

## Seventh Quarterly Progress Report

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



The Bionic Ear Institute

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## 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 paper was presented during the quarter.

Fallon J.B. The auditory brain and cochlear implants. Lions Club International National Australian Convention, Melbourne, Australia.

### **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 system 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.

In the [previous quarter](#) we reported on our results of implantation of the mouse cochlea with our custom designed electrode array and fully implantable stimulator. We found no detrimental effects on SGN survival as a consequence of the surgical procedure that included stapedial artery cauterization. In addition, mice that were deafened and implanted with the electrode array, but did not receive chronic ICES, exhibited a significant loss of SGNs throughout the cochlea compared to the contralateral control cochlea.

We are continuing with the histological analysis of this project. The next phase of the project is to continue to implant mice and deliver chronic ICES and evaluate the effects of chronic electrical stimulation on auditory function (EABRs) and cochlear histology.

#### **2.2.2. Rat**

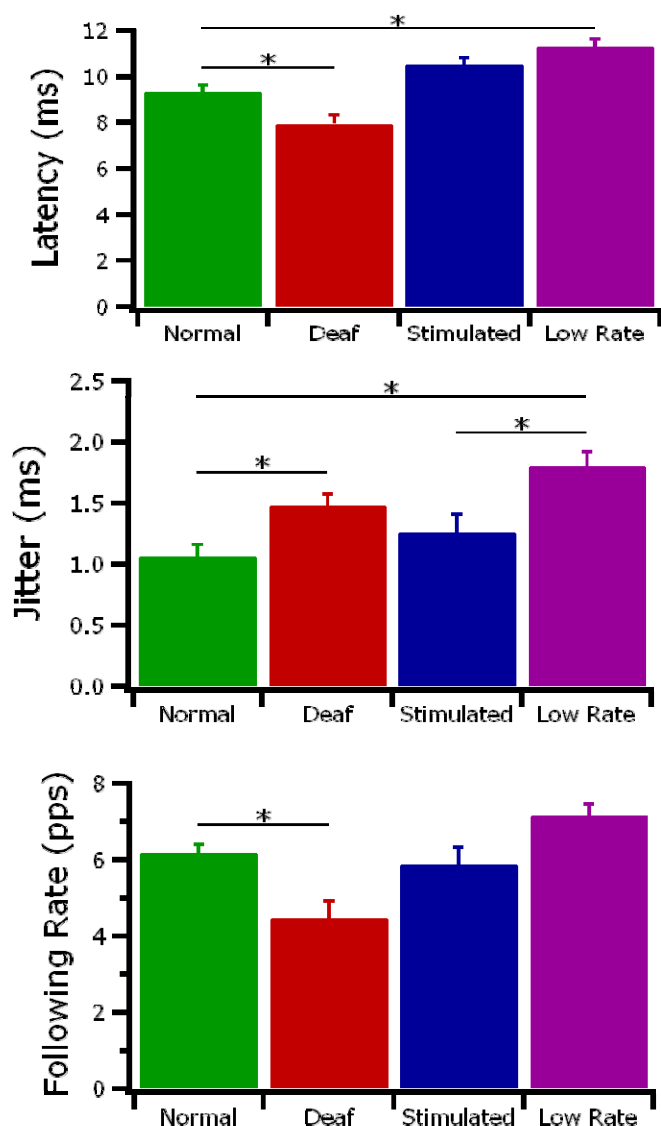
As well as providing an additional species to study the effect of ICES on neural survival, the rat provides a useful model to study the effects of temporally challenging ICES on the adult deafened auditory pathway. The small size of the rat cochlea limits the number of intra-cochlea electrodes that can 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.

During this quarter we finalized our acute multi-channel electrophysiological recording and analysis techniques in the inferior colliculus, and are currently extending our approach to include simultaneous recording in the auditory cortex, details of which will be presented in a following quarter. We have also implanted one normal-hearing animal with a chronic epidural recording electrode, placed over the auditory cortex, to facilitate awake evoked potential recordings. We are currently investigating the use of the Middle Latency and Mismatch Negativity Response in tracking the time course of any plasticity induced by chronic ICES in long-term deafened animals.

### **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 environmentally derived chronic ICES can exert a plastic influence on the developing central 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 to run the five animals in our late intervention cohort, all have begun their stimulation regime (500 pps/electrode, monopolar stimulation on all 7 intracochlear electrodes using the SPEAK<sup>®</sup> speech processing strategy) at approximately 8 months of age. Three acute electrophysiological experiments were performed this quarter (two late intervention animals and a normal hearing aged matched control). Analysis of existing AI data for the standard rate and low-rate stimulation groups is underway and will be reported in detail in a following quarter. Preliminary analysis of the 'temporal processing' data (latency, jitter and maximum following rate) is complete and is presented in Figure 1. Chronic environmentally derived ICES at 500 pps/electrode, but not 50 pps/electrode, was able to completely reverse the effects of long-term deafness. Following the completion of the acute electrophysiological experiments, the cochleae and CNS were harvested and prepared for subsequent analysis.



**Figure 1** Changes in the temporal processing of the primary auditory cortex after long-term deafness (Deaf), long-term deafness and chronic ICES (Stimulated) and long-term deafness and low-rate (50 pps/electrode) chronic ICES (Low Rate). \*  $p < 0.05$  (One-way ANOVA).

We have also begun to develop techniques for chronic recording of electrically evoked potentials in awake animals using indwelling electrodes and the telemetry capabilities of the clinical cochlear implants. We have therefore begun development of a modified electrode assembly to allow both stimulating and recording.

### 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 incorporation of NTs into a material that has been designed for implantation in to the cochlea, and engineered for optimal NT release kinetics would offer a potentially elegant solution.

We have [previously reported](#) a collaboration with Prof. F. Caruso and Dr. Y. Wang from the University of Melbourne resulting in the successful encapsulation of NTs in nanoparticles produced using the layer-by-layer technique. These nanoparticles have been shown to deliver biologically active NTs *in vitro* for periods of in excess of 30 days. We have unilaterally injected BDNF-encapsulated nanoparticles into the inner ear of rats 3 weeks after aminoglycosides-induced deafness, with the contralateral ear serving as a control. Two animals have successfully completed a 2 month post-surgery period and an example of the cochlea histology is shown in Figure 2. Additional animals will be implanted in a following quarter and the subsequent analysis of the histological data will be reported.

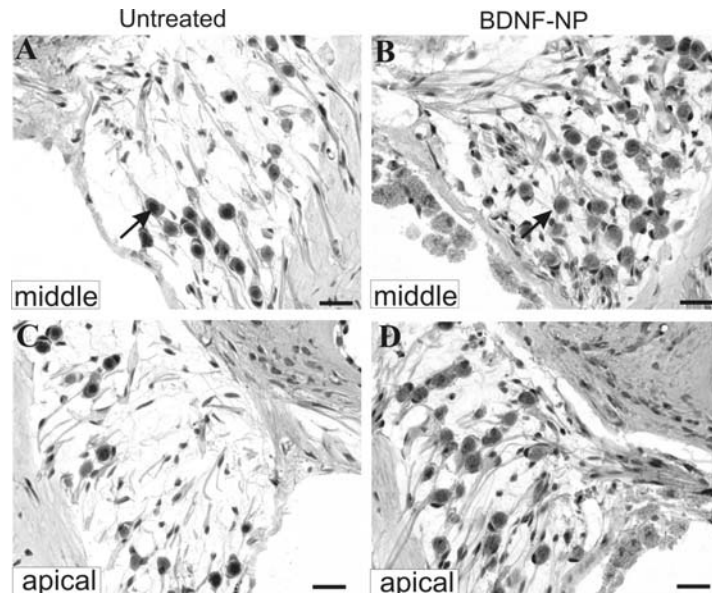
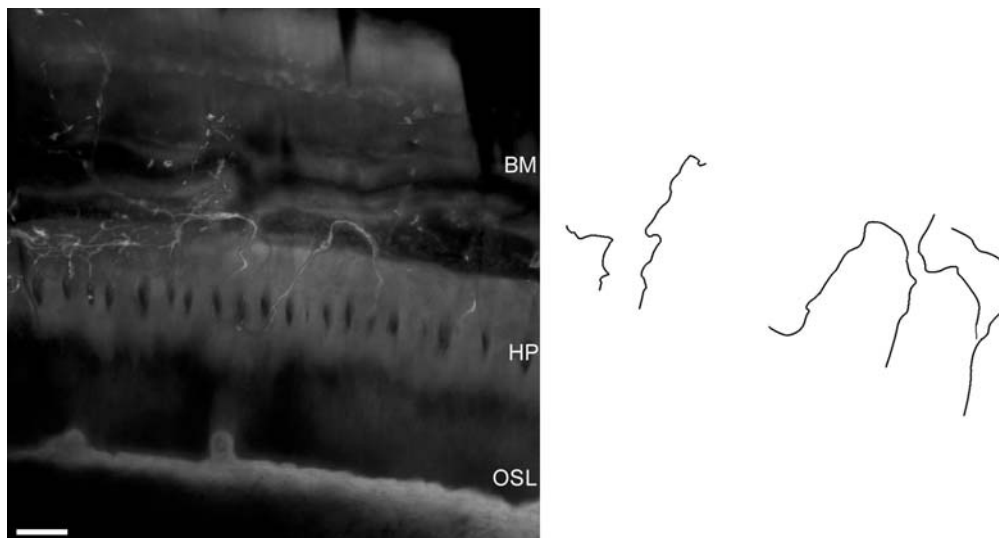


Figure 2 Increased numbers of primary auditory neurons (arrows) can be seen in both the middle (B) and apical turns (D) 2 months following delivery of BDNF-encapsulated nanoparticles (BDNF-NP), compared to the untreated contralateral ear (A, middle; C, apical). Scale bar = 20  $\mu$ m

### 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.

During this quarter all remaining treatment groups finished their treatment periods and acute experiments were performed on fourteen treated animals and two normal hearing controls. Data from the electrophysiological experiments is currently being analyzed and will be presented in a following quarter. We have also continued cochlea tissue processing and histological data collection including surface preparation confocal images (Figure 3). Detailed analysis of the histological data will be reported in a following quarter.



**Figure 3.** A collapsed confocal image of the basal turn surface preparation of a neurotrophin/chronic ICES treated guinea pig is shown on the left. SGN peripheral fibers that have been labeled by biotinylated dextran amine injected into the auditory nerve are visible, as well as structural features. An example of tracing of labeled fibers are shown on the right. BM = basilar membrane, HP = Habenula Perforata, OSL = osseous spiral lamina. Scale bar = 20 $\mu$ m.

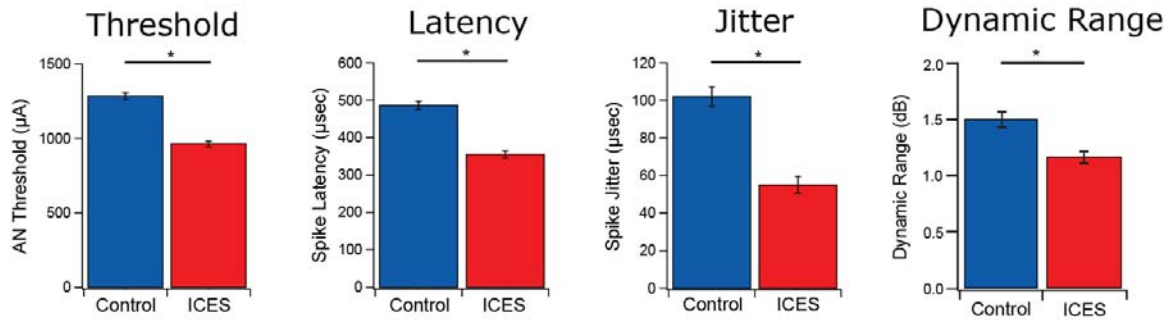
During this quarter we continued to develop the protocols for tissue processing and image analysis that will be used in our study of the ultrastructural changes to the cells in the cochlea as a result of chronic neurotrophin administration and electrical stimulation. We are working with Professor Remy Pujol (Universite de Montpellier, France) on this aspect of project and will continue to collect control cochleae tissue in the following quarter.

### 2.3.3. Cat

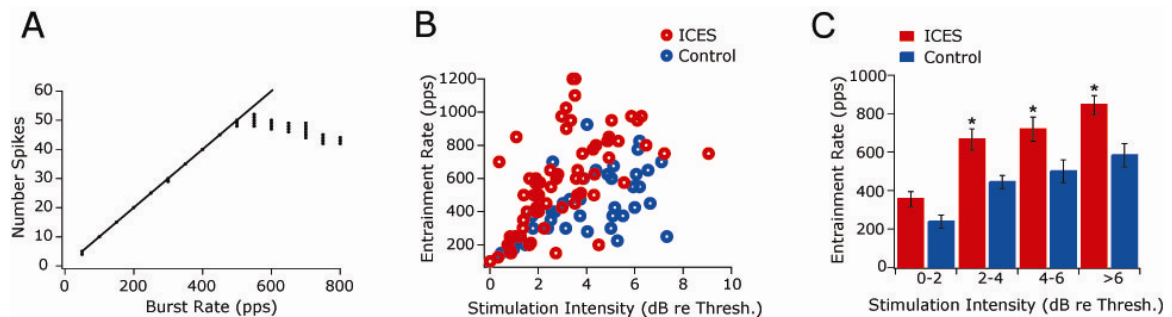
It is well established that ICES and NT delivery can promote SGN survival over periods of up to one month; however, from a clinical perspective it is important to examine the effects of long term ICES and NT delivery. Therefore, we are using [LCT Pty Ltd's](#) NT-cell<sup>®</sup> - a porcine derived 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.

During this quarter we continued the histological analysis and quantification of SGNs from all of the experimental cohorts (normal, ICES only, NT only and ICES+NT). A detailed analysis of this data will be presented in a following quarter.

We have completed the acute electrophysiology experiments and analyzed a subset of the data. In these experiments we are interested in the capacity of single auditory neurons (ANs) to encode spatial and temporal information from a multichannel intracochlear electrode array and how deafness and chronic ICES might affect AN function. Some of the results from these experiments are shown below. These data are now being prepared for publication.



**Figure 4** Basic Response Properties Data was collected from a total of 405 ANs. Input-Output functions were generated for each AN to determine threshold - the current required for 0.5 firing probability; spike latency – the interval between the electrical stimuli and the spike; jitter – the standard deviation of the latency; and the dynamic range - the current range required for firing probabilities of 0.1 to 0.9. In response to monopolar electrical stimulation thresholds, latency, jitter and dynamic range were significantly lower in ICES cats compared to control cats ( $p < 0.001$ ).



**Figure 5** Maximum Entrainment Rate (A) AN entrainment was examined by measuring responses to electrical stimulation at rates up to 1.2kpps. The maximum entrainment rate was defined as the maximum rate of stimulation that the AN could respond with 1:1 firing; 500pps in example (A). A scatter plot (B) and summary data (C) from 107 ANs is shown. ANs from the ICES group (red) could entrain at significantly higher stimulation rates than ANs in the Control group (blue) (ANOVA  $P < 0.001$ ).

### **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.
- k) Continued development of techniques to trace single SGN peripheral fibers.
- l) Continued development of protocols for TEM examination of cochlea tissue.

### **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 VIIIth 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.