Sixth Quarterly Progress Report

NIH-N01-DC-0-2109

Protective Effects of Patterned Electrical Stimulation On the Deafened Auditory System

J. B. Fallon, J. M. Crook, A. Serruto, and R. K. Shepherd

Department of Otolaryngology
University of Melbourne
32 Gisborne Street
East Melbourne 3002,
Victoria, AUSTRALIA

January 1 – March 31, 2002

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 to prolonged electrical stimulation of the auditory nerve following a neonatal sensorineural hearing loss (SNHL). This work is designed to provide insight into the protective effects of electrical stimulation on the auditory nerve (AN) in addition to investigating the plastic response of the central auditory system (CAS) to temporally challenging stimuli presented chronically to one or two sectors of the AN.

(b) The neurophysiological and neuroanatomical response to the AN and CAS of deafened animals following prolonged 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 AN survival. This work will also provide insight into the role of neurotrophins in improving synaptic efficiency in the deafened auditory pathway.

(c) The neurophysiological and neuroanatomical response to acute electrical stimulation of the auditory nerve following a neonatal SNHL. These studies are designed to provide insight into the acute response of the AN and CAS to intracochlear electrical stimulation in deafened animals with little prior auditory experience.

While these studies are designed to provide insight into the plastic response of the deaf 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:

- Deafened an additional five kittens to act as controls for the chronically electrically stimulated cats.
- Continued our terminal acute electrophysiological studies on the first group of cats that have been chronically stimulated.
- Deafened six adult guinea pigs. Some of these animals will be used as deafened un-implanted controls. Others will receive a delivery of artificial perilymph into the cochlea, while the remaining animals will receive electrical stimulation and simultaneous delivery of artificial perilymph.

3. Chronic electrical stimulation studies in neonatally deafened cats

A major undertaking during the quarter was the continuation of the terminal electrophysiological experiments we commenced mid 2001 (*Fourth Quarterly Progress Report*). We performed an additional three experiments during the quarter, bringing the total number of experiments completed to nine. All animals had been chronically stimulated for periods of up to eight months.

In partial-hearing animals we recorded single neuron activity from the primary auditory cortex in response to both acoustic and electrical stimulation. This work was performed in collaboration with Prof. D. Irvine and Dr. M. Brown of Monash University. Analysis of the response properties of single and multi-unit data from the primary auditory cortex has been used to construct both acoustical and electrical cortical maps. More details of the cortical results will be provided in a future report.

3.1 Effects of long-term SNHL on neurons in the Inferior Colliculus

Our previous work on the response of inferior colliculus (IC) neurons to electrical stimulation of the auditory nerve following long-term SNHL demonstrated several significant changes in their response properties (Shepherd et al., 1999). The absence of normal afferent input to the CAS during development resulted in a significant decrease in temporal resolution, increased latencies and an increase in dynamic range of the IC neurons. However, the rudimentary cochleotopic organization of the IC was not affected by the long-term SNHL, suggesting that this organization is laid down during development even in the absence of normal afferent input. The cochleotopic organiation of the IC does show some degree of plasticity, as chronic intracochlear electrical stimulation of neonatally (Snyder et al., 1990) and adult (Moore et al., 2002) deafened cats can result in expansion of the IC represenation of the chronically stimulated cochlear sector.

Therefore, in a second series of experiments we recorded from the IC of chronically stimulated, neonatally deafened animals to study spatial and rate plasticity in the central nucleus of the inferior colliculus (ICC). An additional three experiments were performed this quarter bring the total number of single and multi-units that have been recorded to date to 665. An additional three more experiments are planned for the following quarter. At the completion of these experiments each animal was killed with an overdose of anesthetic and its cochleae and brain were prepared for histological (Hardie and Shepherd 1999) and immunohistochemical examination (*Fifth Quarterly Progress Report*). Histopathological examination of the cochleae and brainstem will be performed over the next few quarters.

3.2 Analysis software upgrade

A significant upgrade to the software used to analyze the single and multi-unit data recorded from the IC has been substantially completed. As over 600 units have been recorded to date, and each additional experiment generates over 80 units, much of the data analysis has been automated or streamlined,

and all analysis is now performed within Igor Pro (Wavemetrics) using custom written analysis procedures.

3.2.1 Spike Selection

Recordings of IC single and multi-unit responses are made using tungsten microelectrodes (impedances 0.8 – 2.0 MΩ at 1 kHz). Unit activity is differentially amplified (DAM-5A, WPI) and band-pass filtered (150 Hz – 3 kHz; Khrone-Hite 3750) before being displayed on an oscilloscope (Tektronix 465). The stimulus artifact is typically eliminated using a sample-and-hold circuit, allowing output trigger pulses from the oscilloscope (adjusted to discriminate the leading edge of the action potentials) to be used to indicate the precise timing of each action potential. The resultant trigger pulse was fed to a PC-controlled in-house stimulus generation and data acquisition system. An Excel based software system was used to control the experiment and provide real-time peristimulus time, interspike interval and period histograms while hardware modules were responsible for synchronising components and recording event times (Tucker-Davis Technologies).

Stimuli consisted of 100 μs per phase charge balanced biphasic current pulses with a 10 μs interphase gap, and variable stimulus intensities up to 3 mA peak. An in-house, optically isolated, current source stimulator generated the stimuli. Recording electrodes were advanced in 2 μm steps in the presence of an electrical search stimulus.

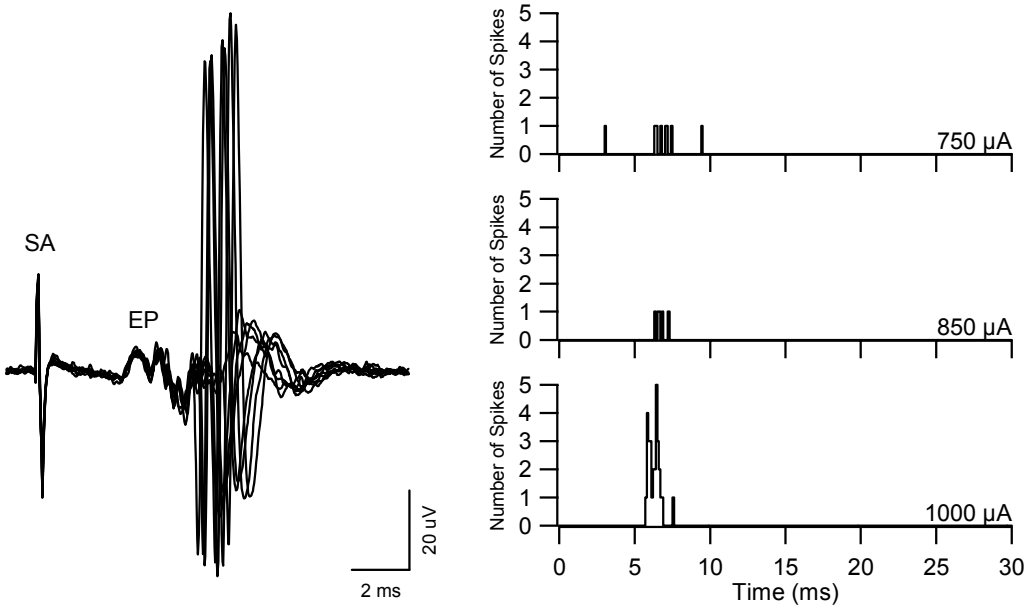

Figure 1. Representative example of an extracellular IC (recording depth, 1910 μm) single-unit response in a chronically stimulated neonatally deafened animal. The stimulus artifact (SA) is typically blanked to avoid contamination of single-unit activity, and adjustment of the oscilloscope trigger level can be used to eliminate the evoked potential (EP). Three poststimulus time histograms (PSTHs) at supra-threshold stimulus currents illustrate the relative levels of spike entrainment with increasing stimulus current.

Single and multi-unit activity in the IC in response to supra-threshold electrical stimulation of the auditory nerve typically have latencies of between 5 and 20 ms (Figure 1). As some units exhibited spontaneous activity (activity unrelated to the electrical stimulus, e.g. the event at approximately 3 ms in the 750 μA response in Figure 1), all analysis is limited to activity that occurs within an adjustable latency window of the electrical stimulus (typically set 5 to 20 ms).

3.2.2 Input-Output and Latency Curves

For each single or multi-unit an input-output (IO) function and Latency curve are determined (Figure 2). The PSTHs are used to determine the probability of firing and the mean and standard deviation of the latency of firing. In the case of multi-unit activity, or single-units that response with more than one action potential per stimulus, all measurements are made on the first spike only.

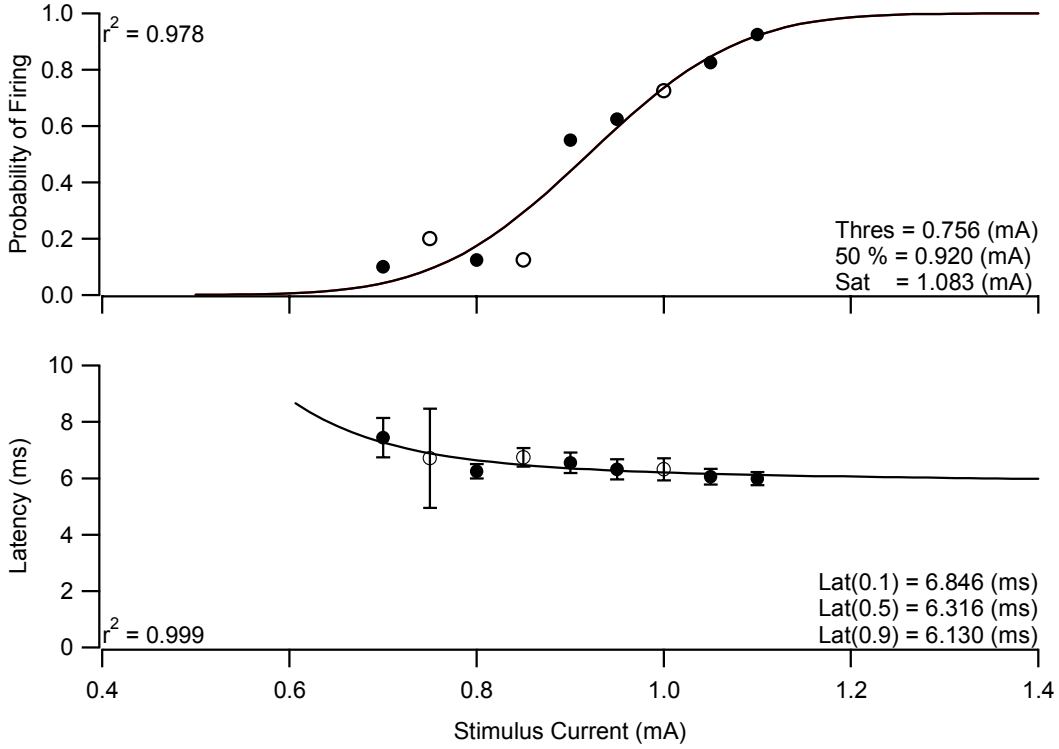

Figure 2. Representative example of an IO function and Latency curve for the same single unit as illustrated in Figure 1. Open symbols represent points calculated from the PSTHs illustrated in Figure 1.

The IO function is fitted with a saturating Gaussian function (Sachs and Abbas, 1974) using a least-squares fitting procedure. The resultant fitted curve is then used to estimate the stimulus current at threshold (probability of firing = 0.1), saturation (probability of firing = 0.9) and mid-dynamic range (probability of firing = 0.5). A feature of the new analysis software is that it is possible to fit a double saturating Gaussian function (Eqn. 1) to non-monotonic IO functions that are occasionally seen for single and multi-units in the IC, particularly in the lateral nuclei (Figure 3).

Eqn. 1

$$\mathrm{Pr} = \tfrac{1}{2}\mathrm{erf}\left(\frac{I - \mathrm{k}_1}{\sqrt{2}\,\mathrm{k}_2} + 1\right) - \tfrac{1}{2}\mathrm{erf}\left(\frac{I - \mathrm{k}_3}{\sqrt{2}\,\mathrm{k}_4} + 1\right)$$

where I is the stimulus current and k_1, k_2, k_3 and k_4 are constants.

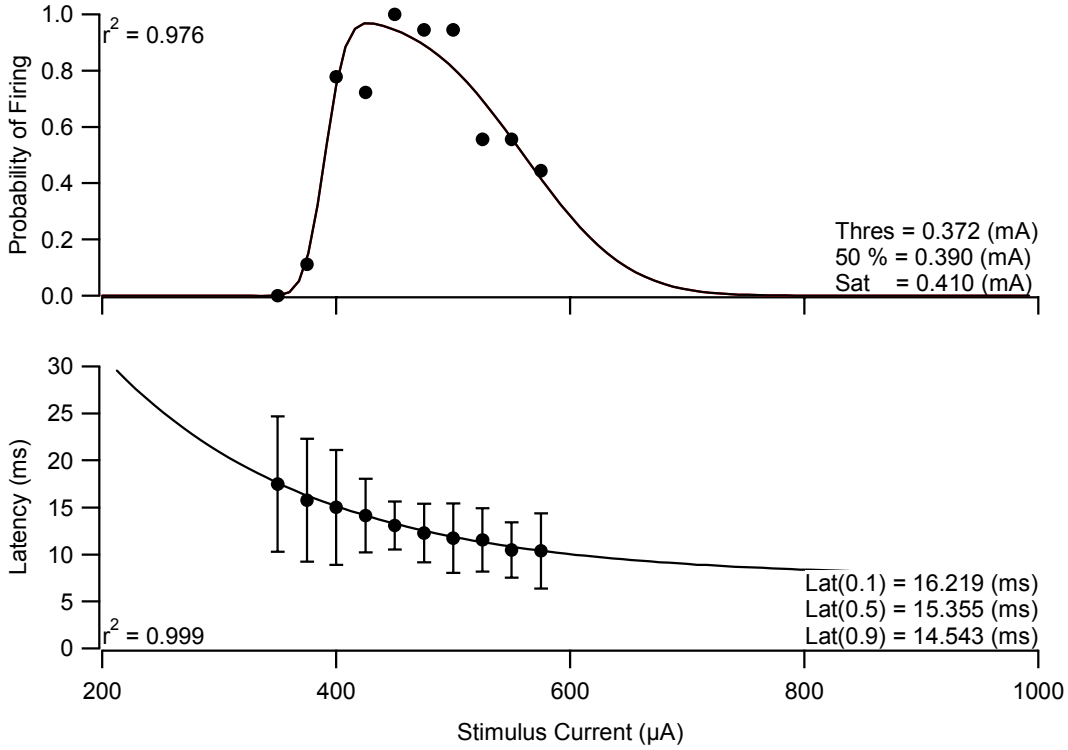

Figure 3. A double Gaussian function can be used to fit the non-monotonic IO function of this multi-unit activity in the lateral nuclei of the IC (recording depth, 1372 μm) of the same animal as Figure 1 and 2. Note that the Latency curve is still accurately modeled by the double-exponential function.

The mean first spike Latency curve is fitted with a double-exponential function (Eqn. 2) using a least-squares fitting procedure. The resultant fitted curve is used to estimate the mean response latencies at threshold, saturation and mid-dynamic range (Figure 2 & Figure 3).

Eqn. 2

$$\mathrm{Latency} = k_1 \exp^{-k_2 I} + k_3 \exp^{-k_4 I}$$

where I is the stimulus current and k_1, k_2, k_3 and k_4 are constants.

The new analysis software has streamlined the generation of PSTHs, IO functions and Latency curves, and has dramatically reduced the time required to produce estimates of threshold, saturation and mid-dynamic stimulus currents and latencies. The new software also gives greater control of the fitting procedures, allowing:

- IO curves to be fitted to non-saturated IO functions

- IO curves to be fitted to non-monotonic IO functions
- constraining IO curves to have a minimum dynamic range
- removal of spontaneous activity from IO functions before fitting
- weighting of the Latency curve fitting, using the latency standard deviations

3.2.3 Temporal Response Properties

The temporal response properties of each single-unit are examined by recording a series of IO functions at different stimulus rates (Figure 4). The new software allows all the IO functions to be loaded and analyzed together, greatly reducing the time required to analyze each unit. The stimulus current required to reach saturation at each rate is determined and displayed. The maximum stimulus rate at which it is possible to saturate the response of the unit can then be determined.

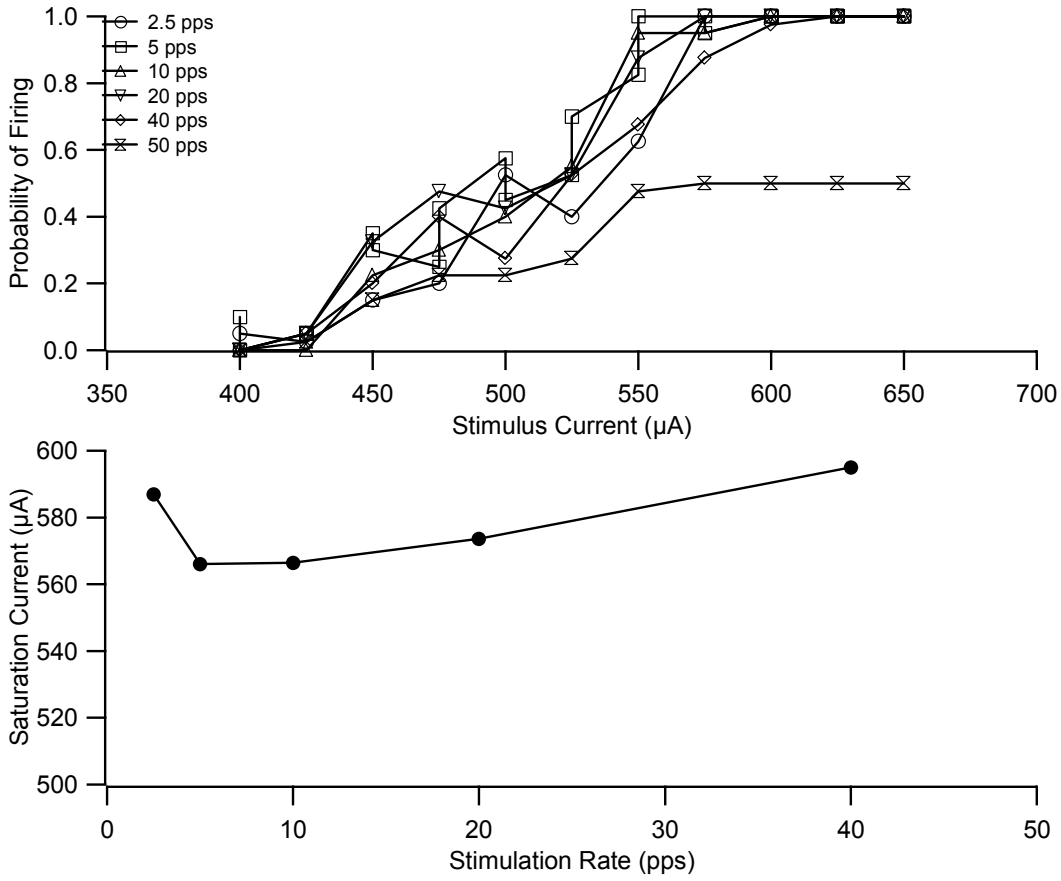

Figure 4. The temporal response properties of an IC single-unit (recording depth: 3146 μm, same animal as Figure 1, 2 and 3) can be determined by measuring several IO functions using different stimulation rates. For this unit, the maximum stimulus rate at which the response saturated was 40 pps.

3.2.4 Spatial Tuning Curves

Once all the single or multi-unit data has been analyzed for each penetration of the IC, a spatial tuning curve (STC) can be constructed based on the IO function of each unit. Historically, threshold STCs have been constructed, whereby the threshold stimulus current is plotted against recording depth (Snyder et al., 1990). As the stimulus current for mid-dynamic range is a more robust measure than either threshold or saturation when determined using the least-squares fitting procedures, the new analysis software allows either threshold, saturation or mid-dynamic range stimulus currents to be plotted against recording depth (Figure 5). It is also possible to exclude units from the STC based on the r^2-value of their fitted IO curve, with typical minimal accepted r^2-values being above 0.95.

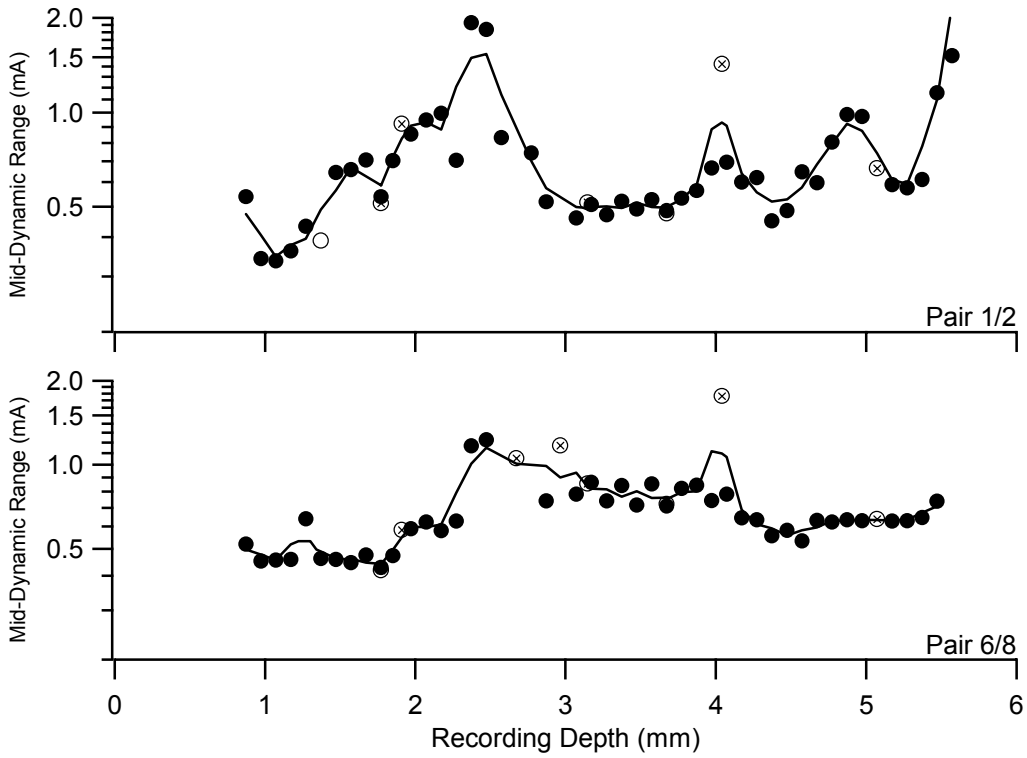

Figure 5. Spatial tuning curves for mid-dynamic range stimulus current for single and multi-units from a single penetration of the IC in the same animal as Figure 1, 2, 3 and 4. Spatial tuning curves for both a basal pair (Pair 6/8) and a more apical pair (Pair 1/2) of stimulating electrodes are shown. Closed symbols represent multi-unit recordings, crossed symbols single-unit recordings and open symbols units with non-monotonic IO functions. The solid curve represents a 3-point smoothed spatial tuning curve.

3.2.5 Auditory Brainstem Response

An upgrade to the software used to analyze auditory brainstem responses (ABRs) was also begun this quarter. This software will significantly improve the analysis of the ABR data generated as part of the Chronic electrical

stimulation studies in neonatally deafened cats, and Electrical stimulation and neurotrophin administration in deafened guinea pigs study. Details will be presented in a future report.

3.3 Preliminary results

A preliminary analysis of the response of IC neurons to electrical stimulation of the auditory nerve following chronic electrical stimulation and long-term SNHL has been made. A more complete analysis will be performed once the upcoming additional experiments have been performed, and data from control animals has been recorded.

The most striking preliminary result is the alteration of the STCs of the chronically stimulated animals, as illustrated by the example in Figure 5. The STCs appear to be shallower and wider than previously reported for normal, neonatally deafened and other chronically stimulate animals (see Shepherd et al., 1999; Snyder et al., 1990; Moore et al., 2002). This may in part be due to the differences in the stimuli used (charge balanced biphasic current pulses compared to continuous sinusoids) and the recording configurations (single-unit activity compared to single and multi-unit activity) of the various studies. Nevertheless, initial analysis appears to indicate that this is from an increased representation of the chronically stimulated cochlea sector, rather than a general decrease in the efficacy of the cochlea stimulation.

The maximum stimulus rates at which it is possible to saturate the response of single-units (25 $\pm$ 15 pps, Mean $\pm$ STD) appears to be lower than the values for normal animals, but higher than values for neonatally deafened un-stimulated animals that have previously been reported. Similarly, the mean first spike latencies appear to be longer than for normal animals, but shorter than for neonatally deafened un-stimulated animals.

These preliminary results suggest that the chronically stimulated, neonatally deafened animals have responses that are intermediate between normal and un-stimulated neonatally deafened animal. However, further electrophysiological experiments are required before any of these results can be confirmed. The histological and immunohistochemical results are also required to allow any correlations between the extent of morphological changes and functional changes to be assessed. Cochlear histology from a number of our chronically stimulated animals is now available and quantitative analysis will commence during the next quarter.

4. Electrical stimulation and neurotrophin administration in deafened guinea pigs

Six adult guinea pigs were profoundly deafened using a single intravenous injection of 100 mg/kg of Frusemide followed by a subcutaneous injection of 400 mg/kg of Kanamycin. Five days after deafening, two of the animals were implanted with an electrode array/mini-osmotic pump assembly (Figure 1; *First Quarterly Progress Report*). Surgical details are outlined in our *Fourth Quarterly Progress Report.* One animal was chronically stimulated, while both animals received artificial perilymph over a 28-day implant period. The second

animal will form part of our chronically implanted un-stimulated control group. Upon completion of this period, functional studies will be performed using electrically-evoked auditory brainstem responses on four of the animals; the animals will then killed with an overdose of anesthetic before preparation of the cochleae and brain for histological and immunohistochemical examination (*Fourth & Fifth Quarterly Progress Reports*). The remaining two deafened guinea pigs will undergo functional studies after an additional 28-days, as will a group of longer term deafened control animals. Quantitative analysis of guinea pig cochleae, including measurements of spiral ganglion neuron density will commence in the next quarter. Both the electrophysiological and histological data will be presented in a future report.

5. Personnel

During the quarter Miss Stephanie Epp joined the team and is employed as a Research Assistant. Stephanie has a B.Sc., with Honours, in Physiology from Monash University. She has experience in animal surgery, molecular biology (including gene expression studies), laboratory management and data collection and analysis. Stephanie will play a key role in all aspects of this project.

6. Publications

During the quarter the following paper was written and submitted to hearing Research:

Shepherd R.K., & Xu, J. A multichannel scala tympani electrode array incorporating a drug delivery system for chronic intracochlear infusion.

In addition a chapter was completed for the forthcoming book “Neuroprosthetics: Theory and Practice”. K. Horch & G. Dhillon (Eds), World Scientific Publishing. Shepherd, R.K. Central Auditory Prostheses.

7. Plans for Next Quarter

- Continue our chronic stimulation studies in deafened kittens and guinea pigs.
- Continue the manufacture of guinea pig and feline electrode assemblies.
- Continue terminal acute electrophysiology experiments on chronically stimulated cats and guinea pigs.
- Continue histological preparation and analysis of cochleae and auditory brainstem structures in cats and guinea pigs following completion of the chronic stimulation program.
- Continue developing our immunochemistry protocols.

8. Acknowledgements

We gratefully acknowledge the important contributions made by our Veterinarian Dr Sue Pierce, Elisa Borg for management of our animal house, Damon Shepherd for research assistance, Dr Jin Xu and Helen Feng for

electrode manufacture, Maria Clarke for histology support and Rodney Millard and Frank Nielsen for engineering support.

9. References

Hardie, N. A., Shepherd, R. K., 1999. Sensorineural hearing loss during development: morphological and physiological response of the cochlea and auditory brainstem. Hear Res. 128, 147-165.

Moore, C. M., Vollmer, M., Leake, P. A., Snyder, R. L., Rebscher, S. J., 2002. The effects of chronic intracochlear electrical stimulation on inferior colliculus spatial representation in adult deafened cats. Hear Res. 164, 82-96.

Sachs, M. B., Abbas, P. J., 1974. Rate versus level functions for auditory-nerve fibers in cats: tone- burst stimuli. J Acoust Soc Am. 56, 1835-1847.

Shepherd, R. K., Baxi, J. H., Hardie, N. A., 1999. Response of inferior colliculus neurons to electrical stimulation of the auditory nerve in neonatally deafened cats. J Neurophysiol. 82, 1363-1380.

Snyder, R. L., Rebscher, S. J., Cao, K. L., Leake, P. A., Kelly, K., 1990. Chronic intracochlear electrical stimulation in the neonatally deafened cat. I: Expansion of central representation. Hear Res. 50, 7-33.