

Second 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 conferences

The following papers were accepted for publication.

Heffer, L.F. and Fallon, J.B. 2008. A novel stimulus artifact removal technique for high-rate electrical stimulation. *Journal of Neuroscience Methods* 170, 277-84. Attached as Appendix A.

The following papers were presented during the quarter and the abstracts, where available, are attached as Appendix B.

Fallon, J.B., Wise, A.K. and Shepherd, R.K. Factors affecting neural response telemetry recordings in the chronically stimulated cat. *Proceedings of the Thirty-First Annual Midwinter Research Meeting of the Association for Research in Otolaryngology* Phoenix, Arizona, USA, 16-21 February.

Hartley, D., Isaiah, A., Schnupp, J., Dahmen, J., Fallon, J.B., Shepherd, R.K. and King, A. Sensitivity to interaural time delays in the auditory cortex of ferrets: Investigating potential benefits of half-wave rectified stimuli to individuals with bilateral cochlear implants. *Proceedings of the Thirty-First Annual Midwinter Research Meeting of the Association for Research in Otolaryngology* Phoenix, Arizona, USA, 16-21 February.

Heffer, L.F. and Fallon, J.B. A novel stimulus artifact removal technique for high-rate electrical stimulation. *Proceedings of the Australia Neuroscience Society Inc., 28th Annual Meeting* Hobart, Tasmania, Australia, 27-30 January 2008.

Heffer, L.F. and Fallon, J.B. A novel stimulus artifact removal technique for high-rate electrical stimulation. *Proceedings of the 5th Australasian Auditory Neuroscience Workshop* Hobart, Australia.

Landry, T.G., Wise, A.K., Fallon, J.B. and Shepherd, R.K. Functional effects of exogenous neurotrophins in the deafened cochlea. *Proceedings of the 5th Australasian Auditory Neuroscience Workshop* Hobart, Australia.

Perry, D.W.J., Fallon, J.B., Grayden, D.B., Millard, R.E. and Shepherd, R.K. Research cochlear implant for small laboratory animals. *Proceedings of the Australia Neuroscience Society Inc., 28th Annual Meeting* Hobart, Tasmania, Australia, 27-30 January 2008.

Perry, D.W.J., Fallon, J.B., Grayden, D.B., Millard, R. and Shepherd, R.K. Research cochlear implant for small laboratory animals. *Proceedings of the 5th Australasian Auditory Neuroscience Workshop* Hobart, Australia.

Shepherd, R.K., Epp, S.B. and Coco, A. Electrical stimulation maintains spiral ganglion neurons following removal of exogenous neurotrophins. *Proceedings of the Australia Neuroscience Society Inc., 28th Annual Meeting* Hobart, Tasmania, Australia, 27-30 January 2008.

Shepherd, R.K., Coco, A., Andrew, J., Wise, A.K. and Pettingill, L.N. Delivery strategies for neurotrophin delivery into the inner ear for SGN protection following deafness. *Proceedings of the Thirty-First Annual Midwinter Research Meeting of the Association for Research in Otolaryngology* Phoenix, Arizona, USA, 16-21 February.

Tan, J., Widjaja, S., Xu, J. and Shepherd, R.K. Cochlear implants stimulate activity-dependent CREB pathway in the deaf auditory cortex: Implications for molecular plasticity induced by neural prosthetic devices. *Proceedings of the Thirty-First Annual Midwinter Research Meeting of the Association for Research in Otolaryngology* Phoenix, Arizona, USA, 16-21 February.

Wimberley, C.J., Fallon, J.B., Irvine, D.R.F. and Shepherd, R.K. Cochleotopic organisation of the central auditory pathway in the neonatally deafened cat. *Proceedings of the Australia Neuroscience Society Inc., 28th Annual Meeting* Hobart, Tasmania, Australia, 27-30 January 2008.

Wise, A.K., Fallon, J.B., Heasman, J.M. and Shepherd, R.K. Factors affecting neural response telemetry recordings in the chronically stimulated cat. *Proceedings of the Australia Neuroscience Society Inc., 28th Annual Meeting* Hobart, Tasmania, Australia, 27-30 January 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.

Due to the small size of the mouse, significant modifications to our standard animal electrode assembly and implantation techniques have been required (described in [HHS-N-263-2007-0053-CQPR1](#)). This quarter seven mice were implanted with our new mouse stimulator assemblies, and we were able to successfully record magnetically driven, electrically evoked auditory brainstem responses (mEABRs) from two of these animals (Figure 1). Further refinements to both the electrode assembly and fixation of the leadwire and stimulator assembly will continue in the coming quarter. The two animals with mEABRs and addition animal that we could not record a mEABR from then received four weeks of chronic stimulation (~ 6 dB above mEABR threshold; 200 pps; 4 hours/day) before being sacrificed. The tissue from these animals along with the other implanted animals will undergo histological analysis in the coming quarters.

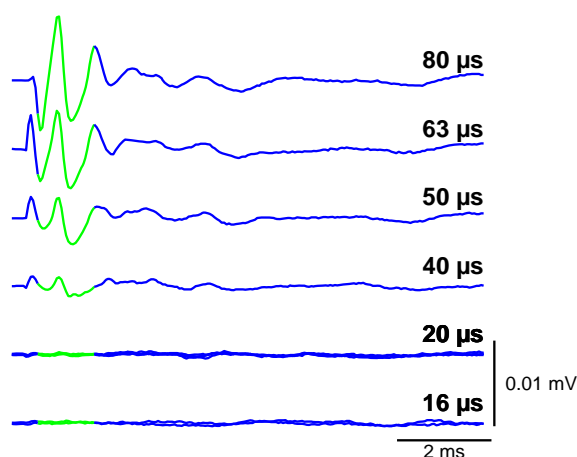


Figure 1 mEABRs recorded from a chronically implanted mouse.

A mEABR series recorded from a chronically implanted mouse. Green region shows the mEABR wave III used for determination of threshold. In this example, mEABR threshold was ~ 20 μ s.

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.

In our previous contract ([N01-DC-3-1005](#)) we attempted to assess the discriminative capacities of chronically deaf rats to electrical stimulation using a modified T-maze task. Whilst the animals were able to discriminate pulse trains of different durations, they struggled with more challenging discriminations like pulse rate. We believe this reflects the use of an appetitive procedure, which limited animal motivation. As such, we are currently developing a conditioned avoidance procedure, where a combination of appetitive (water delivery) and aversive (a mild shock) manipulations can be used to optimize performance. This approach has been used successfully by other groups in implanted cats (e.g. Vollmer et al., 2001). An experimental apparatus has been constructed in a small self-contained test chamber (Figure 2). The apparatus is currently being validated with normal hearing animals to obtain behavioral audiograms, and later, gap and amplitude modulation (AM) detection thresholds. Once we are confident that the apparatus and training procedure is effective, we will begin testing with chronically implanted animals. An initial pilot study will examine AM and gap detection, measures correlated with speech perception in implanted humans (Busby and Clark, 1999; Fu and Shannon, 2002).

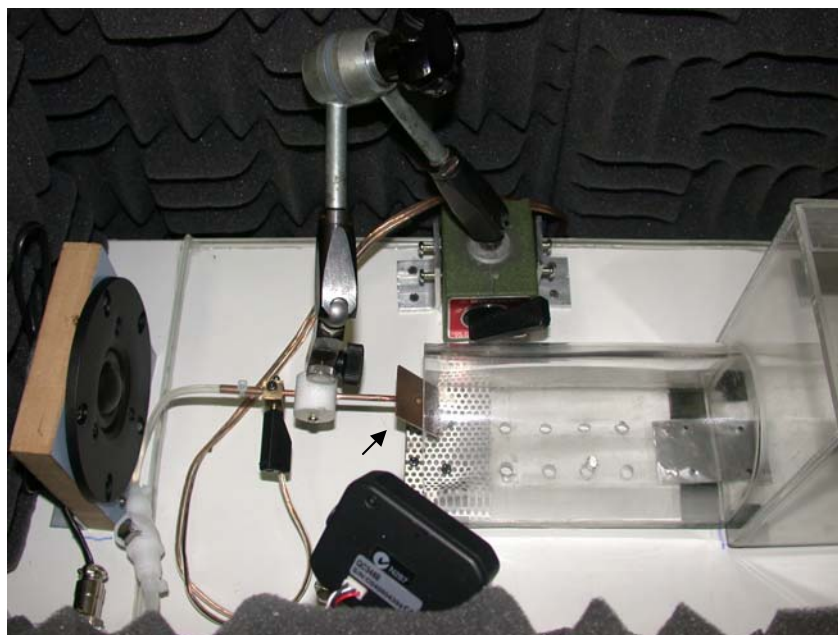


Figure 2 Conditioned avoidance testing apparatus.

Water is provided to the animal via a copper lick plate (arrow). Water flow is controlled by a programmable peristaltic pump, which is turned on when animal contact is detected. The acrylic chamber housing the animal is designed such that an external coil can be used to wirelessly power an implanted stimulator during testing.

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 neonatally deafened an additional three animals; and these animals are scheduled for implantation with our standard intracochlear electrode arrays and extracochlear ball electrode in the next quarter. Currently, two animals are receiving low-rate (50 pps/electrode) monopolar stimulation on all 7 intracochlear electrodes using the SPEAK[®] speech processing strategy. Two additional animals are in our late intervention cohort and 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. One acute electrophysiological experiment was performed this quarter on a chronically stimulated (low-rate) animal. Following the completion of the acute electrophysiological experiment, the cochleae and CNS were harvested and prepared for subsequent analysis.

2.2.3.1. Cochlear Nucleus

This work aims to address whether ICES of neonatally deafened cats is able to preserve the volume of the cochlear nucleus (CN). In particular, we are interested in the changes of the anteroventral cochlear nucleus (AVCN) as it is the site for direct synapses from the endbulbs of Held that are known to be affected by ICES (Ryugo et al., 2005). Light microscopy was performed on coronal sections (Figure 3) of CNs of 19 animals (3 normal hearing controls, 5 long-term deaf controls, and 11 chronically stimulated animals). The subdivisions of the CN were outlined using NIH Image following the criteria of Osen (1969) and subsequent volumes were calculated.

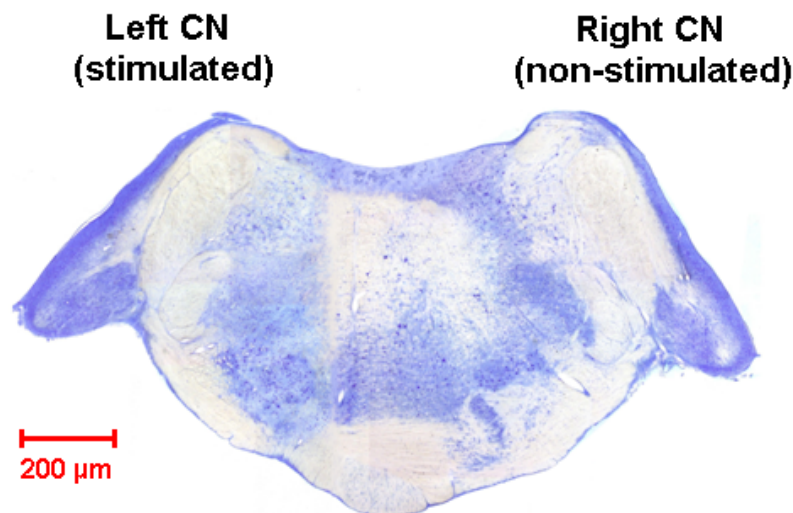


Figure 3 Cochlear nuclei in a chronically stimulated animal.

Coronal section of the medulla showing the cochlear nuclei on the lateral borders stained with Thionin for light microscopy. Section is from a neonatally deafened cat that was implanted on the left side and received 6 months of chronic ICES.

Analysis of the data revealed that long-term deafness resulted in a significant reduction in AVCN volume ($5.148 \pm 0.137 \text{ mm}^2$; mean \pm S.E.M.) compared to normal hearing controls ($8.421 \pm 0.128 \text{ mm}^2$; One-way ANOVA, Holm-Sidak Post Hoc, $p < 0.001$; Figure 4). This observation is consistent with previous reports (Hardie and Shepherd, 1999; Lustig et al., 1994). This reduction in AVCN volume was also reflected in the non-stimulated side of the chronically stimulated animals ($5.19 \pm 0.12 \text{ mm}^2$), which was not significantly different to the deafened cats ($p=0.783$). However, the volume of the stimulated (left) AVCN of these animals ($5.700 \pm 0.142 \text{ mm}^2$) was significantly greater than the non-stimulated side ($p=0.008$), as well as the deafened controls ($p=0.005$). Other regions of the CN including the dorsal cochlear nucleus (DCN), posteroventral cochlear nucleus (PVCN) and the granular cell layer (GCL) were also examined; however there was no significant difference between the stimulated and non-stimulated groups (all p 's > 0.05).

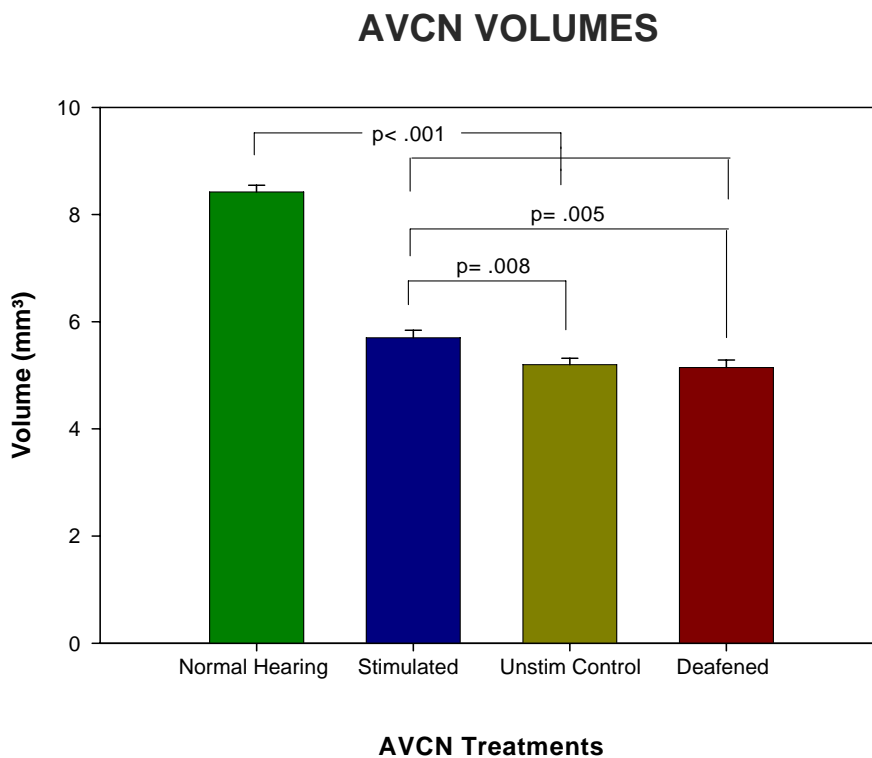


Figure 4 AVCN volumes

Mean AVCN volumes of normal hearing (green), deafened/chronically stimulated (stimulated = blue; unstimulated = gold) and long-term deafened (red) subjects. Statistics obtained by One-way ANOVA, Holm-Sidak Post Hoc. Statistical significance is indicated by the lines above the bars. Error bars represent SEM's. The left AVCN of the stimulated cats is significantly greater than both the right AVCN ($p=0.008$) and the deafened controls ($p=0.005$). Both deafened and stimulated groups were significantly lower than the normal hearing subjects ($p<0.001$).

Chronic ICES was able to prevent some of the atrophy in the AVCN caused by long-term deafness; however, it was not able to maintain the same AVCN volume seen in the normal hearing controls.

2.3. Neurotrophins

2.3.1. Guinea Pig

The pro-survival effects of NT delivery (with or without ICES) following aminoglycoside-induced deafening are well established. What are 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.

There is profuse dendritic resprouting following aminoglycoside-induced deafening and NT delivery; however the consequences of this resprouting on the functional cochleotopic organization of the central auditory system are unclear. This quarter, pilot studies have continued to optimize single SGN peripheral fiber tracing techniques using the tracer tetramethylrhodamine dextran (TMRD) to determine the extent of aberrant peripheral fiber regrowth following NT and / or ICES treatment. Following several time comparison experiments, we have concluded that 3 hours is a sufficient length of time for the dye to infiltrate the peripheral processes of basal and some middle turn SGNs (the target cells of cochlear implants). Surface preparations have been viewed using confocal microscopy to reduce the interference of autofluorescence when tracing peripheral fibers. Although the effect of autofluorescence was greatly reduced, the signal in the fibers was too weak to be reliably traced when viewed at 20X magnification. Peripheral fibers could be clearly seen extending from SGN somata at 40X magnification, but the thickness of the preparations prevented viewing planes of focus beyond this level. Therefore, future pilot studies will attempt to reduce the thickness of sections.

Spatial tuning curves have been successfully recorded from the central nucleus of the inferior colliculus to different acoustic frequencies in normal hearing animals using 32 channel [NeuroNexus](#) arrays. In the next quarter we will be repeating this and the peripheral fiber tracing protocol in guinea pigs that have been acutely or chronically deafened, and acutely stimulated with a cochlear implant. The auditory nerve will be partially degenerated following chronic deafness so it is prudent to determine how successfully data can be collected from these worst case scenarios.

2.3.2. 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 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, we neonatally deafened and implanted an additional three animals with empty alginate capsules and our standard intracochlear electrode arrays and extracochlear ball electrode. These animals complete our cohort of stimulated control animals. All animals in this study will receive monopolar stimulation on all 7 intracochlear electrodes at 400 pps/electrode using the SPEAK[®] speech processing strategy. NRT recordings are taken fortnightly and EABR recordings monthly on all animals in this study.

Acute electrophysiology experiments have begun; these experiments are designed to evaluate the effect of neurotrophin delivery (via the encapsulated NT-cells) and chronic electrical stimulation on the function of the auditory nerve. These experiments utilize the new artifact removal technique we recently developed (see [Heffer and Fallon](#)). We have been able to record detailed input/output functions from single auditory neurons in these long-term deaf stimulated control animals (Figure 5). A detailed analysis and report of these data will be presented in the coming quarters. Following the completion of the acute electrophysiological experiments, the cochleae and CNS of all animals were harvested and prepared for subsequent analysis. These data will be statistically analyzed and prepared for publication in the coming quarters.

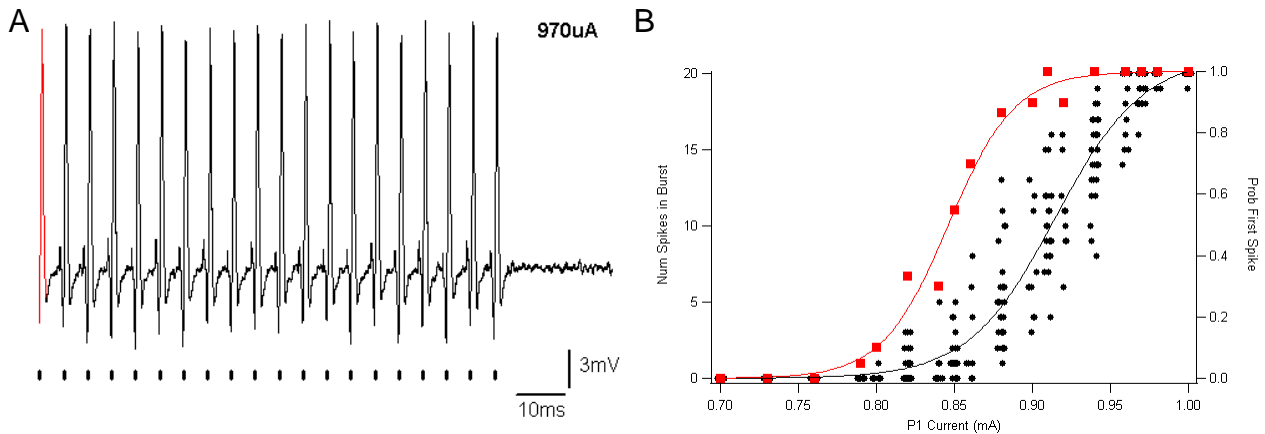


Figure 5 Recording from a single auditory nerve fiber.

A) Example of action potentials from a single auditory neuron measured with a glass micropipette. This neuron was responsive to each electrical stimuli delivered via the intracochlear electrode at a rate of 200 pulses per second (depicted with the stimulus bars below the trace). B) Decreasing the intensity of the stimulus caused a decrease in the probability of the neuron firing in response to each stimuli. The first pulse of the neuron in the sequence of stimuli (red squares) was more reliable in firing to a given stimulus current level (ie a shift in the curve to the left). It is anticipated that the functional properties of the auditory neurons will be affected by chronic stimulation and / or neurotrophin delivery.

3. Additional activities

Prof. David Ryugo visited our group during the quarter to further discuss our ongoing collaboration in the study of the ultrastructural changes in the cochlea nucleus of long-term deaf and chronically stimulated animals.

This quarter we had a significant redevelopment of our contract website (<http://www.bionicear.org/oto/nih/>).

4. 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) Continued development of new behavioral test apparatus, using conditioned avoidance techniques, for testing temporal processing in the rat.
- f) Implant additional animals for ICES studies in the cat (both with and without NT delivery).
- g) Continue chronic ICES programs in deafened/implanted cats.
- 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 of techniques to trace single SGN peripheral fibers.

5. Personnel

Dr Fergal Glynn has joined the group this quarter as a visiting research fellow working on encapsulating BDNF into biocompatible biodegradable nanoparticles. Dr Glynn is an Ear Nose and Throat surgeon from Ireland, who will be working with our group while completing his MCh thesis. He is will be working both in the institute and at the Department of Biomolecular and Chemical Engineering at University of Melbourne, perfecting the encapsulation process.

Meera Ulaganathan commenced work this quarter as a part-time Research Assistant with our group. Meera completed her Bachelor of Science with Honours at the University of Melbourne in 1999. She has previously worked as a Research Assistant for the Institute of Child Health (University College London) and Department of Medicine (University of Melbourne). Meera has been trained to carry out and assist with various tasks, which include;- daily monitoring of chronically stimulated cats, recording of EABRs and NRTS, tissue processing and staining, cell culture and other routine laboratory duties.

Michael Giummarra has joined the group this quarter as a UROP (Undergraduate Research Opportunity Program) student and is currently completing his Bachelor of Biomedical Science degree. His UROP project will focus on the effects of chronic electrical stimulation in the cochlear nucleus of the cat.

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; 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.

7. References

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Appendix A (attached)

Heffer, L.F. and Fallon, J.B. 2008. A novel stimulus artifact removal technique for high-rate electrical stimulation. *Journal of Neuroscience Methods* 170, 277-84.

8. Appendix B (attached)

Conference abstracts published during the quarter.