectivity of ICES of SGNs) are similar for normal, untreated deaf, and neurotrophin treated deaf cochleae. However, the multi unit thresholds in animals that were untreated appear to be higher than normal and neurotrophin treated animals, a finding that is consistent with our hypothesis.

We have carried out experiments that aim to label newly formed cells using 5-bromo-2'-deoxyuriodine (BrdU). Guinea pigs received two injections of BrdU (50mg/kg) per week for the four week treatment period following cochlear implantation and one injection 3h prior to sacrifice. We have been optimizing the protocol for immunodetection and have obtained positive, albeit variable, control results (Figure 2) that are consistent with those reported by previous researchers (Heine et al. 2004; Guidi et al. 2005; O'Hara and Sharkey 2007).

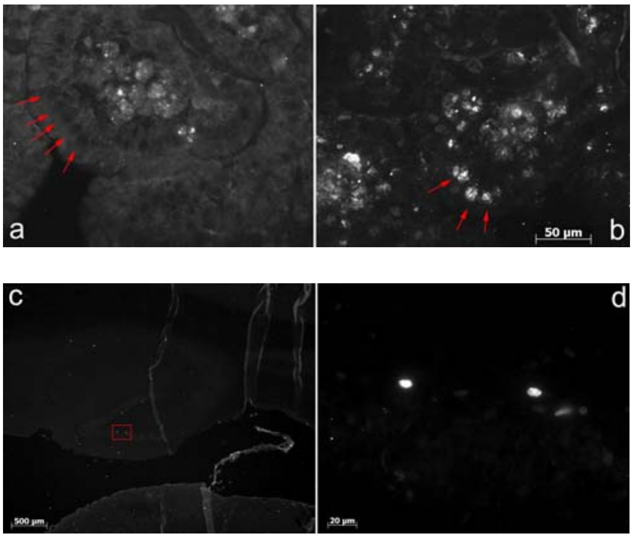


**Figure 2.** BrdU exposed control tissue. **a** & **b**: Gut tissue showing cell nuclei (arrows) that are unlabelled in tissue not incubated with anti-BrdU antibody (**a**) and labeled in tissue exposed to the antibody (**b**). **c** & **d**: Sagittal brain sections showing the hippocampus. The boxed area in **c** is shown enlarged in **d**, showing positively labeled cells. Although labeled cells were scattered across locations, the most consistent localization was in the subgranular zone, as expected. The success of labeling was highly variable, even within the same animal.

### 2.3.3. Cat

It is well established that ICES and NT delivery can promote SGN survival over periods of up to one month; however, from a clinical perspective it is important to examine the effects of long term ICES and NT delivery. Therefore, we are using LCT Pty Ltd's NT-cell® - a porcine derived choroid plexus cell product encapsulated in alginate. The NT-cell has been shown to express multiple NTs over an extended period of time – in combination with ICES in our ototoxically deafened cat model to asses the effects of combined ICES and NT delivery on the developing nervous system and the ability for ICES to maintain SGNs in deafened cochleae following cessation of NT delivery.

We are nearing completion of the acute electrophysiology experiments on the remaining animals. Six of these experiments have been completed recently and we have been able to collect a significant amount of data. We are interested in the capacity of single auditory neurons to encode spatial and temporal information from a multichannel intracochlear electrode array and how neurotrophin treatment might affect auditory neuron function. Some preliminary results from these experiments are shown in figure 3. The cochleae from the cats in which acute experiments were performed have been harvested and are undergoing histological processing. A complete data set will be available once all of the cochleae have been processed for histology and examined.

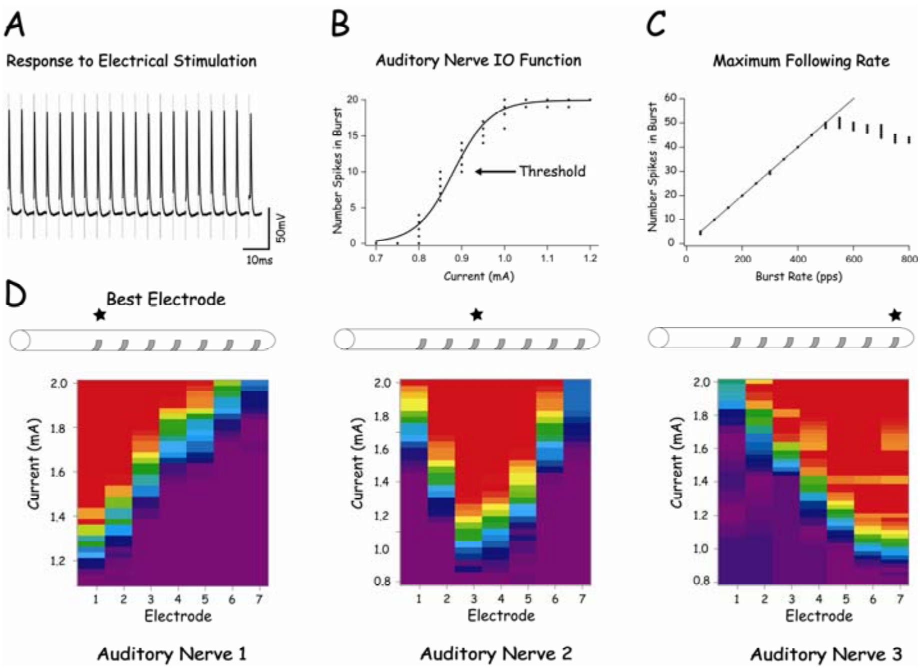


**Figure 3. (A)** Preliminary data showing recordings from a single AN in response to electrical stimulation delivered in MP configuration. Stimuli were delivered with a current sufficient to evoke a firing probability of 1.0 at a stimulation rate of 200pps. Stimulus artefact (grey line) is eliminated using a technique developed in a recent paper (see publications) so that action potentials can be readily identified. **(B)** IO curves are generated for each intracochlear electrode to establish activation thresholds – defined as the current required for 0.5 firing probability. **(C)** maximum rate at which a single AN can phase lock to electrical stimulation delivered at rates varying from 50pps to 800pps. This AN was able to fire in response to each stimulus at stimulation rates of up to 550pps. **(D)** An electrically evoked response area (ERA) is generated to identify the best electrode and the extent of AN activation spread across the array. Preliminary data are shown for three different ANs that were most responsive to electrode 1 (Auditory nerve 1), electrode 3 (Auditory nerve 2) and electrode 7 (Auditory nerve 3) stimulated in an MP electrode configuration. Colours are firing probability (purple-blue = 0 to red = 1.0). From Wise, Fallon & Shepherd, unpublished data.

We have analyzed the available evoked data collected during the treatment period (EABR and NRT data - see Figure 4). Our results indicated that it was possible to record NRTs in only 57% of cases where there was an EABR. However, electrode impedance, electrode location, and threshold were not contributing factors for the absence of NRT on some electrodes. Once these acute experiments are completed we will have a complete data set ready for analysis.

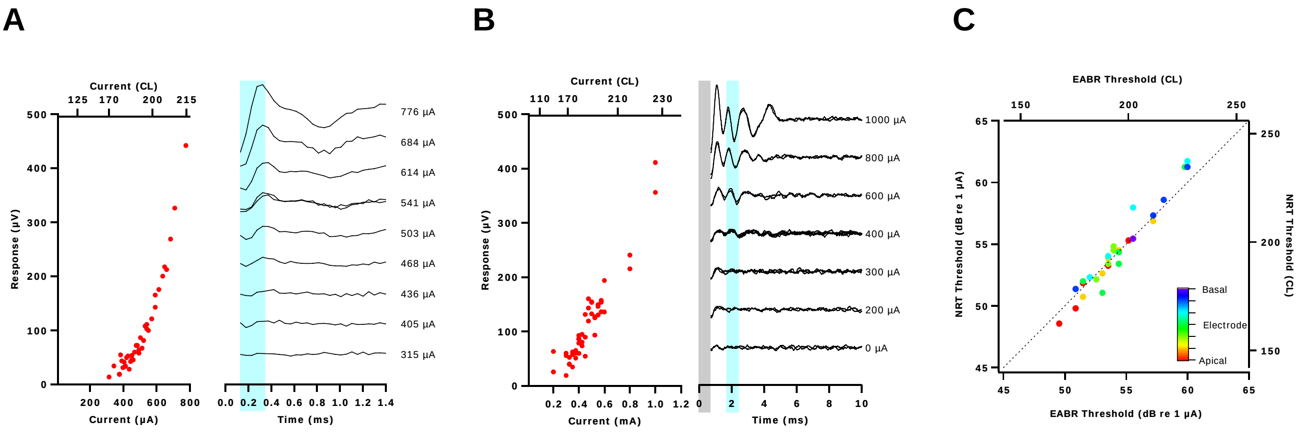


**Figure 4.** (**A**) An NRT growth function (left panel) and individual NRT traces (right panel) for a range of stimulus currents. The blue highlighted region shows the N1/P1 response and the NRT was calculated as the peak to peak value within this region. In this example the NRT threshold was ~400µA. (**B**) An EABR growth function (left panel) and individual EABR traces (right panel) for the same electrode as shown in **A**. The blue highlighted region shows the EABR wave III response measured as the peak to peak value. In this example the EABR threshold was ~400µA. (**C**) Plot of EABR thresholds and NRT thresholds for the 28 electrodes in which NRT could be measured. Thresholds have been colour-coded for cochlear position. NRT and EABR thresholds were not statistically different.

## 3. Plans for next quarter

Plans for the following quarter include:

a) Continued manuscript writing and submission, and preparation for attending conferences.

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

c) Continued fabrication of fully implantable stimulators for the mice and rats and further refinements to both the electrode assembly and fixation of the leadwire and stimulator assembly for use in the mouse studies.

d) Quantification of the mouse histology.

e) Implant additional animals for ICES studies in the cat.

f) Continue chronic ICES programs in deafened/implanted cats.

g) Preparation for chronic recording experiments.

h) Analysis of data from the deafened, chronically stimulated cats, including acute electrophysiological data.

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).

j) Continued development and testing of nanoparticles.

    a. We will analyse the distribution of the fluorescently-labeled nanoparticles in the cochlea.

    b. We will also prepare deafened rats which have received BDNF-encapsulated nanoparticles in the round window 2 months post-delivery.

k) Continued development of techniques to trace single SGN peripheral fibers.

l) Acute experiments on chronically deaf guinea pigs and the initiation of the treatment and control groups for neurotrophin treatment and chronic ICES.

m) Develop protocols for TEM examination of cochlea tissue.

n) Begin experiments that aim to examine the effects of chronic ICES, with or without neurotrophin treatment, in the deafened guinea pig cochlea.

## 4. 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; Dr David Sly and Prof. Stephen O'Leary for collaboration on VIII$^{th}$ nerve recordings; The Department of Biomolecular Engineering and Nanoscience Technology, The University of Melbourne; Living Cell Technologies Pty Ltd; and 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.

## 5. References

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Heine, V. M., S. Maslam, et al. (2004). "Prominent decline of newborn cell proliferation, differentiation, and apoptosis in the aging dentate gyrus, in absence of an age-related hypothalamus-pituitary-adrenal axis activation." Neurobiol Aging **25**(3): 361-75.

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## 6. Appendix A (attached)

Landry, T.G., Fallon, J.B., Wise, A.K. and Shepherd, R.K. Effects of exogenous neurotrophins in the deaf stimulated cochlea. *Proceedings of the Medical Bionics - a new paradigm for human health.* Lorne, Victoria, Australia, 16-19 November 2008. Awarded MiniFab Best Poster Prize.

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