Eighth Quarterly Progress Report

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Protective Effects of Patterned Electrical Stimulation on the Deafened Auditory System

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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 eighth quarter the following activities were completed:

2.1. Publications and conferences

The following papers were accepted for publication and an additional six manuscripts are currently in the review process.

Gillespie, L.N. and Shepherd, R.K. Clinical application of neurotrophic factors: the potential for primary auditory neuron protection. European J. Neuroscience 22: 2123-2133, 2005. (Appendix A)

Hildebrand, M.S., Dahl, H-H.M., Hardman, J., Coleman, B., Shepherd, R.K. and de Silva, M.G. Survival of partially differentiated mouse embryonic stem cells in the scala media of the guinea pig cochlea. JARO (in press). (Appendix B)

Ass Prof Shepherd was a Session Chair at the 2005 Conference on Implantable Auditory Prostheses and gave the following presentation:

"Electrode technology and design" Session chair's Introduction, 2005 Conference on Implantable Auditory Prostheses, Asilomar CA, USA August 2005.

In addition, the following presentations were made at the same meeting (Appendix C):

O'Leary, S.J., Richardson, R.T., Wise, A., Hardman, J., Sly, D., Heffer, L., Shepherd, R.K. & Clark, G.M. "Design considerations for new implant electrodes". Conference on Implantable Auditory Prostheses, Asilomar CA, USA August 2005. p44.

Sly, D.J., Heffer, L.F., White, M.M., Shepherd, R.K. & O'Leary, S.J. "The response of the auditory nerve to electrical stimulation following deafness". 2005 Conference on Implantable Auditory Prostheses, Asilomar CA, USA August 2005. p108.

Heasman, J.M., Prado-Guitierrez, P., Fewster, L.M., McKay, C.M., Shepherd, R.K. "Measurement of the electrically evoked auditory brainstem response and cortical response using the Nucleus freedom cochlear implant" 2005 Conference on Implantable Auditory Prostheses, Asilomar CA, USA August 2005. p204.

Newbold, C.J., Richardson, R., Millard, R.E., Huang, C., Milojevic, D., Seligman, P., Cowan, R., & Shepherd, R.K. "Findings of an in vitro model of the electrode interface: Implications for cochlear implants". 2005 Conference on Implantable Auditory Prostheses, Asilomar CA, USA August 2005. p218.

McGuinness, S.L. & Shepherd, R.K. "Exogenous BDNF rescues rat spira; ganglion neurons *in vivo*". 2005 Conference on Implantable Auditory Prostheses, Asilomar CA, USA August 2005. p227.

2.2. 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.

Counts of SGN density were commenced this quarter, and should be completed in the following quarter. The results of which will be analyzed and prepared for publication in future quarters.

2.3. Chronic electrical stimulation in the cat

This work continues to address the questions of whether chronic depolarization alone, via a cochlear implant, can prevent SGN degeneration. Additionally, the question of whether patterned chronic electrical stimulation of the auditory nerve can produce plastic reorganization within the central auditory pathway is being addressed.

During this quarter, up to seven neonatally deafened, implanted animals received daily chronic electrical stimulation. Each animal had an electrically evoked auditory brainstem responses (EABRs) recorded monthly, with subsequent behavioral testing of maximum comfortable stimulus levels and minor adjustments of chronic stimulation levels as required. Daily impedance monitoring of the chronically stimulated animals is also ongoing. Three of the chronically stimulated animals, along with two normal hearing controls were underwent acute electrophysiological experiments this quarter (see section 3.3), while an additional four animals were neonatally deafened with daily injections of neomycin and will serve as un-implanted deafened controls. Therefore, at the end of the quarter we had four implanted animals that are nearing six months of chronic stimulation and six deafened un-implanted controls.

Following the completion of each acute electrophysiological experiment, the cochlea and CNS from each animal were harvested and prepared for subsequent analysis. Additionally,

the cochlear nuclei from each animal were processed for both light microscopy and transmission electron microscopy and sent to Prof. David Ryugo for ultrastructural analysis of the end bulb of Held.

As we have now completed the acute electrophysiological experiments on our first cohort of chronically stimulated animals we feel, it is appropriate to review our progress and our choice of equipment for our multi-channel recordings. This review has been included in section 3.3.

2.4. Chronic electrical stimulation in the rat

This work aims to address the issue of 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 two deafened, implanted adult rats continued to undergo daily stimulation via their intracochlear electrode arrays. The threshold current for eliciting a magnetically induced EABR (mEABR) has remained relatively stable to-date (Figure 1), giving us confidence that our totally implantable stimulator can be successfully applied chronically as required for our behavioral experiments.

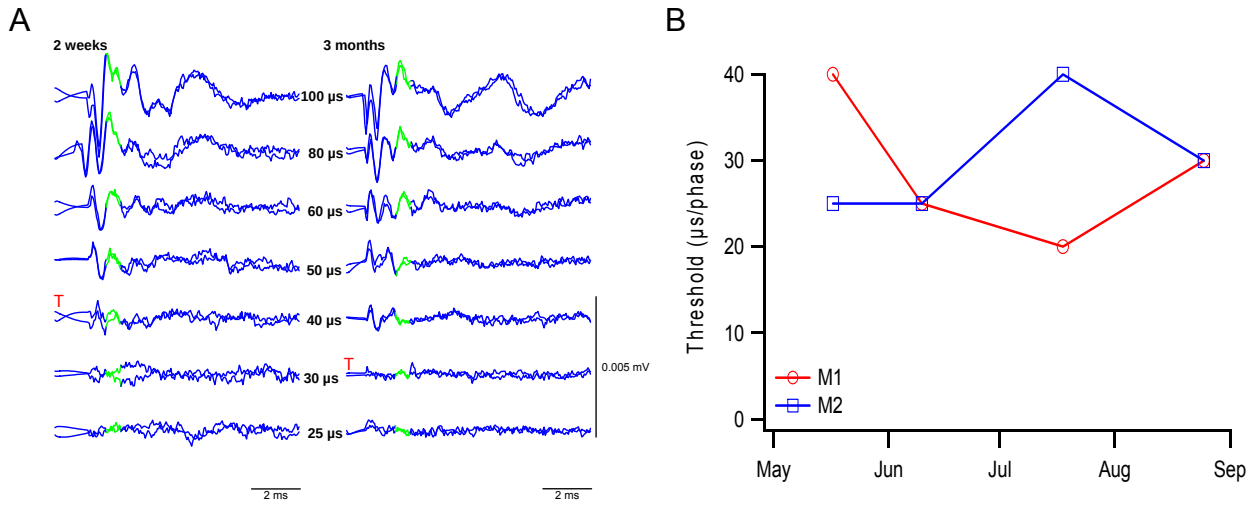


Figure 1 mEABRs over time

A: mEABRs evoked from a deafened, chronically implanted animal (M1) two weeks and three months after implantation. B: The threshold phase duration to elicit an mEABRs from two deafened, chronically implanted animals has not significantly changed over the duration of their implantation. The implantable stimulator induces a charge balanced biphasic current pulse of fixed current (500 µA peak), with charge delivery controlled by varying pulse width.

This quarter has also seen the completion of the modifications of the behavioral training maze and continued testing of normal hearing animals on a variety of discrimination tasks.

2.5. Cellular over-expression of BDNF

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

As indicated in the previous QPR, Schwann cells have been successfully transfected to incorporate DNA to induce over-expression of the neurotrophins BDNF and NT-3. These

initial transfections used five different types of plasmid DNA, resulting in five different Schwann cell lines:

a) EGFP-SCs – Schwann cells were transfected with the pEGFP-N1 expression vector (ClonTech) only, which contains the reporter gene enhanced green fluorescent protein (EGFP), but no neurotrophin DNA; this will serve as a control

b) BDNF-SCs – Schwann cells were transfected with the BDNF gene

c) E-BDNF-SCs – Schwann cells were transfected with the BDNF gene that is conjugated to EGFP, such that any BDNF produced is visible using fluorescence microscopy

d) NT3-SCs – Schwann cells were transfected with the NT-3 gene

e) E-NT3-SCs – Schwann cells were transfected with the NT-3 gene that is conjugated to EGFP

Preliminary *in vitro* experiments were conducted this quarter to determine the survival effects of these neurotrophin-producing Schwann cells on SGNs. Results from these experiments confirm the biological activity of both BDNF and NT-3 produced by these cells, with significantly greater SGN survival resulting from co-culture with each of the neurotrophin-producing Schwann cells, in comparison to normal Schwann cells, EGFP-SCs and untreated controls.

In addition to the *in vitro* experiments, enzyme-linked immunosorbent assays (ELISAs) were performed to quantify the BDNF and NT-3 released from different Schwann cell lines. These experiments indicated that the BDNF-SCs, E-BDNF-SCs and E-NT3-SCs produce significantly greater amounts of the respective neurotrophins than both normal Schwann cells and the EGFP-SCs. Interestingly, despite eliciting a survival effect, the NT3-SCs did not produce detectable amounts of NT-3. Further analysis will be conducted over the coming quarters to resolve this issue.

Finally, experiments to transfect male Schwann cells were commenced this quarter. These cells are preferable for use in the *in vivo* implantation studies, as it will enable us to positively identify the transplanted cells via *in situ* hybridization for the Y chromosome. While the transfections of the male Schwann cells were successful, the selection for stable transformants – those cells that will continuously produce the neurotrophin – was not. The concentration of selective reagent (400 µg/ml, Geneticin G418; Gibco) that was effective for use in the selection of Schwann cells of mixed derivation was too high for the male only Schwann cell cultures. A subsequent ‘kill curve’ has determined that 200 µg/ml will be an appropriate concentration for selection of the male only transformants.

2.6. 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.

In this quarter, efforts have been directed towards exploring quantitative or semi-quantitative means of analyzing gene expression. This requires a gel documentation system that can image and evaluate the change. Applications for additional equipment funding from several philanthropic sources to purchase such a system were submitted this quarter. Funding has been obtained from the Marion and EH Flack Trust, which would enable the purchase of a medium-level gel documentation system. As the system will be

an integral part of our molecular biology facility, a system from SciTech is currently being extensively evaluated.

Additional, this quarter Western blot analysis of cochlear and brain samples to test the specificity of the antibodies and to gain experience using the BioRad gel electrophoresis system was commenced. This technique allows analysis of protein changes of a targeted gene, while confirming that protein levels of a housekeeping gene is unaffected, thus eliminating bias that can arise from using unequal amounts of proteins. The technique is currently operational for cytoplasmic proteins and is expected to being generating useful data in the following quarters.

2.7. 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.

This quarter, final *in vitro* co-culture experiments were completed and statistical analysis of the results showed highly significant effects of co-culture on the differentiation of mouse embryonic stem cells into neurons *in vitro*. We intend to prepare this work for submission to the Journal of Experimental Cell Research. Additionally, a methodological paper resulting from this work is being prepared for submission to the Journal of Neuroscience Methods.

3. Multi-channel recording

The primary aim of the acute electrophysiological experiments that are part of the Chronic electrical stimulation in the cat study is to address the question of can 'patterned chronic electrical stimulation of the auditory nerve produce plastic reorganization within the central auditory pathway'? Specifically, data from the primary auditory cortex (AI) is being collected to answer the following questions:

a) Does chronic electrical stimulation of restricted regions of the cochlea lead to reorganization of the normal cochleotopic map in AI (Spatial Plasticity)?

b) Does chronic electrical stimulation (and the rate of that stimulation) effect the temporal resolution of neurons within AI (Temporal Plasticity)?

c) Does patterned chronic electrical stimulation lead to altered current interactions for closely paired stimuli (Channel Interactions)?

The nature of the experiments, particularly the Spatial Plasticity section, lend themselves to multi-channel recording. Multi-channel recording systems can be broadly subdivided into two sections, multi-channel electrode arrays and multi-channel data acquisition systems. In the following sections, a brief summary of our requirements, the available options and our selected option and its performance to-date will be given. Finally, a brief summary of some preliminary results from our initial acute electrophysiological experiments will be presented.

3.1. Multi-electrode arrays

There are many designs of multi-electrodes that have been reported in the literature and/or are available for purchase, however many of them appear to have been designed primarily for chronic recordings from the primate cortex. The suitability of such arrays for use in smaller animal studies (i.e. cat, rat and guinea pig), where electrode spacing and curvature

of the cortex become more significant, does not seem to have been addressed. The ideal multi-electrode array for our acute electrophysiological experiments would:

a) Provide adequate spatial sampling, including sampling across the cortex (and potentially down sulcal banks), at an appropriate insertion depth(s) (or at multiple depths for current source density analysis). In practice, for the cat, AI extends for 5 – 7 mm in the rostrocaudal direction between the tips of AES and PES (Reale and Imig 1980) and in the mediolateral direction is normally considered to be between the SSS and a line 2-3 mm ventral to the tips of AES and PES (Raggio and Schreiner 1999). Therefore, a planar recording area of more than 5 x 5 mm is required; additionally AI extends down the rostral side of PES for several millimeters. The ideal recording depth within cat AI is approximately 1 mm, while for rat is it approximately 0.8 mm.

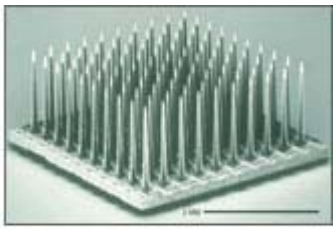
b) Allow for independent positioning of each electrode.

c) Be easily inserted and allow for adjustment of electrode placement if required.

d) Be suitable for multiple reuse and not be prohibitively expensive.

None of the existing multi-electrode arrays currently fulfil all of these requirements. The existing arrays can be broadly divided into two main categories: linear arrays (typified by the Michigan array) and planar arrays which can be further divided into solid-state arrays (typified by the Cyberkinetics array) and flexible arrays (typified by µ-wire arrays (e.g. Rennaker et al. 2005)).

3.1.1 Cyberkinetics array



The Cyberkinetics / Utah array is a solid-state, silicon substrate array that was developed at the University of Utah (Nordhausen et al. 1996) that is now marketed by Cyberkinetics and is one of our current arrays of choice. In its present form, the arrays are available as up to 10x10 arrays with 400 µm spacing between electrodes of either 1 or 1.5 mm length. Each electrode has a conductive tip that is approximately 50 µm long and less than 8 µm wide with impedances in the order of 100s of kΩ.

Due to the curvature of the cat cortex between AES and PES, and the fixed length of the electrodes (1 mm), it is not feasible to use a single Cyberkinetics array to record from the entire cat AI. We are currently employing up to two 5x5 or 6x6 arrays that cover a total of up to approximately 5 x 2.5 mm (Note: this does not sample any of AI extending down PES). The fixed length of the recording electrodes, with a single active site at their tip, also means that the precise depth recorded from varies across the extent of the array. The fixed 1 mm depth is also often too deep for recording in rat AI. The arrays are an “insert once” type, whereby it is not possible to adjust the position (or depth) of the electrodes once the array is inserted.

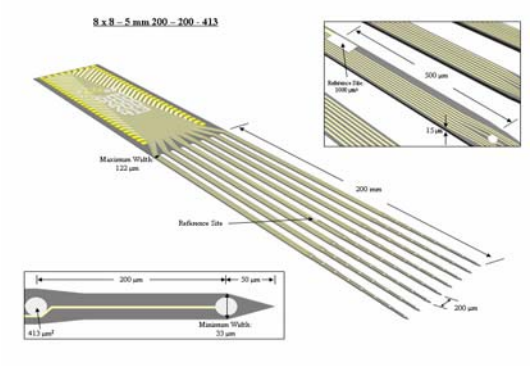
10 x 10 versions of these arrays have been used by others to record AI maps, although only with limited success, as “the most common problem was the sparsity of drivable units that could be reliably recorded from the UEA [Utah Electrode Array] implanted in AI (Normann et al. 2004b).” Out of 16 attempted experiments, two were reported as excellent, one as good (70% of electrodes exhibited activity), two as poor and four as the arrays may not have been deeply enough inserted (Normann et al. 2004c) , it appears that 25 – 30% activity is about the usual outcome (Normann et al. 2004a).

3.1.2 µ-wire array

There are a variety of µ-wire arrays described in the literature (see for example deCharms et al. 1999; Rennaker et al. 2005). The arrays typically consist of a number of fine tungsten or iridium wires that are held together in a fixed configuration. Generally, the spacing of the electrodes is in the order of 250 – 350 µm and assembled into arrays of up to 10 x 10 electrodes. Some designs allow for independent control of insertion depth (deCharms et al. 1999), but none allow for independent spatial positioning.

These arrays seem to offer a few advantages over the Cyberkinetics array in that it is possible to control the depth of insertion in some configurations, there appears to be a greater flexibility of spatial configurations and they appear to be a simpler construction. However, the arrays themselves do not appear to be as mechanically robust, and they are still not capable of recording from multiple sites within the rostral bank of PES in the cat. Multiple electrodes inserted to multiple depths may be an option in the future. The arrays also allow for repositioning of the array to a new / different recording site.

3.1.3 Michigan array



The Michigan array is a solid-state, silicon substrate array developed at the University of Michigan that is now marketed by NeuroNexus Technologies and is our current array of choice for recording from the sulcal bank in cat AI. The arrays are available in a variety of forms, including linear arrays with 16 recording sites spaced 150 µm apart and 200 µm spaced recording sites, on up to 8 shanks that are spaced also at 200 µm.

These arrays offer the advantage of being able to record at multiple depths, potentially at several different sites, although only along a single axis. The single shank probes have been used by Middlebrooks et al. (see for example Bierer and Middlebrooks 2004) to record along the tonotopic axis of the guinea pig AI, and by Synder et al. (for example Bonham et al. 2004) to record along the tonotopic axis of cat IC. While it might be possible to attempt a similar insertions in the rat, either multiple insertions or multiple arrays would be required to record from the entire cortical extend of cat AI. The arrays also allow for repositioning of the array to a new / different recording site.

3.1.4 Array performance

Our initial results with the Cyberkinetics arrays were promising, with activity on 80-90% of electrodes immediately after insertion of the array. Subsequent experiments were not as successful, often with less than 50% of electrodes exhibiting activity, resulting in significant areas of AI that were unable to be recorded from. After significant discussions with Cyberkinetics (including a site visit), a combination of changes in our experimental protocol and further experience in using the arrays has resulted in our recent experiments achieving recordable activity on more than 75% of electrodes. The results with the Michigan array (when used) have been similar to those reported by others. The combination of Cyberkinetics arrays and Michigan arrays appears to be satisfactory, if not ideal, for cat AI recordings. However, the spacing of the electrodes on Cyberkinetics arrays is too coarse for rat AI. We are therefore exploring the possibility of either µ-wire arrays or a multi-pronged Michigan array for planned rat AI experiments.

3.2. Data Acquisition System

The Neuroshare website contains a list of approximately 25 suppliers of data acquisition / software vendors, with many more not listed. To assess the suitability of the various multi-channel data acquisition options, we identified the key features that such a system must have for our electrophysiological experiments. The features can be broadly separated in hardware and software requirement and include:

a) Hardware
   a. High signal-to-noise ratio (SNR) amplification with good common-mode rejection (CMR) and low channel cross-talk.
   b. Compatibility with a variety of electrode arrays.
   c. Rapid recovery from electrical stimulus artifact.
   d. Sufficient precision and timing in the data acquisition, not less than: 16 bit resolution; 30 kSample/s; and 60 channels.
   e. Ability to record / control other experimental signals, including integration into the current recording system and future adaptability.

b) Software
   a. Easy to use / control
   b. Ability to automate recording of standard protocols such as I/Os and RAs.
   c. Option to store either / both raw and processed data.
   d. On-line spike discrimination / artifact rejection.
   e. Support / documentation.

Of the systems available at the start of 2005, only four systems had specification that approximated our requirements. Of these, only the Cerebus system from Cyberkinetics appeared to be a mature product that was likely to meet our needs.

		Plexon		**Cyberkinetics**	**RC Electronics**	**Neuralynx**
		MAP	Recorder	Cerebus	DataMAXII	Cheetah
Online	Channels	64		96		~ 64
	Rate (kS/s)	40		30		~39
Offline	Channels	32	64	64	64	~64
	Rate (kS/s)	20	40	30	50	~39
Resolution (Bits)		12	12	16	16	12
Other Inputs	Digital	16		16	8	32
	Analogue			16		
External Trigger		No	No	No	Yes	
Waveform views online		All	One	All	Eight	

Table 1 Multi-channel data acquisition systems

3.2.1 Cerebus system performance

Our experience to date with the Cerebus system has generally been positive. While there have been a few initial 'teething' problems, they have been satisfactorily dealt with, with good support from Cyberkinetics. While the system has reliably performed during the

majority of experiments, producing good quality recordings, there are a few aspects of the system that continue to be less that ideal. Some specific issues with the system include: no 'pre-trigger' facility; poor integration of digital input; and poor integration with none Cyberkinetics electrodes. All these issues are being addressed with a variety of 'in-house' and Cyberkinetics based solutions.

3.3. Preliminary results

Acute electrophysiological experiments have been attempted in two normal hearing animals and six deaf, chronically stimulated animals. Two of the experiments in the chronically stimulated animals were terminated without recording neuronal data, due to complications related to anesthesia. Detailed analysis of the data from the remaining chronically stimulated animals and control animals is underway, and will be the subject of a future QPR.

Preliminary analysis of response area data from one of the normal hearing animals, recorded using a 6 x 6 Cyberkinetics array is presented in Figure 2. The corner electrodes of the array were not wired, resulting in 32 active electrodes, based on the post-stimulus time histograms (PSTH), 29 of the 32 active electrodes had driven activity in response to acoustic stimulation (Figure 2A). The 'response areas' of these multi-unit clusters are illustrated in Figure 2B and exhibit tonotopic organization, with the caudal units being most sensitive to frequencies of approximately 20 kHz, while the most rostral units were preferentially excited by frequencies of approximately 30 kHz. This change in best frequency with rostrocaudal distance is in good agreement with that reported by Raggio et al. (1999).

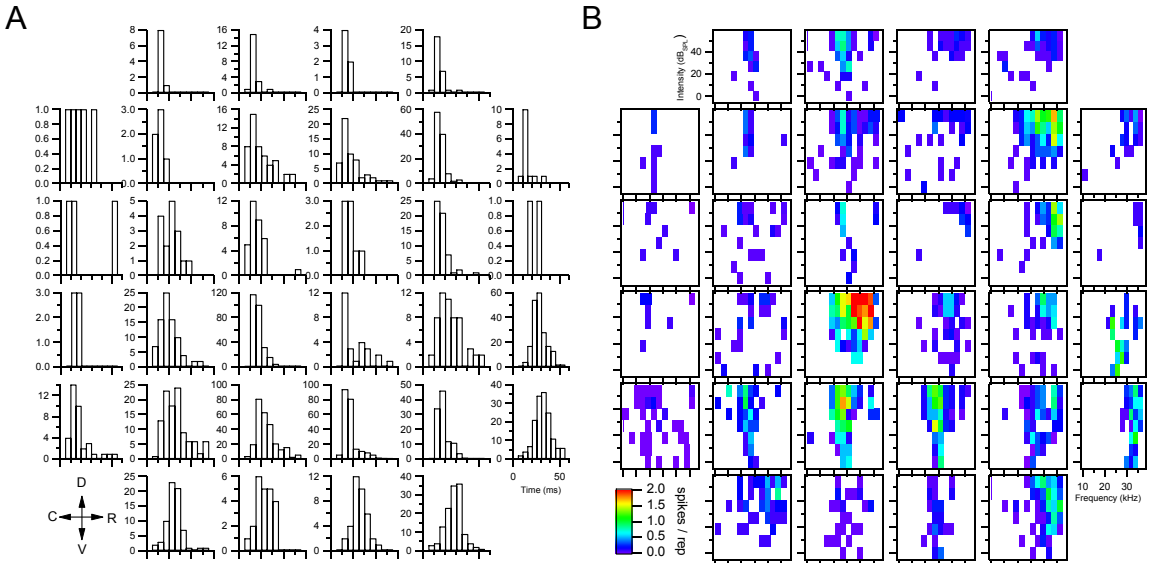


Figure 2 Response to acoustic stimulation

A: Post-stimulus time histograms (PSTH) recorded from each electrode on a 6 x 6 Cyberkinetics array (inter-electrode spacing = 400 µm) inserted into the left AI of a normal hearing cat illustrate that the majority of electrodes (29 / 32) recorded driven activity. B: 'Response Areas' constructed from 20 presentation of each acoustic intensity / frequency combination demonstrate tonotopic organization (high frequencies preferentially activate more rostral electrodes) of AI.

The animal was subsequently unilaterally deafened by perfusion of the left cochlea with 10 % w/v Neomycin. A standard 8-ring stimulating electrode was then inserted into the cochlea, and confirmed to be operational by recording electrically evoked auditory brainstem responses (EABRs). All electrical stimulation was performed using a common-

ground electrode configuration. Figure 3 illustrates the recorded cortical responses to electrical stimulation of the cochlea. Based on the PSTH at least half of the active electrodes had clear indications of driven activity in response to electrical stimulation (Figure 3A). The 'cortical maps' of these multi-unit clusters are illustrated in Figure 3B along with the EABR threshold for each stimulating electrode. The regions of the cortex that responded to the electrical stimulation correspond to a best frequency range of 15 – 25 kHz (compare Figure 2 and Figure 3), which is in good agreement with the estimated cochlear location of the stimulating electrode (Brown et al. 1992). However, unlike the responses to acoustic stimulation, no finer grain cochleotopic organization differentiating between individual stimulating electrodes is evident using this coarse analysis of the responses to electrical stimulation.

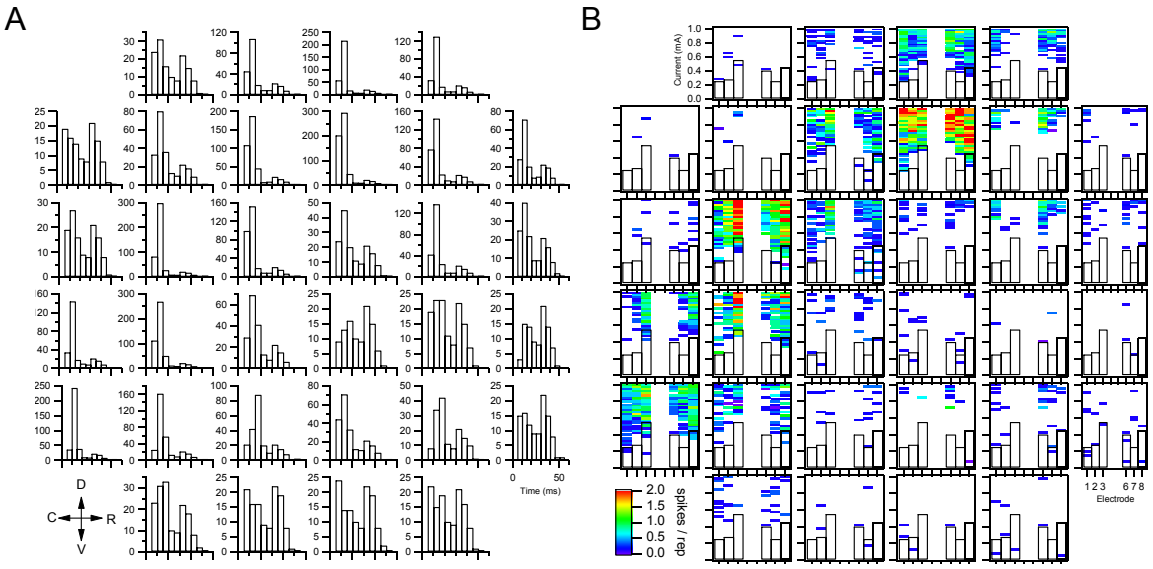


Figure 3 Response to electrical stimulation

A: Post-stimulus time histograms (PSTH) illustrating driven activity in response to electrical stimulation of the cochlea. B: 'Cortical maps' constructed from 5 presentation of each electrode / current level combination (White bars (black outlined) represent EABR threshold for each electrode).

3.4. Conclusion

The preliminary results from our first acute electrophysiological experiments using our new multi-channel recording system have produced data that confirms the well known tonotopic organization of cat AI. In doing so, we are confident that we now have a multi-channel data acquisition system that will be able to record appropriate data to address the questions of plastic reorganization within the central auditory pathway.

What is not as clear at this point is the suitability of the currently available multi-channel electrode arrays. Given the centre-to-centre spacing of the electrodes on our simulating arrays (750 µm), the expected normal rostrocaudal representation of each stimulating electrode in cat AI is of the order of 100 µm (Raggio and Schreiner 1999). Therefore, the sampling of the cortex available with the Cyberkinetics array (400 µm spacing) may ultimately be too coarse to adequately examine any small scale reorganization of the cochleotopic map of AI. While other arrays currently on the market offer spacing of down to 150 µm, even this may be too coarse to observe small changes in the reorganization of the normal cochleotopic map.

4. Plans for next quarter

Plans for the following quarter include:

a) Continued manuscript writing and submission, and preparation for attending conferences.

b) Continued counting of SGN density and analysis of data from the Chronic electrical stimulation and neurotrophin delivery in the guinea pig study.

c) Continued studies in the auditory cortex of deafened, chronically stimulated cats, including four planned acute electrophysiological experiments using our multi-channel Cerebus data acquisition system and analysis of data from the first two cohorts of chronically stimulated animals.

d) Sacrifice of the animals that are part of the Chronic electrical stimulation in the rat studies to assess the condition of the implanted stimulators.

e) Continued fabrication of electrode assemblies for use in our chronic stimulation studies.

f) Complete an auditory discrimination study in normal hearing rats where the animals will be required to discriminate between acoustic click trains presented at different rates.

g) Finalize *in vitro* studies examining the survival activity of neurotrophin producing Schwann cells on SGN cultures.

h) Refinement of the protocols for the transfection and selection for stable transformants of the male Schwann cells.

i) Continues investigation of the short- and long-term effects of deafness on neuronal and trophic markers in the cochlea neurons.

j) Commencement of animal experimentation for final in vivo studies of The application of stem cells for SGN regeneration, including exploration of alternative surgical approaches for delivery of those cells.

5. Personnel

David Perry joined the group as an 'Undergraduate Research Opportunities Program' student via the Bio21 program. He will be working approximately one day per week as he continues his undergraduate studies in electrical engineering and physiology. His duties include daily monitoring of our chronically stimulated cats, software development for analysis of our chronic electrode impedance data and assisting Rodney Millard in optimization and construction of the fully implantable stimulators used in the chronic rat stimulation studies.

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; Prof. Trevor Kilpatrick and Dr. Simon Murray 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.

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8. Appendix A (attached)

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9. Appendix B (attached)

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10. Appendix C (attached)

Presentations made at the 2005 Conference on Implantable Auditory Prostheses, Asilomar CA, USA August 2005.