15th Quarterly Progress Report

April 1 to June 30, 2007

Neural Prosthesis Program Contract N01-DC-3-1006

Protective and Plastic Effects of Patterned Electrical Stimulation on the Deafened Auditory System

Submitted by:

Patricia A. Leake, Ph.D., Principal Investigator
Stephen J. Rebscher, MA
Alexander M. Hetherington, BS
Russell L. Snyder, Ph.D.
Ben H. Bonham, Ph.D.

Epstein Hearing Research Laboratories
Department of Otolaryngology, Room U490
University of California, San Francisco
San Francisco, Ca 94143-0526

SUMMARY OF WORK COMPLETED DURING THE PAST QUARTER.

1) During the past quarter, work has continued on our chronic experimental series evaluating the effects of brain derived neurotrophic factor (BDNF) delivered to the cochlea via an osmotic pump and – in half the animals – combined with electrical stimulation (ES) via a cochlear implant. Histological preparation of the cochleae and cochlear nuclei in these subjects (4 neonatally deafened animals and 5 animals deafened at 30 days of age) is continuing and data analysis will continue during the coming quarter. A current focus of this work is to compare 3 different methods for quantitative analysis of spiral ganglion cell survival. Because cell size is markedly changed with BDNF treatment, we feel it is critical to use one of the newer, statistically unbiased stereological methods, the "physical dissector" technique, for assessing the survival of SG neurons following BDNF treatment and to define the relative contributions of cell size vs. cell number to increased SG density after treatment. In order to compare results to our prior findings, initial data are being evaluated with both the new method and our previous area fraction (volume ratio) technique. In addition, during this past quarter, terminal electrophysiological experiments were carried out in two subjects (one neonatally deafened and one 30-day deafend) following 14-15 weeks of chronic electrical stimulation combined with BDNF infusion. Analysis of these data will continue into the next quarter.

2) A new paper on work supported in part by this Contract was submitted during this past quarter and is now being revised to address the reviewers' comments:
   Leake PA, Hradek GT, Bonham B, Snyder RL. Degraded topographic specificity of auditory nerve projections to the cochlear nucleus in cats after neonatal deafness and electrical stimulation. J. Comp. Neurol.

3) Another previously submitted manuscript is also currently being revised to address the reviewers suggestions:
   Vollmer M, Snyder RL, Beitel RE, Rebscher SJ, Leake PA. (Submitted) Spatial Selectivity in the Inferior Colliculus is Degraded Following Long-Term Deafness in Cats. J. Neurophysiol.

4) During the past quarter, 5 abstracts on work supported by this Contract were submitted and accepted for presentation at the biennial Conference on Implantable Auditory Prostheses. The abstracts are included at the end of this report.

5) The main scientific report for this Quarterly Report comprises a manuscript that is currently in press in the Journal of Neuroscience Methods that is entitled "**Design and Fabrication of Multichannel Cochlear Implants for Animal Research**" by SJ Rebscher, AM Hetherington, RL Snyder, PA Leake and BH Bonham. Due to possible copyright infringement issues, the completed manuscript is being submitted to the NIH Project Officer as an appendix, and we are requesting that it not be posted on the NIH website. The abstract is included below, and interested individuals may contact the first author to request: srebscher@ohns.ucsf.edu.

Design and Fabrication of Multichannel Cochlear Implants for Animal Research

ABSTRACT

The effectiveness of multichannel cochlear implants depends on the activation of perceptually distinct regions of the auditory nerve. Increased information transfer is possible as the number of channels and dynamic range are increased and electrical and neural interaction among channels is reduced. Human and animal studies have demonstrated that specific design features of the intracochlear electrode directly affect these performance factors. These features include the geometry, size, and orientation of the stimulating sites, proximity of the device to spiral ganglion neurons, shape and position of the insulating carrier, and the stimulation mode (monopolar, bipolar, etc.).

Animal studies to directly measure the effects of changes in electrode design are currently constrained by the lack of available electrodes that model contemporary clinical devices. This report presents methods to design and fabricate species-specific customizable electrode arrays. We have successfully implanted these arrays in guinea pigs and cats for periods of up to 14 months and have conducted acute electrophysiological experiments in these animals. Modifications enabling long-term intracochlear drug infusion are also described. Studies using these scale model arrays will improve our understanding of how these devices function in human subjects and how we can best optimize future cochlear implants.

Keywords: Cochlear implant, electrode, neural prosthesis, electrical stimulation, drug delivery, neurotrophin.

It should be noted that subsequent to completing the work summarized in this paper, additional modifications have been made in the drug delivery system of these electrodes. Specifically, initial results of chronic experiments in cats using these electrodes to infuse brain-derived neurotrophic factor (BDNF) directly into the cochlea suggested that more or even most of the drug effect was observed in the basal cochlea. Therefore, additional development was done to extend the polyimide cannula for drug delivery through the body of the intracochlear electrode in order to provide a drug delivery port at the apical tip of the electrode (Fig. 1). The apical end of these electrodes is positioned on average at an insertion depth of 12.3 mm from the basal end of the basilar membrane (in a recent series of 12 chronic ES cats). Since the basilar membrane averages 24 mm in the cat, this means apical drug delivery port is positioned at >50% distance from the base of the cochlea. We hypothesize that moving the drug delivery to this site in the upper basal/lower middle cochlear turn will provide more uniform distribution of drugs throughout the cochlea.

Moreover, the insertion depth achieved with these custom-made electrodes places the apical stimulating contact almost 360^{o} from the round window, with a represented frequency of ~4 kHz. We believe that one of the major reasons we have seen consistent trophic effects of ES in our studies is because this relatively deep insertion allows the electrode to encircle the auditory nerve and stimulated to substantially lower frequencies than is possible with straight electrodes

or in studies of smaller lab animals that have smaller, tightly coiled cochleae. This device has now been implanted chronically in the latest subject in our current pilot BDNF experimental series: a neonatally deafened cat implanted at 4 weeks of age, which has been now been undergoing BDNF infusion for 6 weeks. Figure 1 illustrates this latest generation device.

Figure 1

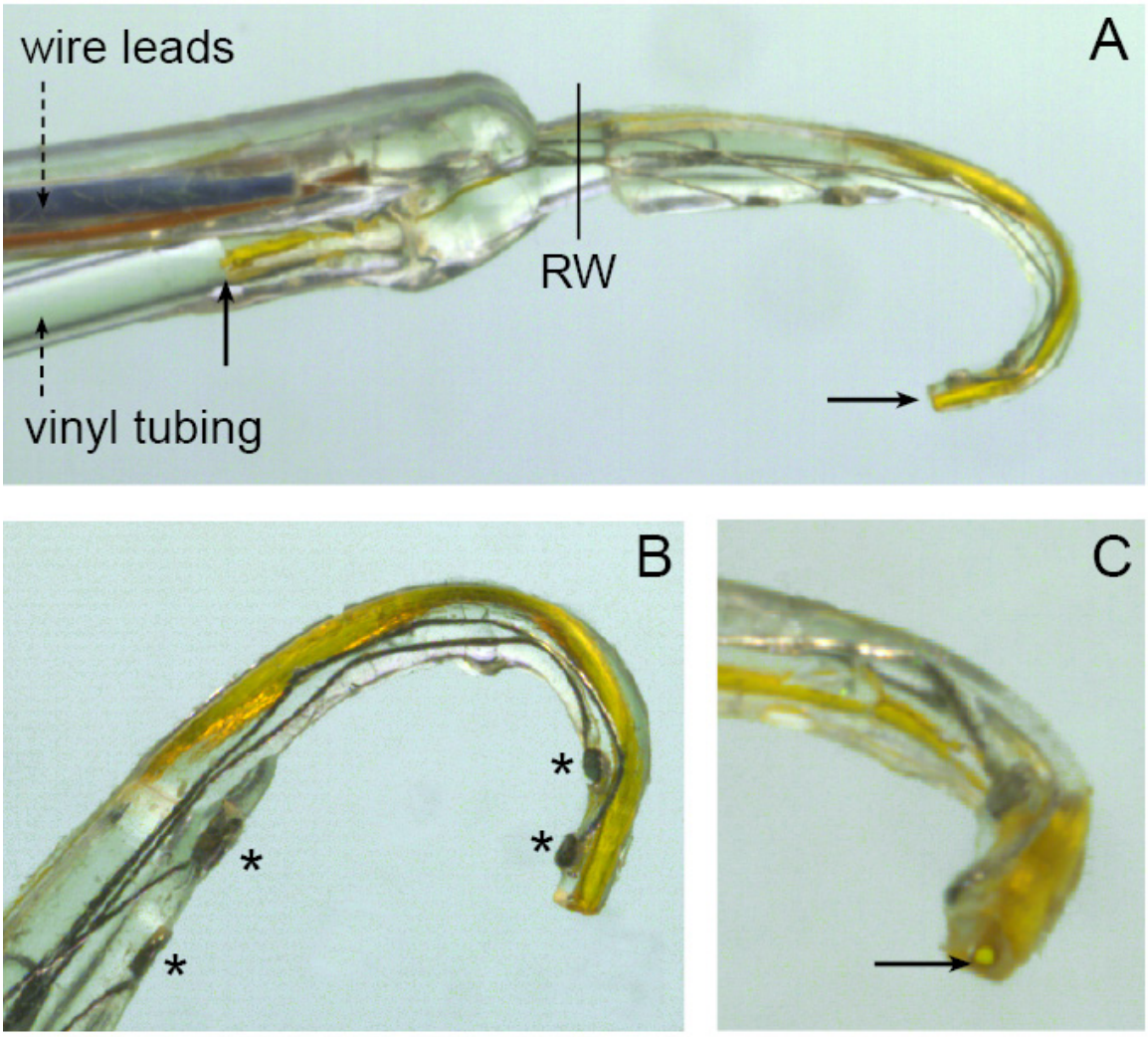

Figure 1. *These images Illustrate the most recent generation of electrode arrays for chronic drug delivery and concurrent electrical stimulation in cats, which has a port at the apical tip of the electrode for drug delivery. Electrodes can be fabricated with a combination of multiple ports, both basal and apical. Arrows indicate the polyimide cannula (0.0044" ID) which runs from the mold hub to the apex of the array. This electrode array was made with four disk stimulation contacts (*), one basal pair and one apical pair for bipolar stimulation.*

WORK PLANNED FOR THE NEXT QUARTER.

1) One additional neonatally deafened animal currently undergoing BDNF infusion and concurrent electrical stimulation (ES) will be studied and euthanized during the next quarter after 19 weeks of BDNF treatment and 15 weeks of applied ES from a two-channel cochlear implant. A terminal electrophysiological experiment will be conducted to determine the efficacy of electrical stimulation (area in the inferior colliculus that is activated by stimulated channels) and to examine temporal processing after chronic stimulation delivered via an Advanced Bionics CII speech processor. Cochlear and cochlear nucleus specimens will be prepared for histology and these analyses will be undertaken as soon as possible.

2) One or 2 litters kittens are expected during the coming quarter and these additional animals will be deafened and implanted as additional subjects for the two BDNF series, i.e., neonatally deafened and 30-day deafened series. In this series, neonatally deafened animals are now being implanted about 1 week after deafening (i.e., at about 4 weeks of age) in order to initiate BDNF infusion during the rapid phase of SG degeneration seen immediately after deafening as reported in our recent study (Leake et al. 2007, J. Comp. Neurol 501:837-853). BDNF treatment continues until the animals are 6-7 weeks of age, when ES is initiated at the usual time in half the animals. In recent experiments, BDNF administration has been extended up to 18 weeks, combined with 15 weeks of electrical stimulation. This protocol is designed to compare more prolonged treatment coupled with ES to our initial data obtained on the short-term (6 weeks) effects of neurotrophin infusion.

3) Work will continue on efforts to compare neurostereological methods for analyses of cochlear and cochlear nucleus histopathology in these BDNF-treated subjects. In the past cell size has not been a major issue for evaluating effects of electrical stimulation because we have seen only very little, if any, difference in cell soma size between stimulated vs. control deafened ears. However, exogenous administration of BDNF elicits marked increases in neuron size, both in culture and *in vivo*. Therefore, we are currently conducting parallel analyses of SG histological material from the BDNF series, directly comparing 1) our standard morphometric method for quantifying area fraction (volume ratio), 2) cell counts applying the Abercrombie correction and 3) the “physical dissector” method. For the cochlear nucleus, we are using an optical dissector method to evaluate neuronal number and density in frozen, along with higher resolution measurements made possible by treating selected frozen sections in osmium tetroxide and embedding the sections in epoxy.

4) Work will continue on several manuscripts in various stages of preparation, submission or revision:
    a. Dr. Vollmer’s J. Neurophysiol. paper entitled “ Spatial Selectivity in the Inferior Colliculus is Degraded Following Long-Term Deafness in Cats” (currently being revised after initial review).
    b. Dr. Leake’s J. Comp. Neurol. manuscript entitled “Degraded Topographic Specificity of Auditory Nerve Projections to the Cochlear Nucleus in Cats after

Neonatal Deafness and Electrical Stimulation" (currently being revised after initial review).

c. Dr. Beitel's manuscript entitled "Neural-Perceptual Model for Auditory Thresholds in Electrical Hearing," (still in preparation).
d. Dr. Stakhovskaya's paper entitled " Effects of Age at Onset of Deafness and Electrical Stimulation on the Developing Cochlear Nucleus (in preparation).

5 ABSTRACTS of work supported by this Contract that were accepted for presentation at the 2007 Conference on Implantable Auditory Prostheses are included below:

1) DEGRADED TOPOGRAPHIC SPECIFICITY OF SPIRAL GANGLION PROJECTIONS TO COCHLEAR NUCLEUS IN CATS AFTER NEONATAL DEAFNESS AND ELECTRICAL STIMULATION

Patricia A. Leake, Gary T. Hradek, Ben H. Bonham and Russell L. Snyder
University of California San Francisco, Epstein Laboratory, Department of Otolaryngology-Head and Neck Surgery, San Francisco, CA USA

We have previously reported that the primary afferent projections from the cochlear spiral ganglion (SG) to the cochlear nucleus (CN) exhibit clear cochleotopic organization in adult cats deafened as neonates. However, the topographic specificity of the CN projections in deafened animals is proportionately broader than normal. This study examined the SG-to-CN projections in adult cats that were deafened as neonates and then received a unilateral cochlear implant (CI) at 6-8 weeks of age.

After >6 months of intracochlear electrical stimulation, SG projections were studied by making focal injections of a neuronal tracer neurobiotin™ directly into Rosenthal's canal to label a small sector of the SG. Clear organization of the SG projections into frequency-band laminae was evident in these deafened animals despite severe auditory deprivation from birth. When normalized for the smaller CN size following deafness, however, AVCN, PVCN and DCN projections from the stimulated ears were broader by 32%, 34% and 53%, respectively, than projections in normal adults. Further, there was no difference between projections from the stimulated and contralateral cochleae. These findings suggest that early normal auditory experience is essential for the normal development (and/or subsequent maintenance) of the topographic precision of SG-to-CN projections. After early deafness, the CN is markedly smaller than normal and the spatial selectivity of SG projections that underlie frequency resolution in the central auditory system is reduced. Further, electrical stimulation from a CI introduced at 8 weeks of age failed to ameliorate, reverse or exacerbate these degenerative changes.

If similar principles pertain in the human auditory system, our results suggest that the selectivity of the neural connections underlying cochleo-topic organization in the central auditory system is likely intact even in congenitally deaf individuals. However, the degraded spatial resolution observed in our studies suggests that there may be inherent limitations in the efficacy of CI stimulation in congenitally deaf subjects, and that spatial (spectral) selectivity of stimulation delivered on adjacent CI channels may be poorer due to the greater extent of overlap of SG central axons representing nearby frequencies within the CN. Such CI users may be more dependent upon temporal features of electrical stimuli, and it may be advantageous to enhance the salience of such cues, e.g., by removing some electrodes from the processor "map" to reduce channel interaction, or by using moderate rates of stimulation to enhance spectral contrasts.

Supported by NIDCD Grant R01-DC00160 & Contract N01-3-DC-1006.

2) NEURAL-PERCEPTUAL MODEL FOR AUDITORY THRESHOLDS IN ELECTRICAL HEARING

Ralph E. Beitel[1], Maike Vollmer[2], Patricia A. Leake[1] and Russell L. Snyder[1]
[1]*Otolaryngology-HNS, UCSF, San Francisco, CA, USA*
[2]*University of Würzburg, Würzburg, Germany*

Electrical hearing thresholds were measured psychophysically in neonatally deafened cats using intra-cochlear trains of biphasic current pulses that varied in duration and intensity. Similar stimuli were used to evoke responses from sustained-response neurons in the central nucleus of the inferior colliculus. Histological analysis revealed complete bilateral degeneration of the organ of Corti; hair cells were not present in the deaf cats. Behavioral detection thresholds decreased when the stimulus duration was increased, and for each neuron studied, the total number of spikes increased directly with stimulus duration and stimulus intensity.

A model is presented that predicts behavioral detection when the neuronal response reaches or exceeds a threshold number of spikes. For short stimulus durations (≤ 100 ms), the accumulation of spikes is dependent on spatial-temporal integration (spatial summation across the activated population of neurons; temporal integration over the duration of the stimulus). For longer duration stimuli, spikes are accumulated during the duration of the stimulus at minimum neuronal threshold intensity by simple spatial summation. The model also predicts that behavioral detection of supra-threshold stimuli and perceptual loudness levels are dependent on accumulation of spikes.

Together, the results and model suggest that spatial-temporal integration and simple summation of neuronal responses are features of central auditory processes underlying detection of electrical signals by cochlear implant listeners.

Support provided by NIH-NIDCD contract # N01-DC-3-1006

3) CUSTOMIZATION OF ACUTE AND CHRONIC MULTICHANNEL COCHLEAR IMPLANTS FOR ANIMAL RESEARCH

Alexander M. Hetherington, Stephen J. Rebscher, Russell L. Snyder, Patricia A. Leake, and Ben H. Bonham
University of California San Francisco, Department of Otolaryngology – Head and Neck Surgery, San Francisco, CA, USA

The efficacy of multichannel cochlear implants depends on the activation of perceptually distinct regions of the auditory nerve. Human and animal studies have demonstrated that specific design features of the intracochlear array directly affect various aspects of device performance. These features include the geometry, size, and orientation of the stimulating contacts, the proximity to spiral ganglion neurons, and the shape and position of the insulating carrier. Further, as studies continue to examine complex stimulation strategies (higher rate multichannel stimulation, virtual channel stimulation, modulated signals, bipolar and tripolar configurations) and channel interaction, the ability to manipulate the locations and size of the stimulating sites may become increasingly valuable. The direct measurement of neural responses to intracochlear electrical stimulation in animals provides a basic understanding of how the auditory system responds to electrical stimuli and a framework for the development of intracochlear arrays and signal processing strategies for use in human cochlear implant systems.

Here we describe a simple, cost-effective method for the design and fabrication of species-specific intracochlear electrode arrays for use in chronic and acute animal experiments. The techniques presented allow accurate modeling of current clinical devices and provide the flexibility to both create novel configurations and test new technologies (e.g. polyimide arrays). We describe modifications that enable chronic intracochlear delivery of therapeutic agents via single or multiple ports (basal and apical) in the electrode arrays. We have also taken advantage of high speed machining and smaller cutting tools, which are now widely available from independent contract vendors, to create smaller feature sizes and a smoother surface finish in our molds. The two-part molds are designed to permit the manipulation of various design features (e.g. shape and size of the carrier, the position, number, and spacing of stimulation sites, and insertion depth), thereby providing the versatility needed to customize devices to meet the requirements of individual experiments. We have successfully implanted various designs of these latest arrays acutely in guinea pigs and chronically in cats for periods of up to 6 months and have conducted acute electrophysiological experiments in these animals.

Support provided by the NIH-NIDCD Contracts #N01-DC-2-1006 and #N01-DC-3-1006.

4) FAST RECOVERY AMPLIFIER FOR MULTICHANNEL NEURAL RECORDING USING HIGH RATE ELECTRICAL STIMULATION

Matthew C. Schoenecker, Olga Stakhovskaya, Russell L. Snyder and Ben H. Bonham
University of California San Francisco, Epstein Laboratory, Department of Otolaryngology-Head and Neck Surgery, San Francisco, CA USA

In neurophysiological studies of neural prostheses, it is often desirable to measure neural responses to amplitude-modulated, electrical pulse trains at relatively high carrier rates. However, long amplifier recovery times following electrical artifacts typically limit usable carrier frequencies to a few hundred pulses per second (pps), especially for monopolar stimulation. We have developed a fast-recovery recording system that can be used to record central responses to intracochlear stimuli at carrier rates above 1000 pps. The amplifier is relatively simple and is designed to be a component in an implantable multichannel recording device. Fast amplifier recovery is achieved by using a relatively wide recording bandwidth, 2 Hz to 40 kHz. The 2-Hz low-frequency cutoff prevents the baseline from shifting excessively after each biphasic stimulus and enables recording of low-frequency local field potentials. The 40-kHz high-frequency cutoff allows the amplifier to recover from each monopolar intracochlear stimulation artifact within 100 μs.

We successfully validated function of the system by recording data from the central nucleus of the inferior colliculus (ICC) in anesthetized guinea pigs while stimulating the cochlea with unmodulated and sinusoidally amplitude-modulated pulse trains. We recorded data from multiple locations in the guinea pig ICC while applying monopolar intracochlear electrical stimuli at 1000 pps. After artifact removal, regions of the ICC with appropriate tonotopic tuning showed evoked neural activity while other regions showed no evoked activity despite comparable or larger stimulus artifacts. Comparison of pre- and postmortem data confirm that the waveforms are of biological origin and not merely electrical artifacts.

To highlight one potential application for this system, we measured spatial tuning curves for sustained responses to 1000-pps pulse trains. We also measured the modulation index of responses to these same pulse trains amplitude-modulated at 100 Hz.

The system presented here will enable us to study interactions among multiple cochlear implant channels during interleaved stimulation at relatively high rates. Furthermore, the system is simple to implement and can find application in multichannel recording devices, both for neuroprosthesis and basic neuroscience applications.

Research supported by NIH-NIDCD Contract N01-3-DC-1006.

5) COCHLEAR NUCLEUS ALTERATIONS FOLLOWING ELECTRICAL STIMULATION IN CATS DEAFENED AT DIFFERENT AGES

Olga Stakhovskaya, Gary H. Hradek, Russell L. Snyder, Patricia A. Leake
University of California San Francisco, Epstein Laboratory, Department of Otolaryngology-Head and Neck Surgery, San Francisco, CA, USA

The goal of this study was to examine whether a brief initial period of normal auditory experience would affect either the vulnerability of the cochlear nucleus to auditory deprivation and/or influence the trophic effects of subsequent chronic electrical stimulation delivered by a cochlear implant. Morphological characteristics of the CN (CN area, and spherical cell size, number and density) were compared in neonatally deafened animals and animals deafened at 30 days of age and studied either at 8 weeks of age or after several months of electrical stimulation.

In animals deafened at 30 days and studied at 8 weeks of age, CN cross-sectional area was about 90-95% of age-matched normal animals. In the neonatally deafened group at this age, CN size was already significantly smaller, about 75-80% of normal. In older deafened animals studied at 36 weeks of age, a significant increase in CN size was observed in both neonatally- and 30 day-deafened groups, suggesting that the CN continued to grow during this 6 month period. However, CN size never reached that of normal hearing animals, remaining at about 75% of normal in the neonatally deafened animals and 85% in 30 day deafened animals. No significant difference was observed in CN size between the stimulated and non-stimulated sides in either deafened group.

In animals studied at both 8 weeks and 36 weeks of age, the mean cross-sectional area of AVCN spherical cells was significantly smaller in the neonatally deafened group than in the 30 day deafened group. Electrical stimulation in both older deafened groups resulted in an increase of about 6% in cell size in the

stimulated CN, with the spherical cells reaching 90% of normal in 30 day deafened animals and 80% in neonatally deafened animals.

Numerical density of AVCN spherical cells was significantly higher in both neonatally and 30 day deafened animals as compared to normal animals. The total number of AVCN spherical cells did not significantly differ from normal values in the 30 day deafened group and was slightly smaller in the neonatally deafened group (90% of normal). No significant difference between stimulated and non-stimulated sides was observed.

The data suggest that most of the differences in CN morphology between the two deafened groups are due to the brief period of normal auditory experience early in life. Electrical stimulation did not alter the retarded growth of CN or the number of AVCN neurons but promoted an increase in spherical cell size in both deafened groups.

Supported by NIDCD Grant R01-DC00160 & Contract N01-3-DC-1006.