First 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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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 accepted for publication.

Shepherd, R.K., Epp, S.B., Coco, A. Neurotrophins and electrical stimulation for protection and repair of spiral ganglion neurons following sensorineural hearing loss. Hearing Research (in press). Attached as Appendix A.

Pettingill, L.N., Minter, R., Shepherd, R.K. Schwann cells genetically modified to express neurotrophins promote spiral ganglion neuron survival in vitro. Neuroscience (in press). Attached as Appendix B.

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

Irvine, D.R.F. “Auditory System Plasticity And Auditory Prostheses” Workshop on “Bringing together the science and practice of hearing prostheses ” hosted by ARC Human Communication Science Network, Sydney, Australia, November 2007. (Invited Speaker).

Shepherd, R.K., Andrew, J., Wise, A.K., Pettingill, L. Coco A., Epp, S. “Sustainable delivery of neurotrophins to the inner ear” 6th Asia Pacific Symposium on Cochlear Implants and Related Sciences, Sydney, Australia, November 2007. (Invited Speaker).

Perry, D, Grayden, D. Fallon, J.B. “Research Cochlear Implant for Small Laboratory Animals” HCSNet SummerFest, Sydney, December 2007. (Abstract attached).

Wimberley, C., Fallon J.B., Irvine, D.R.F., Shepherd, R. K. “Chronic intra-cochlear stimulation of the deafened cat results in plastic changes within the Primary Auditory Cortex” HCSNet SummerFest, Sydney, December 2007. (Abstract attached).

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 cochlea, significant modifications to our standard animal electrode assembly are required. We have therefore performed anatomical studies on live and dead mice, including measurements to determine the maximum outer dimensions of the intra-cochlear array, and the length of the leadwire assemblies. Based on these measurements, a small animal electrode array die - with a 0.2mm tip diameter - was designed and manufactured. In addition, a new resistance welder from Unitek (Monrovia, CA) was purchased and commissioned to allow reliable low-energy welding on the miniature parts of the new mouse electrode array. Test insertions of the prototype electrode arrays in live mice were performed and confirm the new design as feasible for implantation in mouse models. Finally, two mouse stimulator assembles (described in N01-DC-3-1005QPR12) have been manufactured to use in initial chronic implantation trials.

Chronic implantation in the mouse presents significant new challenges compared to implantation in the rat, due to the additional reduction in size required. Therefore, we have initiated a study to investigate the surgical issues relating to implant surgery in the mouse including cauterization of the stapedial artery and fixation of the leadwire and stimulator assembly. This quarter, stapedial artery surgery has been performed on eleven wild-type mice, and a unilateral deafening protocol using local perfusion of a solution of neomycin through the cochlea has been performed on three additional animals. Further surgeries and histological analysis of the effects of both surgeries will proceed in the following quarter.

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.

This quarter has seen the completion of two projects designed to extend our capabilities to provide temporally challenging ICES to the rat. To date, our fully implantable small laboratory animal stimulator has only been capable of delivering naïve stimulation. Catriona Wimberley, a final year engineering student working in our group, has recently developed a system that is capable of modulating the stimulus being delivered to an animal based on tracking the location of the animal within the chronic stimulation chamber. The system can modulate either the frequency and / or intensity (via changes in pulse duration) of the stimulation based on the location of the animal within the stimulating chamber. The resulting stimulation, although not derived from the acoustic environment of the animal, may at least contain some relevant environmental cues. Such environmentally derived cues within the stimulation are considered important for studies involving high centers including the auditory cortex. The second project was a complete redesign of the fully implantable stimulator and associated controllers to allow for near real-time adjustment of all stimulus parameters and stimulation on multiple electrodes. This project was David Perry’s final year engineering project, and a detailed description of this project will be the subject of a future report.

In our previous contract we used a modified T maze task to assess the ability of chronically deaf rats to distinguish between electrical pulse trains of different duration and / or rates. While we were able to observe discrimination of pulse trains of different durations, we were unable to get the animals to discriminate different stimulation rates. We believe this was due to the appetitive task being used; and have therefore proposed to use a conditioned avoidance technique. We have received approval from out local ethics committee for the use of negative reinforcement and have begun construction of a new behavioral test apparatus which will be described in more detail in a future report.

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 three animals; and implanted two of these animals with our standard intracochlear electrode arrays and extracochlear ball electrode. Currently, two animals are receiving low-rate (50 pps/electrode) monopolar stimulation on all 7 intracochlear electrodes using the SPEAK® speech processing strategy. The remaining animal will be the first animal in our late intervention cohort and begin its stimulation regime at approximately 8 months of age. Three acute electrophysiological experiments were performed this quarter (2 chronically stimulated animals and one normal hearing animal). Following the completion of each acute electrophysiological experiment, the cochleae and CNS from each animal were harvested and prepared for subsequent analysis. These data will be statistically analyzed and prepared for publication in the coming quarters.

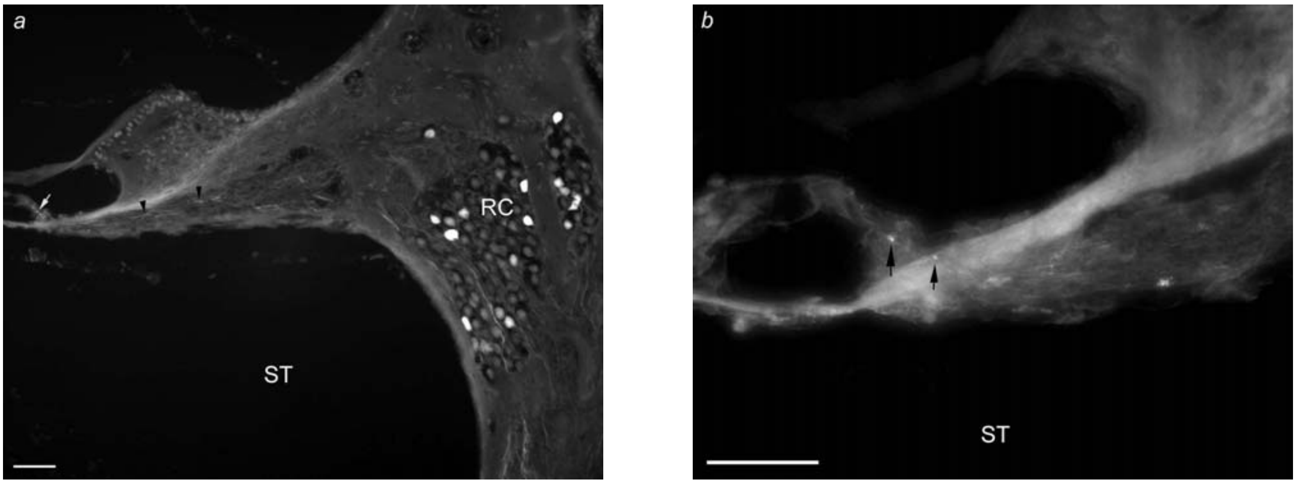
2.3. Neurotrophins

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 (particularly acoustic overstimulation) 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 (Wise et al., 2005); however the consequences of this resprouting on the functional cochleotopic organization of the central auditory system are unclear. As part of his PhD project, Tom Landry has begun assessing the viability of applying tetramethylrhodamine dextran (TMRD) to the auditory nerve in order to retrogradely trace single SGN peripheral fibers, to determine the extent of aberrant peripheral fiber regrowth following NT and / or ICES treatment. Iontophoresis was initially used as the method of application, but resulted in complete blockage of the micropipette tip with cellular debris because of the relatively large tip diameter required, preventing movement of the TMRD. Therefore, we have begun exploring the use of pressure injection.

The results of two pilot studies using pressure injection are shown in Figure 1. Labeling of some, but not all SGN peripheral fibers was achieved as hoped, although clear labeling was only seen in neurons in the basal turns of the cochlea. Labeled peripheral fibers could be seen in the osseous spiral lamina and innervating inner hair cells at the organ of Corti in midmodiolar sections of the basal cochlea. The portion of the peripheral fibers passing through the osseous spiral lamina was not seen in cochlear wholemounts. This was likely because of the large degree of background fluorescence in that tissue. In the future we will observe the wholemount sections using confocal microscopy to overcome this, as well as take steps to reduce autofluorescence in the tissue.



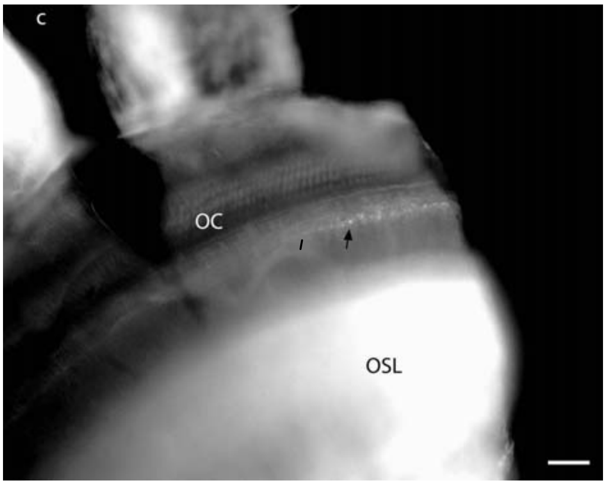


Figure 1. Cochlear sections following pressure injection of TMRD into the guinea pig auditory nerve. Midmodiolar sections of the upper basal turn are shown in *a* and *b*. Peripheral fibers can be seen at the OC (arrows), and passing through the OSL (arrowheads). The selective labeling of SGN somata within RC can be seen in *a*. Peripheral SGN fibers at the OC in a basal turn wholemount are shown in *c* (arrow). OC = organ of Corti, OSL = osseous spiral lamina, RC = Rosenthal's canal, ST = scala tympani. Scale bars = 20 µm.

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 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 neonatally deafened and implanted three animals with empty alginate capsules and our standard intracochlear electrode arrays and extracochlear ball electrode. These animals have begun receiving monopolar stimulation on all 7 intracochlear electrodes at 400 pps/electrode using the SPEAK® speech processing strategy and will serve as stimulated control animals. NRT recordings will be taken fortnightly and EABR recordings monthly on all animals in this study.

3. Additional activities

Mr. David Perry submitted his final year engineering thesis entitled “Research Cochlear Implant for Small Laboratory Animals” for which he received "The Mathworks Prize for Best Final Year Project" at the University of Melbourne, Department of Electrical and Electronic Engineering's Endeavour 2007 project exhibition. David subsequent received first class honors for his engineering degree and will be commencing a PhD with our group at the start of 2008.

Ms. Catriona Wimberley submitted her final year engineering thesis entitled “Lab Animal Tracking with Adaptive Stimulation” for which she received a first class honors mark. Catriona will be starting a new job working for the Australian Nuclear Science and Technology Organisation in 2008 and therefore will be leaving our group.

Prof. Patricia Leake visited our group during the quarter and we outlined our research proposal for this contract to her and discussed areas of collaboration.

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) Additional mouse surgeries to examine the effects of cauterization of stapedial artery and the efficacy of local perfusion of neomycin will be performed. Histological analysis of the effects of both surgeries will be performed.

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

Alison Evans has joined our group as a full-time Research Assistant having recently completed a Bachelor of Science degree with Honors under the supervision of Dr. Rachael Richardson. During the quarter, Alison has been trained to undertake daily monitoring of our chronically stimulated cats, the recording of EABRs and NRTs and other laboratory tasks.

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

Wise, A.K., Richardson, R., Hardman, J., Clark, G., O'Leary, S. 2005. Resprouting and survival of guinea pig cochlear neurons in response to the administration of the neurotrophins brain-derived neurotrophic factor and neurotrophin-3. J Comp Neurol 487, 147-65.

8. Appendix A (attached)

Shepherd, R.K., Epp, S.B., Coco, A. Neurotrophins and electrical stimulation for protection and repair of spiral ganglion neurons following sensorineural hearing loss. Hearing Research (in press).

9. Appendix B (attached)

Pettingill, L.N., Minter, R., Shepherd, R.K. Schwann cells genetically modified to express neurotrophins promote spiral ganglion neuron survival in vitro. Neuroscience (in press).

10. Appendix C (attached)

Conference abstracts published during the quarter.