Sixth Quarterly Progress Report

NIH-N01-DC-3-1005

Protective Effects of Patterned Electrical Stimulation on the Deafened Auditory System

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January 1 – March 31, 2005

Contents:

1. Introduction

The goal of this contract is to develop methods of protecting the remaining portions of the auditory system from degeneration after loss of hair cells and to improve its effectiveness in extracting information provided by auditory prostheses. We have taken a broad neurobiological approach to this goal in order to study both the short and long-term response of the auditory system to loss of hair cells and the subsequent introduction of afferent input via an auditory prosthesis. Our studies are divided into three major areas of investigation:

(a) The neurophysiological and neuroanatomical response of spiral ganglion neurons (SGNs) and the central auditory system (CAS) following chronic intracochlear electrical stimulation in combination with neurotrophic support of the auditory nerve. This work is designed to investigate whether electrical stimulation and chronic administration of neurotrophins act in synergy to promote auditory nerve (AN) survival in both guinea pig and other mammalian models of sensorineural hearing loss (SNHL). This work will also provide insight into the role of neurotrophins in improving synaptic efficiency in the deafened auditory pathway.

(b) The neurophysiological and neuroanatomical response to prolonged electrical stimulation of the auditory nerve following a neonatal SNHL. This work is designed to provide insight into the protective effects of electrical stimulation on SGNs and the plastic response of the CAS to temporally challenging stimuli presented chronically to one or two sectors of the AN. This work will also examine the ultrastructural changes evident at the AN/cochlear nucleus synapse in response to a neonatal SNHL and to chronic electrical stimulation of the AN.

(c) The application of cell based therapies for rescue and regeneration of SGNs following SNHL. These studies are designed to provide insight into the potential clinical application of cell-based therapies in the severe and profoundly deaf prior to cochlear implantation.

While these studies are designed to provide insight into the plastic response of the deafened auditory pathway to re-activation via an auditory prosthesis, a major objective of this work is to apply our findings to the clinical environment.

2. Summary of activities for the quarter

During the sixth quarter of this contract the following activities were completed:

Publications and conferences

Manuscript preparation: During the quarter two manuscripts were accepted for publication and have been appended to this application (Appendix A and B):

> Shepherd, R.K., Coco, A., Epp, S.B. and Crook, J.M. Chronic depolarization enhances the trophic effects of BDNF in rescuing auditory neurons following a sensorineural hearing loss. J. Comp. Neurol (in press).

Lu, W., Xu, J. and Shepherd, R. K. Cochlear implantation in rats: A new surgical technique. Hear. Res. (in press).

Three additional manuscripts are currently in the review process. In addition, the following invited book chapter was submitted (Appendix C):

Shepherd, R.K., Meltzer, N.E., Fallon, J.B. and Ryugo, D.K. Consequences of electrical stimulation on the peripheral and central auditory system. In: Cochlear Implants, 2nd Edition, S. B. Waltzman & J. T. Roland (Eds), Thieme, New York.

- Drs. Lisa Gillespie and Justin Tan were invited speakers at the 2nd Australian Auditory Neuroscience Workshop held at the University of Western Australia, Perth on 29 Jan 2005. Dr. Gillespie's presentation was titled "Neurotrophic factors: the potential for hearing recovery" and Dr. Tan's paper was titled "Gene expression in spiral ganglion neurons - Activity-dependent and neurotrophic genes" (no abstracts available).

- The following papers were presented at the Australian Neuroscience Society annual meeting in Perth, February 2005 (Appendix D):

McGuinness, S. L. & Shepherd, R. K. Exogenous BDNF rescues rat spiral ganglion neurons *in vivo*. Proc. Aust. Neurosci. Soc. p95, 2005.

Milojevic, D.M., Tasche, C.T., Coco, A., Epp, S.E., Parker, J.P. & Shepherd, R.K. Acute modulation of electrical thresholds in the auditory periphery following pharmacological intervention. Proc. Aust. Neurosci. Soc. p95, 2005.

Sly, D., Heffer, L., White, M., Shepherd, R. & O'Leary, S. The effect of neurotrophins on auditory nerve function after hearing loss. Proc. Aust. Neurosci. Soc. p27, 2005.

- The following poster was presented at the Assoc. Res. Otolaryngol. annual meeting in New Orleans, February 2005 (Appendix D):

Shepherd, R.K., Roberts, L, & Paolini, A. Long-term sensorineural hearing loss induces functional changes in the rat auditory nerve. Assoc. Res. Otolaryngol. , Abs.: 1387, 2005.

Chronic electrical stimulation and neurotrophin delivery in the guinea pig

This work aims at developing techniques for SGN rescue based on the exogenous delivery of neurotrophins in combination with chronic depolarization via a cochlear implant.

- The 10 guinea pigs implanted during the previous quarter completed their BDNF and chronic electrical stimulation program during this quarter. Their cochleae are currently being processed for histological analysis.

Chronic electrical stimulation in the cat

As noted in our 3rd QPR, this work uses Nucleus® CI24 cochlear implants in combination with Nucleus® ESPrit 3G behind-the-ear speech processors. The cochlear implants are *not implanted* but are hardwired to connect directly with the animal's percutaneous leadwire system.

- During the present quarter we continued daily chronic electrical stimulation of five neonatally deafened animals. These animals have now been chronically stimulated for periods of 7-8 months using a behaviorally relevant stimulation regime.
- Continued development of a direct interface to the Nucleus® ESPrit 3G processors that will allow us finer control over the Nucleus® CI24 and Nucleus® 22 cochlear implants. As part of this process the feasibility of using the Nucleus® processors and implants for performing basic electrophysiological measurements, particularly EABRs, and behavioural testing will be assessed.
- Hosted a visit from our consultant Prof. David Ryugo from Department of Otolaryngology, Johns Hopkins University. Under this collaboration we are studying the synaptic plasticity in the auditory nerve/cochlear nucleus (end bulb of Held) in our neomycin deafened/chronically stimulated cats. During this quarter Dr. Ryugo visited the laboratory and tissue specimens taken from one chronically implanted/chronically stimulated cat and two control animals were prepared for light and transmission electron microscopy.
- Repeated behavioral testing of comfort levels for the implanted cats and subsequent adjustment of chronic stimulation levels based on EABR and comfort level measurements.
- Received our new 96-channel Cerebus data acquisition system from Cyberkinetics and initiated testing and integration of the system with our existing recording and stimulating systems.
- Performed practice insertions of the dummy multi-electrode arrays from Cyberkinetics Inc. in both the rat and cat.
- Hosted a visit from Jorge Ramirez, a representative from Cyberkinetics Inc. who provided advice regarding electrode insertion techniques, and discussed possible future collaborations.
- Continued analysis of histological and electrophysiological data from the previous series of experiments on the effects of long-term chronic intracochlear electrical stimulation.

Chronic electrical stimulation in the rat

The aim of this project is to develop a small fully implantable stimulator that can provide chronic electrical stimulation of the cochlea in small laboratory animals such as the rat. This work will examine the trophic effects of electrical stimulation in a third animal species. Moreover, it will allow us to perform behavioral studies on animals that have received chronic stimulation and compare their performance at rate and pitch discrimination with implanted, un-stimulated control animals.

To this end, the aim of this component of the project is to determine whether early experience with simple forms of electrical stimulation enhances the ability to perceive differences between more complex patterns of electrical stimulation later in life. The experiments to examine this issue will use a rat behavioral model in which rats with implanted stimulators are trained to discriminate different patterns of stimulation in a specially-designed T maze apparatus.

- The test apparatus has now been installed in our animal house. A preliminary study has been initiated to establish procedures for training the animals to perform discrimination tasks in the apparatus using visual and auditory discriminations, prior to deafening and the investigation of electrical discrimination.
- A group of three (normal hearing) rats have been trained to perform the task and have subsequently been trained on two visual discrimination tasks – a simple discrimination between a low-spatial frequency display and a blank display, and a more difficult discrimination between low- and high-spatial frequency displays. All rats reached criterion on the simple discrimination and two of the three are approaching criterion on the complex discrimination.

Cellular over-expression of BDNF

The aim of this study is to use cell transplantation techniques to deliver long-term/ongoing neurotrophic support to SGNs in animal models of deafness.

- Frozen Schwann cell stocks were obtained from our collaborators at the Howard Florey Institute. Dr. Gillespie has subsequently become competent in maintaining these cells, in terms of growing cultures up from frozen stocks, regularly sub-culturing the cells, and freezing down cells for storage.
- A new Science Honours student commenced research studies with our group, under the supervision of Drs. Gillespie and Shepherd. Miss Ricki Minter will be involved in our cell therapy investigations; specifically, she will investigate the survival-promoting effects of neurotrophin-3 (NT-3) producing Schwann cells on SGNs *in vitro*. During this quarter Miss Minter was successfully trained in the Schwann cell culturing techniques, and has also developed competence in dissection of the cochlea from early postnatal rats. The next quarter will see Miss Minter perform SGN cultures and begin the molecular biology aspects of genetically modifying Schwann cells to produce NT-3.
- The Schwann cells currently being utilized are a mixed population of cells – from both male and female animals – and are being used for training purposes only. Time constraints pertaining to Miss Minter's Honours project (which ends in October) have led us to this alternative. Once the male Schwann cells cultures are successfully established, similar techniques will be employed to genetically modify these cells for use in the *in vivo* studies (the y-chromosome of the male Schwann cells will be used to identify them following inoculation into female guinea pigs).

Analysis of gene-specific markers altered by deafening in the cochlea

The aim of this study is to investigate how the expression of genes related to neuronal survival and function in the mammalian auditory system are modified by sensorineural hearing loss and by re-activation via a cochlear implant. During this quarter we:

- Mapped the expression of patterns neurotrophins and their receptors in the adult normal hearing rat cochlea using immunohistochemical techniques. Using a newly developed fixation/decalcification protocol, we detected the presence of NT-3 and BDNF proteins in both hair cells and supporting cells of the organ of Corti. This staining pattern could be abolished by pre-incubation with the relevant blocking peptide, indicating antibody specificity. This suggests that deafening protocols (acoustic- or aminoglycoside-based), by primarily damaging the hair cells, remove this source of neurotrophic support, resulting in a secondary death of SGNs. This effect is ameliorated when exogenous NT-3 or BDNF proteins are delivered into the cochlea, suggesting that cellular compartments within the cochlea must produce the relevant tropomyosin-related kinase (trk) receptors to mediate the signaling pathways of these neurotrophins.
- Examined the expression pattern of trkA, trkB, trkC and p75NGFR in the normal hearing rat cochlea and found that radial fibers in the osseous spiral lamina and SGNs express primarily trkB and p75NGFR receptors. The specificity of these staining patterns was underlined by co-localization experiments with known markers for afferent fibers in the cochlea – NF-200, synaptophysin and GAP43 – as well as using specific antibodies that recognize rat p75NGFR but not mouse p75NGFR. We could not detect trkA expression either in the fibers or SGNs but saw strong expression in neighboring vestibular ganglion neurons, suggesting that the absence of trkA expression is specific to auditory neurons. Relative to trkB and p75NGFR staining patterns, SGNs appear to express trkC at a much reduced level, which can be abolished by pre-incubation with the blocking peptide.
- We are still in the process of determining how these neurotrophins and their receptors in the cochlea are affected by deafening. Towards this end, we may consider analyzing molecules that are directly affected by the signaling pathways of these neurotrophins as this would have more physiological relevance.

The application of stem cells for SGN regeneration

The aim of this study is to develop clinically feasible techniques for the application of stem cell therapy for SGN regeneration in the profoundly deaf.

- Quantification of *in vitro* cultures and statistical analyses was performed during the quarter. It would appear that SGN and stem cell co-cultures primarily produce cells with glial morphology.
- Hair cell explants were co-cultured with stem cells differentiated for 8-days with retinoic acid. The analysis of these *in vitro* data is in progress.

- Professor Alan Makay-Sim, a consultant on our contract, visited our laboratory this quarter to provide advice on exogenous cell identification and other issues associated with this project.

3. Plans for next quarter

- Continue manuscript writing and submission, and preparation for attending conferences.
- Commence studies in the auditory cortex of hearing animals using our multi-channel Cerebus data acquisition system.
- Continue our chronic electrical stimulation studies in the cat.
- Deafen and implant additional cats for further chronic stimulation studies.
- Install a computer-based facility to capture electrode voltage and current waveforms from our chronically implanted cats.
- Continue to investigate short- and long-term effects on neuronal and trophic markers in the cochlea neurons.
- Continue *in vitro* and *in vivo* studies directed at further differentiating stem cells towards SGNs.
- Continue to fabricate electrode assemblies for use in our chronic stimulation studies.
- Initiate a behavioral training program using auditory discrimination in normal hearing rats. Initially they will be required to discriminate between low- and high-frequency tone bursts, and subsequently between pulse trains at different rates. This acoustic study will precede a similar study in deafened rats using cochlear implants.

4. Personnel

Stephanie Epp, a Research Assistant on this contract has accepted post-graduate entry to the School of Nursing at the University of Melbourne and completed her activities on this contract at the end of this quarter. Stephanie provided excellent support on all aspects of this contract and we wish her well in her new endeavors. We have appointed Ms. Lauren Donley as our new Research Assistant to this contract. Lauren completed a BSc degree with first class Honours in 2004 from Melbourne University, majoring in Physiology and Clinical Pharmacology.

5. 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; Prof. Trevor Kilpatrick, Dr. Simon Murray and Tania Cipriani from the Howard Florey Institute for their collaboration in obtaining Schwann cells, and Dr. Tony Paolini from La Trobe University for advice in using the rat test chamber.

6. Appendix A (attached)

Shepherd, R.K., Coco, A., Epp, S.B. and Crook, J.M. Chronic depolarization enhances the trophic effects of BDNF in rescuing auditory neurons following a sensorineural hearing loss. J. Comp. Neurol (in press)

7. Appendix B (attached)

Lu, W., Xu, J. and Shepherd, R. K. Cochlear implantation in rats: A new surgical technique. Hear. Res. (in press).

8. Appendix C (attached)

Shepherd, R.K., Meltzer, N.E., Fallon, J.B. and Ryugo, D.K. Consequences of electrical stimulation on the peripheral and central auditory system. In: Cochlear Implants, 2nd Edition, S. B. Waltzman & J. T. Roland (Eds), Thieme, New York.

9. Appendix D (attached)

Abstracts from the Australian Neuroscience Society annual meeting in Perth, Western Australia, February 2005 and the Association for Research in Otolaryngology's mid-winter meeting in New Orleans, February 2005.