**FINAL REPORT**

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**Neural Prosthesis Program Contract #N01-DC-0-2108**

# *Protective Effects of Patterned Electrical Stimulation on the Deafened Auditory System*


**Submitted by:**

**Patricia A. Leake, Ph.D., Principal Investigator**

**Russell L. Snyder, Ph.D.**
**Gary T. Hradek, M.S.**
**Stephen J. Rebscher, M.A.**
**Maike Vollmer, M.D., Ph.D.**
**Charlotte M. Moore, Ph.D.**
**Raph E. Beitel, Ph.D.**
**Christoph E. Schreiner, M.D., Ph.D.**

**Epstein Hearing Research Laboratories**
**Department of Otolaryngology-Head and Neck Surgery**
**533 Parnassus Avenue, Room U490**
**University of California, San Francisco**
**San Francisco, Ca 94143-0526**

## INTRODUCTION

This Final Report summarizes results from work conducted during the past three years with the support of this Contract. Based upon these results and as required by the Work Statement of the original Request for Proposals, the Report includes recommendations for future research and development in this area. The primary objectives of this research, as stated in the Technical Specifications of the original RFP (#260-03-01), were to evaluate "*how certain forms of chronic electrical stimulation of selected portions of the auditory system, and neurotrophic agents, maintain and possibly enhance the anatomical and physiological viability of the remaining auditory system after loss of hair cells, in a manner compatible with preserving and possibly extending the function of an implanted auditory prosthesis.*" Further, the studies were to be conducted in appropriate animal models of human acquired deafness using both single and multiple channel stimulation for periods sufficient to evaluate their protective effects on neural structures. Possible protective effects on neural structures were to be evaluated by histopathological examination of the cochlea, auditory nerve and cochlear nuclei. Changes in auditory function and underlying mechanisms were to be studied using neurophysiological, and, if appropriate, behavioral measures of auditory system activity. Before summarizing specific studies and findings, it is important to review our premises for the design of these experiments.

Cochlear implants have revolutionized the rehabilitation of individuals with severe to profound sensorineural hearing loss. Most adult cochlear implant recipients enjoy significantly enhanced lip-reading capabilities, and a majority of those using the latest speech processors score above 80-percent correct on high-context sentences, even without visual cues (65). In addition, thousands of hearing-impaired children, including congenitally deaf children, are now receiving cochlear prostheses at an early age, and increasing numbers of these children are being mainstreamed into public education settings. In contrast, however, many pediatric cochlear implant recipients lag far behind in language development (76,105) and some cannot even discriminate between the most basal and most apical electrodes of their implants (13). Thus, in addition to the significant bioengineering challenges in maintaining a device for the lifetime of an implanted child, there are important neurobiological and developmental issues concerning the effects of electrical stimulation on the immature auditory system (43,46,47, 48,97,98). The rationale for providing cochlear implants to deaf children at an early age stems from the tenet that there is a critical period for language acquisition (85,16). This belief is based upon the profound effects of auditory deprivation seen in congenitally deaf children and adults and is supported by numerous animal studies indicating that auditory deprivation during maturation is especially harmful in causing degeneration and reorganization in the central auditory system (see Critical Periods, Section I). It is generally accepted that normal speech and language acquisition occur during a critical period that occurs early in life, since earlier and more severe auditory deprivation has a greater impact on oral language development. The most profound effects are seen when hearing loss occurs at or around birth, and the severity of these effects diminishes if impairment occurs toward the end of the second year (84). With cochlear implants in young deaf children, therefore, it is assumed that restoring input during this critical period will be more effective in ameliorating detrimental effects of deprivation and that the immature auditory system will be more plastic, better able to adapt to the unnatural signals delivered by the implant.

However, it is important to recognize that the increased plasticity characteristic of critical periods of nervous system development might also have potentially negative consequences. Electrical signals delivered in a particular format might entrain the immature auditory system into an idiosyncratic organization that might be suboptimal for effective processing of other patterns or formats of stimulation introduced subsequently. It is clear from

studies in other sensory systems that early restricted or aberrant inputs can have profound effects on central nervous system development that are irreversible due to developmental critical periods. For example, in the immature visual system widely distributed, synchronous input to the retina elicited by electrical stimulation of the optic nerve or stroboscopic illumination causes profound alterations in the central processing of visual information that are not reversible when normal visual input is later restored (e.g.,1,63,104,123).

Thus, our overall premise in this research has been that because so many children, including congenitally deaf and very young (<12 months) children, are now receiving cochlear prostheses, it is imperative to better understand the consequences of electrical stimulation in the developing auditory system. Our work has focused largely on defining the consequences of total auditory deprivation and subsequent highly controlled, unilateral chronic electrical stimulation in neonatally deafened animals and acutely-deafened adult control subjects. Significant progress has been made over the past three years, although many important questions remain.

**A. <u>Several appropriate deafened animal models have been developed</u>.** To address the issues outlined above, we have developed several appropriate animal models in which to examine the functional and anatomical consequences of auditory deprivation and chronic stimulation in the developing mammalian auditory system. Studies have been conducted primarily in cats that are neonatally deafened by daily injections of the ototoxic drug neomycin sulfate (60 mg/kg IM) administered for the first 16 to 21 days after birth. Kittens are deaf at birth due to the immaturity of their auditory system (for review see 117), and the neomycin destroys the cochlear hair cells and causes profound hearing loss by an age when adult-like hearing sensitivity would normally develop, i.e., at about 21 days postnatal (44,115,116). Thus, these animals have no normal auditory experience and model congenital or very early-acquired bilateral profound hearing loss.

In virtually all deafness etiologies, including ototoxic drug damage, hair cell degeneration leads to secondary degeneration of the primary afferent spiral ganglion (SG) neurons and their central axons, which form the auditory nerve (23, 32,64,69,101,127). This degeneration is progressive and continues for many months to years (39), although initial ganglion cell loss occurs as rapidly as 3 weeks postnatal in these ***neonatally deafened animals*** (44). Figure 1 illustrates the time course of SG degeneration in the control (unstimulated) cochleae of neonatally deafened cats. Although considerable variation is seen among individual subjects in the extent of neural degeneration for a specific duration of deafness, decreasing SG survival is strongly correlated to duration of deafness.

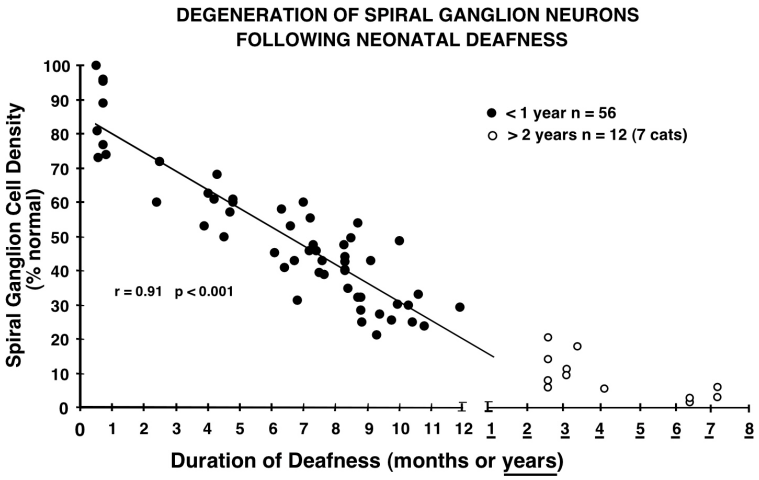



**Figure 1.** Data illustrate SG degeneration in control (unstimulated) cochleae of cats that were deafened neonatally by daily injections of neomycin sulfate beginning the day after birth. The mean SG cell density (averaged value for entire cochlea) is expressed as percent of normal. Decreasing SG survival is strongly correlated with longer durations of deafness, but there is considerable individual variability in the extent of degeneration for a given duration of deafness.

It is important to note that cochlear pathology is highly symmetrical in the two ears of individual animals (38, 44,45). The consistent bilateral symmetry of cochlear pathology and relatively rapid, progressive neuronal degeneration allow systematic study of effects of unilateral electrical stimulation over a reasonable time-frame using within-animal paired comparisons.

Figure 1 also shows SG data from subjects studied at very long durations (>2.5 years) following neonatal deafening (open symbols). SG pathology is very severe in this group, and residual neural survival averages about 9% of normal. Thus, we consider these ***long-deafened subjects*** to comprise a *separate* and highly valuable animal model, in which we have studied the long-term effects of *severe* auditory nerve degeneration upon efficacy and selectivity of electrical stimulation delivered by multichannel cochlear implants. We have also conducted studies in ***adult-deafened cats***, modeling postlingual or adult-onset deafness, and we have obtained preliminary data from ***animals deafened at 30 days postnatal*** rather than neonatally (see below).

**B. <u>Chronic intracochlear electrical stimulation promotes improved survival of spiral ganglion neurons in neonatally deafened cats</u>**. Early anatomical studies of cochlear implants focused primarily on issues of trauma and safety (see 40 for review), or effects of relatively short-term implantation and stimulation. Subsequent research, however, has demonstrated that chronic electrical stimulation of the cochlea can partially prevent the degeneration of the spiral ganglion neurons which otherwise occurs after deafness (22,41,42,43, 45,46,53,54). Our previous studies evaluated the histopathological and functional consequences of both intra- and extracochlear electrical stimulation and using various signals and stimulation modes (e.g.,monopolar vs. bipolar) in neonatally deafened cats (41,42,43,45,46). In our most recent publications (45, 46, 48) we have reported data from neonatally deafened animals that received a unilateral cochlear implant at 7 to 10 weeks of age and then underwent chronic electrical stimulation via bipolar intracochlear electrodes for periods of 8-9 months, using signals explicitly designed to be temporally challenging to the central auditory system. Morphometric studies of cochlear spiral ganglion (SG) cell density demonstrated significantly higher neuronal survival in the stimulated cochleae as compared to the contralateral control deafened ears. Figure 2 shows unpublished SG data from a group of 12 subjects, in which electrical stimulation with a cochlear implant elicited an increase of about 20% of the normal SG population.

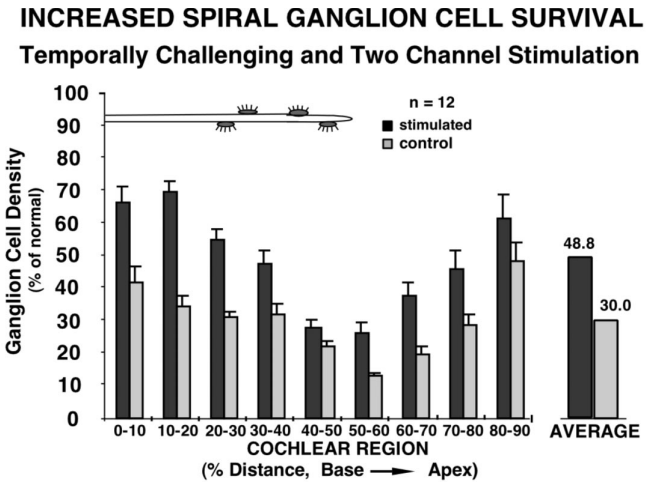



**Figure 2.** Pooled data from 12 cats that were deafened neonatally by daily injections of neomycin sulfate immediately after birth, received a cochlear implant at 6-9 weeks of age, and underwent chronic electrical stimulation with temporally-challenging and amplitude-modulated signals delivered by bipolar electrodes in the basal cochlea for 8-9 months. Data are shown as mean SG cell density for the stimulated (black) and control deafened (shaded) ears, expressed as percent of normal for each cochlear region. It should be noted that slight trauma during surgical insertion of the electrodes in several animals caused the noticeable reduction in survival in the stimulated ears in the 40-50% cochlear region. SG cell density was almost 20% higher in the stimulated ears, and this difference was highly significant (P< 0.001; Student's paired t-test).

In addition, paired comparisons of SG cell diameters in these deafened animals showed only a slight (although significant) difference between stimulated and control ears, indicating that changes in cell density observed after stimulation were due primarily to higher numbers of surviving neurons (45).

**C. <u>Stimulation mode, temporal characteristics and duration of stimulation are important factors in maximizing the neurotrophic effects of chronic electrical stimulation on the spiral ganglion neurons</u>.** Several other research groups also have reported neurotrophic effects of electrical stimulation in promoting SG neuronal survival. Lousteau (60), Hartshorn et al. (22), and Miller and Altschuler (53,54) demonstrated increased SG cell survival after chronic electrical stimulation in guinea pigs deafened by ototoxic drugs and implanted as young adults. Other investigations, however, have failed to demonstrate such trophic effects *in vivo*. For example, Shepherd and co-workers (95,3) found no difference in SG cell survival after chronic stimulation in cats deafened at an early age (although the latter study did report a significant increase in the *size* of SG cells in the stimulated cochleae). Finally, a study by Li et al. (49) reported an increase in ganglion cell density after chronic monopolar stimulation in guinea pigs, but these authors concluded that the density increase was due to a stimulation-induced *narrowing* of Rosenthal's canal, rather than an increase in the actual number of surviving neurons. These conflicting results have led to controversy about whether or not stimulation by a cochlear implant can provide trophic support of SG neurons *in vivo* after deafness. Thus, one important focus of our research has been to define the specific conditions necessary to induce the protective effects of electrical stimulation in maintaining the SG neurons that we have demonstrated.

Figure 3 presents data for a large group of individual subjects studied in three different chronic stimulation experiments, and comparing the increase in SG survival as a function of duration of stimulation. Subjects that were stimulated using a ball-type monopolar electrode positioned near the round window (triangular symbols) clearly comprise a separate group, showing a smaller affect of stimulation on SG survival as compared to other experimental groups stimulated for equivalent periods. We have suggested, therefore, that this mode of stimulation may preferentially activate the SG neurons via their central axons within the modiolus, rather than at more peripheral locations (43), and that such antidromic stimulation may not be as effective in inducing the trophic effects on the parent SG cell somata.

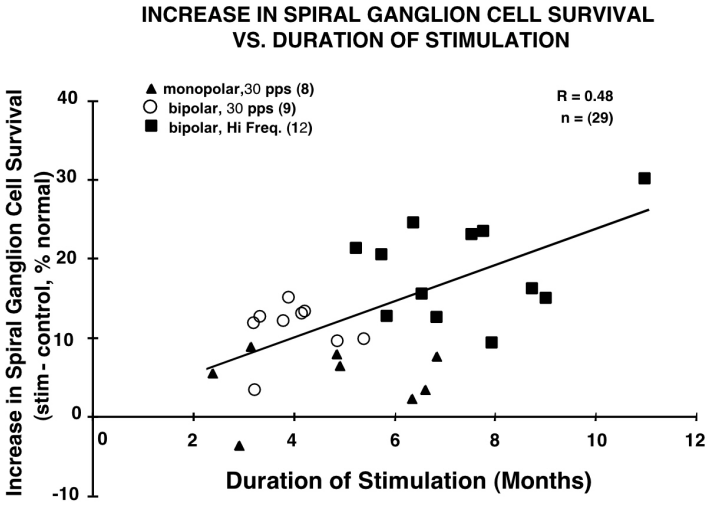



**Figure 3.** Increase in SG density is shown for individual subjects in 3 different experimental groups as a function of duration of stimulation. Subjects that were stimulated using a monopolar electrode near the round window (triangular symbols) clearly show less effect on SG survival for a given duration of stimulation. In the remaining intracochlear stimulation groups, greater increase in SG survival is significantly correlated with longer duration of stimulation (R=0.51), but the data also suggest that higher frequency stimulation may elicit a greater effect than low frequency stimulation (30 pps) for similar durations.

If we now compare efficacy of stimulation in the remaining intracochlear bipolar stimulation groups shown in Figure 3, animals stimulated using higher frequency, modulated signals (square symbols) clearly show greater trophic effects of stimulation than subjects stimulated using a continuous simple low frequency (30 pps) pulse train (circular symbols). However, in addition to the higher frequency signals (e.g., 300 pps amplitude modulated at 30 Hz, or an analogue speech processor) applied, animals in the former group also were stimulated for longer periods. The correlation of duration of stimulation with increases in survival (r=0.48) suggests that duration is another important factor in determining the extent of neurotrophic effects. On the other hand, age-matched comparisons suggest that higher frequency signals may be more effective than 30 pps. Although these data do not define the specific or relative contributions of duration and stimulus frequency/complexity, they demonstrate that prolonged stimulation using temporally challenging signal elicits highly significant neurotrophic effects, and that both factors likely contribute.

It is interesting to note that in these recent experiments, electrical stimuli were delivered at relatively low current levels with reference to evoked response (EABR) thresholds (i.e., 2 dB above EABR threshold). When final inferior colliculus (IC) electrophysiological experiments were conducted in these animals, and responses were mapped at chronic stimulation levels, stimuli appeared to excite more limited sectors of the spiral ganglion than the region over which increased neuronal survival was seen. For example, activation of bipolar electrode pair 1,2 at 2 dB above EABR threshold on average excited roughly one quarter of the central nucleus of the IC, yet chronic stimulation at that level in the same subjects elicited significantly increased SG density throughout the entire cochlea (Fig. 2). This suggests the possibility that subthreshold electrical currents may play a role in promoting survival, e.g., via modulation of neurotrophic factors (see below). It is obviously important to resolve this issue, because determination of the direct cause(s) of spiral ganglion conservation will allow development of more efficacious practical devices that produce optimal benefits in young children.

**D. <u>GM1 ganglioside promotes modest enhancement of SG neural survival after neonatal deafness and its effects are additive with trophic effects of chronic stimulation.</u>**
As described above, *appropriate* electrical stimulation delivered over several months can promote increases in SG survival that are highly significant; but it is also clear that stimulation only *partially* prevents degeneration resulting after early deafness (Fig. 2.) Thus, we are very interested in exploring other procedures and treatments that may further promote neural survival. Recent studies of cultured SG neurons by Green et al. (20,25) suggest that there are multiple mechanisms underlying the protective effects of depolarization *in vitro*, including a cyclic-AMP pathway, autocrine neurotrophin expression, and at least one other pathway. Neurotrophins are of particular interest because they are involved in the development and maturation of the central nervous system and also because exogenous administration of neurotrophins can promote survival of neurons following injury, including SG neurons (24,33,36,55,61,88,94,102,125, 126,128,129, 130).

Particularly relevant to our work is a study by Walsh and Webster (118) suggesting that exogenous administration of GM1 ganglioside significantly ameliorated atrophy of SG neurons in mice after conductive hearing loss. Further, Parkins et al. (71) reported that GM1 treatment produced a marked 77% increase in SG cell survival in guinea pigs deafened acutely by ototoxic drugs. GM1 ganglioside is a glycosphingolipid that has been shown to promote neuronal survival after injury by potentiating the activity of neurotrophins (15,78). GM1 has been the subject of a number of clinical trials in humans suggesting that it has beneficial effects in the treatment of stroke, spinal cord injuries and Alzheimer disease. Based upon these

findings, we hypothesized that GM1 treatment in our neonatally deafened animals would ameliorate SG degeneration that occurs prior to the time when electrical stimulation is initiated.

During this Contract funding period we have conducted two experiments with GM1. Figure 4a shows data from 11 neonatally-deafened animals that received GM1 treatment: Six subjects received daily subcutaneous injections of GM1 (30 mg/kg, SID) at 2-3 weeks of age, immediately *after* ABR testing confirmed profound hearing loss; 5 additional subjects received GM1 at 20 mg/kg (SQ SID) concomitant with the neomycin injections beginning *immediately after birth*. Injections in both groups continued until each animal underwent implant surgery at 7-8 weeks of age. In all subjects chronic stimulation on 2 channels of the implant was delivered at 2 dB above EABR threshold for 6-8 months. The SG data were very similar in both groups, so they have been pooled here. The mean overall SG density on the stimulated side was about 54% vs. 37% for the control side. Figure 4b illustrates data from a comparison group of 7 neonatally deafened animals that did not receive GM1 but were selected to match the stimulation histories and duration of deafness of the GM1 group. This group shows overall SG survival of 46% of normal on the stimulated side vs. 30% in the control ears. Thus, it appears that GM1 ganglioside may promote a modest enhancement of neural survival in the control deafened ears, which is *additive* to the effects of electrical stimulation in promoting SG survival.

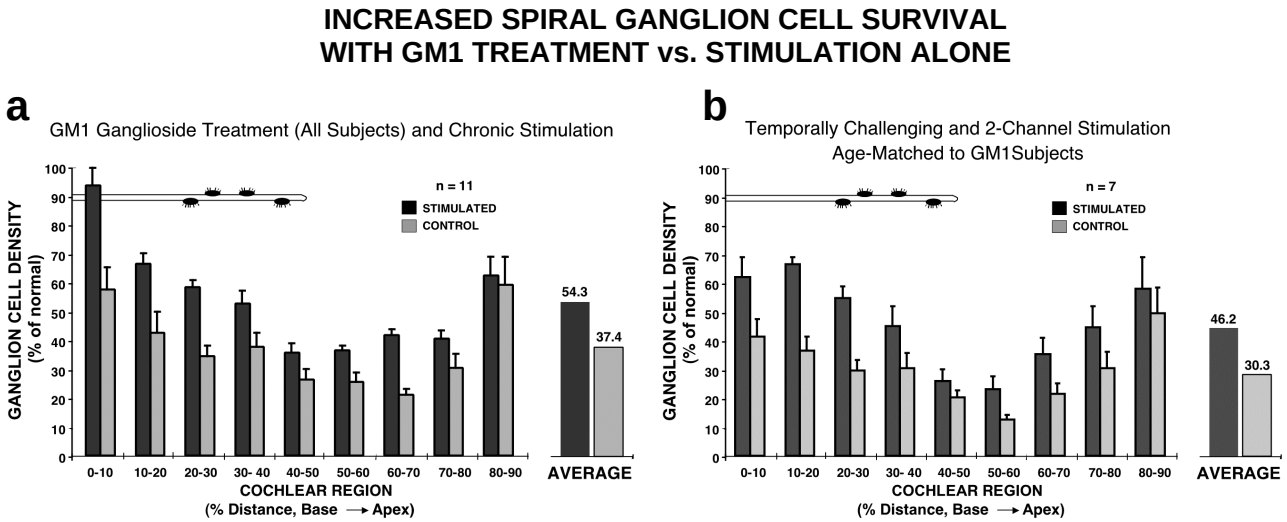



**Figure 4.** **a.** Pooled SG data from all GM1 subjects. 11 neonatally deafened cats that were treated with GM1 ganglioside prior to receiving a cochlear implant and after 6-8 months of chronic electrical stimulation. Overall, SG neural survival is 54% of normal. This is about 8% higher than what is seen in the comparison experimental group (**b**) that underwent chronic stimulation but did NOT receive GM1. This comparison group is comprised of neonatally deafened cats that were selected to be age-matched to the GM1 group and had

Since SG survival appears to be improved in both stimulated and control ears in the GM1 group, we can average values for the *2 sides* in each group to provide an overall estimate of the GM1 effect. The mean bilateral SG density in the GM1 group was 45.85% of normal, whereas the matched non-GM1 group had a mean value of 38.23 % of normal. This difference of about 8% in neural survival was statistically significant ($P<0.05$, Student's unpaired t-test), although relatively modest. It is interesting also to note that in control neonatally deafened kittens studied immediately after GM1 treatment at about 8 weeks postnatal (at the time their littermates were implanted), neural survival averaged 78.4% of normal (n=5), 20% higher than in

a non-GM1 control group at the same age (66.2%). Clearly this effect was NOT fully maintained over a subsequent prolonged period of chronic electrical stimulation with the cochlear implant. This suggests that GM1 might be more effective if treatment were continued throughout the subsequent chronic electrical stimulation period. While the data suggest that GM1 ganglioside can help to ameliorate the initial SG degeneration resulting from ototoxic drug insult, it will be important in further studies to determine if this survival-promoting effect can be maintained over the long-term in conjunction with stimulation via a cochlear implant. *Otherwise, GM1 and other strategies for modulating neurotrophic factors may be of little practical value clinically if "rescued" neurons are not viable over the long term.*

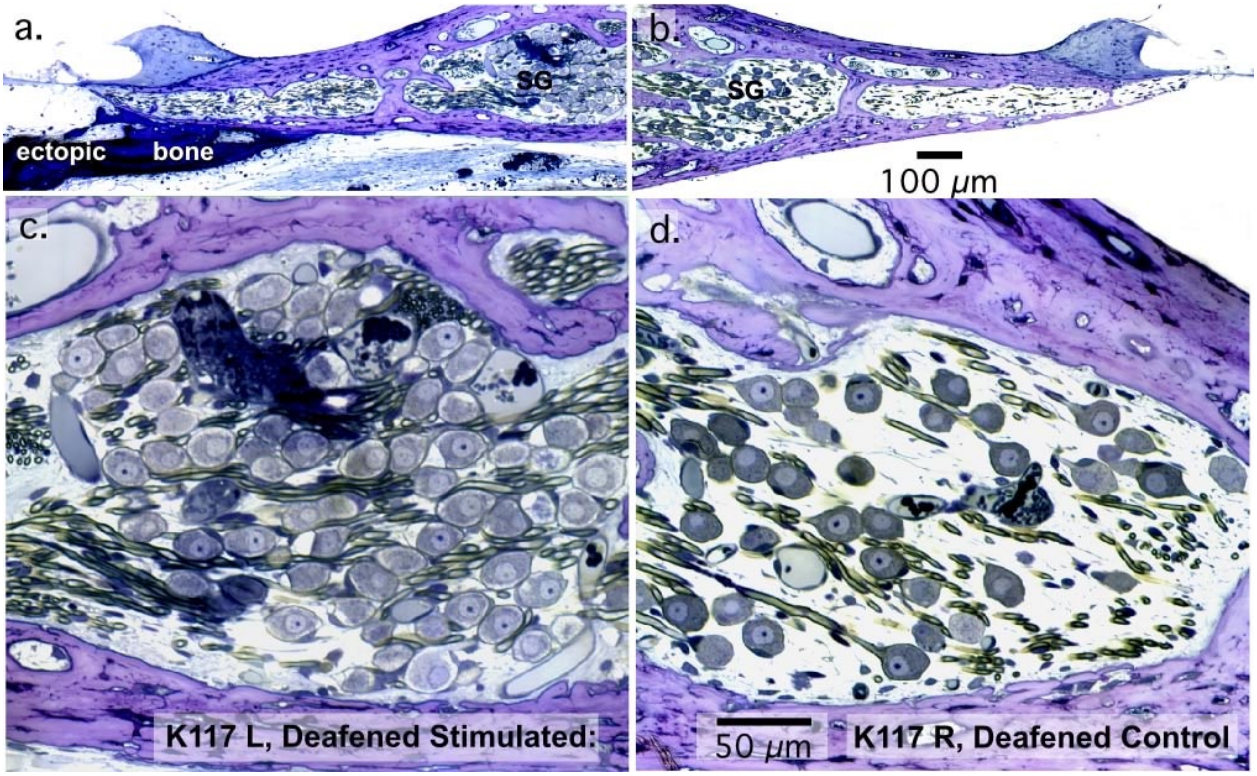
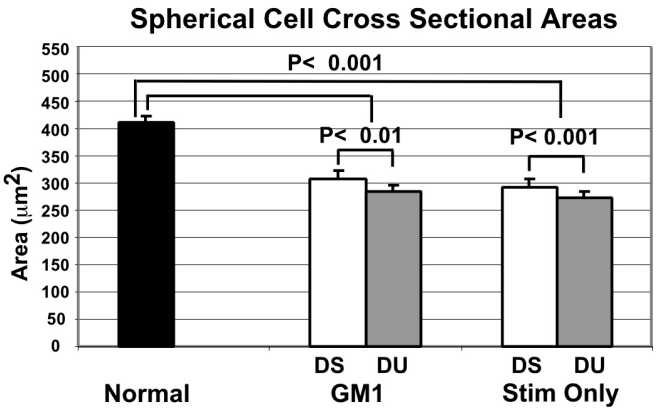



**Figure 5.** Histological sections illustrating the trophic effects of combined GM1 treatment and chronic electrical stimulation on SG neural survival. The mean SG density in the cochlear region shown (**20-30%** from the base) was about 80% of normal in the stimulated cochlea (**a,c** micrographs on left) as compared to about 44% in the same region of the control deafened, unstimulated ear (**b, d**, right) in this neonatally deafened subject studied after 7 months of stimulation. The upper micrographs also illustrate the finding that more of the myelinated radial nerve fibers (peripheral processes of SG neurons) survived within the osseous spiral lamina in the stimulated cochlea.

**E. <u>Neonatal deafness results in marked degenerative changes in the cochlear nuclei; combined GM1 treatment and chronic electrical stimulation have only a modest effect on this degeneration.</u>** Histological studies of the cochlear nuclear complex (CN) in neonatally deafened, chronically stimulated cats have demonstrated profound degenerative changes in the CN -- changes that are progressive for many months after deafening (61). As compared to data from normal adult cats, the cochlear nuclei of neonatally deafened cats showed: i) the total volume of the CN was markedly reduced to about 76% of normal ii) the mean cross-sectional area of AVCN spherical cells was reduced to $\leq$75% of normal. These degenerative changes are completely consistent with many previous studies showing that neonatal sound deprivation or deafening results in profound adverse effects within the cochlear nucleus (2,8,9,82,83,107,108,119,120,121,122).

Comparisons between stimulated and control CN in these animals revealed *no significant difference* in nuclear volume due to chronic stimulation. However, for the cross-sectional area of spherical cells in the AVCN, a modest but significant increase (6%) was observed in the stimulated CN of both stimulation-only and GM1-treated groups (27,46,51,68). CN data in Figure 6 (data from Osofsky et al., 2001 [68]) obtained from cats in our recent temporally challenging stimulation experiments that showed a mean increase in SG neural survival of about 20%. It is unclear why stimulation elicits only such a modest effect in preventing or reversing the pronounced degenerative CN changes in these animals.

**Figure 6.** Cross-sectional areas of spherical cells in the rostral AVCN: Data show a marked reduction in cell size after neonatal deafness in both GM1 and stimulation-only groups. In the **GM1 group** spherical cells in the stimulated CN (DS) were significantly larger (75% of normal; 309μm$^2$) than cells on the deafened control (DU) side (69% of normal; 284μm$^2$). In the **stimulation-only** group, cells were again significantly larger on the stimulated side (71% of normal; 294μm$^2$) than in the control CN (66% of normal; 273μm$^2$). The 5-6% difference due to stimulation seen in both groups was significant but modest, given that these same animals showed increases in SG cell survival >20% in the stimulated ears vs. controls. Thus, CN changes do not appear to parallel the SG maintenance induced by stimulation. Finally, there was no significant difference between GM1 and stimulation-only groups, although both DS and DU values were slightly higher in the GM1 group.




One possible explanation is the delay that occurs before chronic stimulation is initiated in our experiments (68). In Larsen's (37) classic study of the development of the cat CN, she described an early growth phase in the AVCN with rapid increase in nuclear and cytoplasmic cross-sectional areas during the first postnatal month; this is followed by a second, longer period of maturation during which the neurons gradually reach mature sizes, by about 12 weeks postpartum. In our neonatally deafened cats, the ototoxic drug administration occurs during this early period of rapid development. In the temporally challenging stimulation experiments, electrical stimulation of the cochlea was initiated at an average age of 9-10 weeks postnatal, i.e., well after this initial rapid growth period. Thus it is possible that intervention with electrical stimulation in these animals took place too late in development to prevent or reverse the profound effects of early deafness. These findings suggest that there is a critical period of development, after which the cochlear nucleus changes due to deafness are largely irreversible.

In this regard, it should be noted that Matsushima et al. (52) have reported data from a similar study of 4 chronically stimulated cats that were deafened *at 1 month of age rather than neonatally*. Their results on CN cell density suggest that chronic electrical stimulation in these animals was more effective in preventing degenerative changes in the CN, as compared to our results in neonatally deafened cats; however, they did not see any difference in SG survival. This finding also suggests that the age at time of deafening may be a critical parameter in determining whether the CN is sensitive to stimulation-induced "protective" effects in the CN. However, given the disparate results and relative paucity of data currently available, this is clearly an area requiring additional study in the future.

**F. Chronic electrical stimulation induces significant changes in spatial selectivity (i.e., distortion of cochleotopic maps) in the auditory midbrain of neonatally deafened cats.** In addition to the anatomical studies outlined above, our group also has evaluated the functional consequences of neonatal deafening and chronic stimulation delivered by a cochlear implant. Acute electrophysiological experiments have been conducted in the auditory midbrain or inferior colliculus (IC) to examine the topographic organization and temporal patterns of neuronal responses evoked by cochlear electrical stimulation (46,47,97,98, 99,100,113). Figure 7 shows representative data illustrating our methods used to examine spatial selectivity and the effects of electrical stimulation in the IC. These studies have been conducted in: a) animals that are deafened, implanted as adults and studied acutely as controls; b) neonatally deafened, chronically stimulated cats -- including initial experimental groups that were stimulated on a single bipolar channel of the cochlear implant and recent experimental groups in which subjects were stimulated on 2 channels; and c) neonatally deafened but *unstimulated* control animals examined at the same age and duration of deafness as the stimulated group. Data from this latter unstimulated group suggest that at least the basic cochleotopic (frequency) organization of the central nucleus of the IC develops normally and is maintained into adulthood despite the complete lack of normal auditory input during development in these animals. The spatial selectivity elicited with our standard bipolar intracochlear electrodes, at a standard intensity re: threshold in this group is not significantly different from normal (Fig. 8).

In contrast, when neonatally deafened animals are chronically stimulated at a young age on a single channel of a cochlear implant, IC spatial selectivity is markedly altered. Our earlier published studies showed that chronic electrical stimulation delivered at a single intracochlear location by a pair of bipolar electrodes, induces significant expansion of the central representation of the stimulated cochlear sector and degrades the IC cochleotopic organization in neonatally deafened animals (46,47,97,98). Specifically, the area within the ICC excited by the chronically activated electrodes is significantly expanded and on average is almost double that of identical electrodes in either unstimulated control deaf littermates, or in acutely-deafened adults (compare Fig. 7b,c). These results indicate that the developing central auditory system is capable of substantial plasticity and functional remodeling. The initially restricted area excited by the stimulated cochlear neurons expands over time as the central auditory system adapts to the only available afferent input. However, such expansion also represents a significant distortion and degradation of the cochleotopic organization (frequency selectivity) of the central auditory system (43,46,47,97,98).

Figure 7d shows representative data from a subject that received chronic stimulation on 2 adjacent bipolar intracochlear channels of the cochlear implant. ***Alternating*** stimulation of 2 channels and use of highly controlled electrical signals (amplitude modulated, higher frequency pulse trains, with intensity set at 2 dB above EABR threshold for each channel) is effective in maintaining or perhaps even sharpening selectivity of central representations of stimulated cochlear sectors. Figure 8 summarizes the data from these 2-channel experiments. These results suggest that competing inputs driven by electrical stimulation delivered on 2 adjacent channels, can ***maintain*** the selective representations of each activated cochlear sector within the central auditory system and prevent the expansion and degradation of frequency selectivity seen after single channel stimulation (46,47).

## SPATIAL (FREQUENCY) SELECTIVITY OF ELECTRICAL STIMULATION

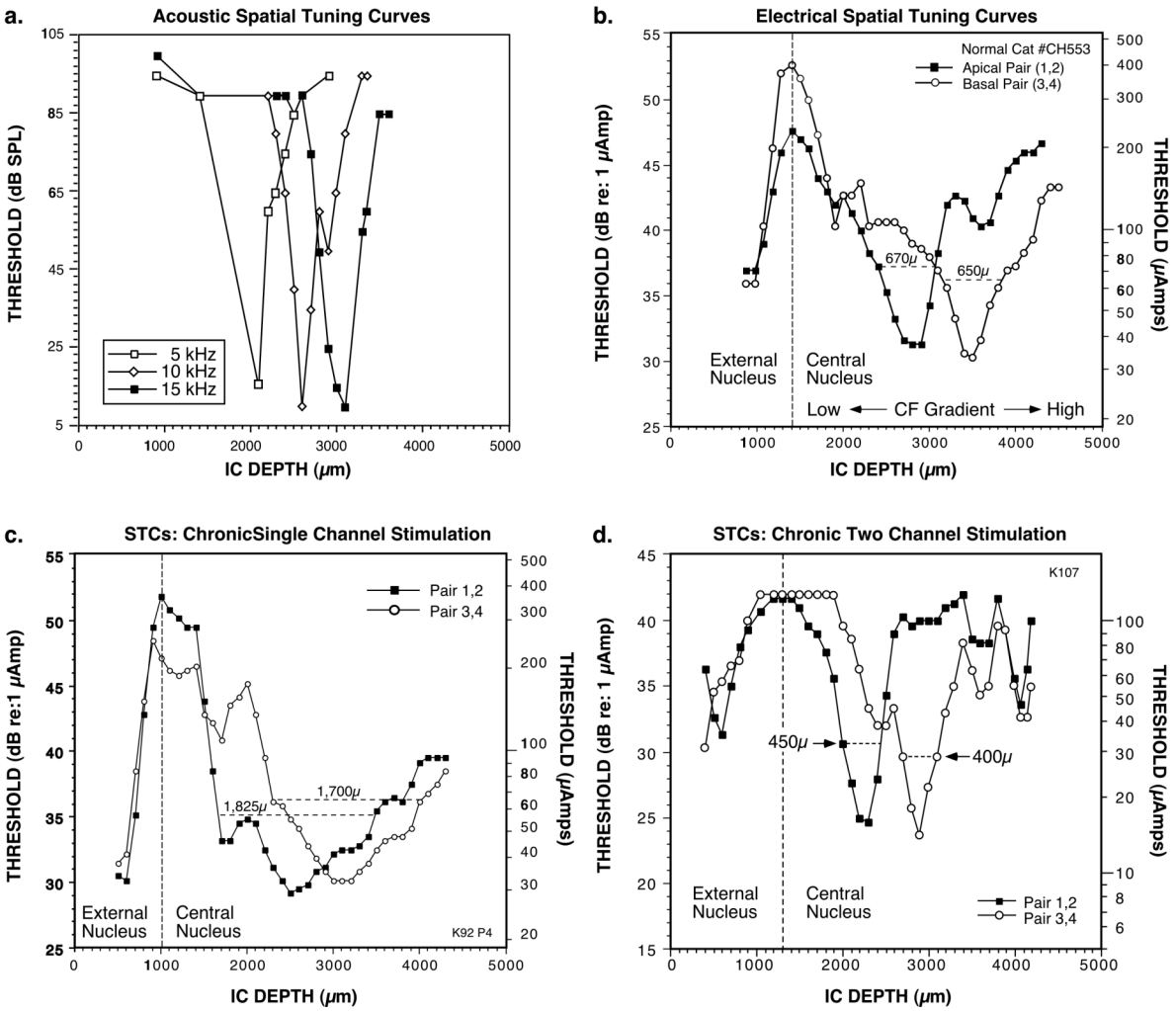



**Figure 7.** Plots of the frequency gradient recording in a standardized trajectory through the central nucleus of the inferior colliculus [ICC] show the precise frequency organization in normal cats, which is the basis for 'mapping' the selectivity of electrical stimulation in deaf cats. **a.** Plots of threshold as a function of IC depth in a normal cat using three tonal frequencies (5, 10, and 15 kHz). These acoustic spatial tuning curves (STCs) show spread of excitation across the IC as a function of stimulus intensity for frequencies corresponding to the cochlear locations of our cat cochlear implant electrodes (apical channel ≈5 kHz; basal channel ≈15 kHz)**. v.** Electrical spatial tuning curves in a control cat, acutely deafened and implanted as an adult. Thresholds (intermingled single units and multi-unit clusters) for the apical and basal bipolar channels of the implant are shown as a function of depth for one penetration through the IC. The apical channel (1,2) has its threshold minimum at a more superficial location due to its lower frequency location in the cochlea. The 2 channels excite completely independent, non-overlapping areas at 6 dB above threshold and have spatial tuning curve bandwidths of <700 μm. This corresponds to an STC bandwidth evoked by an acoustic tone delivered at roughly 50-60 dB SPL. **c.** Altered STC from a cat deafened at birth and chronically stimulated on a single bipolar channel (apical electrodes 1,2). The area in the midbrain excited by the chronically activated channel is greatly expanded (STC width=1.5mm), and at 6 dB above threshold it substantially overlaps the area activated by the basal channel. **d.** STC data from a subject that received stimulation on 2 channels using higher frequency, modulated pulse trains and stimulating 1 channel at a time, *alternating* between channels. Both channels maintained highly selective STC widths (450 and 400 μm), and the mean for all penetrations was 700 μm, actually more selective than the mean STC width seen in normal cats. This is a striking contrast to the STC expansion seen with single channel stimulation (46,47).

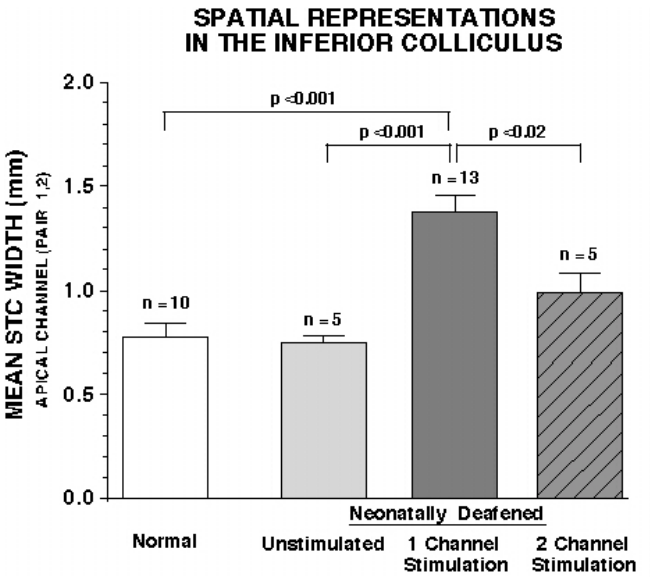



**Figure 8.** Summary graph of means for electrical STC widths in 4 experimental groups (6 dB width, apical channel, averaged for all recording sites in each cat; error bars = SD). STC width in 10 prior-normal controls was 0.78 mm; the mean in 5 neonatally deafened, unstimulated cats was virtually identical at 0.74 mm. The single-channel intracochlear stimulation group had a mean STC width of 1.39 mm; and the 2-channel stimulation group had a mean of 1.00 mm. *Thus, the mean STC width for single-channel stimulated animals was expanded to almost double that of controls and deafened, unstimulated subjects**, but chronic 2-channel stimulation maintained more selective STC widths that were not significantly different from normal*** **(47).**

It should be noted that Figure 8 shows representative data from highly controlled 2-channel stimulation experiments in which the thresholds on the 2 channels were well-matched. In contrast, in other subjects when ***simultaneous*** stimulation was delivered on 2 channels of a model analogue cochlear implant processor or thresholds on the 2 channels differed greatly, stimulation often *failed to maintain* channel selectivity and elicited marked expansion and overlap or fusion of the central representations of the activated channels. These potentially important findings (47) suggest that under suboptimal conditions the central auditory system may not discriminate simultaneous inputs from 2 adjacent implant channels as distinct, resulting in pronounced expansion or even fused central representations. Taken together, our results suggest that electrical stimulation from a cochlear implant in neonatally deafened animals can induce dramatic functional plasticity and reorganization at the level of the auditory midbrain. As we have emphasized in our published reports, central representations in these animals can be highly variable and idiosyncratic, apparently because they are ***dramatically influenced*** by intersubject variables like threshold, neural degeneration, individual stimulation history, and especially the selectivity of initial stimulation elicited by individual channels of the cochlear implant.

Research in other sensory systems (particularly the visual system), has demonstrated that the initial sensory input during early development initiates a ***critical period***, after which organizational changes driven by aberrant or distorted initial inputs are largely irreversible (see 12 for review; and section I. below). If the changes in the auditory midbrain seen in our single-channel experiments and in the 2-channel analog processor subject above were ***irreversible***, they would likely limit the effectiveness of subsequent selective multichannel stimulation. Unfortunately, this clearly may be a problem in very young children using a cochlear implant, because fitting a processor and setting channel loudness levels is so difficult. If one channel is set at too loud in intensity, it may dominate the input, perhaps producing the type of distortions seen in our single-channel experiments (46,47). Given the potential importance of these findings, we believe that future studies should further examine the consequences of chronic stimulation. Specifically, it is important to determine whether expansion of central auditory representations elicited by *initial* single-channel stimulation after neonatal deafening is ***reversible*** if competing inputs from multiple channels of an implant are introduced subsequently.

**G. Experiments conducted in primary auditory cortex (A1) indicate that alterations in the spatial input selectivity also occur at the cortical level in neonatally deafened cats.** In collaboration with Drs. Christoph Schreiner and Marcia Raggio electrophysiological studies of responses in primary auditory cortex (AI) to electrical stimulation of the cochlea have been conducted in many of the same experimental animals for which IC data and SG survival data are described above (79,80,81,92). Following the IC electrophysiological experiment, a second craniotomy is made to expose AI and the cortical experiment is conducted. With current procedures and monitoring equipment, such double experiments have been completed successfully in many animals studied during the current Contract period, usually with no apparent compromise in the physiological status of the cats. In these cortical studies, high-resolution spatial "maps" in AI are constructed by making numerous (80-150), closely-spaced microelectrode penetrations and systematically determining response threshold and temporal response properties at each location. Each map is composed of a series of recording locations made across the frequency gradient of AI (i.e., across the caudal-to-rostral axis of the middle ectosylvian gyrus), and a series of penetrations made across the isofrequency gradient of AI (across the ventral-to-dorsal axis), focusing on the main thalamo-cortical input layers III and IV.

Results in normal cats (deafened, implanted as adults) show that stimulation of an intracochlear bipolar electrode pair produces two regions of higher sensitivity (lower threshold) in AI: one is located dorsally in AI and the second one more ventrally. These regions are separated by a narrow "ridge" of lower sensitivity (high response thresholds) that is oriented caudal-rostrally. Each of the lower-threshold regions shows cochleotopic organization: the minimum threshold locations for apical electrodes are located caudally and shift progressively more rostral with excitation of more basal electrode pairs on the cochlear implant. The positions of these preferential locations for different electrodes are consistent with the known tonotopic organization of AI to acoustic stimulation, indicating that tonotopic organization also occurs with electrical stimulation. In contrast, however, in neonatally deafened animals studied after long term deafness (2-5 years) this selectivity is degraded or even completely absent, resulting in broad regions of equally low response thresholds without clear cochleotopic organization (80,81). Analysis of the spatial extent of the highly sensitive regions revealed that the size of the activated area depends on the stimulus waveform. Sinusoidal stimulation resulted in a spatially more sharply tuned activation than pulsatile stimulation. Differences in threshold behavior and cortical response distributions between the sinusoidal and pulsatile stimulation suggest that stimulus waveform shape plays a significant role in the activation of cortical activity, especially near threshold, that is not accounted for simply by the difference in charge per phase of the applied stimuli. Differences in the activation pattern for short-term and long-term deafness may reflect deafness-induced reorganization based on factors such as differences in excitatory and inhibitory balance that are affected by the stimulation parameters (81). In the future, an area of particular interest will be in examining in detail the *effects of chronic electrical stimulation* on these cortical representations. Preliminary data indicate that the extent of the central ridge and the degree of threshold elevation may vary with an extended duration of deafness or after chronic electrical stimulation (Figure 9). This suggests that the initially inaccessible central region of AI may be accessible for processing of electrical stimuli under appropriate circumstances.

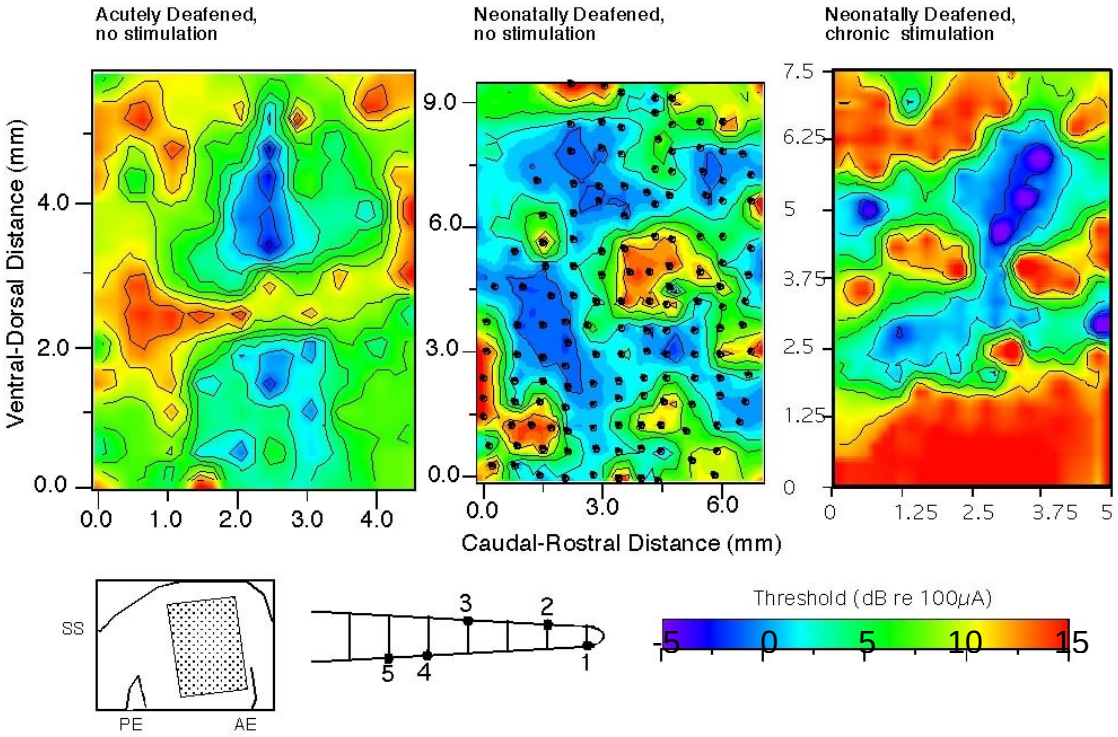



**Figure 9**: Three cortical maps of AI obtained with electrical stimulation of a nearly radial electrode pair in the cochlea. Left map: Acutely deafened animal mapped immediately following deafening procedure. Main features are a narrow low-threshold region (blue) in the vertical, iso-frequency domain of AI separated by a horizontal, high-threshold ridge (orange-red). Middle map: Neonatally deafened animal mapped 2.5 years after deafening. The animal received no chronic electrical stimulation. Main features are a disorganized cortical map with no focal low-threshold region and no clear high-threshold ridge. Right map: neonatally deafened animal that received chronic electrical stimulation for ≈7 months on two adjacent channels. Mapping after end of behavioral training on a signal detection task. Main features are the presence of a vertical low-threshold region separated by a horizontal high-threshold ridge. Chronic electrical stimulation combined with behavioral training appears to have preserved many organizational features of normal AI. (The dots in the middle map indicate actual recording site density.)

**H. <u>Temporal characteristics of chronic electrical stimulation determine the temporal response properties of IC neurons in neonatally deafened subjects.</u>** In addition to the studies of spatial representations of electrical signals in the central auditory system as described above, electrophysiological studies have also analyzed temporal properties of single neurons in the IC responding to electrical stimuli. Initial work showed that many temporal characteristics of IC unit responses to electrical signals are very similar to their responses to acoustic stimulation. In control subjects (animals deafened and studied acutely as adults), all major response types can be identified, and first spike latencies and phase-locking capacities appear to be very similar to responses to acoustic signals (98,99). However, quantitative analysis of response patterns (peristimulus time histograms, PSTH) in cats deafened at a young age revealed significant alterations in the ***temporal*** responses of midbrain neurons. Specifically, the temporal resolution of IC neurons (i.e., the ability of these neurons to phase lock to or follow repetitive signals), is altered both by severe sensory deprivation during development (long-term neonatal deafening) and by controlled, temporally-stereotyped electrical stimulation. When frequency transfer functions for all IC neurons were analyzed quantitatively for adult deafened "normal" control animals, the average maximum following (phase locking) frequency is about 100 pps (pulses per second). Neonatally deafened, unstimulated cats, studied after prolonged intervals (long-deafened animals) showed a significant decrease in the temporal resolution of IC

neurons to an average of 82 pps (Fig. 10).

In contrast to long-deafened subjects, Dr. Vollmer's recent analyses of data from chronically stimulated cats showed either maintenance of normal temporal resolution or an ***increase*** in temporal resolution, depending upon the temporal properties of the electrical signals used for chronic activation of the implant (113). Animals stimulated exclusively with a simple low frequency signal (30 pps) exhibited only a slight increase in temporal resolution (mean maximum following frequency of 109 pps), suggesting maintenance of normal temporal resolution. In contrast, higher frequency, amplitude-modulated and in some cases behaviorally-relevant electrical stimulation resulted in highly significant *increases* in temporal resolution with an average maximum following rate (Fmax) of 134 pps. These increases in temporal resolution were restricted to neurons in the central nucleus of the IC. Neurons in the external nucleus showed poorer temporal following, and their temporal response characteristics were not significantly altered by chronic stimulation (Fig. 10). Parallel changes in latencies were also observed, i.e., shorter latencies in subjects with higher temporal resolution (113). Thus, experience with these electrical stimuli can markedly alter temporal response properties of central auditory neurons in neonatally deafened animals, and the magnitude of these effects is dependent upon the specific temporal properties of the signals delivered by the implant.

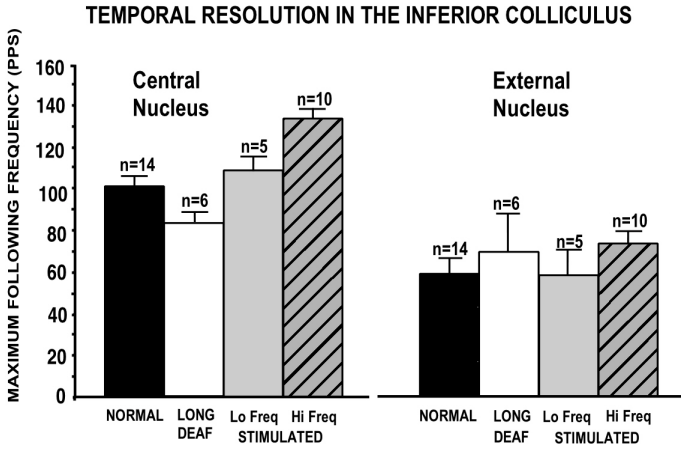



**Figure 10.** The mean maximum following (phase-locking) frequencies for neurons in the central and external nuclei of the IC in three groups of cats: a) acutely-deafened prior-normal adult control subjects; b) neonatally-deafened cats that were chronically stimulated using 30 pps trains of pulses; and c) neonatally-deafened cats stimulated chronically with temporally challenging signals (e.g., 300 pps amplitude modulated at 30 pps; or analog processor.

These experience-dependent effects of chronic stimulation that modulate the capacity of the midbrain neurons to resolve relatively fast temporal events may be important in understanding differences in performance among cochlear implant subjects and in understanding how subjects improve over time. Does this ability to follow electrical pulse trains at higher-than-normal frequencies underlie the success of the latest CIS speech processor designs, which utilize amplitude modulation of high frequency (>5,000 pps) pulse trains? Is the poorer speech recognition capability of some implant subjects related to an inability of their central auditory system to entrain to higher frequencies (e.g., due to specific deafness pathology)? These issues now can be addressed in future studies by systematic examination of the functional consequences of various parameters of chronic electrical stimulation applied in appropriate deaf animal models.

**I. <u>Psychophysical thresholds for higher frequency, modulated stimuli can be significantly lower than EABR thresholds; this difference varies in individual animals.</u>**
A conditioned avoidance paradigm has been developed for the relatively rapid estimation of psychophysical thresholds to electrical stimuli in chronically implanted cats. Cats are trained to lick a metal spoon on "safe" trials to obtain a preferred food reward (meat puree) and to interrupt licking on "warning" trials to avoid a mild electrocutaneous shock. With the implementation of this method it is possible to determine behavioral thresholds during chronic stimulation periods. Thresholds to a number of different electrical signals (30 pps biphasic pulses, 0.2 msec/phase; 100 Hz sinusoids of varying durations; 300 pps pulse trains both simple and AM modulated at 30 Hz) have been obtained in many animals that were subsequently studied in acute physiological experiments. The reports published by our group during the current Contract funding period have directly compared psychophysical data and single- and multi-unit electrophysiological data ***in the same animals*** (4,5,114). Findings showed that behavioral thresholds to intracochlear electrical stimulation were virtually identical to IC and AI single unit thresholds measured in the same cat. EABR thresholds were higher than psychophysical thresholds (mean difference =6.5 dB), but the two threshold measures were directly correlated. This is important because it validates use of the EABR threshold as an indication of perceptual threshold and an appropriate metric for setting levels of chronic stimulation (e.g., at 2 dB above EABR threshold). Behavioral studies were also extended during our current Contract funding period to train several deaf subjects to *discriminate* between changes in the modulation frequency of successive SAM electrical signals, e.g., 300pps/8 Hz AM vs. 300/30 (114).

One of the objectives of our most recent studies has been to apply chronic stimulation in animal models using ***higher frequency modulated signals*** that more closely model signals used in current clinical CIS cochlear implant processors (which use amplitude modulation of carrier rates ranging from about 800 to 5,000 pps). However, studies in both human cochlear implant subjects and in animals have demonstrated that perceptual thresholds become slightly lower with increasing stimulus frequency (4,5,75). Figure 11 shows threshold data for 8 neonatally deafened, chronically stimulated cats that were also behaviorally trained in order to estimate their detection thresholds for an electrical stimulus (100 pps/30 Hz or a 300 pps/30 Hz AM). EABR thresholds (shaded data bars) are compared to psychophysical thresholds (black data bars). In all subjects, the behavioral threshold was lower than the EABR threshold, but the magnitude of this difference varied from 1.9 to 8.5 dB (mean 4.68 dB $\pm$ 0.77SE) in individual subjects, presumably reflecting individual variation in dynamic ranges.

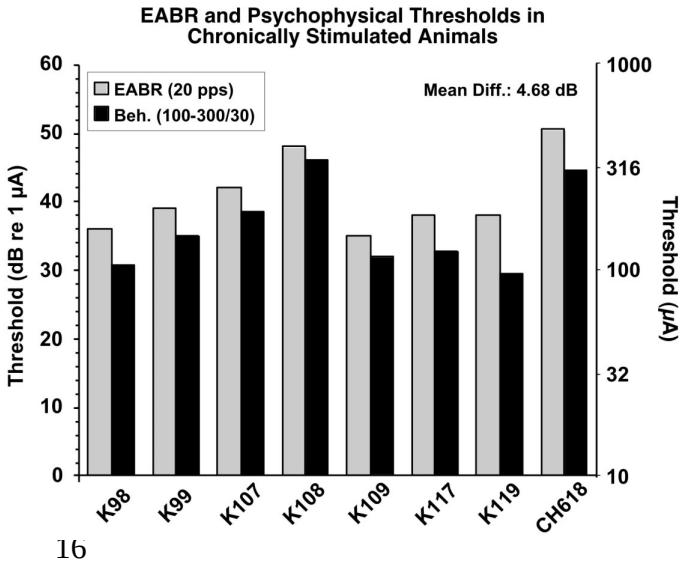



**Figure 11.** EABR thresholds (shaded data bars) and psychophysical thresholds for a 300pps/30Hz electrical stimulus (black bars) obtained in 8 individual behaviorally trained subjects during their chronic stimulation periods. Differences between EABR and behavioral thresholds vary from about 2 to 8.5 dB, presumably reflecting the individual dynamic ranges for electrical stimulation.

EABR thresholds must be estimated using single pulses (due to electrical artifact). Therefore, we expect the difference between EABR and perceptual thresholds to be somewhat greater for higher frequency signals (due to summation in the latter). Thus, the optimum method to set levels for higher frequency stimulation, would be to determine psychophysical threshold for a particular channel and stimulus, then set intensity relative to this value for chronic stimulation. This would be particularly true in experiments in which 2 or more channels will be stimulated in order to ensure selective stimulation by individual channels, a critical feature to test the hypothesis that competitive, multichannel stimulation can prevent de-tuning of the central auditory system and maintain selective central representations. Moreover, setting intensity relative to perceptual thresholds models more appropriately models the fitting adjustments made in human cochlear implant subjects.

Many previous behavioral studies have been conducted to study cochlear implant function in animals, including several studies by Pfingst and colleagues in the monkey; and disparities between behavioral and physiological thresholds have long been suggested (29,30,70,73,74,75,110,111). However, very few direct comparisons of behavioral, single unit and EABR thresholds have been made in the same animals. Comparisons across research groups are confounded by differences between electrodes, animal models, modes of stimulation, and different acute or chronic stimulation histories. *Conducting both psychophysical and electrophysiological studies in the same animals, in a subset of our experiments, has allowed us to study directly the neurophysiological mechanisms that underlie the psychophysical findings and the differences among individual animals.* It will be extremely valuable in future experiments to determine the effects of chronic stimulation using signals more like those used in current clinical CIS processors, and to relate the central representations of such signals to psychophysical discriminability and physiological thresholds for these higher frequency, complex stimuli.

J. Plasticity in Profoundly Deafened Adult Cats. Another study completed during this Contract period examined the functional effects of chronic stimulation in cats that were *deafened as adults after a lifetime of normal auditory experience* (57). Adult cats with normal hearing thresholds received a single injection of kanamycin (300 mg/kg, injected subcutaneously) followed by intravenous infusion of ethacrynic acid (1 mg/min.) as described by Xu et al. (124). Click evoked ABRs were recorded to monitor hearing loss, and the infusion was stopped (10-25 mg/kg total dose) when thresholds rose above 105 dB SPL. Subjects then received 6 months of chronic electrical stimulation on a single channel of the implant, using the 300 pps/ 30 Hz AM signal that induced marked increases in SG survival, expanded STC, and significant increases in temporal resolution of IC neurons in previous neonatally deafened animals.

In terminal physiology experiments, response thresholds to electrical pulses and sinusoidal signals were recorded within the IC using the previously described methods. Spatial tuning curves (STCs) were constructed and their widths measured to infer spatial selectivity. The data showed that chronic electrical stimulation of a single bipolar channel elicited spatial expansion of the IC representation of the stimulated cochlear sector. Findings in these adult animals were not significantly different from results obtained after similar chronic stimulation in neonatally deafened animals, suggesting that similar degrees of plasticity were induced in both animal models (57). Preliminary histological data from these subjects showed that electrical stimulation resulted in an average increase in SG survival of about 10%. This is only about half the increase in SG neural survival seen in neonatally deafened cats stimulated for equivalent periods and using equivalent signals (see Fig.2, Page 3), in which chronic stimulation resulted in a

mean increase of about 20%. This finding suggests that the trophic effects of electrical stimulation in promoting SG survival may be age-dependent. However, these results are viewed preliminary due to the small number of subjects, and because 2 of animals in the study had a chronic infection in the implanted cochlea that may have further compromised histological results in this series. Future studies should seek to resolve this potentially important issue. As mentioned previously, conflicting results among studies in different laboratories have led to controversy as to whether or not stimulation by a cochlear implant can provide trophic support of SG neurons *in vivo*. Additional studies in adult-deafened cats are required to determining whether disparities across studies reported to date are due to species differences, different deafening procedures or critical period effects.

**K. <u>The role of developmental "critical periods" in the stimulation-induced functional alterations seen in these studies is unknown.</u>** There is extensive evidence from research on other sensory systems that input activity, especially synchronized activity, can exert a powerful organizing influence in the developing nervous system. For example, the development of refined connections in the visual system is believed to be dependent upon correlated activity from local retinal locations (1,7,10,14,63,103,103,104; and see 56,89,90,91,96 for review). Development of normally refined connections in these regions can be prevented by introducing widely distributed, synchronous inputs into the retina, for example by electrical stimulation of the optic nerve (103,123), or by stroboscopic illumination that results in nearly synchronous inputs from both eyes (10,17,34,72,89,90). Stroboscopic stimulation during development modifies the receptive field properties and enlarges receptive fields of midbrain and cortical neurons in the cat and maintains the enlarged receptive fields of regenerating retinotectal fibers in goldfish. Moreover, as may be relevant to our 2-channel cochlear electrical stimulation experiments, segregation of inputs from the two eyes can be sharpened by exaggerating the temporal decorrelation of their inputs, for example by introducing a prism or diffuser over one eye (107,109,112) or by alternate monocular deprivation (1,26,109). These results are interpreted as evidence that the underlying competitive processes which act to segregate different neural populations that are driven by *uncorrelated* inputs in the developing nervous system.

There is a limited 'critical period' for these coincidence-based developmental effects in the visual system, but this period can be delayed or extended substantially if experimental animals are profoundly deprived of normal sensory inputs (11,12,62,63). Once normal vision is restored, a critical period is initiated which results in reorganization that generally stabilizes over a period of 6 to 8 weeks in animal models and is largely irreversible after this time. If the central auditory system is governed by similar developmental principles, *then a period of chronic electrical stimulation with an implant over an extended postnatal period in a congenitally deaf child might be expected to generate parallel organizational changes*. As in the visual system, this stimulation might initiate the onset of a delayed critical period, which would render these stimulus-induced changes permanent.

Many animal studies have provided evidence that early sound exposure is critical for normal development and maturation of the auditory pathways in mammals (6,16,82,83,85), and that neonatal sound deprivation is especially deleterious, causing profound adverse effects on the central auditory system. After neonatal deafening or conductive hearing loss, animals show severe atrophy of neurons in the cochlear nucleus (CN) (8,59,60,108,121,122), decrease in the volume of the CN (9,108,120), physiological changes (e.g., 18), as well as transneuronal changes at higher levels of the auditory system (19,31,66,67,77). Other studies have shown that neonatal cochlear lesions can result in substantial functional reorganization (21,28) as well as modification in the anatomical projections from the contralateral CN to the superior olivary

complex and inferior colliculus (35,58,59,67,86). Further, many studies suggest that deprivation occurring later in development (e.g., after 36 days postnatal in the rat and 45 days in the mouse) does not have the same profound impact on the central auditory system (6, 107,119). Thus, deprivation during early development clearly results in profound changes, and there is evidence for the existence of critical periods for such changes (16,82,84). However, these studies have been conducted in many different species, and in various models of deprivation and deafness. Therefore, the specific nature and timing of auditory critical periods as would apply in our neonatally deafened cats (or in a congenitally deaf child) are unclear.

In the neonatally deafened kitten model studied by our group, ototoxic drug treatment extends over the period during which spontaneous activity normally develops in the auditory nerve and during which the organ of Corti and cochlear innervation patterns are undergoing considerable maturation (87; see 117 for review). Clearly, these kittens are severely deprived of normal auditory experience. On the other hand, electrical stimulation is not initiated in these studies until the animals are weaned at 6 weeks postnatal. The critical or sensitive periods in normal auditory system development might be completed by this age; and whereas critical periods in visual system development may be delayed by bilateral deprivation (as discussed previously), such a mechanism has not yet been defined in auditory system development.

We conclude from our findings that whereas early chronic stimulation may result in positive conservation of the auditory nerve in children, *sub-optimal forms of stimulation may also have negative functional consequences within the central auditory nervous system.* Thus, we suggest that future studies evaluating chronic electrical stimulation as a possible means of maintaining the viability of the auditory nerve for optimum function of a cochlear implant must *necessarily* include evaluation of potentially deleterious functional consequences of such stimulation.

Several studies in guinea pigs (27,38,60,65) have shown that chronic electrical stimulation can induce protective effects on SG neurons in adult animals. However, other investigators found no difference in SG survival after chronic stimulation in cats deafened at one month of age by co-administration of kanamycin and ethacrynic acid (3,106) or in adult guinea pigs (58). Given these conflicting results, we believe that it is still premature to draw definite conclusions regarding the age-dependence of the protective effects of chronic electrical stimulation. It is clear that deafness caused by hair cell loss induces degeneration of spiral ganglion neurons in adult animals (46) as well as in neonates. This retrograde degeneration is a slow atrophic process which continues over many months to years. The nature and sequence of pathologic changes in neurons are quite similar in adults and neonates, so it seems likely that electrical stimulation can forestall degeneration in both. This issue, however, remains controversial. *It remains to be determined whether the highly significant, long-term conservation of spiral ganglion neurons seen following chronic electrical stimulation in neonatally deafened cats can be also be induced in animals deafened after a period of normal auditory experience (e.g., animals deafened at 30 days of age) and/ or in adult deafened cats.* Moreover, very few functional studies of the effects of chronic stimulation have been reported in adult-deafened animals. Additional studies in the future are required to determine unequivocally whether the protective effects in the cochlea and functional alterations in central nervous system are age-dependent.

**Summary and Conclusions**. In studies conducted at UCSF with the support of this Contract, it has been demonstrated that chronic stimulation using temporally challenging stimuli results in significant conservation of the spiral ganglion neurons in a pediatric deafness model. These studies also suggest the possibility that the behavioral importance of inputs, or alternatively, the stimulus frequency and/or waveform complexity may influence spiral ganglion protection. Modulation of protective effects by varying stimulus frequency, complexity or behavioral significance is consistent with the observation that the cochlear area over which ganglion cell conservation is observed is substantially broader than the estimated region of directly excited cells. This interpretation is consistent with the hypothesis that stimulation-induced ganglion cell conservation is mediated by indirect factors such as modulation of neurotrophic factors. However, given the paucity of data on the selectivity (re: CF) of electrical stimulation in the auditory nerve with cochlear implant devices, it is also possible that measurements in the auditory midbrain somehow underestimate the region of the spiral ganglion actually excited by the electrical stimulus. It is important to resolve these issues, as an understanding of the fundamental mechanism(s) underlying neural protection is obviously critical to maximizing protection in a child with early-onset deafness.

While it appears likely that optimized stimulation of the cochlea can result in substantially positive spiral ganglion cell conservation in deaf children, it is important to determine whether this phenomenon is age-dependent. Moreover, our electrophysiological studies have shown that there are potential deleterious effects of sub-optimal formats of chronic stimulation, as it can result in substantially negative functional distortions of cochleotopic representations in the auditory midbrain and cortex that may be irreversible. Thus, pediatric cochlear prostheses must be optimized not only to conserve the spiral ganglion neurons, but also the topographic and temporal representations within the central auditory system. We do not yet understand the anatomical bases of these representational distortions. Nor do we know if these striking effects of chronic stimulation in pediatric animals are age-dependent, or if they are reversible. If these effects are not reversible, as visual system studies suggest, then certain forms of electrical stimulation may result in a functional degradation of the auditory system that would compromise the effectiveness of a multichannel prosthesis. On the other hand, if the refinement of auditory system connections reflects coincidence-based competitive processes, then early stimulation with discrete, patterned non-coincident stimuli (e.g., alternating among channels) may result in a positive refinement of central auditory representations, while at the same time conserving the spiral ganglion neurons.

**LIST OF PUBLICATIONS** **Contract #N01-DC-0-2108**

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

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1. Beitel, R.E., M. Vollmer, R.L. Snyder, C.E. Schreiner, P.A. Leake (2000) Behavioral and neurophysiological threshold for electrical cochlear stimulation in the deaf cat. Audiology and Neuro-Otology 5:31-38.

2. Leake, P.A., R.L. Snyder, S.J. Rebscher, G.T. Hradek, C. M. Moore, M. Vollmer, and M. Sato. (2000a) Long term effects of deafness and chronic electrical stimulation of the cochlea. In: *Cochlear Implants.* S.B. Waltzman and N. Cohen (eds.) Thieme, NY, pp. 31-41.

3. Beitel, R.E., R.L. Snyder, C.E. Schreiner, M.W. Raggio and P.A. Leake (2000) Electrical cochlear stimulation in the deaf cat: Comparisons between psychophysical and central auditory neuronal thresholds. J. Neurophysiol. 83: 2145-2162.

4. Snyder, R.L., M. Vollmer, C. Moore, S. Rebscher and P.A. Leake (2000) Responses of inferior colliculus neurons to amplitude modulated intracochlear electrical pulses in deaf cats. J. Neurophysiol. 84: 166-183.

5. Leake, P.A., R.L. Snyder, S. J. Rebscher, C. M. Moore and M. Vollmer (2000b) Plasticity in central representations in the inferior colliculus induced by chronic single- vs. two-channel electrical stimulation by a cochlear implant after neonatal deafness. Hearing Res. 147:221-241.

6. Rebscher, S., R. Snyder, P. Leake. (2001) The Effect of Electrode Configuration on Threshold and Selectivity of Responses to Intracochlear Electrical Stimulation. JASA 109:2035-2048.

7. Osofsky, M.R., C.M. Moore, and P.A. Leake (2001) Does exogenous GM1 ganglioside enhance the effects of electrical stimulation in ameliorating degeneration after neonatal deafness? Hearing Res. 159:23-35.

8. Vollmer, M, R.E. Beitel and R.L. Snyder (2001) Auditory detection and discrimination in deaf cats: Psychophysical and neural thresholds for intracochlear electrical signals. J. Neurophysiol. 86:2330-2343.

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

10. Raggio, M.W. and C.E. Schreiner (2003) Neuronal Responses in Cat Primary Auditory Cortex to Electrical Cochlear Stimulation: IV. Activation Pattern for Sinusoidal Stimulation. J. Neurophysiol. 89:3190-3204.

11. Leake, P.A., and S.J. Rebscher (In Press) Anatomical Considerations and Long-Term Effects of Electrical Stimulation. In: *Auditory Prostheses.* F.-G. Zeng, A.N. Popper and R.R. Fay (eds.), Springer Handbook of Auditory Research. Springer-Verlag, New York, NY.

## Literature Cited:

1. Altman, L., Luhmann, H.J., Greuel, J.M. and Singer,W. 1987. Functional and neuronal binocularity in kittens raised with rapidly alternating binocular occlusion. J. Neurophysiol. 58:965-980.
2. Apfel, S.C., Lipton, R.B. Arezzo, J.C. Kessler, J.A. 1991. Nerve growth factor prevents toxic neuropathy in mice. Ann. Neurol. 29:87-89.
3. Araki, S., K. Atsushi, H.L. Seldon, R.K. Shepherd, S. Funasaka, G.M. Clark 1998. Effects of chronic electrical stimulation on spiral ganglion neuron survival and size in deafened kittens. Laryngoscope 108: 687-695.
4. Beitel, R.E., M. Vollmer, R.L. Snyder, C.E. Schreiner, P.A. Leake 2000a. Behavioral and neurophysiological threshold for electrical cochlear stimulation in the deaf cat. Audiology and Neuro-Otology.5:31-38.
5. Beitel, R.E., R.L. Snyder, C.E. Schreiner, M.W. Raggio and P.A. Leake 2000b. Electrical cochlear stimulation in the deaf cat: Comparisons between psychophysical and central auditory neuronal thresholds. J. Neurophysiol.83:2145-2162.
6. Blatchley, B.J., Williams, J.E., and Coleman, J.R. 1983. Age-dependent effects of acoustic depri-vation on spherical cells of the rat anteroventral cochlear nucleus. Exp. Neurol., 80:81-93.
7. Chapman, B., Jacopson, M.D., Reiter, H.O. and Stryker, M. P. 1988. Ocular dominance shift in kitten visual cortex caused by imbalance of retinal electrical activity. Nature 324:154-156.
8. Coleman, J.R. and O'Connor, P. 1979. Effects of monaural and binaural sound deprivation on cell development in the anteroventral cochlear nucleus of rat. Exp. Neurol., 64: 533-566.
9. Coleman, J., Blatchley, B.J. and Williams, J.E. 1982. Development of the dorsal and ventral cochlear nuclei in rat and effects of acoustic deprivation. Dev. Brain Res., 4:119-123.
10. Cremieux, J., G. Orban, A., Duysens, J., and Amblad, B. 1987. Response properties of area 17 neurons in cats reared in stroboscopic illumination. J. Neurophysiol. 57:1511-1535.
11. Cynader, M. and Chernenko, P. 1976. Abolition of direction selectivity in the visual cortex of the cat. Science, 193:504-505.
12. Cynader, M. and Mitchell, D.E. 1980. Prolonged sensitvity to monocular deprivation in dark-reared cats. J Neurophysiol. 43:1026-1040.
13. Dawson, P.W. and Clark, G.M. 1997. Changes in synthetic and natural vowel perception after specific training for congenitally deafened patients using a multichannel cochlear implant. Ear and Hearing 18: 488-501.
14. Dubin, M.W., Stark, L. A. and Archer, S. M. 1986. A role for action-potential activity in the development of neuronal connections in the kitten retinogeniculate pathway. J. Neurosci. 6:1021-1036.
15. Duchemin, A. M., Ren, Q., Mo, L.. Neff, N. H., Hadjiconstantinou, M. 2002. GM1 ganglioside induces phosphorylation and activation of Trk and Erk in brain J Neurochem 8: 696-707.
16. Eggermont, J.J., and Bock, G.R. (Eds.). 1986. Critical periods in auditory development. Acta Otolaryngol. (Stockholm) Suppl. 429: 1-64.
17. Eisele, L.E., and Schmidt, J.T. 1988. Activity sharpens the regenerating retinotectal projections in Goldfish: Sensitive period for strobe illumination and lack of effect on synaptogenesis and on ganglion cell receptive field properties. J. Neurobiology, 19: 395-411.
18. Evans J.W., Webster D.B., Cullen, J.K. Jr. 1983. Auditory brainstem responses in neonatally sound deprived CBA/J mice. Hear. Res. 10: 269-277.
19. Feng A.S., Rogowski, BA. 1980. Effects of monaural and binaural occlusion on the morphology of neurons in the medial superior olivary nucleus of the rat. Brain Res. 189: 530-534.
20. Hansen, M.R., X.-M. Zha, J. Bok and S.H. Green (2001) Multiple distinct signal pathways, including an autocrine neurotrophic mechanism contribute to the survival-promoting effect of depolarization on spiral ganglion neurons *in vitro*. J. Neuroscience:21:2256-2267.
21. Harrision, R.V., Ibrahim., D., Stanton., S., Mount, R.J., 1996. Reorganization of frequency maps in Chinchilla auditory midbrain after long-term basal cochlear lesions induced at birth. In: Salvi, R., Henderson, D., Fiorino, F., Colletti, V. (eds), Auditory System Plasticity and Regeneration. Thieme, New York, N.Y., pp 283-255.
22. Hartshorn, D.O., Miller, J.M., and Altschuler, R.A. 1991. Protective effect of electrical stimulation in the deafened guinea pig cochlea. Otolaryngol. Head Neck Surg. 104: 311-319.
23. Hawkins, J.E., Jr., Stebbins, W.C. , Johnsson, L.-G., Moody, D.C. and Muraki, A. 1977. The patas monkey as a model for dihydrostreptomycin ototoxicity. Acta Otolaryngol. 83, 123-129.
24. Hefti, F. 1986. Nerve growth factor (NGF) promotes survival of septal cholinergic neurons after

fimbrial transections. J. Neurosci. 6: 2155-2162.
25. Hegarty, J.L., A.R. Kay and S.H. Green. 1997. Trophic support of cultured spiral ganglion neurons by depolarization exceeds and is additive with that by neurotrophins or cAMP and requires elevation of $[CA^{2+}]i$ within a set range. J. Neurosci. 17: 1959-1970.
26. Hubel,. D.H. and Wiesel T.N. 1965. Binocular interaction in striate cortex of kittens reared with artificial squint. J. Neurophysiol. 28:1041-1059.
27. Hultcrantz, M., Snyder, R.L., Rebscher, S.J., and Leake, P. 1991. Effects of neonatal deafening and chronic intracochlear electrical stimulation on the cochlear nucleus. Hearing Res. 54:272-282.
28. Irvine, D.R.F. and Rajan, R. 1994. Plasticity of frequency organization in inferior colliculus of adult cats with unilateral restricted cochlear lesions. Abstr. 17th Assoc. Res. Otolaryngol. 21.
29. Javel, E. 1989. Acoustic and electrical encoding of temporal information. In: *Cochlear Implants: Models of the Electrically Stimulated Ear*. eds. J.M. Miller and F.A.Spelman, pp.247-295, New York, Springer-Verlag.
30. Javel, E., Tong, Y.C., Shepherd, R.K., Clark, G.M. 1987. Responses of cat auditory nerve fibers to biphasic electrical current pulses. Ann. Otol. Rhinol. Laryngol., 96, Suppl. 128, 26-30.
31. Jean-Baptist, M. and Morest, D.K. 1975. Transneuronal changes of synaptic endings and nuclear chrom-atin in the trapezoid body following cochlear ablations in cats. J. Comp. Neurol. 162:111-133.
32. Johnsson, L.-G, Hawkins, J.E., Jr., Kingsley, T.C., Black, F.O and Matz, G.J. 1981. Aminoglycoside-induced inner ear pathology in man, as seen by microdissection. In: Lerner, S.A., Matz, G.J. and Hawkins, J.E., Jr. (Eds.), Aminoglycoside Ototoxicity, Little, Brown and Co., Boston, pp. 389-408.
33. Kanzake, S., Stover, T., Kawamoto, K., Priskorn, D.M., Altschuler, R. A., Miller, J.M., and Raphael, Y. (2002) GDNF and chronic electrical stimulation prevent VIII cranial nerve degeneration following denervation. J.Comp Neurol 454: 450-360.
34. Kennedy, H., Flandrin, J.M. and Amblard, B. 1980. Afferent visual pathways and receptive field properties of superior colliculus neurons in stroboscopically reared cats. Neurosci. Lett. 19:283-288.
35. Kitzes, L. 1996. Anatomical and physiological changes in the brainsem induced by neonatal ablation of the cochlea. In: R.J. Salvi & D. Henderson (eds.), Auditory System Plasticity and Regeneration (pp. 256-274). New York: Thieme Medical Publishers.
36. Lalwani, A. K., Han, J. J., Castelein, C. M., Carvalho, G. J., Mhatre, A. N. (2002) In vitro and in vivo assessment of the ability of adeno-associated virus-brain-derived neurotrophic factor to enhance spiral ganglion cell survival following ototoxic insult. Laryngoscope: 112: 1325-1334.
37. Larsen, S.A. 1984. Postnatal maturation of the cat cochlear nuclear complex. Acta Otolaryngol. Suppl. 217:1-43.
38. Leake, P.A., Snyder, R.L., Schreiner, C.E. 1987. Cochlear pathology of sensorineural deafness in cats: Coadministration of kanamycin and Aminoooxyactetic acid. Ann. Otol. Rhinol. and Laryngol. 96:48-50.
39. Leake, P.A. and Hradek, G.T. 1988. Cochlear pathology of long term neomycin induced deafness in cats. Hearing Res., 33:11-34.
40. Leake, P.A., Kessler, D.K. and Merzenich, M.M. 1990. Cochlear prostheses application and safety In: Neural Protheses. W.F. Agnew and D.B. McGreery (eds). Prentice Hall, Inc. pp. 253-296.
41. Leake, P.A. and Hradek, G.T., Snyder, R.L., Rebscher, S.J. 1991. Chronic intracochlear electrical stimulation induces selective survival of spiral ganglion cells in neonatally deafened cats. Hearing Res. 54:251-271.
42. Leake, P.A.,Snyder, R.L., Hradek, G.T., and Rebscher, S.J. 1992. Chronic intracochlear electrical stimulation in neonatally deafened cats: Effects of intensity and stimulating electrode location. Hearing Res. 64:99-117.
43. Leake, P.A., Snyder, R.L., Hradek, G.T. and Rebscher, S.J. 1995. Consequences of chronic extra-cochlear electrical stimulation in neonatally deafened cats. Hearing Res. 82:65-80.
44. Leake, P.A., A.L. Kuntz, C.M. Moore and P.L. Chambers. 1997. Cochlear pathology induced by aminoglycoside ototoxicity during postnatal maturation in cats. Hearing Res.
45. Leake, P.A., G.T. Hradek and R.L. Snyder. 1999. Chronic electrical stimulation by a cochlear implant promotes survival of spiral ganglion neurons in neonatally deafened cats. J. Comp. Neurol. 412:543-562.
46. Leake, P.A., R.L. Snyder, S.J. Rebscher, G.T. Hradek, C. M. Moore, M. Vollmer, and M. Sato. 2000a. Long term effects of deafness and chronic electrical stimulation of the cochlea. In: *Cochlear Implants.* S.B. Waltzman and N. Cohen (eds.) Thieme, NY, pp. 31-41.

47. Leake, P.A., R.L. Snyder, S. J. Rebscher, C. M. Moore and M. Vollmer. 2000b. Plasticity in central representations in the inferior colliculus induced by chronic single- vs. two-channel electrical stimulation by a cochlear implant after neonatal deafness. Hearing Res. 147:221-241.
48. Leake, P.A., and S.J. Rebscher (In Press) Anatomical Considerations and Long-Term Effects of Electrical Stimulation Springer Handbook of Auditory Research. In: *Auditory Prostheses* F.-G. Zeng, A.N. Popper and R.R. Fay (eds.) Springer-Verlag, New York, NY.)
49. Li, L. Parkins, C.W., and Webster, D.B. 1999. Does electrical stimulation of deaf cochleae prevent spiral ganglion degeneration? Hearing Res., 133:27-39.
50. Lousteau, R.J. 1987. Increased spiral ganglion cell survival in electrially stimulated deafened guinea pig cochleae. Laryngoscope 97 (7): 836-842.
51. Lustig, L.R., Leake, P.A., Snyder, R.L. and Rebscher, S.J. 1994. Changes in the cat cochlear nucleus following neonatal deafening and chronic intracochlear electrical stimulation. Hearing Res. 74: 29-37.
52. Matshushima, J.I., Shepard, Seldon, H.L., Xu, S.A. and Clark, G.M. 1991. Electrical stimulation of the auditory nerve in kittens: Effect on cochlear nucleus morphology. Hearing Res. 56: 133-142.
53. Miller, J.M. and R.A. Altschuler 1995. Effectiveness of different electrical stimulation conditions in preservation of spiral ganglion cells following deafness. Ann. Otol. Rhinol. Laryngol. 166:57-60.
54. Miller, J.M., Altschuler, R.A., Dupont, J., Lesperance, M., & Tucci, D. 1996. Consequences of deafness and electrical stimulation on the auditory system. In: R.J. Salvi & D. Henderson (eds.), *Auditory System Plasticity and Regeneration* (pp. 378-391). New York: Thieme Medical Publishers.
55. Miller, J.M., Chi, D.H. O'Keefe, L.J., Kruszka, P., Raphael, Y. and Altschuler, R.A. 1997. Neurotrophins can enhance spiral ganglion cell survival after inner hair cell loss. Int. J. Devl. Neurosci. 15: 631-643.
56. Miller, K.D., Keller, J. B. and Stryker, M. P. 1989. Ocular dominance column development: Analysis and simulation. Science 245:605-615.
57. Moore, C.M., M. Vollmer, P.A. Leake, R.L. Snyder and S.J. Rebscher 2002. The effects of chronic intracochlear electrical stimulation on inferior colliculus spatial representation in adult deafened cats. Hear. Res.164:82-96.
58. Moore, D.R. and Kitzes, L.M. 1985. Projections from the cochlear nucleus to the inferior colliculus in normal and neonatally cochlea-ablated gerbils. J. Comp. Neurol., 240:180-195.
59. Moore, D.R. and Kowalchuk, N.E. 1988. Auditory brainstem of the ferret: Effects of unilateral cochlear lesions in cochlear nucleus volume and projections to the inferior colliculus. J. Comp. Neurol., 272:503-515.
60. Moore, J. 1986. Cochlear Nuclei: Relationship to the Auditory Nerve. in: R.A. Altschuler, D.W. Hoffman and R.T. Bobbin, eds., Neurobiology of Hearing: The Cochlea. Raven Press, Inc. N.Y. pp.284-301.
61. Mou, K., Hunsberger, C. L., Cleary, J. M., Davis, R. L. 1997. Synergistic effects of BDNF and NT-3 on postnatal spiral ganglion neurons. J. Comp Neurol. 386: 529-539**.**
62. Mower, G.D., Berry, D., Burchfiel, J. L. and Duffy, F. H. 1981. Comparison of dark-rearing and binocular suture on development and plasticity of cat visual cortex. Brain Res. 220:255-267.
63. Mower, G.D., and Cristen, W. G. 1985. Role of visual experience in activating critical period in cat visual cortex. J. Neurophysiol. 53:572-587.
64. Nadol, J.G., Jr. 1981. Histopathology of human aminoglycoside ototoxicity. In: S.A. Lerner, G.J. Matz and J.E. Hawkins, Jr. (Eds.), Aminoglycoside Ototoxicity, Little, Brown and co., Boston, pp. 389-408.
65. NIH Consensus Statement. 1995. Cochlear Implants in Adults and Children. 13(2):1-30.
66. Niparko, J.K., and Finger, P.A. 1997. Ascending projections to the inferior colliculus following unilateral cochlear ablation in the neonatal gerbil, Meriones unguiculatus. J. Comp. Neurol., 214:144-153.
67. Nordeen, K.W., Killackey, H.P., and Kitzes, L.M. 1983. Ascending projections to the inferior colliculus following unilateral cochlear ablation in the neonatal gerbil, Meriones unguiculatus. J. Comp. Neurol., 214:144-153.
68. Osofsky, M.R., C.M. Moore, and P.A. Leake. 2001. Does exogenous GM1 ganglioside enhance the effects of electrical stimulation in ameliorating degeneration after neonatal deafness? Hearing Res. 159:23-35.
69. Otte, J., H.F. Schuknecht and A.G. Kerr 1978. Ganglion populations in normal and pathological human cochlea: Implications for cochlear implantation. Laryngoscope 88, 1231-1246.
70. Parkins, C. W. 1989. Temporal response patterns of auditory nerve fibers to electrical stimulation in deafened squirrel monkeys. Hearing Res., 41:137-168.

71. Parkins, C.W., Li., L., and Webster, D.B. 1999. Comparison of GM1 ganglioside treatment and electrical stimulation for preventing spiral ganglion cell loss in pharmacologically deafened guinea pigs. Abst. of ARO 22nd Midwinter Research Meeting, 660:167.
72. Pasternak, T., and Leinen, L.J. 1986. Pattern and motion vision in cats with selective loss of cortical directional selectivity. J. Neuroscience 6:938-945.
73. Pfingst, B.E., D. Sutton, J.M. Miller and B.A. Bohne. 1981. Cochlear implants: relation of psychophysical measures to histological findings. Acta Otolaryngol. (92): 1-13.
74. Pfingst, B.E. 1988. Comparisons of psychophysical and neurophysiological studies of cochlear implants. Hearing Research, 34:243-252.
75. Pfingst, B.E., and Morris, D.J. 1993. Stimulus features affecting psychophysical detection thresholds for electrical stimulation of the cohclea. II: Frequency and interpulse interval. J. Acoust. Soc. Am. 94:1287-1294.
76. Ponton CW, Don M, Eggermont JJ, Waring MD, Kwong B, Masuda A. 1996. Auditory system plasticity in children after long periods of complete deafness. Neuroreport. 20:8(1):61-65.
77. Powell, T.P.S. and Erulkar, S.D. 1962. Transneuronal cell degeneration in the auditory relay nuclei of the cat. J. Anat., Lond.: 96:249-268.
78. Rabin, S. J.,Bachis, A., Mocchetti, I. 2002. Gangliosides activate trk receptors by inducing the release of neurotrophins. J Biol Chem 277 (51):49466 –49472.
79. Raggio, M.W. and Schreiner, C.E. 1994. Neuronal responses in cat primary auditory cortex to electrical cochlear stimulation: Intensity dependence of firing rate and onset latency. J. Neurophysiol. 72:2334-1359.
80. Raggio, M.W. and C.E. Schreiner 1999. Neuronal responses in cat primary auditory cortex to electrical cochlear stimulation. III. Activation patterns in short- and long-term deafness. J. Neurophysiol. 82:3506-3526.
81. Raggio, M.W. and C.E. Schreiner (In Press) Neuronal Responses in Cat Primary Auditory Cortex to Electrical Cochlear Stimulation: IV. Activation Pattern for Sinusoidal Stimulation. J. Neurophysiol.
82. Rubel, E.W., Born, D.E., Deitch, J.S. and Durham, D. 1984. Recent advances toward understanding auditory system development. In: Hearing Science, pp. 109-157. Editor: C. Berlin. College-Hill Press, Inc. San Diego, CA.
83. Rubel, E.W., Hyson, R.L. and Durham, D. 1990. Afferent regulation of neurons in the brain stem auditory system. J. Neurobiol. 21:169-196.
84. Ruben, R.J. 1986. Unsolved issues around critical periods with emphasis on clinical application. Acta Otolaryngol. Suppl. 429: 61-64.
85. Rubens, R.J., and Rapin, I. 1980. Plasticity of the developing auditory system. Ann. Otol. Rhinol. Laryngol. 89: 303-311.
86. Russell, R.A. & Moore, D.R. 1995. Afferent reorganization within the superior olivary complex of the gerbil: Development and induction by neonatal unilateral cochlear removal. J. Comp. Neurol. 352: 607-625.
87. Sato, M., P.A. Leake and G.T. Hradek 1999. Postnatal development of the organ of Corti in cats: A light microscopic morphometric study. Hearing Res. 127:1-13.
88. Schindler, R.A., Gladstone, H.B., Scott, N., Hradek, G.T., Williams, H., and Shah, S.B. 1995. Enhanced preservation of the auditory nerve following cochlear perfusion with nerve growth factor. Am. J. Otol. 16: 304-309.
89. Schmidt, J.T., and Eisele, L.E. 1985. Stroboscopic illumination and dark rearing block the sharpening of the retinotectal map in the goldfish. Neurosci. 14:535-546.
90. Schmidt, J.T. and Tieman, S.B. 1985. Eye specific segregation of optic afferents in mammals, fish, and frogs: The role of activity. Cell. Molec. Neurobiol., 5:5-34.
91. Schmidt, J.T. and Tieman, S.B. 1989. Activity, growth cones and the selectivity of visual connections. Comments Develop. Neurobiol., 1:11-2.
92. Schreiner, C.E. and Raggio, M.W. 1996. Neuronal responses in cat primary auditory cortex to electrical cochlear stimulation: II. Repetition rate coding. J. Neurophysiol. 75:1283-1300.
93. Shatz, C.J., and Stryker, M.P. 1988. Prenatal tetrodotoxin infusion blocks segregation of retinogeniculate afferents. Science 242:87-89.
94. Shepherd, R.K. 2003. Protective effects of electrical stimulation and neurotrophin delivery on auditory neurons *in vivo*: Implications for cochlear implants. Assoc. Res. Otolaryngol. Abs. 26: #770:195.
95. Shepherd, R.K., Matsushima, J., Martin, R.L. & Clark, G.M. (1994). Cochlear pathology following chronic electrical stimulation of the auditory nerve: II deafened kittens. *Hearing Research 81*, 150-166.

96. Singer, W. 1987. Activity-dependent self-organization of synaptic connections as a substrate of learning. IN: The Neural and Molecular Bases of Learning. J. P. Changeux and M. Konishi, eds., J. Wiley, Chichester U.K., pp. 301-336.
97. Snyder, R.L., Rebscher, S.J., Cao, K., Leake, P.A. 1990. Effects of chronic intracochlear electrical stimulation in the neonatally deafened cat. I.: Expansion of central spatial representation. Hearing Res. 50:7-33
98. Snyder, R.L., Rebscher, S.J., Leake, P.A. Kelly, K., Cao, K. 1991. Chronic electrical stimulation in the neonatally deafened cat. II: Temporal properties of neurons in the inferior colliculus. Hearing Res. 56:246-264.
99. Snyder, R.L., P.A. Leake, S.J. Rebscher and Beitel, R. 1995. Temporal resolution of neurons in cat inferior colliculus to intracochlear electrical stimulation : Effects of neonatal deafening and chronic stimulation. J. Neurophysiol. 73:449-467.
100. Snyder, R.L., M. Vollmer, C. Moore, S. Rebscher and P.A. Leake (2000) Responses of inferior colliculus neurons to amplitude modulated intracochlear electrical pulses in deaf cats. J. Neurophysiol.84:166-183.
101. Spoendlin, H. H. 1975. Retrograde degeneration of the cochlear nerve. Acta Otolaryngol. 79, 266-275.
102. Staecker, H. Kopke, R., Malgrange B., LefebvreP., Van de Water, TR. 1996. NT-3 and/or BDNF therapy prevents loss of auditory neurons following loss of hair cells. Neuroreport 7:889-894.
103. Stryker, M.P. and Strickland, S.L. 1984. Physiological segregation of ocular dominace columns depends on the pattern of afferent electrical activity. Invest. Opthal. MVis. Sci. 25:278.
104. Stryker, M.P., and W. A. Harris 1986. Binocular impulse blockage prevents the formation of occular dominance columns in cat visual cortex. J. Neurosci. 6:2117-2133.
105. Svirsky MA, Robbins AM, Kirk KI, Pisoni DB, Miyamoto RT. (2000) Language development in profoundly deaf children with cochlear implants. Psychol Sci.11(2):153-158.
106. Tieman, S.B., Tumosa, N. 1997. Alternating monocular exposure increases the spacing of ocularity domains in area 17 of cats. Visual Neurosci. 14, 929-938.
107. Tierney, T.S., Russell, F.A., Moore, D.R. 1997. Susceptibility of developing cochlear nucleus neurons to deafferentation-induced death abruptly ends just before the onset of hearing. J. Comp. Neurol. 378:295-306.
108. Trune, D. 1982. Influence of neonatal cochlear removal on the development of mouse cochlear nucleus: Number, size and density of its neurons. J. Comp. Neurol. 209: 409-424.
109. Tumosa, N., S.B. Tieman, and H.V.B. Hirsch. 1980. Unequal alternating monocular deprivation causes asymmetric visual fields in cats. Science, 208:421-423.
110. van den Honert, C. and Stypulkowski, P.H. 1987a. Single fiber mapping of spatial excitation patterns in the electrically stimulated auditory nerve. Hearing Res., 29:195-206.
111. van den Honert, C. and Stypulkowski, P.H. 1987b. Temporal response patterns of single auditory nerve fibers elicited by periodic electrical stimuli. Hearing Res., 29:195-206.
112. van Sluyters, R.C. and F.B. Livitt. 1980. Experimental strabismus in the kitten. J. Neurophysiol. 43:686-698.
113. Vollmer, M., R.L. Snyder, P.A. Leake, R.E. Beitel, C.M. Moore and S.J. Rebscher 1999. Temporal properties of chronic cochlear electrical stimulation determine temporal resolution of neurons in cat inferior colliculus. J. Neurophysiol. 82:2883-2902.
114. Vollmer, M., Beitel, R.E., Snyder, R.L.(2001) Auditory detection and discrimination in the deaf cat: Psychophysical and neural thresholds for temporally modulated intracochlear electrical stimulation. J. Neurophysiol. 86: 2330-2343.
115. Walsh, E.J. and McGee, J. 1986. The development of function in the auditory periphery. In: *Neurobiology of Hearing: The Cochlea*. R.A. Altschuler, D.W. Hoffman and R.P. Bobbin, eds. pp. 247-269, Raven Press, N.Y.
116. Walsh, E.J. and McGee, J. and Javel, E. 1982. Development of auditory evoked potentials in the cat. I. Onset of response and development of sensitivity. J. Acoust. Soc. Am., 79:745-754.
117. Walsh, E.J., and Romand, R. (1992) Functional development of the cochlea and the cochlear nerve. In: Development of Auditory and Vestibular Systems 2. R. Romand (ed.), Chapter 6, pp. 161-219., Elsevier.
118. Walsh, M.E. and D.B. Webster. 1994 Exogenous GM1 ganglioside effects on conductive and sensorineural hearing losses. Hearing Res. 75: 54-60.
119. Webster, D.B. 1983. Late onset of auditory deprivation does not affect brainstem auditory neuron soma size. Hear. Res 12:145-147.

120. Webster, D.B. 1988. Conductive hearing loss affects the growth of the cochlear nuclei over an extended period of time. Hearing Res. 32: 185-192.
121. Webster, D.B. and Webster, M. 1977. Neonatal sound deprivation affects brain stem auditory nuclei. Arch. Otolaryngol. 103: 392-396.
122. Webster D.B. and Webster M. 1979. Effects of neonatal conductive hearing loss on brain stem auditory nuclei. Ann. Otol.,Rhinol., Laryngol; 88: 684-688.
123. Weiliky, M. , & Katz, L.C. 1997. Disruption of orientation tuning in visual cortex by artifically correlated neuronal activity. *Nature 386 (6626):* 680-685.
124. Xu, S-A., Shepherd, R.K., Chen, Y. and Clark, G.M. 1993. Profound hearing loss I the cat following the single co-administration of kanamycin and ethacrynic acid. Hearing Res. 71:205-215.
125. Yan, Q. Elliott, J.L., Snider, W.D. 1992. Brain-derived neurotrophic factor rescues motoneurons from axotomy induced cell death. Nature 360: 753-755.
126. Yagi M, Kanzaki S, Kawamoto K, Shin B, Shah PP, Magal E, Sheng J, Raphael Y (2000) Spiral ganglion neurons are protected from degeneration by GDNF gene therapy. JARO 1:315-325.
127. Ylikoski, J. 1974. Correlative studies of the cochlear pathology and hearing loss in guinea pigs after intoxication with ototoxic antibiotics. Acta Otolaryngol. 79, 266-275.
128. Zha, X. M., Bishop, J. F., Hansen, M. R., Victoria, L., Abbas, P. J., Mouradian, M. M., Green, S. H. 2001 BDNF synthesis in spiral ganglion neurons is constitutive and CREB-dependent. Hear Res: 156: 53-68.
129. Zheng, J.L., Stewart, R.R. and Gao, W-Q. 1995. Neurotrophin-4/5 enhances survival of cultured spiral ganglion neurons and protects them from cisplain ototoxicity. J. Neurosci. 7:5079-5087.
130. Zheng, JL., Gao, W-Q. 1996. Differential damage to auditory neurons and hair cells by ototoxins and neuroprotection by specific neurotrophins in rat cochlear organotypic cultures. Eur. J. Neurosci. 8: 1897-1905.