

Fourth Quarterly Progress Report

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



The Bionic Ear Institute

J.B. Fallon, F. Glynn, J. Tan, T.G. Landry, D.W.J. Perry, A.K. Wise, F. Caruso and  
Y. Wang J. Xu, A.J. Evans, M. Ulaganathan, M. Giummarra, M. Trotter, J. Andrew,  
A. Coco, D.R.F. Irvine, R.E. Millard and R.K. Shepherd

**The Bionic Ear Institute**  
**East Melbourne, Victoria 3002, AUSTRALIA**  
*Affiliated with The University of Melbourne*

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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 papers were accepted for publication.

Backhouse, S., Coleman, B. and Shepherd, R. in press. Surgical access to the mammalian cochlea for cell-based therapies. *Experimental Neurology*. Attached as Appendix A.

Coleman, B., Rickard, N.A., de Silva, M.G. and Shepherd, R.K. in press. A protocol for cryoembedding the adult guinea pig cochlea for fluorescence immunohistology. *Journal of Neuroscience Methods*. Attached as Appendix B.

Fallon, J.B., Irvine, D.R.F. and Shepherd, R.K. in press. Cochlear implant use following neonatal deafness influences the cochleotopic organization of the primary auditory cortex in cats. *Journal of Comparative Neurology*. Attached as Appendix C.

The following papers were presented during the quarter and the abstracts are attached as Appendix D.

Fallon, J.B., Irvine, D.R.F. and Shepherd, R.K. Changes in the cochleotopic organization of primary auditory cortex resulting from chronic deafness and cochlear implantation. *Proceedings of the 2nd Queensland Brain Institute, Brain Plasticity Symposium*. Brisbane, Queensland, Australia, 9 – 12 September, 2008.

Hartley, D.E.H., Isaiah, A., Schnupp, J.W.H., Dahmen, J.C., Fallon, J.B., Shepherd, R.K. and King, A.J. Potential benefits of half-wave rectified stimuli to individuals with bilateral cochlear implants. *Proceedings of the British Society of Audiology Meeting*. University of Nottingham, 9-11 September 2008.

## 2.2. Chronic intracochlear electrical stimulation

### 2.2.1. Mouse

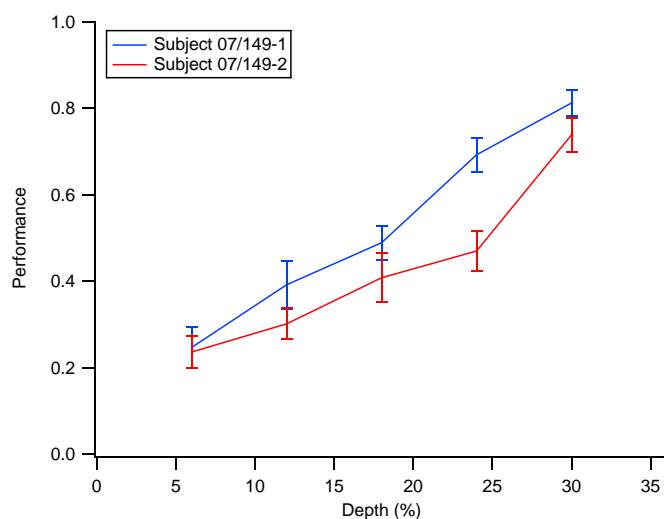
Mutations in specific genes account for approximately 50% of childhood deafness. In the past decade, deafness genes in mouse mutants have been identified, providing a platform to study the mechanisms of genetically based deafness in humans. We are seeking to determine whether the auditory systems of 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.

Two animals successfully completed their chronic stimulation program (~ 6 dB above magnetically driven, electrically evoked auditory brainstem response (mEABR) threshold; 200 pps; 4 hours/day). The tissue from these animals along with the other implanted animals was processed 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 developed and testing of [conditioned avoidance procedures](#) for testing psychophysical thresholds in chronically implanted adult rats was concluded this quarter. Two normal hearing animals were tested on an acoustic AM detection task until their performance had stabilized, they were tested for 15 days (not all consecutive), using a descending series of five depths repeated five times. The average performance level for each animal is shown in Figure 1.



**Figure 1 AM detection thresholds**

Average performance level over 15 days (75 warning trials) for two normal hearing animals performing an acoustic AM detection task. Error bars =  $\pm$ SEM.

The average threshold (i.e. modulation depth giving 50% performance) for subject 07/149-1 was  $18.3 \pm 6.3$  %, and  $24.7 \pm 7.5$  %, for 07/149-2 (95% C.I.). The threshold for the first subject is within the range reported by previous studies, whilst the threshold for the second subject is slightly above what has been previously reported (Kelly, Cooke et al. 2006; Cooke, Zhang et al. 2007). We are confident that these results are valid for these particular animals, as we were unable to detect any significant trend in performance at any depth over the 15 day period (linear regression;  $p > 0.05$ ), or between 5 day periods (one-way ANOVA;  $p > 0.05$ ).

Work is also continuing on a means to provide behaviorally relevant ICES to chronically implanted rats. The enclosure used to house the animals during stimulation is relatively small, which places unique constraints upon the sorts of tasks that may be undertaken while inside. We are currently investigating a simple operant conditioning task (for a preferred food reward) to provide a degree of behavioral relevance to the chronic stimulation and have begun to collect movement / activity data for normal hearing rats placed in the chronic stimulation enclosure.

### **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.

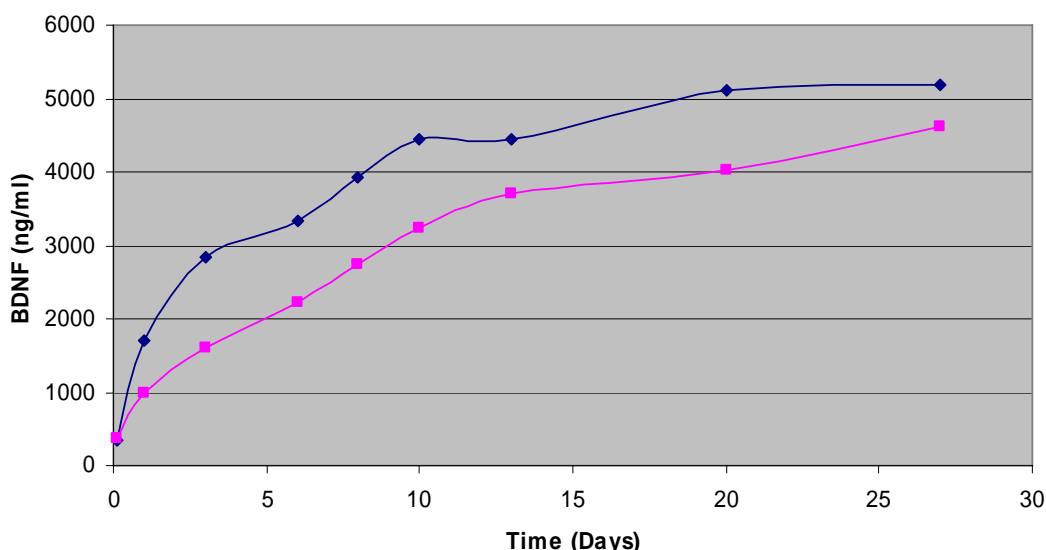
During this quarter, we implanted three neonatally deafened animals with our standard intracochlear electrode arrays and extracochlear ball electrode. Currently, one animal is receiving low-rate (50 pps/electrode) monopolar stimulation on all 7 intracochlear electrodes using the SPEAK<sup>®</sup> speech processing strategy. Five animals are in our late intervention cohort, two 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. One acute electrophysiological experiment was performed this quarter on the first of our late intervention cohort. Analysis of the data from this animal is underway. 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.

This quarter we began the development of bio-compatible bio-degradable nanoparticles containing BDNF which will be capable of delivering a sustained slow release of neurotrophin to the cochlea. These nanoparticles released measureable amounts of BDNF over a period of a month (Figure 2).



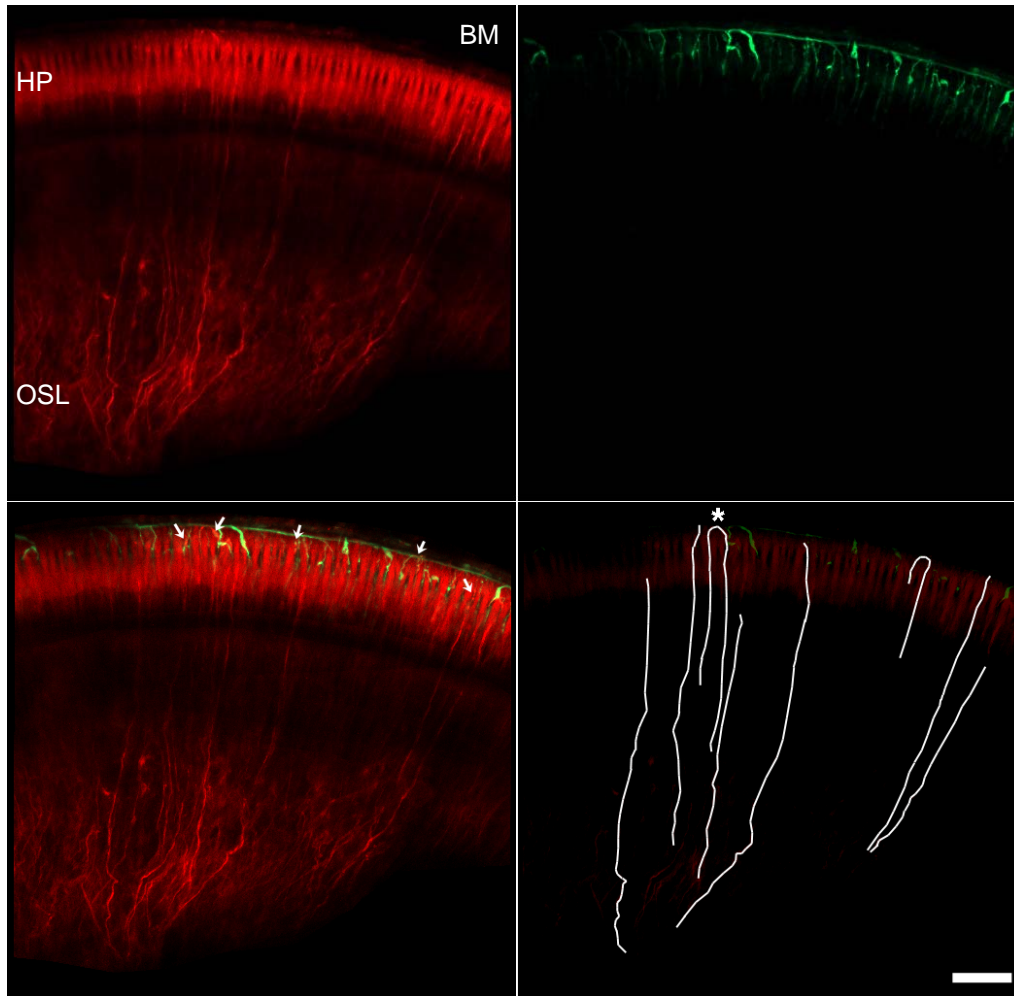
**Figure 2 Cumulative BDNF release**

BDNF release was measured by repeated sampling of the supernatant of a solution of nanoparticles followed by an enzyme-linked immuno-adsorbant assay (ELISA). Data for repeated preparations of the nano-particles (two lines) indicated the methods produce consistent release kinetics.

### 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, four deafened animals were implanted with an intra-cochlear electrode array and drug delivery system. Following implantation, two animals received chronic ICES, with the other two animals serving as unstimulated controls. Acute electrophysiological experiments were performed on three of the chronically treated animals and an additional three normal hearing controls. During these experiments biotinylated dextran amine (BDA) or tetramethylrhodamine dextran (TMRD) was injected into the auditory nerve in order to retrogradely trace peripheral processes within the osseous spiral lamina following long-term deafness with or without chronic treatment (Figure 3).



**Figure 3 Surface preparation of the basal turn of a guinea pig that has been deaf for 6 weeks viewed using confocal microscopy.**

The red channel (top left) shows the injected neural tracer TMRD in some SGN peripheral fibers. The green channel (top right) shows immunolabelled neurofilament protein (200kDa), which typically only labels the unmyelinated distal portion of the fibers. The bottom left merged image shows double labeling (white arrows) in fibers that were infiltrated by TMRD. The bottom right image shows SGN fiber tracings. The asterisk indicates a long looped tracing that is most likely two separate fibers that contacted each other in the periphery. Viewing and manipulating reconstructed optical slices in three dimensions was instrumental in identifying fiber continuity within the high background fluorescence level. Scale bar = 50µm; BM = basilar membrane; HP = Habenula Perforata; OSL = osseous spiral lamina.

### 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 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.

This quarter our second (capsules containing NT-cells<sup>®</sup>; n = 6) and third (capsules containing NT-cells<sup>®</sup> and chronic ICES; n = 6) cohorts of animals continued their chronic treatment programs. These animals are progressing well and we monitor the electrode impedance daily. We record evoked activity from the auditory nerve (neural response telemetry - NRT) fortnightly and electrically evoked brainstem responses (EABRs) monthly.

Analysis of the data from the acute electrophysiology experiments that have already been completed continues, and a detailed analysis of this data will be reported on in the coming quarters. Histological processing of the cochlea, using our recently developed [cryosectioning techniques](#), from our first cohort of ICES with empty capsules continues. A complete data set will not be available until the cochleae from the current cohorts of animals have also been processed so a detailed analysis of this data will be reported on in the coming quarters.

### **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.
- d) Further refinements to both the electrode assembly and fixation of the leadwire and stimulator assembly for use in the mouse studies.
- e) Implant additional animals for ICES studies in the cat.
- f) Continue chronic ICES programs in deafened/implanted cats.
- 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 nano-particles.
- j) Continued development of techniques to trace single SGN peripheral fibers.
- k) Acute experiments on chronically deaf guinea pigs and the initiation of the treatment and control groups for neurotrophin treatment and chronic ICES.

#### **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<sup>th</sup> 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 VIII<sup>th</sup> nerve/cochlear nucleus synapse.

#### **5. References**

Cooke, J. E., H. Zhang, et al. (2007). "Detection of sinusoidal amplitude modulated sounds: deficits after bilateral lesions of auditory cortex in the rat." Hear Res **231**(1-2): 90-9.

Kelly, J. B., J. E. Cooke, et al. (2006). "Behavioral limits of auditory temporal resolution in the rat: amplitude modulation and duration discrimination." J Comp Psychol **120**(2): 98-105.

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

Backhouse, S., Coleman, B. and Shepherd, R. in press. Surgical access to the mammalian cochlea for cell-based therapies. *Experimental Neurology*.

#### **7. Appendix B (attached)**

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#### **9. Appendix D (attached)**

Fallon, J.B., Irvine, D.R.F. and Shepherd, R.K. Changes in the cochleotopic organization of primary auditory cortex resulting from chronic deafness and cochlear implantation. *Proceedings of the 2nd Queensland Brain Institute, Brain Plasticity Symposium*. Brisbane, Queensland, Australia, 9 – 12 September, 2008.

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