Final Report

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Protective Effects of Patterned Electrical Stimulation on the Deafened Auditory System

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This Final Report summarizes results from work conducted during the past three years 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 specified in the Technical Specifications, were to determine how electrical stimulation delivered by a cochlear implant can be optimized to preserve the anatomical and functional integrity of the deafened auditory system; and ultimately, to determine how such stimulation might be applied in young deaf children in a way that is compatible with preserving and possibly extending the function of a multiple channel auditory prosthesis.

1. Further characterization of an animal model of congenital profound hearing loss. Our studies have been conducted primarily in cats that are neonatally deafened by systemic administration of the ototoxic drug neomycin sulfate (60 mg/kg IM) for the first 16 to 21 days after birth. Kittens are born deaf due to the immaturity of their auditory system (for review see 96), and the ototoxic drug destroys the cochlear hair cells inducing a profound hearing loss by an age when adult-like hearing sensitivity would normally develop, i.e., at about 21 days postnatal (40). Thus, these animals have no normal auditory experience and are a model of congenital or very early-acquired bilateral profound hearing loss.

It is well-known that in virtually all deafness etiologies, including deafness caused by ototoxic drugs, hair cell degeneration results in subsequent secondary degeneration of the primary afferent spiral ganglion (SG) neurons and their central axons which form the auditory nerve (21,29,58, 85, 105). This degeneration is progressive and continues for many months to years (35), although initial ganglion cell loss is seen as soon as 3 weeks postnatal in these neonatally deafened animals (40). Figure 1 illustrates the time course of SG degeneration in the control (unstimulated) cochleae of neonatally deafened cats. Although there is considerable variation among animals in the extent of neural damage for a given duration of deafness, decreasing SG survival is strongly correlated to duration of deafness. Moreover, cochlear pathology is highly symmetrical in the two cochleae of individual animals (33,34,40).

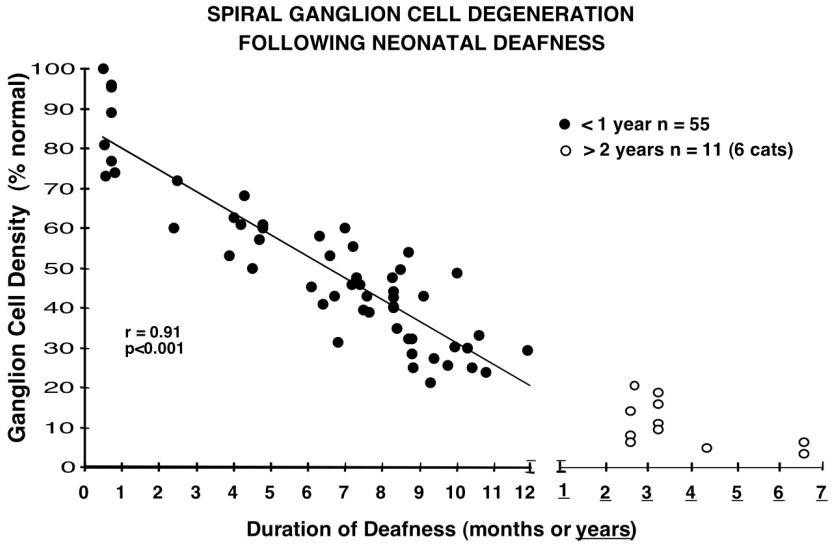

Figure 1. Data from the control, unstimulated cochleae of cats that were deafened neonatally by daily injections of neomycin sulfate beginning the day following birth. The data are shown as the mean SG cell density (averaged over all cochlear sectors), expressed as percent of normal. SG survival is strongly correlated with duration of deafness, although there is considerable individual variability in the extent of degeneration for a given duration of deafness.

The consistent bilateral symmetry of cochlear pathology and the relatively rapid, progressive neuronal degeneration allow the systematic study of the effects of unilateral electrical stimulation using within-animal paired comparisons.

2. <u>Chronic intracochlear electrical stimulation promotes survival of spiral ganglion neurons in neonatally deafened cats</u>. Early studies of the morphological effects of chronic cochlear electrical stimulation focused primarily on issues of safety and damage (e.g.,see 36), or effects of relatively short term implantation and stimulation. More recently, however, several studies have demonstrated that chronic intracochlear electrical stimulation of the cochlea can partially prevent the degeneration of the spiral ganglion neurons which otherwise occurs after deafness (20,37,38,39,42,48). In work supported by this Contract, we have evaluated the histopathological and functional consequences of chronic intra- and extracochlear electrical stimulation using various signal parameters in neonatally deafened cats (37,38,39,41,42). In our most recently published study (41), neonatally deafened animals ranging in age from 6 to 9 weeks were implanted unilaterally with multichannel cochlear prostheses which model those employed in human subjects. The animals were stimulated for several months (mean: 35 weeks) by activation of one or two bipolar intracochlear channels of the implant, using signals designed to be temporally challenging to the central auditory system. Morphometric studies of cochlear spiral ganglion cell density demonstrated that neuronal survival was markedly better in the stimulated cochleae as compared to the contralateral control deafened cochleae. Data pooled from 8 animals demonstrated a highly significant increase of more than 20% in overall neuronal density induced by stimulation (Figure 2). In addition, paired comparisons of SG cell diameters 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.

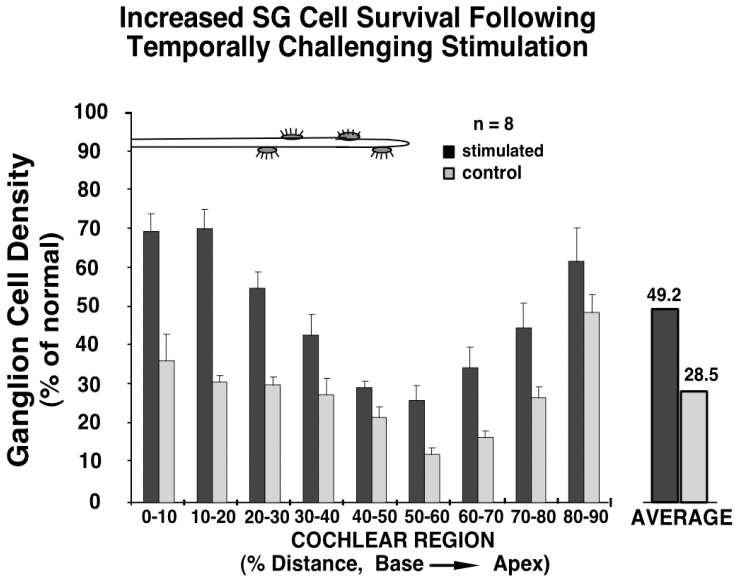

Figure 2. Pooled data from 8 cats that were deafened neonatally by daily injections of neomycin sulfate immediatedly after birth, implanted unilaterally (6-9 weeks of age) with a model cochlear implant, and chronically stimulated using higher frequency, amplitude-modulated electrical signals for 8-9 months delivered by a pair of bipolar electrodes in the basal cochlea. The data are shown as the 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 segment. SG cell density was more than 20% higher in the stimulated ears, and this difference was highly significant ($P<0.001$; Student's paired t-test).

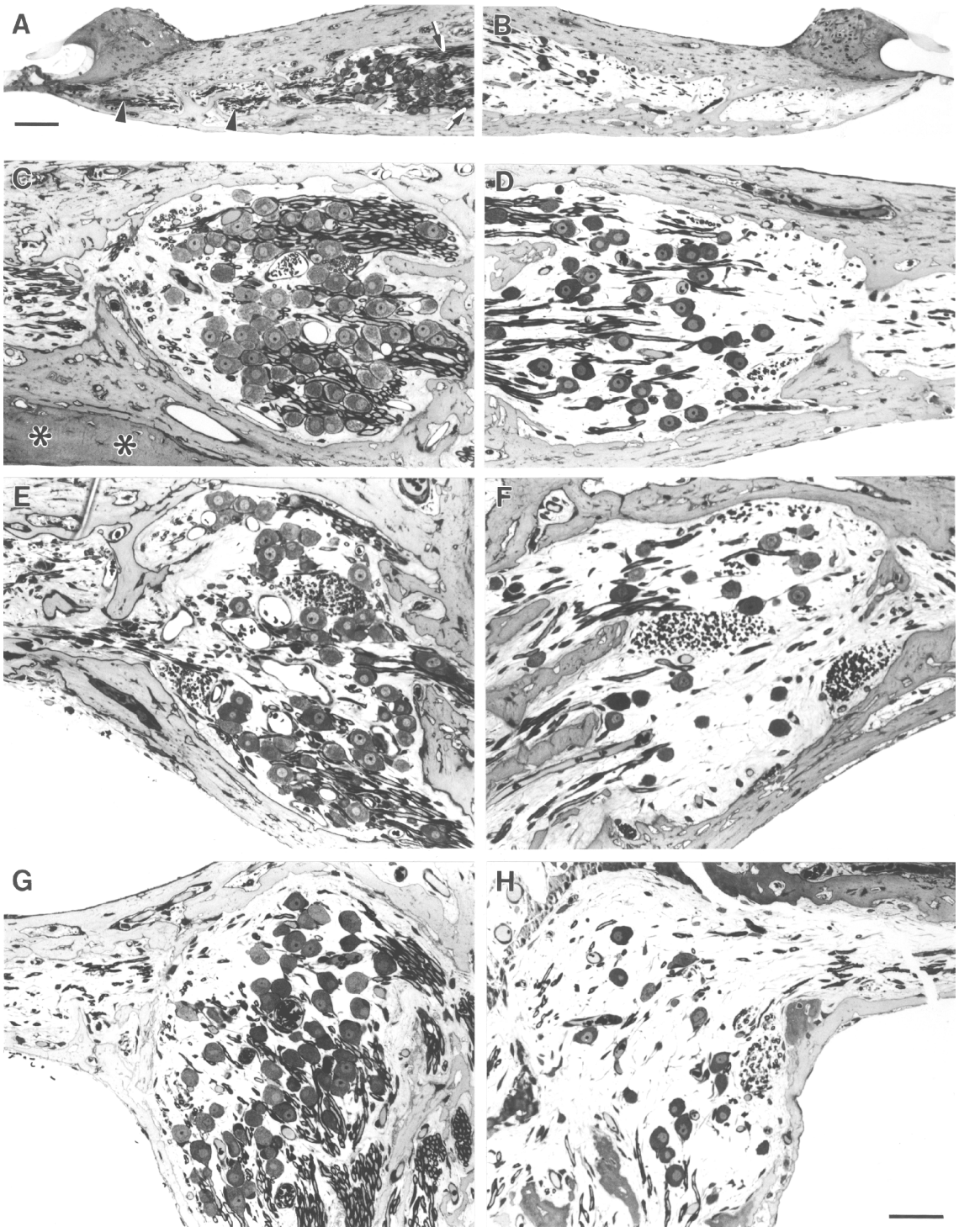

Figure 3. Histological sections showing examples of the differences in SG cell survival between the stimulated cochleae (micrographs on left) and the same regions of the contralateral control ears (right) in 4 different cats following several months of temporally challenging chronic stimulation. Also note that myelination and other morphologic features appear more normal in the stimulated cochlea. Sections in **A,B**: 10-20% region, SG density 75% vs. 31% of normal; Many more peripheral processes of SG cells are seen in the osseous spiral lamina of the stimulated cochlea (arrowheads) than on the control side. **C,D:** 20-30% region, SG density 68% vs. 38%. Asterisks, new bone adjacent to electrode in scala tympani. **E,F**: 30-40% region, SG density 55% vs. 25%; **G,H**: 70-80% region, SG density 68% and 26%. Scale bars =100 μm in A (applies to A and B); 50 μm in H (applies to C-H).

3. <u>GM1 ganglioside appears to modestly enhance SG neural survival after deafness and is additive with trophic effects of chronic stimulation</u>. Recent studies by Green et al. in cultured SG neurons (18,22) suggest that there are multiple mechanisms underlying the neural protective effect of electrical stimulation, one of which is an autocrine neurotrophin response. Our hypothesis is that higher frequency modulated signals may be more effective in driving the mechanisms which underlie the trophic influence of depolarization. Moreover, it is also known that neurotrophins can protect SG neurons against various types of insult, including ototoxic drugs (17, 49). In view of these findings, we conducted a study of GM1 ganglioside which is known to promote neural survival by potentiating growth factors (97), and which is administered exogenously, facilitating potential application in a clinical protocol.

Figure 4A presents data from 6 neonatally deafened animals that received GM1 ganglioside (30 mg/kg, daily subcutaneous injections) after ABR testing had confirmed profound hearing loss at 2-3 weeks of age. Daily injections were continued until the animals underwent implant surgery at 7-8 weeks. Chronic stimulation on 2 channels of the implant was delivered at 2 dB above EABR threshold for 6-8 months. The SG data demonstrate markedly improved neural survival in the stimulated ears, especially in the basal one-third of the cochlea where mean survival is > 70% of normal in the stimulated ears. The mean overall SG density on the stimulated side was about 55% of normal, as compared to 34% for the control side. Figure 4B illustrates data from a comparison group of 6 neonatally deafened animals that did not receive GM1 but had stimulation histories and duration of deafness similar to the GM1 group. This group shows overall SG survival of 49% of normal on the stimulated side vs. 32% in the control ears. Thus, it appears that GM1 ganglioside provides a modest enhancement of neural survival and is *additive* to the effects of electrical stimulation in promoting SG survival, resulting in an increase of about 7% in the GM1-stimulated ears over the effects of stimulation alone.

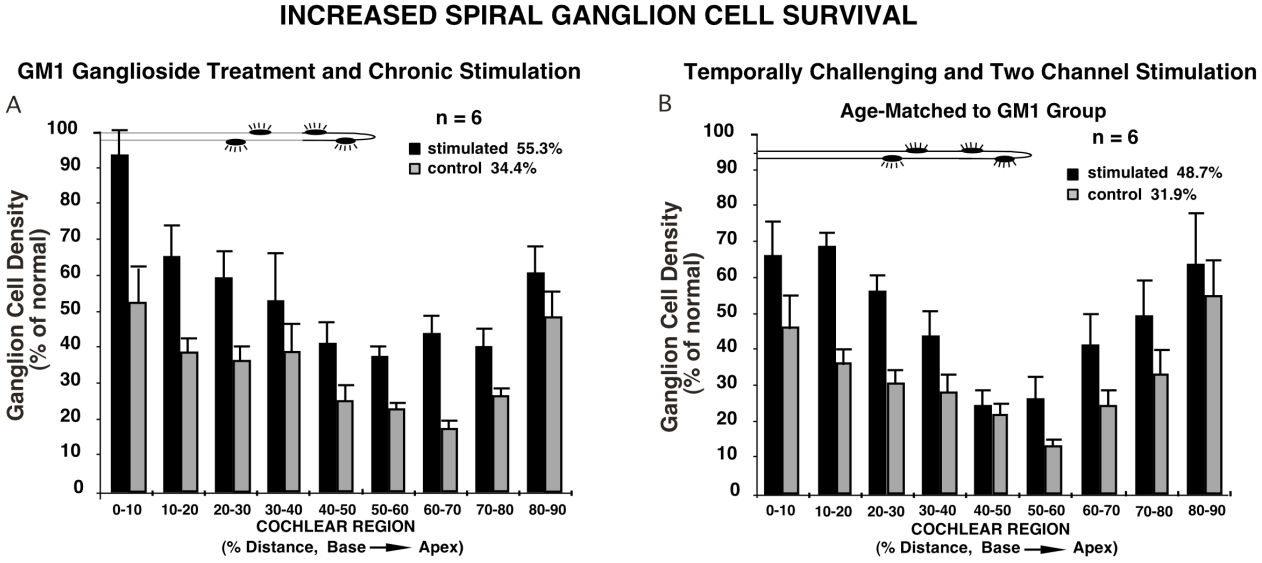

Figure 4. Preliminary data illustrating SG survival in 6 cats that were treated with GM1 ganglioside in the interim period following neonatal deafening and prior to receiving a cochlear implant and 6-8 months of chronic electrical stimulation (**A**). Note that particularly good neural survival is observed in the basal one-third of the cochlea. Overall, SG neural survival is 55% of normal. This is about 7% higher than survival in the comparison experimental group (**B**), that received only chronic stimulation. This comparison group is comprised of neonatally deafened cats that were selected to be age-matched to the GM1 group and had similar histories of chronic stimulation.

4. <u>Stimulation mode, temporal characteristics and duration of stimulation are critically important in maximizing the neurotrophic effects of chronic electrical stimulation on the auditory nerve</u>. As mentioned previously, several other studies also have reported neurotrophic effects of electrical stimulation on the SG neuronal survival. Lousteau (45), Hartshorn et al. (20), and Miller and Altschuler (48) 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 et al. (80) and Araki et al. (2) found no difference in SG survival after chronic stimulation in cats deafened at an early age (although the latter study did report a significant increase in the size of spiral ganglion cells after chronic stimulation). Finally, a recent study by Li et al. (44) reported an increase in ganglion cell density after chronic monopolar stimulation in guinea pigs, but these authors concluded that the density increase resulted from 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 some controversy as to whether or not stimulation by a cochlear implant can promote survival of SG neurons *in vivo* after deafness. Thus, an important focus of our research has been elucidating the specific conditions necessary to induce the protective effects of electrical stimulation in maintaining the SG neurons that we have demonstrated.

Figure 5 presents data for a large group of individual subjects studied in four different chronic stimulation experiments, 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. These subjects showed very little effect of stimulation on SG survival as compared to other experimental groups stimulated for equivalent periods. We have suggested that this mode of stimulation may preferentially excite the SG neurons via their central axons within the modiolus, rather than at more peripheral locations (39), and that such antidromic stimulation may not be as effective in inducing the trophic effects on the SG cells**.**

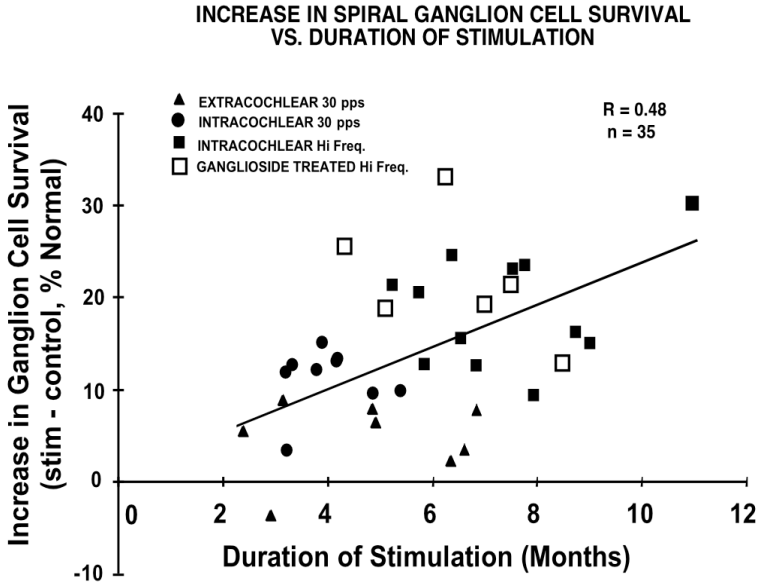

Figure 5. Increase in SG density is shown for individual subjects in 4 different experimental groups as a function of duration of stimulation. Subjects that were stimulated using a monopolar electrode near the round window (triangular symbols) clearly showed less increase in SG survival than other groups. In the remaining intracochlear stimulation groups, greater increase in SG survival is significantly correlated with longer duration of stimulation (R=0.48).

In the remaining intracochlear bipolar stimulation groups, animals stimulated using higher frequency, amplitude 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, the subjects in which higher frequency signals were used also received stimulation for longer periods. The correlation between duration of stimulation and increase in neural density (R=0.48) suggests that duration is an another important factor in determining the extent of neurotrophic effects induced. On the other hand, age-matched comparisons of individuals suggest that higher frequency signals may be more effective than 30 PPS. Thus, although these data do not define the specific contributions of duration vs. stimulus frequency/complexity, our findings suggest that prolonged stimulation using temporally challenging signals induces highly significant neurotrophic effects, and that both factors contribute to this result. The GM1 subjects (unfilled squares) do not stand out in these plots as showing an increased effect, but 5 of the 6 subjects do fall above the regression line for the entire group of subjects, again suggesting a modest improvement in neural survival.

In these recent experiments, electrical stimuli were applied at relatively low current levels with reference to evoked response (EABR) thresholds (2 dB above EABR threshold). When final inferior colliculus experiments were conducted in these animals to map responses to electrodes at chronic stimulation levels, these stimuli appeared to excite more limited sectors of the spiral ganglion than the sectors in which ganglion cell conservation was seen. Thus, for example, activation of a given bipolar electrode pair at 2 dB above EABR threshold might excite roughly one quarter of the central nucleus of the inferior colliculus, while chronic stimulation at that level in the same cat resulted in significant ganglion cell conservation over all or almost all of the cochlea (Fig. 2). These observations suggest the possibility that *direct chronic activation of spiral ganglion cells may not be the direct or only cause of ganglion cell conservation*. A number of different mechanism(s) must be considered as possible contributors to this conservation. Reflexive mechanisms such as vascular changes (e.g., mediated by sympathetic innervation), chronic activation of the efferent system or modulation of neurotrophic factors by subthreshold electrical currents may play a role. It is obviously important to resolve these questions, because determination of the direct cause(s) of spiral ganglion conservation will allow application of practical devices in young children which produce optimal benefits.

5. <u>Neonatal deafness causes marked degenerative changes in the cochlear nuclei; chronic electrical stimulation has only a modest effect on this degeneration even when pronounced increases in spiral ganglion cell survival are observed.</u> Histological studies of the cochlear nuclear complex (CN) in these neonatally deafened, chronically stimulated cats have demonstrated profound degenerative changes in the CN -- changes that are progressive for many months after deafening (46). As compared to data from normal adult cats, the cochlear nuclei of neonatally deafened cats showed: i) marked shrinkage in the volume of the CN; ii) a signficant reduction in the density (number of cells/unit area) of spherical cells within the anteroventral cochlear nucleus (AVCN); and iii) a significant reduction in the mean cross-sectional area of AVCN spherical cells. 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 (7,8, 69,90,99,100,101).

Comparisons between stimulated and control CN in these animals revealed no significant differences in either nuclear volume or spherical cell density due to chronic stimulation. However, for one histological measure, the cross-sectional area of spherical cells in the AVCN, a modest but significant increase (6%) was demonstrated in the stimulated CN (24, 46). Figures 6 and 7 present data from cats in our recent temporally challenging stimulation

experiments, in which we demonstrated a mean increase in SG neural survival of more than 21%. These subjects with marked increases in SG survival induced by chronic stimulation showed CN results that were virtually ***identical*** to data we reported previously for animals in initial experiments with more modest differences in SG survival. Figure 6 illustrates the lack of stimulation effects in preventing shrinkage of the CN after neonatal deafness, and Figure 7 shows the modest (again, mean 6%) increase in spherical cell area induced by chronic stimulation. It is unclear why the cochlear nucleus showed relatively little prevention or reversal of the pronounced morphological consequences of deafening. One possible explanation for the relatively modest effect of stimulation in preventing or reversing the degenerative CN changes in these animals is the delay that occurs before chronic stimulation is initiated in our experiments (42).

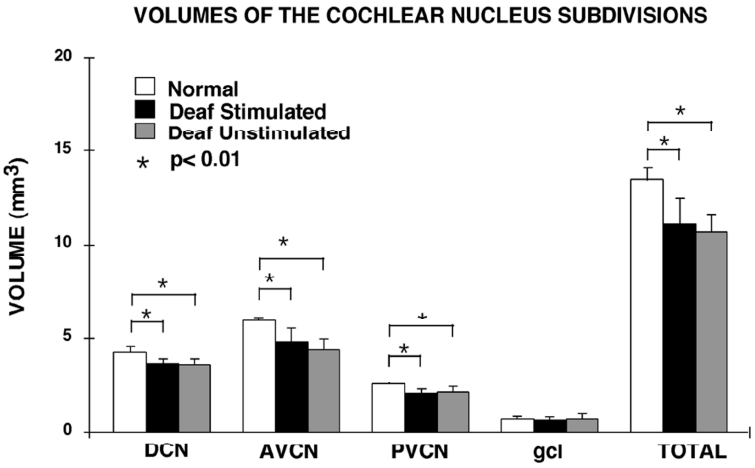

Figure 6. Volumes of the cochlear nucleus and it's component subdivisions in normal cats and in a group of 4 neonatally deafened, chronically stimulated cats. Both deafened, stimulated (black bars) and unstimulated (shaded bars) cochlear nuclei are significantly smaller than normal. This difference is due mainly to reduction in the volume of the anteroventral (AVCN) and posteroventral (PVCN) subdivisions. This effect of deafening is not significantly ameliorated by stimulation. DCN, dorsal cochlear nucleus; GCL, granular cell layer.

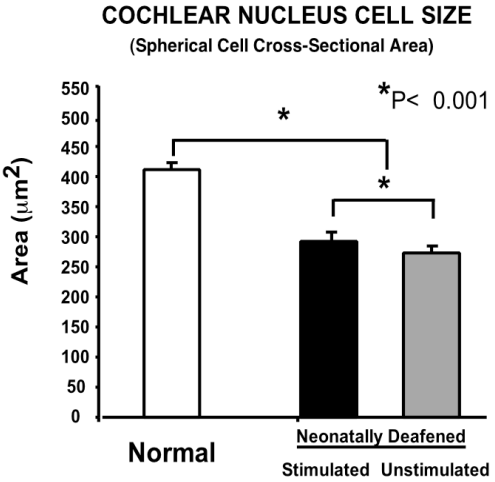

Figure 7. Cross-sectional areas of spherical cells in the rostral AVCN. Data demonstrate a marked reduction in cell size as a consequence of neonatal deafness. Cells on the deafened control side had a mean area of 273 μm (66% of normal), and cells in the stimulated AVCN were significantly larger (294 μm, 71% of normal). This 6% increase due to stimulation is quite modest, considering that these animals showed large increases in SG cell survival (>20%) in the stimulated cochleae as compared to the control deafened ears. Thus, the CN changes do not appear to parallel the degree of SG maintenance induced by stimulation.

Larsen (32) has studied the development of the CN in cats and reported that in the AVCN there is an early growth phase with rapid increase in nuclear and cytoplasmic cross-sectional areas during the first four weeks of postnatal life. This is followed by a second, longer period of development during which the neurons gradually reach mature sizes, by about 12 weeks postpartum. In our neonatally deafened cats, the ototoxic drug is administered during the period when this early, rapid development normally occurs. In the temporally challenging stimulation experiments, intracochlear electrical stimulation was initiated at an average age of 8-9 weeks, well after this initial rapid growth period. Thus, one possibility is that intervention with electrical stimulation in these animals took place too late in development to prevent or reverse

the profound consequences of early deafness. These findings indicate that there is a critical period of development, after which the cochlear nucleus changes induced by deafness are largely irreversible.

In this regard, it should be noted that Matsushima et al. (47) 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 initiated at a similar age was more effective in preventing degenerative changes in the CN in these animals; however, they did not see any difference in SG survival. This 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. However, given the disparate results and relative paucity of data currently available, this is clearly another area requiring additional study. Future studies should directly address these important issues by examining CN data from adult-deafened animals, from kittens deafened at later ages after initial normal development, and from neonatally deafened kittens treated with GM1 ganglioside during and after deafness is induced. These data would provide important information about auditory critical periods in this species.

6. Chronic electrical stimulation markedly alters spatial selectivity (i.e., cochleotopic maps) in the auditory midbrain of neonatally deafened cats. In addition to the anatomical studies outlined above, acute electrophysiological experiments conducted by our group have examined the topographic organization and the temporal patterns of neuronal responses evoked by cochlear electrical stimulation within the auditory midbrain (IC) (35,37,42, 43,67,68,69,95,84). Studies have been conducted in: a) animals that were deafened, implanted as adults and studied acutely as controls (Fig. 8B); b) neonatally deafened, chronically stimulated cats -- including both the initial experimental groups stimulated on a single bipolar channel of the cochlear implant and more recent experiments in which subjects were stimulated on 2 channels (Figs. 8C,D); and c) neonatally deafened but unstimulated controls examined at the same ages as the stimulated group. Data from this latter neonatally deafened/unstimulated group suggest that the precise cochleotopic organization of the central nucleus of the IC (ICC) develops normally and is unaltered despite the lack of normal auditory input during development in these animals. That is, the spatial selectivity elicited with our standard bipolar intracochlear electrodes is normal in this group (Fig. 9).

On the other hand, when neonatally deafened animals are chronically stimulated at a young age on a single channel of a cochlear implant, spatial selectivity assessed in the midbrain was markedly altered. Our previous 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 cochleotopic organization of the IC in neonatally deafened animals. Specifically, the area within the ICC excited by the chronically activated electrodes is significantly expanded and on average is almost double the area for identical electrodes in either unstimulated control deaf littermates, or in acutely-deafened adults (39,82) (Fig. 8C). These results are interpreted as evidence 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, this expansion actually represents a significant distortion and degradation of the cochleotopic organization (frequency selectivity) of the central auditory system (39,42,43, 82).

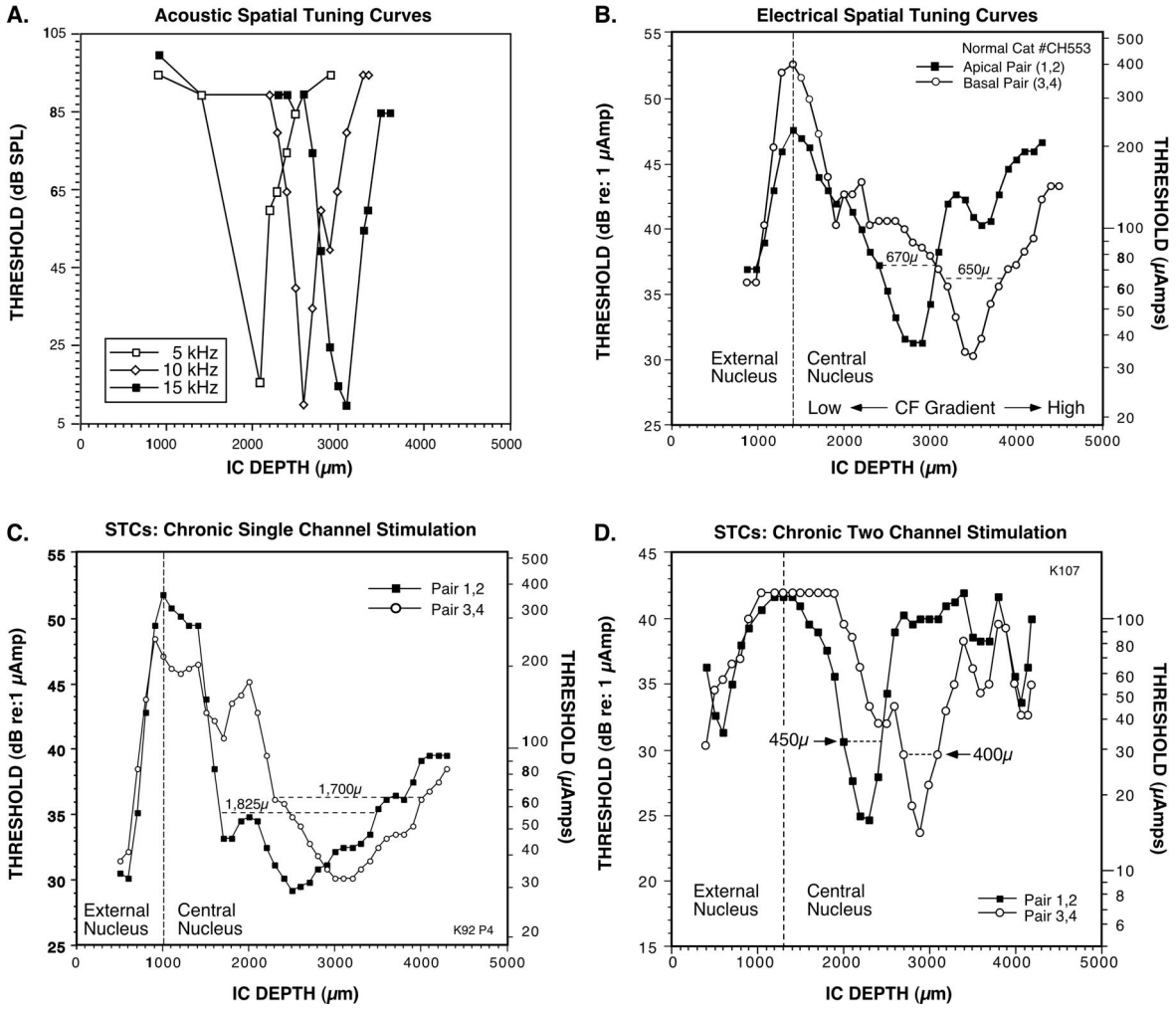

Figure 8. Plots of the frequency gradient in penetrations made in a standardized trajectory through the central nucleus of the inferior colliculus [ICC] demonstrate the precise frequency organization in normal cats. This uniform topographic organization of the IC has been exploited as a basis for 'mapping' the relative selectivity of excitation of the cochlea evoked by stimulation with intracochlear electrodes in deaf cats. **A.** Plots of threshold as a function of depth across the ICC in a normal cat using three tonal frequencies (5, 10, and 15 kHz). These acoustic spatial tuning curves (STCs) illustrate the distribution of excitation across the IC as a function of stimulus intensity for these frequencies which correspond to about the same cochlear locations that would be excited by our intracochlear electrodes (apical channel =5 kHz; basal channel =about 15 kHz). **B.** Electrical spatial tuning curves in a prior-normal cat, acutely deafened and implanted as an adult. Plots of stimulus threshold (intermingled single- and multiunits) for the apical and basal channels of the implant as a function of depth for one penetration through the ICC are shown. The apical channel (1,2) has a slightly higher absolute threshold and the most superficially located threshold minimum (corresponding to the lower frequency location in the cochlea). The 2 channels excite completely independent, non-overlapping areas and have selective 6 dB spatial tuning curve bandwidths of <.7 mm, corresponding to an ICC spatial tuning bandwidth evoked by a stimulus 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=1.5 mm), and at 6 dB above threshold it substantially overlaps the area activated by the basal channel. **D.** STC data from a cat in our most recent 2-channel chronic stimulation experiments. This subject 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 (.45 and .4 mm), and the mean for all penetrations was .7mm, actually more selective than normal cats and a striking contrast to single channel stimulation which induced marked expansion of STC. These data suggest that competitive stimulation of 2 active channels may act to segregate inputs, maintaining distinct response areas.

Figure 8D shows data from a subject in our most recent experimental series of neonatally deafened cats 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) in this subject was effective in maintaining or perhaps even sharpening selectivity of central representations of stimulated cochlear sectors. Figure 9 shows pooled data from these 2-channel experiments. Results suggest that competing inputs elicited by electrical stimulation delivered by 2 adjacent channels, can ***maintain*** 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.

Finally, data from another experimental subject indicate that ***simultaneous*** stimulation using 2 channels of a model analogue cochlear implant processor failed to maintain channel selectivity and resulted in marked expansion and fusion of the central representations of the stimulated channels (K117). This potentially important result suggests that under certain conditions the central auditory system may fail to discriminate simultaneous, overlapping inputs from adjacent cochlear implant channels as distinct, resulting in pronounced expansion and creation of highly overlapping or fused central representations. Taken together, findings from these experiments 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. Central representations in these animals are highly variable and idiosyncratic, because they are ***dramatically influenced*** by intersubject variables like individual stimulation history, threshold, and extent of neural degeneration especially with respect to the selectivity of individual channels of the cochlear implant.

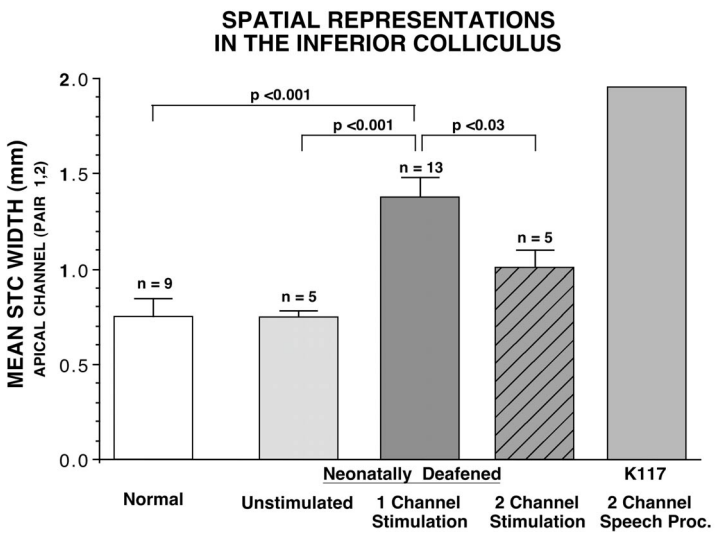

Figure 9. Summary figure illustrating mean and standard deviation for electrical STC widths in various experimental groups (6 dB width, apical channel, averaged for all penetrations in each cat). STC width in 9 prior-normal control cats was 0.78 mm; the mean in neonatally deafened, unstimulated cats was 0.74 mm (n=5). The single-channel intracochlear stimulation group (n=13) had a mean STC width of 1.39 mm; and the 2-channel stimulation group (n=5) had a mean of 1.00 mm. *Thus the average STC width of single-channel stimulated animals was expanded to almost double that of prior-normal animals and neonatally deafened, unstimulated animals**, but 2-channel stimulation maintained selective STC widths that were not significantly different from normal.*** The final data bar shows preliminary results from a single subject that received chronic electrical stimulation delivered simultaneously on 2 channels using inputs from an analog cochlear implant processor. STC were extremely broad, suggesting that the central auditory system failed to distinguish the 2 channels as distinct, resulting in pronounced expansion and highly over-lapping central representations.

Research in other sensory systems (particularly the visual system), has demonstrated that the initial input activity during development initiates a ***critical period***, after which organizational changes driven by aberrant or distorted initial inputs are largely irreversible. If the changes in the auditory midbrain demonstrated in our single channel experiments and in the 2-channel analog processor experiment were ***permanent***, they would clearly limit the possibility for selective multichannel stimulation. Unfortunately, this actually may be a problem in very young children using the cochlear implant, because fitting a processor and setting channel loudness levels is so difficult. If one channel is set at too loud an intensity, it could dominate the input, and perhaps produce the type of distortions seen in our single-channel experiments (53).

Given the potentially important implications of these findings, we believe that it is important to substantiate these results and to further examine the effects of various format of chronic multichannel stimulation. In particular, it is critical in future research to determine whether or not distortions induced by initial stimulation in these young animals are irreversible later in life.

Moreover, the potential implications of these results in animal experiments for clinical pediatric implants should not be overlooked. Specifically, we have suggested that the marked intersubject variability and the severe (and possibly irreversible) distortions in the central cochleotopic (frequency) organizations seen in some subjects emphasizes the importance of the initial fitting of cochlear implants in the naive, developing auditory system. ***Our results suggest that there may be specific ways of introducing stimulation in a young deaf child that might optimize setting up appropriately distinct central representations of individual channels of the cochlear implant (43).*** For example, this might be accomplished by introducing the channels one at a time, and encouraging discrimination among pairs of channels, rather than simply turning on all the channels simultaneously.

7. <u>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</u>. 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 cats described above (77, 66). 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 successfully completed in many of the 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 of 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). The recordings are made at a depth of ≈800 μm, focussing on the main thalamo-cortical input layers III and IV.

Results in normal cats (deafened and implanted as adults) show that stimulation of an individual 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 show 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 lost, resulting in broad regions of equally low response thresholds without clear cochleotopic organization (66).

The finding that electrical stimulation produces a central high-threshold region in AI , which forms a "ridge" separating two tuned regions in ventral and dorsal AI, is of particular interest (65,77). Direct comparison of response properties in hearing and deafened animals shows a high degree of ***anti-correlation*** of the acoustic and electric response thresholds in this ridge region. That is, this area has the lowest acoustic response thresholds and the sharpest

frequency tuning of all of AI (Fig. 10), but with electrical stimulation it shows very high response thresholds and hardly any tuning. In other words, the acoustically ***most sensitive*** and selective core area of AI is not appropriately accessed by electrical stimulation.

The high thresholds for electrical stimulation in this central ridge region are likely due to strong inhibitory effects, since this region coincides with the most sharply tuned region in AI of normal hearing animals and contains strong inhibitory side-bands. These side-bands reflect the thalamo-cortical projections to this region, but also may be strengthened by intrinsic cortical mechanisms as suggested by a relatively high local density of GABA-ergic neurons in or near this region. Recent studies of the effects of cortical application of bicuculline, a GABAa antagonist, support this view by demonstrating a clear broadening of the frequency response areas (88,98). This strong inhibitory influence normally results in fairly weak responses to acoustic broad-band stimuli in this sharply-tuned cortical region. Since electrical stimulation is more akin to broad-band stimulation than pure-tone stimulation, it may engage the strong inhibitory system in central AI, effectively shutting down excitatory inputs to that region. It would be of great interest in future experiments to examine the ***effects*** of chronic electrical stimulation on these cortical representations. Preliminary data suggest that the extent of the central ridge and the degree of threshold elevation can vary with duration of deafness and after chronic electrical stimulation (single pair). This suggests that the initially inaccessible central region of AI may be accessible under appropriate circumstances and may be regained for the contribution to signal processing in electrical stimulation.

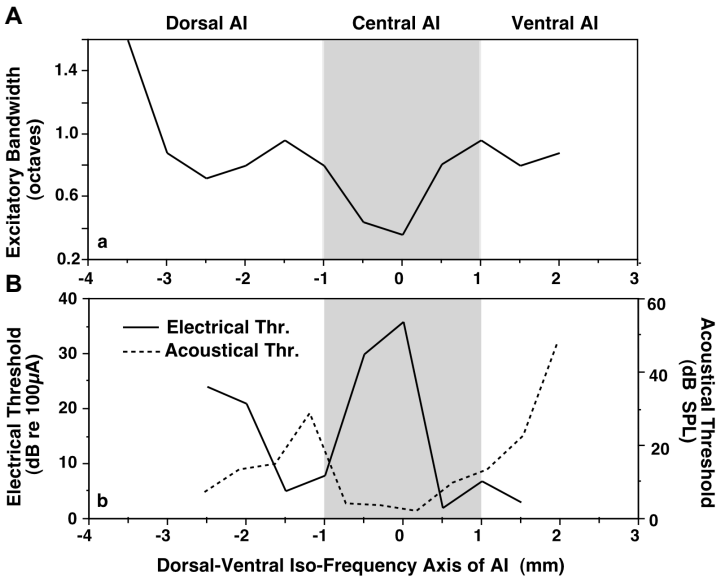

Figure 10. A. Spatial distribution of excitatory bandwidth within the isofrequency domain of AI. Mean excitatory bandwidth of multiple-unit (MU) responses at 40dB above threshold is shown as a function of location along the isofrequency axis. The bandwidth, expressed in octaves, was averaged in bins of 0.5 mm width for several animals. Position 'zero mm' corresponds to the location with the narrowest tuning in each individual case. The shaded areas indicate the approximate extent of the central, narrowly-tuned region of AI, labeled "central AI". The adjacent, more broadly-tuned regions are labeled 'dorsal AI' and 'ventral AI', respectively.

B. Minimum response threshold for cortical sites comparing cochlear electrical stimulation (solid line, left ordinate) and acoustic stimulation (dashed line, right ordinate). Electrical stimuli were single bipolar pulses (200 μsec/phase) delivered on a single bipolar cochlear electrode pair. Acoustical thresholds to CF-tones were measured before implantation of the cochlear electrode. Note that the location of lowest acoustical threshold and sharpest tuning in the center of AI coincides with the region of highest thresholds for electrical stimulation.

8. <u>Chronic electrical stimulation in neonatally deafened cats significantly alters temporal response properties of neurons in the auditory midbrain.</u> In addition to the studies of spatial representations of electrical signals in the central auditory system as described above, electrophysiological studies also have 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 animals deafened and studied as adults, all major response types are identified, and

first spike latencies and phase-locking capacities appear to be very similar (68,83). However, quantitative analysis of response patterns (peristimulus time histograms, PSTH) in cats deafened at a young age revealed significant alterations in the ***temporal*** response properties of midbrain neurons. In particular, 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 (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. Neonatally deafened, unstimulated cats, studied at prolonged intervals after deafening showed a significant decrease in the temporal resolution of IC neurons to an average of 86 pps (83).

In contrast, 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 (95). Animals stimulated exclusively with a simple low frequency signal (30 pps) exhibit only a slight increase in temporal resolution (mean maximum following frequency or Fmax of 109 pps), indicating a maintenance of normal temporal resolution, but not a significant increase above normal. However, higher frequency, modulated and in some cases behaviorally-relevant electrical stimulation resulted in a marked, highly significant *increase* in temporal resolution with Fmax of 134 pps. Moreover, these changes in temporal resolution were restricted to neurons in the central nucleus of the IC, while neurons in the external nucleus showed much lower temporal following and were not significantly modified by chronic stimulation (Fig. 11).

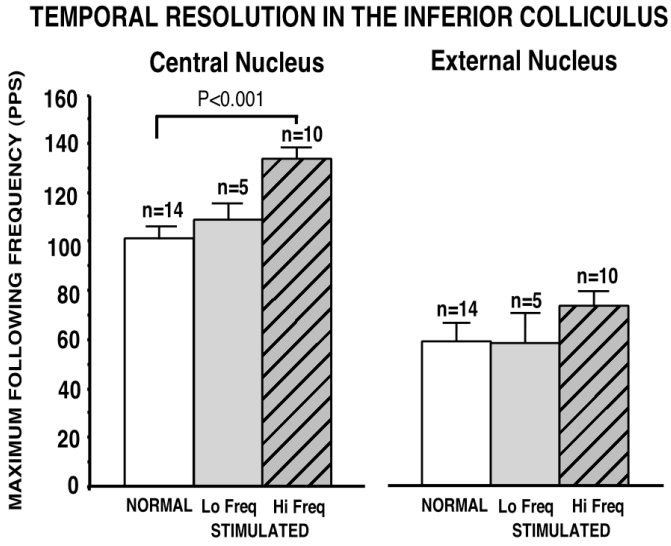

Figure 11 The mean maximum following (phase-locking) frequencies for neurons in the central and external nuclei of the IC in three groups of cats: i) acutely-deafened prior normal adults, ii) neonatally-deafened cats chronically stimulated with 30 pps pulses and iii) neonatally-deafened cats stimulated chronically with temporally challenging signals (e.g., 300 pps amplitude modulated at 30 pps; or analog cochlear implant processor)

Thus, experience with these electrical stimuli in neonatally deafened animals can profoundly alter temporal response properties of central auditory neurons, and the magnitude of these effects is dependent upon the specific temporal properties of the signals delivered by the implant. These frequency-dependent effects of chronic stimulation in increasing the capacity of the midbrain neurons to resolve relatively fast temporal events may be important in understanding differences between the performances of some 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 recent sophisticated CIS speech processor designs which utilize amplitude modulation of high frequency (>800 pps) pulse trains? Is poorer speech recognition capability of 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 should be addressed in future studies by systematic study of the functional effects of various parameters of chronic electrical stimulation in appropriate deaf animal models.

9. Responses of Neurons in the Inferior Colliculus to High Frequency Pulsatile, and SAM Stimuli. Studies conducted during the current Contract period indicate that IC neurons have specific, idiosyncratic combinations of carrier rates and modulation frequencies to which they will respond. As mentioned previously, responses to unmodulated pulses are almost all low-pass functions of pulse rate, with a maximum following frequency (Fmax) of 104 pps. Above Fmax neurons cease to respond, except for an onset burst at the beginning of the stimulus. However, most neurons can respond to much higher pulse rates when pulse trains are amplitude modulated. In fact, about 75% of the IC neurons studied to date were relatively insensitive to differences in carrier rate and would respond to all modulated carriers including those exceeding 600 pps. However, the average maximum modulation frequency (maxFm) that IC neurons follow are only about 35-40 Hz. Thus, temporal resolution of these neurons for modulated frequencies is significantly lower than that for unmodulated pulses. Moreover, these 2 measures of temporal resolution (Fmax and maxFm) appear to be relatively independent, since the correlation value for units in which both values have been determined is low (Fig. 18). Thus for example, unmodulated pulse trains delivered at 800 pps evoke no response or only a very small response in a very few IC neurons. *But almost all IC neurons will respond to this same 800 pps carrier, when it is modulated at 30 Hz.* Thus, IC neurons respond to AM pulse trains when the *modulation frequency falls within their response range* (i.e., at or beneath their maximum following frequency (83,84). With carrier rates above a specific level (i.e., about 300 pps in normal cats, but perhaps higher in chronically stimulated animals), the carrier becomes essentially invisible to the IC, and neurons respond only to the AM envelope of the stimulus. To our knowledge, these data represent the first systematic characterization of the responses of single neurons in the central auditory system to complex electrical stimuli. Obviously, such data are highly relevant to understanding how such signals are utilized by cochlear implant subjects and to optimizing information transfer with the implant.

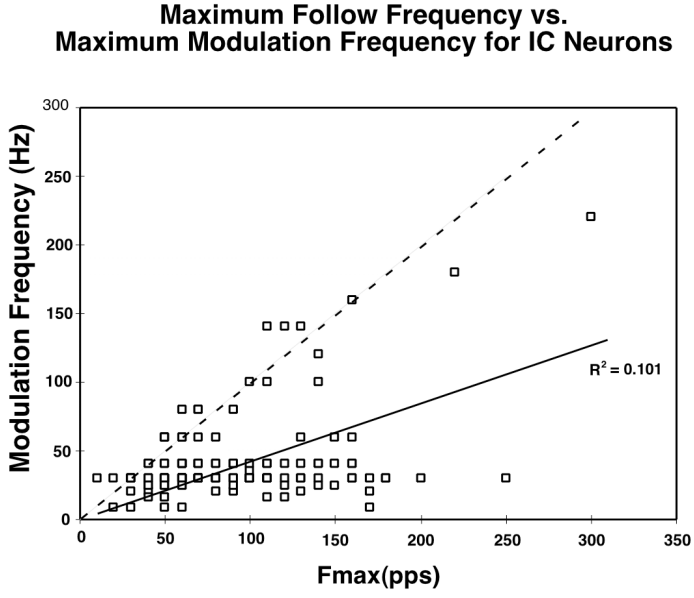

Figure 18. Scatter plot of the maximum unmodulated pulse frequency (Fmax) vs. the maximum modulation frequency for all IC neurons in which both values have been determined. The diagonal dashed line is the equal-frequency contour; the solid diagonal line represents a linear regression analysis of the data. Neurons show specific, idiosyncratic combinations of Fmax and maxFm to which they are most sensitive

10. <u>Psychophysical thresholds for higher frequency, amplitude modulated stimuli can be significantly lower than EABR thresholds; this difference varies in individual animals.</u> A conditioned avoidance paradigm has been developed for 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 electrophysiological experiments (3,4). Initial experiments 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), and 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.)

One important objective of future work with chronic stimulation in animal models should be to examine ***higher frequency modulated signals*** that more closely model signals used in current CIS human cochlear implant processors (which use amplitude modulation of carrier rates up to 2000 pps). Studies in both human cochlear implant subjects and studies conducted in animals have demonstrated that perceptual thresholds become slightly lower with increasing stimulus frequency (4,63,78). Figure 12 shows threshold data for 5 behaviorally-trained, neonatally deafened cats that were chronically stimulated (300 pps/30 Hz AM) with bipolar intracochlear electrodes. EABR thresholds (shaded data bars) are compared to psychophysical thresholds (black data bars) for the chronic 300 pps/30 Hz stimulus. As expected, in all animals for which these data have been collected, the behavioral thresholds for pulses were within a few dB of EABR thresholds, but the magnitude of this difference varies from 3 to 8 dB (mean 5.24 dB $\pm$0.82) in individual cats, presumably reflecting individual variation in thresholds and dynamic ranges.

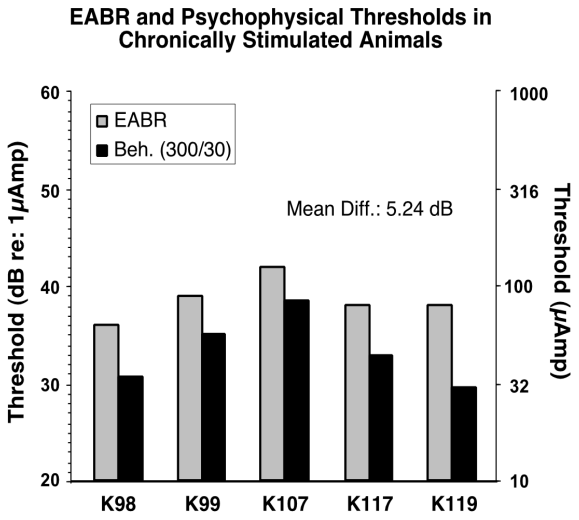

Figure 12 EABR thresholds (shaded data bars) and psychophysical thresholds to the 300pps/30Hz stimulus used for chronic stimulation (black bars) for 5 individual behaviorally trained cats. Differences between EABR and behavioral thresholds vary from 3 to 8 dB, presumably reflecting the individual dynamic range for electrical stimualtion.

Thus, the optimum method to set levels for higher frequency stimulation would be to determine psychophysical thresholds for the particular channel and stimulus, then use these values to set intensity for chronic stimulation. Determination of perceptual thresholds is

important to ensure selective stimulation by individual channels, which is critical in testing the hypothesis that competitive, multichannel stimulation will prevent de-tuning of the central auditory system and maintain selective central representations. Also, setting intensity relative to perceptual thresholds more appropriately models function of a cochlear implant in human subjects.

A number of previous behavioral studies have been conducted to examine cochlear implant stimulation in animals, primarily by Pfingst and colleagues in the monkey, and disparities between behavioral and physiological thresholds have been reported (26,27,59,61,62, 92,93). However, very few direct comparisons of behavioral, single unit and EABR thresholds have been made in the same animals, and such comparisons across research groups are confounded by differences between electrodes, animal models, modes of stimulation, and different acute or chronic stimulation histories. It is important to conduct both psychophysical and electrophysiological studies in the same animals in order to directly study the neurophysiological mechanisms which underlie the psychophysical data and differences among individual animals. It would be very valuable in future experiments to explore the effects of chronic stimulation like that used in current clinical implant processors, and to study the central representations of such signals by defining physiological thresholds for such higher frequency, complex stimuli.

11. <u>Plasticity in Profoundly Deafened Adult Cats</u>. With support of this Contract, Ms. Charlotte Moore, completed her doctoral dissertation in the UCSF Ph.D. program in Speech and Hearing Science. She examined the effects of chronic stimulation in cats that were deafened as adults after a lifetime of normal auditory experience. Adult cats 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. (104). 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. For her thesis, Dr. Moore completed study of 4 adult-deafened subjects. All these subjects 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. However, technical problems in 1 of these subjects clearly compromised the histological results (implant failure required reimplantation, during which the round window was fractured). Figure 13 illustrates the histological data from the remaining 3 subjects, showing 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 may be age-dependent. However, these results are viewed preliminary due to the small number of subjects, and also because one of the 3 remaining 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 would be very valuable in indicating whether disparities across studies reported to date are due to species differences, different deafening procedures or critical period effects.

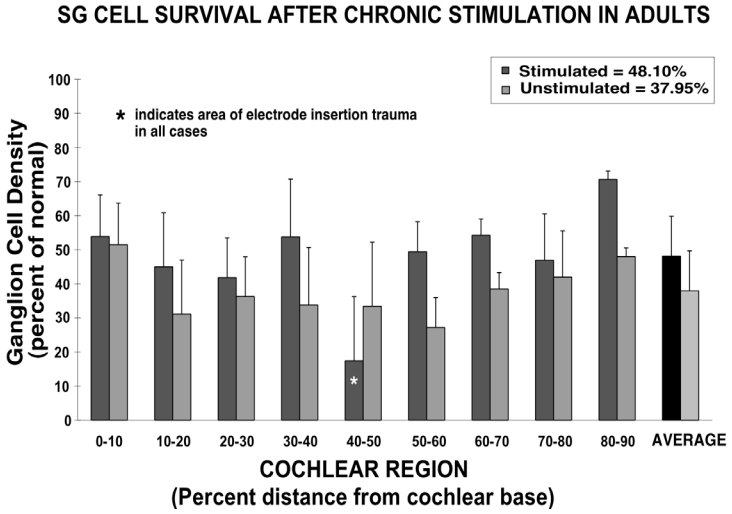

Figure 13. Preliminary data on SG neural survival in adult-deafened cats (n=3) following 6 months of chronic single-channel electrical stimulation. SG density is only about 10% higher in the chronically stimulated ears, as compared to about double this difference in neonatally deafened cats stimulated for similar periods. This suggests that there may be significant age-dependent differences in the 2 animal models. However, chronic infection may have compromised results in one of these subjects and additional studies are required to draw a definite conclusion regarding this important issue.

12. <u>The role of developmental "critical periods" in the stimulation-induced effects documented in these studies is unknown.</u> There is substantial evidence from research on other sensory systems that input activity, especially synchronized activity, exerts 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 (1,6,9,12,55, 79,86, 87; and see 50,75,76,81 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 (86, 103), or by stroboscopic illumination that results in nearly synchronous inputs from both eyes (9, 14,30,60,74,75). 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 is potentially 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 (91,94,89) or by alternate monocular deprivation (1,23,91). These results are interpreted as evidence that the underlying competitive processes act to segregate different neural populations that are driven by uncorrelated inputs in the developing nervous system.

Although there is a normal 'critical period' for these coincidence-based developmental effects in the visual system, this period can be extended substantially if experimental animals are profoundly deprived of normal sensory inputs (e.g., see 10,11,54,55). 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 irreversible.

In addition, it is well-known that early sound exposure is important for development and maturation of the auditory pathways in mammals (13,69,70,71), and that neonatal sound deprivation results in 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) (7,56,90,101,102), decrease in the volume of the CN (8,90,100), physiological changes (e.g., 15), as well as transneuronal changes at higher levels of the auditory system (16,28,64). Other studies have shown that neonatal cochlear lesions can result in substantial modification in the anatomical projections from the contralateral CN to the superior olivary complex and inferior colliculus (19, 25,31,51,52,53,57, 72). Further, many studies suggest that deprivation later during development (e.g., later than 36 days in the rat and 45 days in the mouse) does not have the same profound impact on the central auditory system (5,99). Thus, deprivation during early development clearly produces profound changes, and there is evidence for the existence of critical periods for inducing such changes (13,68,69). However, these studies have been conducted in a wide variety of species, and in many different models of deprivation and deafness. Thus, the specific nature and timing of critical periods and the role of early auditory deprivation for later structural and functional development of the central auditory system as would apply in our pediatric deaf animal model are unknown.

In the neonatally deafened kitten model studied by our group, animals are deafened 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 (96; for review; see 73). Clearly, these kittens are severely deprived of normal auditory experience. On the other hand, electrical stimulation is not initiated in these studies until 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 mechanisms have not yet been defined in auditory system development.

We conclude that while early chronic stimulation may result in positive conservation of the auditory nerve in children, ***it can also have negative consequences for the functional organization of the auditory nervous system***. Thus, future research 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 the potentially deleterious functional consequences of such stimulation.

Three different studies in guinea pigs (20,45,48) have shown that chronic electrical stimulation also can induce protective effects on spiral ganglion neurons in animals that have matured normally and are deafened and implanted as adults. However, other investigators have 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 (2,80) or in adult guinea pigs (44). Given these conflicting results, we believe that it is premature to draw definite conclusions at present regarding the age-dependence of the protective effects of chronic electrical stimulation. It is clear that deafness and hair cell loss induce degeneration of spiral ganglion neurons in both adult animals (35) and 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. Moreover, almost no functional studies of the effects of chronic stimulation have been reported in adult-deafened animals. Future research is required to determine to what degree, if any, the protective effects on the spiral ganglion neurons that we have demonstrated are age-dependent and if parallel alterations in central nervous system representations also occur in adult animals.

Summary of Results and Recommendations. In studies conducted at UCSF with the support of this Contract, it has been demonstrated that chronic passive stimulation using temporally challenging stimuli results in significant conservation of the spiral ganglion in a pediatric deafness model. Results also suggest 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 input 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, direct stimulation effects on the vasculature, or reflexive mechanisms. 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, the age-dependency of these phenomena must be determined. Moreover, our electrophysiological studies have shown that there are potential deleterious effects of chronic stimulation, as it can result in a substantially negative functional remodeling of cochleotopic representations in the auditory midbrain and cortex. Thus, a pediatric cochlear prosthesis must be optimized to conserve not only the spiral ganglion neurons, but also the topographic and temporal representations of 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 mitigate 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 noncoincident 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.

Finally, the potential implications of these results in animal experiments for clinical pediatric implants cannot be overlooked. The finding that a distorted input from a cochlear implant in these young deaf animals can result in profound distortions and degradation in central representations (frequency organization) within the auditory midbrain emphasizes the importance of the initial fitting of cochlear implants in the naive, developing auditory system. ***Results suggest that there may be specific ways of introducing initial stimulation in young deaf children that might optimize setting up appropriately distinct central representations of individual channels of the cochlear implant (43).*** For example, this might be accomplished by introducing one channel at a time and providing feed back to encourage discrimination among pairs of channels. This would to emphasize the segregation of inputs from individual channels rather than simply turning on all the channels simultaneously and requiring the naive auditory system to extract the relevant spatial and temporal information.

VI. REFERENCES 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. Araki, S., Atsushi, K., Seldon, H.L., Shepherd, R.K., Funasaka, S., Clark, G.M. 1998. Effects of chronic electrical stimulation on spiral ganglion neuron survival and size in deafened kittens. Laryngoscope 108:687-695.

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

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

5. Blatchley, B.J., Williams, J.E., and Coleman, J.R. 1983. Age-dependent effects of acoustic deprivation on spherical cells of the rat anteroventral cochlear nucleus. Exp. Neurol. 80:81-93.

6. 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.

7. 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.

8. 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.

9. 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.

10. Cynader, M. and Chernenko, P. 1976. Abolition of direction selectivity in the visual cortex of the cat. Science 193:504-505.

11. Cynader, M. and Mitchell, D.E. 1980. Prolonged sensitivity to monocular deprivation in dark-reared cats. J. Neurophysiol. 43:1026-1040.

12. 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.

13. Eggermont, J.J., and Bock, G.R. (Eds.). 1986. Critical periods in auditory development. Acta Otolaryngol. (Stockholm) Suppl. 429:1-64.

14. 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. Neurobiol. 19:395-411.

15. 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.

16. 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.

17. Gao, W-Q, Zheng, J.L., Karihaloo, M. 1995. Neurotrophin-4/5 (NT-4/5) and brain-derived neurotrophic factor act at later stages of cerebellar granule cell differentiatiation. J. Neurosci. 15: 2656-2667.

18. Hansen, M.R., Zha, X.-M., Bok, J., and Green, S.H. (In Press) Multiple distinct signal pathways, including an autocrine neurotrophic mechanism contribute to the survival-promoting effect of depolarization on spiral ganglion neurons *in vitro*. J. Neurosci.

19. 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: Auditory System Plasticity and Regeneration. R. Salvi, D. Henderson, F. Fiorino, V. Colletti, (eds), Thieme, New York, N.Y., pp. 283-255.

20. 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.

21. 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.

22. Hegarty, J.L., Kay, A.R., and Green, S.H. 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.

23. Hubel, D.H. and Wiesel T.N. 1965. Binocular interaction in striate cortex of kittens reared with artificial squint. J. Neurophysiol. 28:1041-1059.

24. 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. Hear. Res. 54:272-282.

25. 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.

26. Javel, E. 1989. Acoustic and electrical encoding of temporal information. In: *Cochlear Implants: Models of the Electrically Stimulated Ear*. J.M. Miller and F.A.Spelman (eds.) Springer-Verlag, New York, pp. 247-295.

27. 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.

28. Jean-Baptist, M. and Morest, D.K. 1975. Transneuronal changes of synaptic endings and nuclear chromatin in the trapezoid body following cochlear ablations in cats. J. Comp. Neurol. 162:111-133.

29. 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.

30. 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.

31. 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.

32. Larsen, S.A. 1984. Postnatal maturation of the cat cochlear nuclear complex. Acta Otolaryngol. Suppl. 217:1-43.

33. Leake, P.A., Rebscher, S.J. and Aird, D.W. 1985. In: Histopathology of cochlear implants: Safety considerations. R.A. Schindler and M.M. Merzenich, Eds., Cochlear Implants. Raven Press, New York, pp. 55-64.

34. 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.

35. Leake, P.A. and Hradek, G.T. 1988. Cochlear pathology of long term neomycin induced deafness in cats. Hearing Res., 33:11-34.

36. 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.

37. 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.

38. 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.

39. 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.

40. 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. 113:117-132.

41. 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.

42. Leake, P.A., R.L. Snyder, S.J. Rebscher, G.T. Hradek, C. M. Moore, M. Vollmer, and M. Sato. 2000. 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.

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

44. Li, L. Parkins, C.W., and Webster, D.B. 1999. Does electrical stimulation of deaf cochleae prevent spiral ganglion degeneration? Hear. Res., 133:27-39.

45. Lousteau, R.J. 1987. Increased spiral ganglion cell survival in electrially stimulated deafened guinea pig cochleae. Laryngoscope 97 (7): 836-842.

46. 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. (See Appendix)

47. 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.

48. 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.

49. 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.

50. Miller, K.D., Keller, J. B. and Stryker, M. P. 1989. Ocular dominance column development: Analysis and simulation. Science 245:605-615.

51. 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.

52. 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.

53. 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

54. 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.

55. 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.

56. Niparko, J.K., and Finger, P.A. 1997. Cochlear nucleus cell size changes in the dalmationh: Model of congenital deafness. Otolaryngol. Head Neck Surg. 117:229-235.

57. 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.

58. 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.

59. Parkins, C. W. 1989. Temporal response patterns of auditory nerve fibers to electrical stimulation in deafened squirrel monkeys. Hearing Res., 41:137-168.

60. Pasternak, T., and Leinen, L.J. 1986. Pattern and motion vision in cats with selective loss of cortical

directional selectivity. J. Neurosci. 6:938-945.

61. Pfingst, B.E., Sutton, D., Miller, J.M., and Bohne, B.A. 1981. Cochlear implants: relation of psychophysical measures to histological findings. Acta Otolaryngol. 92:1-13.

62. Pfingst, B.E. 1988. Comparisons of psychophysical and neurophysiological studies of cochlear implants. Hear. Res. 34:243-252.

63. 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.

64. 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.

65. 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.

66. 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.

67. Recanzone, G., Allard, T.T., Jenkins, W.M., and Merzenich, M.M. 1990. Receptive field changes induced by peripheral nerve stimulation in SI of adult cats. J. Neurophysiol., 63:1213-1225.

68. Ruben, R.J. 1986. Unsolved issues around critical periods with emphasis on clinical application. Acta Otolaryngol. Suppl. 429: 61-64.

69. Rubel, E.W., Born, D.E., Deitch, J.S. and Durham, D. 1984. Recent advances toward understanding auditory system development. In: Hearing Science, C. Berlin (ed.), College-Hill Press, Inc. San Diego, CA, pp. 109-157.

70. 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.

71. Rubens, R.J., and Rapin, I. 1980. Plasticity of the developing auditory system. Ann. Otol. Rhinol. Laryngol. 89:303-311.

72. 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.

73. Sato, M., P.A. Leake and G.T. Hradek 1999. Postnatal development of the organ of Corti in cats: A light microscopic morphometric study. Hear. Res. 127:1-13.

74. 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.

75. 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.

76. Schmidt, J.T. and Tieman, S.B. 1989. Activity, growth cones and the selectivity of visual connections. Comments Develop. Neurobiol. 1:11-2.

77. 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.

78. Shannon, R.V. 1985. Threshold and loudness functions for pulsatile stimulation of cochlear . Hear. Res. 18:135-143.

79. Shatz, C.J., and Stryker, M.P. 1988. Prenatal tetrodotoxin infusion blocks segregation of retinogeniculate afferents. Science 242:87-89.

80. 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.

81. 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.

82. 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. Hear. Res. 50:7-33

83. 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.

84. Snyder, R.L., M. Vollmer, C.M. Moore, S.J. 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

85. Spoendlin, H. H. 1975. Retrograde degeneration of the cochlear nerve. Acta Otolaryngol. 79:266-275.

86. 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.

87. 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.

88. Taha S, Bonham B, Wong SW, Schreiner CE. 1996. Intra-cortical inhibitory connections refine the specificity of cat AI neuron responsivity. Soc. Neurosci. Abstr. 22:465

89. 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.

90. 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.

91. 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.

92. van den Honert, C. and Stypulkowski, P.H. 1987a. Single fiber mapping of spatial excitation patterns in the electrically stimulated auditory nerve. Hear. Res. 29:195-206.

93. van den Honert, C. and Stypulkowski, P.H. 1987b. Temporal response patterns of single auditory nerve fibers elicited by periodic electrical stimuli. Hear. Res., 29:195-206.

94. van Sluyters, R.C. and F.B. Livitt. 1980. Experimental strabismus in the kitten. J. Neurophysiol. 43:686-698.

95. 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.

96. 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.), Elsevier, Chapter 6, pp. 161-219.

97. Walsh, M.E. and D.B. Webster. 1994 Exogenous GM1 ganglioside effects on conductive and sensorineural hearing losses. Hear. Res. 75:54-60.

98. Wang J, Salvi RJ, Caspary DM, Powers N. 1996. GABAergic inhibition profoundly alters the tuning and discharge rate of neurons in the primary auditory cortex of the chinchilla. Assoc. Res. Otolaryngol. Abstr. 19:156

99. Webster, D.B. 1983. Late onset of auditory deprivation does not affect brainstem auditory neuron soma size. Hear. Res. 12:145-147.

100. Webster, D.B. 1988. Conductive hearing loss affects the growth of the cochlear nuclei over an extended period of time. Hear. Res. 32:185-192.

101. Webster, D.B. and Webster, M. 1977. Neonatal sound deprivation affects brain stem auditory nuclei. Arch. Otolaryngol. 103:392-396.

102. 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.

103. Weiliky, M. and Katz, L.C. 1997. Disruption of orientation tuning in visual cortex by artifically correlated neuronal activity. Nature 386 (6626):680-685.

104. 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. Hear. Res. 71:205-215.

105. 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.