12th Quarterly Progress Report

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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) Work has continued on our new experimental series evaluating the effects the neurotrophin 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. Six subjects now have been implanted with our new UCSF feline cochlear implant incorporating a drug delivery cannula. (See QPR #9, October 1 – December 30, 2005 for description of this device).

    a. SG data analysis has been completed for the first 3 animals in this series:
        - One neonatally deafened animal implanted at 4 weeks of age and studied after 6 weeks of BDNF and ES – an Initial failed experiment in which the BDNF cannula had separated from the intracochlear electrode at the round window. The drug delivery electrode was modified to rectify this problem.
        - Two additional subjects were deafened at 30 days of age and implanted at 7 weeks of age, with the modified BDNF cochlear implants. Electrical stimulation on 2 bipolar channels was initiated via the CII TM processor (Advanced Bionics Corp). Initial small BDNF pumps were replaced after 2 weeks with larger pumps capable of drug delivery for 30 days. One animal was sacrificed for study after 6 weeks of combined BDNF and ES. Histological data evaluating the cochleae have been completed, demonstrating very high SG cell densities in BOTH cochlea, and sprouting of myelinated nerve fibers into the scala tympani, within the connective tissue encapsulation overlying the stimulating electrode. .
        - In the third BDNF animal, the osmotic pump was successfully replaced a total of 4 times with no problems, to continue BDNF delivery combined with ES for a total period of 15 weeks. This animal was studied in a terminal electrophysiological experiment recording from the inferior colliculus to characterize the activation of the central auditory system with the chronically applied ES. Histological results in this animal showed extensive ectopic sprouting of myelinated nerve fibers into the scala tympani over the electrode, but SG survival showed mixed results after prolonged BDNF administration.

    b. Three additional animals were implanted for the BDNF series this last quarter. Unfortunately, one neonatally deafened animal died during the implant surgery, could not be resuscitated and was found on autopsy to have a rare congenital heart defect (endocardial fibroelastosis). Another neonatally deafened kitten and a 30-day deafened animal were implanted successfully and will be studied during the next quarter after 6 weeks of BDNF delivery. Four additional animals were deafened for implantation next quarter (2 neonatal subjects and 2 subjects at 30-days).

2) Ongoing chronic electrical stimulation was continued in another 30-day deafened cat that is scheduled to continue stimulation for a duration of 6 months. This

subject damaged the external connector twice, but it was successfully repaired on both occasions. This animal is the last in the experimental series in which the effects of a short period of normal hearing early in life ("critical" or "sensitive" period) are being evaluated by comparing effects of deafness and chronic stimulation in neonatally deafened vs. 30-day deafened animals. Data being collected in this series will document both SG survival and alterations in the cochlear nucleus.

3) This main scientific report for this Quarterly Report is a full-length manuscript that was recently submitted to the Journal of Comparative Neurology, entitled: "Neurotrophic Effects of GM1 Ganglioside and Electrical Stimulation on Cochlear Spiral Ganglion Neurons in Cats Deafened as Neonates." 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 investigators for a preprint.

ABSTRACT

Previous studies have shown that electrical stimulation of the cochlea by a cochlear implant promotes increased survival of spiral ganglion (SG) neurons in animals deafened early in life (Leake et al., 1999). However, electrical stimulation only partially prevents SG degeneration after deafening, and other neurotrophic agents that may be used along with an implant are of great 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 shown to reduce SG degeneration after hearing loss.

In the present study, animals were deafened as neonates and received daily injections of GM1 (beginning either at birth or after animals were deafened and continuing until the time of cochlear implantation). GM1-treated and deafened control groups were examined at 7-8 weeks of age; separate GM1 and non-GM1 deafened control groups received a cochlear implant at 7-8 weeks of age and at least 6 months of unilateral electrical stimulation. Electrical stimulation elicited a significant trophic effect in both the GM1 group and the non-GM1 group as compared to the contralateral, non-stimulated ears. The results also demonstrated a modest initial improvement in SG density with GM1 treatment, which was maintained by and additive with the trophic effect of subsequent electrical stimulation. However, in the deafened ears contralateral to the implant, SG soma size was severely reduced several months after withdrawal of GM1 in the absence of electrical activation.

4) Finally, during the past quarter Dr. Leake attended the Neural Interfaces Workshop sponsored by the NINDS and the NIDCD on August 21-23 at Bethesda, Maryland. Dr. Leake is a member of the Neural Interfaces Conference Steering Committee, which met immediately following the 2006 workshop to begin work on planning future Neural Interfaces Conferences.

WORK PLANNED FOR THE NEXT QUARTER.

1) Two control cats currently undergoing BDNF infusion only (no ES) will be sacrificed during the next quarter after 6 weeks of BDNF treatment. Cochlear and cochlear nucleus specimens will be prepared for histology and analyses will be completed as soon as possible.

2) Four additional animals are currently being deafened and will be implanted during the upcoming quarter as additional subjects for the two BDNF series, i.e., neonatally deafened and 30-day deafened. 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 an additional 6-week period. This protocol is designed to compare the short -term effects of neurotrophin infusion to more prolonged treatment coupled with ES.

3) The final 30-day deafened subject will be studied in a terminal electrophysiological recording experiment. Recording in the inferior colliculus will focus on characterizing the extent of activation of the central auditory system elicited by the applied chronic stimulation protocol. In addition, 2 channel interaction and forward masking will be studied, and if time permits SAM modulation depth studies will be conducted. After completion of the physiology experiment, the cochleae and brain will be perfused for histological studies of the spiral ganglion and cochlear nuclei.

4) Work will continue on several manuscripts that in various stages of preparation for submission, including:

    a. Dr. Vollmer's J. Neurophysiol. paper entitled " Spatial Selectivity in the Inferior Colliculus is Degraded Following Long-Term Deafness in Cats."
    b. Dr. Leake's J. Comp. Neurol. manuscript entitled "Degraded Topographic Specificity of Auditory Nerve Projections to the Cochlear Nucleus in Cats Following Neonatal Deafness and Electrical Stimulation Delivered by a Cochlear Implant."
    c. Dr. Beitel's JASA paper entitled "Behavioral-Neural Model for Temporal Integration in the Deaf Cat."
    d. Mr. Stephen Rebscher's IEEE paper entitled Design and Fabrication of Multichannel Cochlear Implants for Animal Research"
    e. Dr. Stakhovskaya's paper on alterations in the cochlear nucleus following profound hearing loss induced by different deafening protocols.

5) The Principal Investigator will attend a conference on Bionics and Regeneration of the Ear, which will be held in Melbourne, Australia, November 12-14.

ABSTRACT

Abstract for invited presentation at the 7th International Academic Conference on Immunobiology in Otorhinolaryngology: BIONICS AND REGENERATION OF THE EAR:

Implications of Degeneration and Regeneration for Central Neural Plasticity

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Aims: Studies in animals show significant neurotrophic effects of electrical stimulation (ES) from a cochlear implant in ameliorating degenerative changes after deafness. Several months of ES promotes increases of ≈20% in spiral ganglion (SG) density, but survival is still far from normal. Exogenous administration or modulation of neurotrophins can further improve SG survival, but must be continued for prolonged periods and combined with ES for long-term efficacy.

Methods: Cats deafened at birth or at 30 days of age model congenital and early acquired deafness. Anatomical changes in the SG and cochlear nucleus (CN) are examined and related to functional consequences of ES assessed in electrophysiological experiments recording in the inferior colliculus.

Results: The cochleotopic organization of the SG-to-cochlear nucleus (CN) pathway is intact even in animals deafened at birth, but due to shrinkage of the CN the spatial selectivity and inferred frequency resolution are significantly poorer than normal. Electrophysiological studies indicate that the fundamental cochleotopic organization of the central auditory system, at least to the level of the midbrain, develops normally even after neonatal deafening. After severe SG degeneration, ES can improve degraded temporal resolution but does not reverse the markedly poorer spatial selectivity and dynamic range associated with severe pathology.

Conclusions: Our findings suggest that synchronized neural activity elicited by ES exerts a powerful influence on the deafened auditory system, especially during maturation. Central auditory processing may be profoundly altered by specific patterns of electrical activation, emphasizing the importance of initial input to the deafened, developing auditory system.

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