14th Quarterly Progress Report

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Neural Prosthesis Program Contract N01-DC-3-1006

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

Submitted by:

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SUMMARY OF WORK COMPLETED DURING THE PAST QUARTER.

1) A new paper appeared in the Journal of Comparative Neurology during this past quarter and the pdf reprint is appended to this report:
   Leake PA, Hradek GT, Vollmer M, Rebscher SJ. 2007. Neurotrophic Effects of GM1 Ganglioside and Electrical Stimulation on Cochlear Spiral Ganglion Neurons in Cats Deafened as Neonates. J Comp Neurol 501: 837-853.

2) A second paper has now been published online in Journal of the Association for research in Otolarlyngology and the pdf reprint will be submitted with the next QPR:
   Stakhovskaya O, Sridhar D, Bonham B, Leake PA. (In Press) Frequency Map for the human cochlear spiral ganglion: Implications for cochlear implants. JARO: 501 (in press).

3) A third manuscript submitted to Journal of Neurophysiology has received favorable reviews and is 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) Work has continued on a new experimental series evaluating the effects of brain derived neurotrophic factor (BDNF) delivered directly to the cochea via an osmotic pump and in some cases combined with electrical stimulation (ES) via a cochlear implant. Histological analyses of the cochleae and cochlear nuclei in several subjects (2 neonatally deafened animals and 3 animals deafened at 30 days of age) have been completed and data will be analyzed during the coming quarter. Terminal electrophysiological experiments were carried out in 2 of these animals (one neonatally deafened and one 30 day-deafened subject), after they had completed a 14 week period of chronic electrical stimulation, prior to perfusion for harvesting tissues for histological analyses. Analysis of these data also will be undertaken during the next quarter.

5) During the past quarter Drs. Leake and Stakhovskaya presented posters relating to this Contract research at the annual midwinter research meeting of the Association for Research in Otolaryngology held in Denver, Colorado on February 10-15th. The abstracts are appended at the end of this report.

6) The main scientific report for this Quarterly Report comprises a manuscript about to be submitted to the Journal of the Acoustical Society of America that is entitled "**Neural-Perceptual Model for Auditory Thresholds in Electrical Hearing**," by RE Beitel, M Vollmer, PA Leake and RL Snyder. 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 at beitel@phy.ucsf.edu to request a preprint.

Neural-Perceptual Model for Auditory Thresholds in Electrical Hearing

Ralph E. Beitel, Maike Vollmer, Patricia A. Leake and Russell L. Snyder

ABSTRACT

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. Together, the results and model suggest that spatial-temporal integration and simple summation of neuronal responses are features of central auditory processes underlying the behavioral performance observed in the profoundly deaf cats.

WORK PLANNED FOR THE NEXT QUARTER.

1) Two more animals currently undergoing BDNF and concomitant electrical stimualtion will be studied and euthanized during the next quarter after 14 weeks of treatment. Electrophysiological experiments 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) A litter of 3-4 kittens is 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. One animal of each pair will be studied after 6 weeks of BDNF infusion and the second subject will have the osmotic pump changed and will continue BDNF/ES for additional periods of 8-12 weeks. This protocol is designed to compare the short-term effects of neurotrophin infusion to more prolonged treatment coupled with ES.

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 is known to elicit substantial 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) a new physical dissector method. For the cochlear nucleus, we are comparing earlier cell area measurements in frozen sections 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:
    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. Mr. Stephen Rebscher's IEEE paper entitled Design and Fabrication of Multichannel Cochlear Implants for Animal Research" (currently undergoing minor revision following favorable review).
    c. 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" (in preparation).
    d. Dr. Stakhovskaya's paper on alterations in the cochlear nucleus following profound hearing loss induced by different deafening protocols (in preparation).

ABSTRACTS of presentations at the 2007 Association for Research in Otolaryngology meeting:

Neurotrophic Effects of GM1 Ganglioside and Electrical Stimulation Delivered by a Cochlear Implant on the Cochlear Spiral Ganglion Neurons in Cats Deafened as Neonates

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Several previous studies have shown that electrical stimulation delivered by a cochlear implant promotes increased survival of spiral ganglion (SG) neurons in cats deafened as neonates by daily injections of the ototoxic drug neomycin sulfate (Leake et al, 1999, J Comp Neurol 412:543). However, electrical stimulation only partially prevents the progressive SG degeneration following deafness in these animals. Thus, neurotrophic agents that might be used in conjunction with an implant are of interest. GM1 ganglioside is a glycosphingolipid that has been reported to be beneficial in treating stroke, spinal cord injuries and Alzheimer disease. GM1 activates TrkB signaling and potentiates neurotrophins, and exogenous administration of GM1 has been reported to reduce SG degeneration in deafened guinea pigs (Parkins et al, 1999, ARO abstr #660:167).

In the present study, neonatal kittens were deafened (using the same neomycin protocol as in prior studies) and received daily injections of GM1, beginning either at birth or after animals were deafened and continuing until 7-8 weeks of age. GM1-treated animals examined at this age showed a modest improvement in SG density as compared to non-GM1 deafened controls (74% vs 62% of normal). Additional GM1-treated and non-GM1 control groups received a cochlear implant at 7-8 weeks of age, followed by at least 6 months of unilateral electrical stimulation. Electrical stimulation elicited a significant trophic effect in both GM1 and non-GM1 groups as compared to the contralateral, non-stimulated ears. These long-term results also showed that a modest increase in SG density with GM1 treatment was maintained by and additive with the trophic effects of subsequent electrical stimulation (55% vs 46% of normal). However, in the GM1-treated deafened ears contralateral to the implant, SG soma size was severely reduced several months after withdrawal of GM1 in the absence of electrical activation.

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Effects of Different Deafening Protocols and Electrical Stimulation on the Developing Cochlear Nucleus

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This study examined the development of the cochlear nucleus (CN) in cats that were deafened using three different protocols. One group of animals was deafened by unilateral cochlear nerve section performed at 2-3 days postnatal (P2-P3), several days prior to the onset of hearing. Two other groups of animals were deafened by daily neomycin injections starting either at P1 or P30. Animals from all groups were examined as juveniles at about 2 months of age. At this age after cochlear nerve section at P2-P3, the CN cross-sectional area (as measured in coronal sections just posterior to the cochlear nerve) was about 35-40% of the normal adult CN. At this same age in animals deafened by neomycin injections starting at P1, the CN was about 58% of the normal adult area. The brief period of normal auditory experience in animals deafened at P30 resulted in a significantly larger CN area (67% of normal adult) as compared to both the other groups ($p<0.05$) examined at 2 months of age.

To explore the potential role of electrical stimulation in reducing CN degeneration following deafness, additional animals in both the P1 and P30 neomycin-deafened groups received a cochlear implant and were studied after 5-7 months of unilateral electrical stimulation. In both groups, the CN on the non-implanted side exhibited marked growth over several months of further development and reached 75% of normal in P1-deafened animals and 88% in those deafened at P30. Thus, a significant difference was maintained into adulthood in the deafened CN of the 2 groups. Comparison between stimulated and unstimulated CN revealed no difference in overall CN size, but cross-sectional areas of the neurons in the AVCN were larger on the stimulated sides in both neomycin-treated groups. The data suggest that electrical stimulation did not alter the retarded growth of the CN in deaf animals, but did provide a significant trophic effect on the spherical cells of the AVCN.

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