

# **Ninth 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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## 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 conference abstracts

The following papers on work based on this contract were accepted for publication.

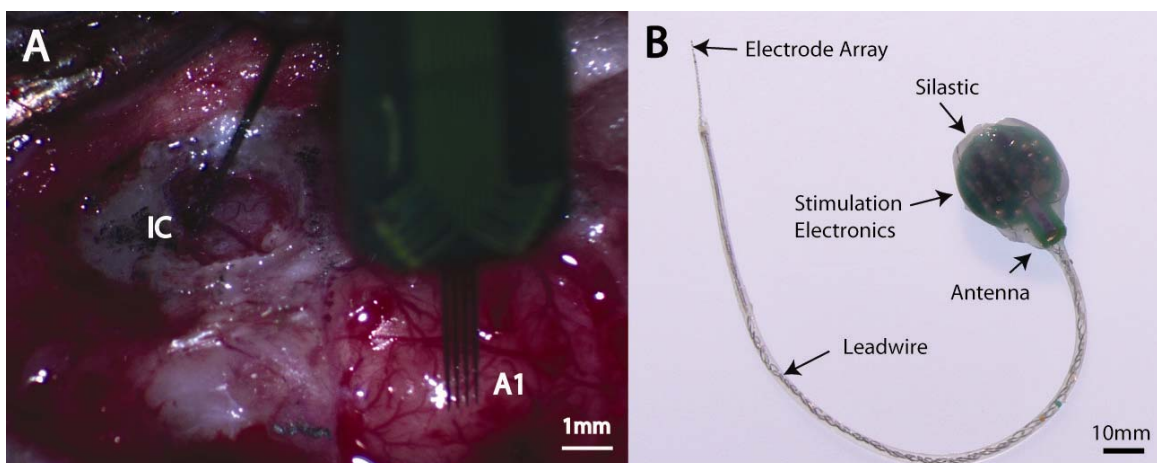
Ryugo, D.K., Baker, C.A., Montey, K.M., Chang, L., Coco, A., Fallon, J.B. & Shepherd, R.K. Synaptic Plasticity after Chemical Deafening and Electrical Stimulation of the Auditory Nerve in Cats. *J. Comp. Neurol* (in press; accepted October 2009). Attached as Appendix A.

### 2.2 Chronic intracochlear electrical stimulation

#### 2.2.1 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 continued to develop our acute multi-channel electrophysiological recording and analysis techniques in the inferior colliculus (IC) and auditory cortex (AI). We have successfully recorded in both regions using multi-channel silicon probes (Neuronexus) using acoustic stimulation, and have completed our first long-term deafened control using ICES (Figure 1A).



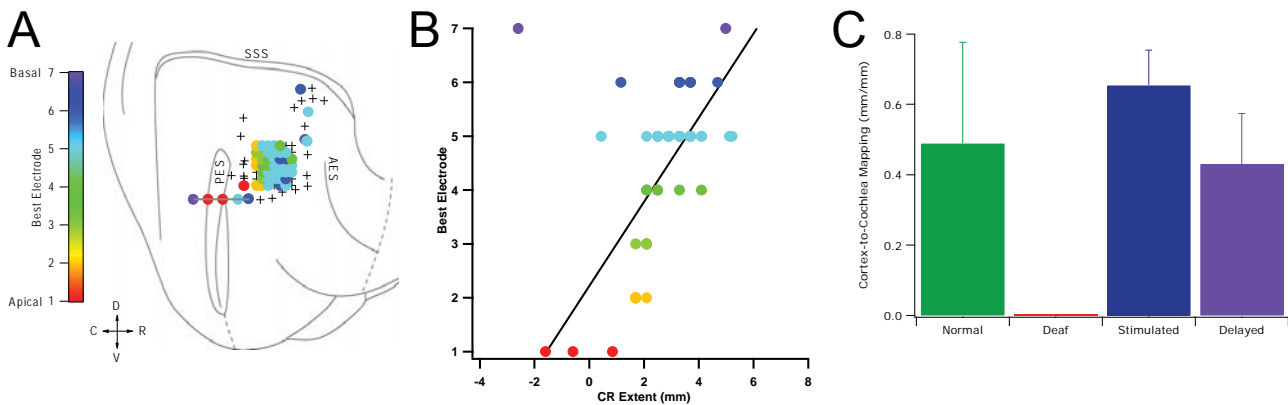
**Figure 1 (A).** Photo showing multi-channel recording setup, using a single shank 32 site probe to reach the IC through the occipital lobe, and four shank 32 site probe inserted along the surface of AI. **(B).** Photo of implant.

We have developed a new fully implantable stimulator (Figure 1B), based upon our earlier design (Millard and Shepherd 2007), which includes two channels of stimulation and adjustable current. Following *in vitro* trials of our updated implant, which showed that the implants could survive in excess of three months immersed in saline, we implanted two Hooded Wistar rats (F, ~250g). Both recovered well, and showed a clear behavioral response to stimulation. Furthermore, the implant was reliable throughout animal movement. However, both implants began to fail after one month, with examination of the implant showing damage due to water ingress. We are currently refining our encapsulation procedure (including curing times and materials) to extend the implant lifespan.

### 2.2.2 Cat

This work addresses 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 (e.g. Fallon *et al.*, 2009); 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 analyzed data from our later intervention cohort. Animals had been deafened as neonates and implanted at two months of age, but did not have their implants activated until 8 months of age. Our previous results have shown that following eight months of profound deafness, the cochleotopic organization of AI is completely scrambled (Fallon *et al.*, 2009). However, in animals that received six months of chronic ICES commencing at this age, the cochleotopic organization of AI appeared near-normal in all animals tested (Figure 2).



**Figure 2** Representative “best electrode” maps of AI in an animal that received delayed chronic ICES (A, B). Filled symbols represent the best electrode at each recording site. Crosses indicate locations for which it was not possible to elicit a response to electrical stimulation. Solid gray line indicate recordings made down the rostral bank of PES. SSS, suprasylvian sulcus; PES, posterior ectosylvian sulcus; AES, anterior ectosylvian sulcus; DVCR indicator 2 mm bar lengths; D, dorsal; V, ventral; C, caudal; R, rostral; CR Extent, caudorostral extent. There was no statistical difference (ANOVA,  $P > 0.5$ ) in the cortex-to-cochlea mapping (C) seen in normal hearing and long-term deaf, chronic ICES animals, regardless of whether the chronic ICES was initiated immediately, or after a delay of 6 months.

During this quarter we completed our first normal hearing animal with a chronic cortical recording array. We are using the same multichannel recording array as we use for our acute experiments, but with a [smaller connector](#) based on an Omnetics range of connectors. An acute electrophysiology experiment was carried out and the animal sacrificed to examine the effects of the chronic implantation on the auditory cortex using a range of histological techniques. Analysis of the histological data, along with analysis of the response data, will be presented in the following quarters.

We are also continuing to develop the 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 developed a modified electrode assembly to allow both stimulating and recording. We have implanted five neonatally deafened cats and have been able to record evoked potentials using our new chronic recording system. These animals are progressing well and due completion in Quarter 11.

## **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 into the cochlea, and engineered for optimal NT release kinetics would offer a potentially elegant solution.

In the previous quarter we examined the osseous spiral lamina (OSL) which contains projecting fibers from the primary auditory neurons to the scar previously occupied by the organ of Corti. We found stronger TrkB immuno-staining in fibers within the OSL from BDNF-treated cochlea compared to untreated, contralateral control. TrkB expression in the OSL decreases after aminoglycoside-induced deafening due to a retraction of fibers (Tan and Shepherd, 2006). The maintenance of TrkB expression in the peripheral fibers receiving BDNF-encapsulated nanoparticles implies a protective effect of these nanoparticles on the degenerating primary auditory neurons.

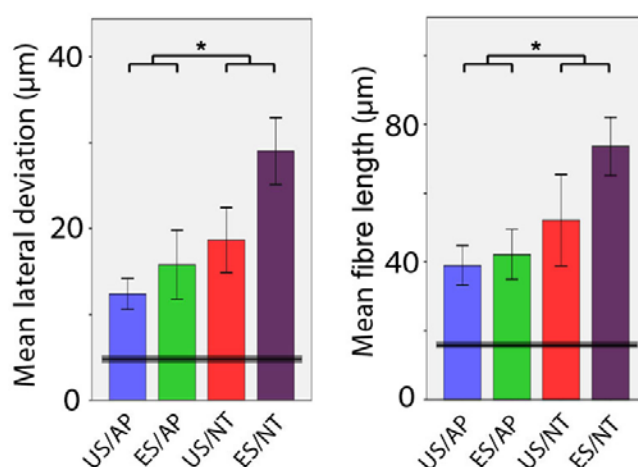
We have continued to analyze the cochlear tissue and have found greater spiral ganglion neuron survival in the cochlea receiving BDNF-encapsulated nanoparticles. Spiral ganglion neuron counts were taken from 4 sections separated by at least 60  $\mu\text{m}$ . In the cochlea receiving BDNF-encapsulated nanoparticles, we counted 1171 neurons/ $\text{mm}^2$  in the upper middle turn and 1188 neurons/ $\text{mm}^2$  in the lower middle turn. In the untreated cochlea, we counted 1081 neurons/ $\text{mm}^2$  in the upper middle turn and 957 neurons/ $\text{mm}^2$  in the lower middle turn. These experiments will be repeated in the following quarter to obtain sufficient numbers for statistical analysis.

### 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 imaging of cochlear surface preparations and tracing of the SGN peripheral fibers was completed in this quarter. Analysis of SGN peripheral fiber spatial deviation was also completed (using methods detailed in [QPR8](#)). Other measures being gathered are the radial deviation, absolute fiber length from the habenula perforata, a qualitative assessment of the directionality (e.g. towards the spiral limbus), and the location within the basal half-turn for each fiber (i.e. basal, middle, or apical aspect).

The tracing data show a significant increase in both the lateral deviation (maximum distance travelled towards the cochlear apex and base) of fibers and in the absolute length from the habenula perforata following neurotrophin treatment (two-way ANOVA, NT main effect  $p < 0.02$ ; Figure 3). This is in contrast to recordings of the spatial activation patterns in the central nucleus of the inferior colliculus to ICES reported in [QPR8](#) that did not show a comparative increase in representation following neurotrophin treatment, although chronic ICES caused increased representation near activation threshold.



**Figure 3** Mean lateral deviation and fiber length (from habenula perforata) for each treatment group. Neurotrophin treatment (NT) had a significant main effect on both measures (two-way ANOVA,  $p < 0.02$ ). Chronic ICES (ES) and interaction effects were not significant. Means for normal hearing fibers are shown as a black line for comparison. Error bars and gray shading  $\pm 1$  S.E.M.

### 2.3.3 Transmission Electron Microscopy

In this project we are collaborating with Professor Remy Pujol (Universite de Montpellier, France) to examine the ultrastructural changes to SGNs and glial cells following chronic ICES with or without NT delivery. In previous quarters we have developed the protocols for tissue processing and image analysis for this project.

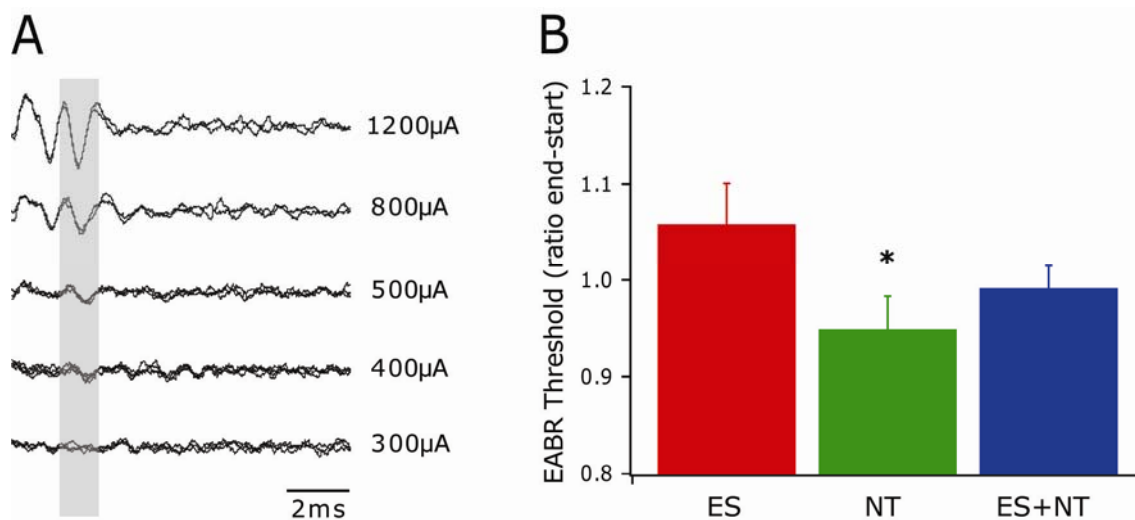
In this quarter we deafened and implanted the guinea pigs for this study. The animals were deafened with our standard aminoglycoside/loop diuretic deafening procedure and two weeks later were implanted with a multichannel cochlear implant containing a drug delivery canula that was attached to a miniosmotic pump (Shepherd and Xu 2002). The pump contained either artificial perilymph (AP) or the neurotrophin BDNF. Clinical speech processors and stimulators were used to deliver chronic intracochlear electrical stimulation (ICES) for a four week period. Five experimental cohorts are being used; i) ICES + BDNF, ii) ICES + AP, iii) BDNF without ICES, iv) AP without ICES, v) normal hearing (unimplanted). The cochleae have been collected and are undergoing TEM processing. We will continue with the processing and imaging of the TEM material in the following quarters.

### 2.3.4 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 assess 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.

Our previous results have shown that cell-based NT delivery can promote SGN survival and that protection can be enhanced with chronic intracochlear electrical stimulation. Although, it must be noted that to date our analysis has been restricted to SGN survival in the upper basal turn. The region of the cochlear most likely to receive maximum benefit from the treatment as it is proximal to both the stimulating electrode array and the NT-Cells.

In this quarter we examined the EABR data that was collected over the 6-month treatment period for these animals. Changes in EABR thresholds over the treatment period were compared for the three experimental cohorts in order to determine whether treatment with encapsulated NT-Cells in combination with a cochlear implant lead to any adverse effects on the electrical thresholds. There was no increase in electrical thresholds over the treatment period, in fact, there was a small but significant decrease with NT-cell treatment (Figure 4).



**Figure 4** Changes in EABR thresholds were calculated by comparing thresholds measured at the end to those at the start of the six month treatment period. (A) Example EABR traces showing the wave III response (grey shaded region) over a range of stimulation intensities (1200µA to 300µA). In this example threshold was 400µA. (B) There was no change in EABR threshold over the treatment period for the ES and the ES+NT groups. A small but significant decrease in EABR threshold (6%) was observed for the NT group (ANOVA,  $P < 0.05$ ).

## **2.4 Collaborations**

In addition to our own direct work on projects during this quarter we have also collaborated with a number of other researchers, including:

- a) Dr. Doug Hartley from Oxford University; with advice for chronic bilateral cochlear implants in ferrets
- b) Dr. Ruth Litovsky and Prof. Tom Yin from University of Wisconsin; with advice, hardware and software for chronic bilateral cochlear implants in cats
- c) Prof. Stephen O'Leary from the University of Melbourne; with software for ABR analysis
- d) Dr. Saparna Pai from the Centenary Institute, Australia with advice on cortical recordings
- e) Dr Ian Bruce from Department of Electrical and Computer Engineering, McMaster University, Canada,; with auditory nerve data for modelling

## **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) Continue chronic ICES programs in deafened/implanted cats.
- f) Continue chronic recording experiments.
- g) Analysis of data from the deafened, chronically stimulated cats, including acute electrophysiological data.
- h) 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).
- i) Continued development and testing of nanoparticles.
- j) Continued tracing single SGN peripheral fibres and quantification of the data.
- k) Continued collection of cochlea tissue for TEM examination.
- l) Imaging of TEM tissue and establishing protocols for quantification.

#### **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.

#### **5. References**

Fallon, J.B., Irvine, D.R.F. and Shepherd, R.K. 2009. Cochlear implant use following neonatal deafness influences the cochleotopic organization of the primary auditory cortex in cats. *Journal of Comparative Neurology*: 512, 101-114.

Millard, R. E. and R. K. Shepherd (2007). "A fully implantable stimulator for use in small laboratory animals." *J Neurosci Methods* 166: 168-177.

Shepherd, R. K. and J. Xu (2002). "A multichannel scala tympani electrode array incorporating a drug delivery system for chronic intracochlear infusion." *Hear Res* 172: 92-98.

#### **6. Appendix A (attached)**

Ryugo, D.K., Baker, C.A., Montey, K.M., Chang, L., Coco, A., Fallon, J.B. & Shepherd, R.K. Synaptic Plasticity after Chemical Deafening and Electrical Stimulation of the Auditory Nerve in Cats. *J. Comp. Neurol* (in press; accepted October 2009).