Final Report
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Fundamental Neurosciences Contract N01-DC-4-2143

Protective Effects of Patterned Electrical Stimulation on the Deafened Auditory System

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THIS QPR IS BEING SENT TO YOU BEFORE IT HAS BEEN REVIEWED BY THE STAFF OF THE NEURAL PROSTHESIS PROGRAM.

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 as to appropriate spatio-temporal patterns of stimulation to protect and possibly enhance auditory system function with auditory prostheses, and recommendations for future research. The primary objectives of this research, as specified in the Technical Specifications, were to determine: 1) whether stimulation has any protective effects on neural structures, and if so which forms of stimulation were most effective; 2) whether stimulation or implantation of electrodes produces damage to the tissues: and 3) whether any functional changes are associated with neuroanatomical alterations in the auditory system.

1. Spiral ganglion neuronal degeneration in an animal model of neonatal deafness. Most earlier studies of the morphological effects of cochlear implants and chronic electrical stimulation focused on issues of safety and damage (see Leake et al., 1990). More recently, however, several studies have shown that chronic electrical stimulation of the cochlea can partially prevent degeneration of the spiral ganglion neurons which otherwise occurs after deafening (Hartshorn et al., '91; Leake et al., '91,'92, '95; Leake and Snyder, '94; Lousteau, '87). Research supported by our previous and current NIH Contracts has evaluated both the histopathological and functional consequences of chronic intra- and extracochlear electrical stimulation in an animal model of early acquired deafness. For these studies, kittens were deafened by injections of the ototoxic drug neomycin sulfate (60 mg/kg I.M.), administered daily for the first 16 to 21 days after birth. Since kittens are born deaf, these animals never develop normal hearing and are profoundly deaf at the age (about 21 days postnatal) when adult-like hearing sensitivity would normally develop. Hearing loss and induced peripheral pathology is bilaterally symmetrical, and Figure 1 characterizes the time course (and inter-subject variability) of degeneration of the primary afferent spiral ganglion neurons in *unstimulated* control cochleas. Although there is considerable scatter in the data, the correlation between spiral ganglion survival and duration of deafness is highly significant ($r=0.88$, $p<0.001$). Moreover, it is clear that significant neural loss occurs very early at 2-3 weeks after deafening.

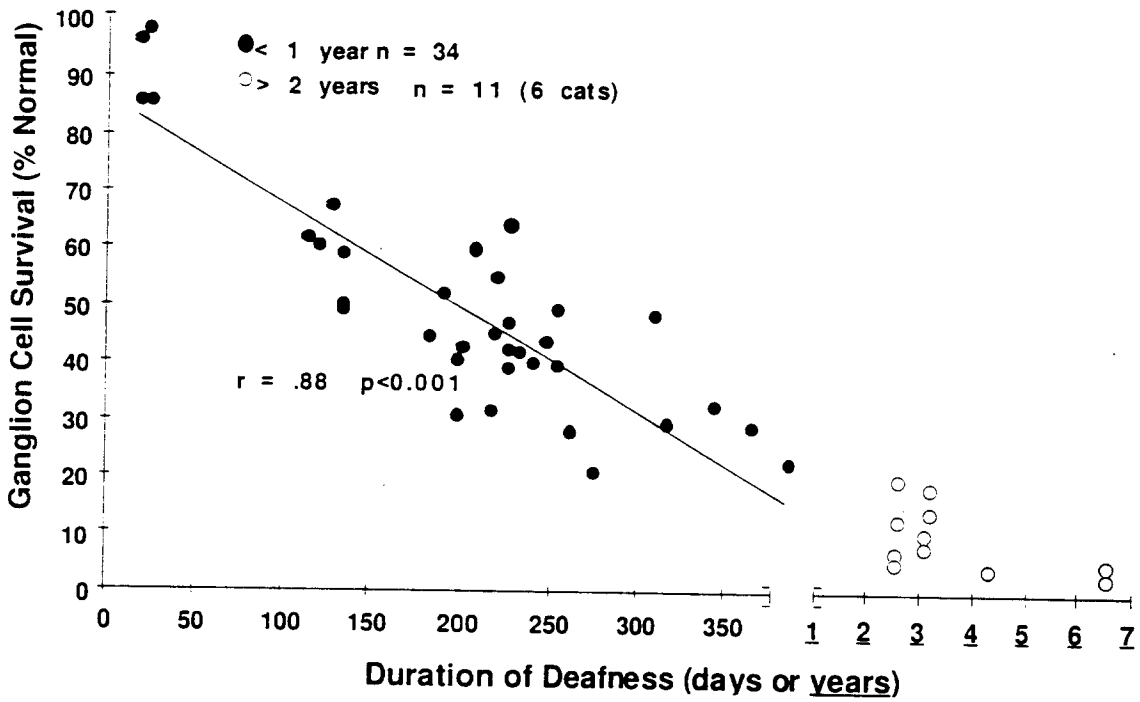

Figure 1. spiral ganglion survival data from control deafened, unstimulated cochleas are shown as a function of duration of deafness. Data are divided into 2 groups. In the first group animals were studied at periods of about 1 year or less (filled circles). The second group we have designated as "long term neonatally deafened" and included animals studied at 2.5 to 6 years of age.

Also shown in Figure 1 are the data from a group of 6 long term deafened animals (open circles). These animals were studied at prolonged intervals ranging from 2.5 to 6.5 years after neonatal deafening, and the data are plotted on a separate scale for duration of deafness (in years rather than days). Note that spiral ganglion degeneration is severe and varies from about 5 to 20% of normal density in the 11 cochleas evaluated in this group.

2. Chronic electrical stimulation with higher frequency, temporally challenging signals results in marked increases in survival of spiral ganglion neurons in neonatally deafened cats.

For studies of the effects of stimulation neonatally deafened cats are implanted with 4-contact scala tympani electrode arrays at 6 to 7 weeks of age (at time of weaning). Chronic stimulation is initiated at 7-8 weeks postnatal. The regression function in Figure 1 suggests that ganglion cell survival would be about 75% of normal at this time, 50-60 days postnatal.

Initial studies conducted under a previous Contract and completed during the initial months of this Contract, demonstrated that chronic electrical stimulation (delivered via both intra- and extracochlear electrodes) using passive and invariant 30 pps stimuli induced a significant protective effect, partially preventing the degeneration of spiral ganglion cells in these neonatally deafened animals. Subsequently, additional experiments have been conducted in which the signals used for chronic electrical stimulation have been varied, in order to begin to define specific parameters that are critical in maximizing the protective effects on the auditory nerve. In our 7th Quarterly Progress Report (April 1, 1996 to June 30, 1996; Contract N01-DC-4-2143), initial histological findings and spiral ganglion morphometric data were reported for animals in this new intracochlear series. Excluding 4 cats in a "stimulation damage" group, we have studied 8 animals in this temporally challenging stimulation group. Table 1 summarizes the experimental histories of these animals. Two cats received chronic passive intracochlear stimulation using continuous pulse trains at 80 pps (200 μs/phase): 3 cats were stimulated with pulse trains (200 μs/phase) at 300 pps which were amplitude modulated (100%) at 30 Hz; and 3 animals received temporally- and intensity-varying stimulation through a single channel speech processor which transduced environmental sounds into an analogue electrical signal. (The subjects in this last group were also behaviorally trained to determine psychophysical thresholds to selected stimuli.)

Table 1. Higher Frequency, Temporally Challenging Stimulation

Cat #	Neomycin mg/kg/days	Age at Initial Stimulation	Stim. Current	Stim. Period	Stim. Frequency	Age at Sacrifice
K83	60/19	10.5 wks	125 μA	21 wks*	80 Hz	32 wks
K84	60/19	10 wks	200–400 μA	35 wks	SP/beh.	45 wks
K85	60/19	10 wks	125 μA	42 wks	80 Hz	52 wks
K86	60/19	9 wks	30-160 μA	44 wks*	SP/beh.	55 wks
K89	50-60/19	10.5 wks	80-100 μA	26.5wks	300/30 Hz	37 wks
K98	60/20	7 wks	50-100 μA	32 wks	SP/beh	39 wks
K99	60-70/25	8 wks	32-100 μA	40 wks	300/30 Hz/beh	49 wks
K101	60/18	7.5 wks	79-125 μA	28.5 wks	300/30 Hz	36.5 wks

Table 1. Individual histories of the 8 animals in the temporally challenging stimulation groups for which spiral ganglion survival showed an increase of 20%.
(*Device failed during chronic stimulation and was replaced.)

Figure 2 presents summary data for this group, showing the regional spiral ganglion cell density (stimulated less control values) in sectors from base to apex of the cochlea, and expressed as percent of normal values. The mean overall spiral ganglion survival was 50% of normal in the stimulated cochleas and 29.5% of normal in the paired control deafened ears. That is, on average more than 20% of the normal neural population was maintained by chronic stimulation, over and above the neural survival in the contralateral control deafened cochleas. Mean increases of 25-35% were documented over the basal one-third of the cochlea, and marked increases in cell survival were observed throughout the cochlea. These data support indicate that a chronic stimulation with higher frequency, temporally challenging signals results in a significantly greater protective effect in maintaining the auditory nerve as compared to results in previous experiments using 30 pps pulse trains with either bipolar intracochlear stimulation (≈12% difference in ganglion cell survival) or monopolar round window stimulation (≈6% difference).

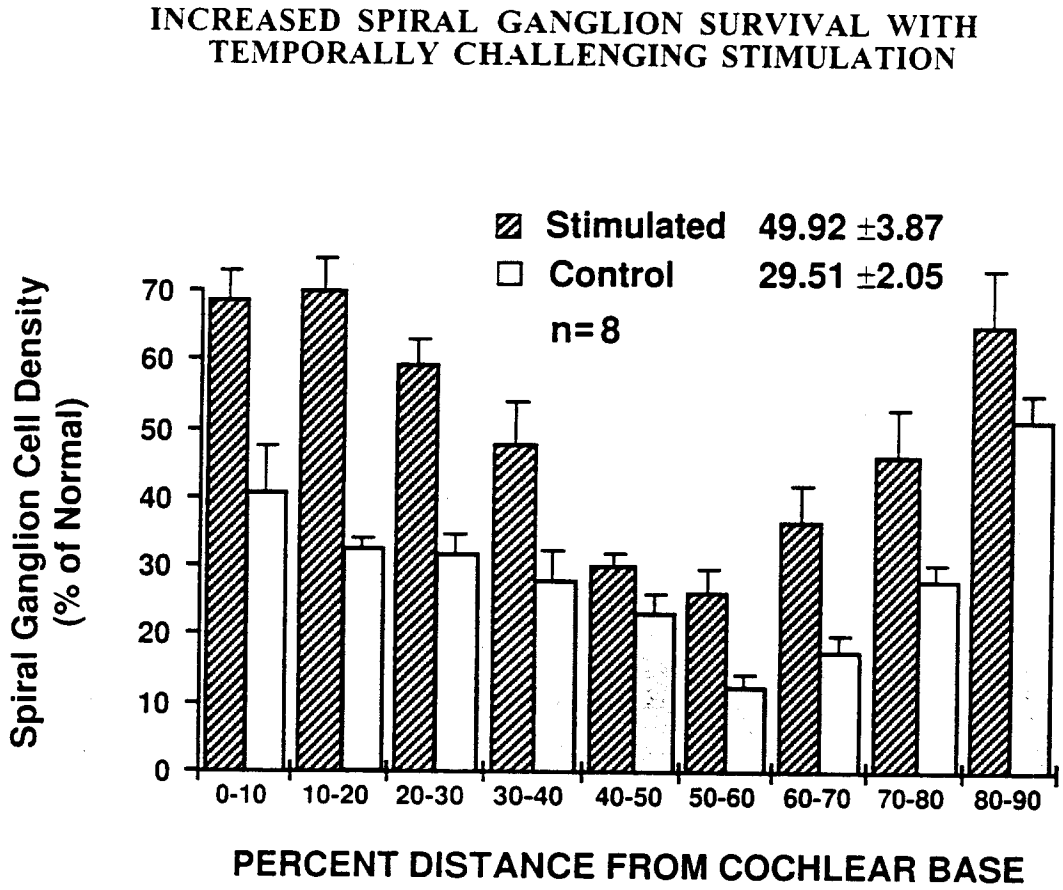

Figure 2. Marked increase in spiral ganglion survival induced by chronic intracochlear electrical stimulation using temporally challenging stimulation in neonatally deafened cats. Data are pooled from 8 animals. The mean stimulated less control values for spiral ganglion cell density are expressed as percent of normal values for each cochlear sector from base to apex and thus represent *% increase* in neuronal survival in the stimulated cochleas. The increased survival was offset by insertion damage which occurred near the tip of the electrode in the 40-50% sector in all cases. Overall, spiral ganglion cell density was increased by about 20%, and this difference was highly significant ($P < 0.001$; Student's t-test, paired).

3. **Different stimulation modes (bipolar intracochlear vs. monopolar extracochlear) and electrical signals with different temporal properties vary in their efficacy in maintaining the auditory nerve.**

We have suggested that these results indicate that the specific parameters of stimulation (frequency of the electrical stimulus and intracochlear bipolar vs. extracochlear monopolar mode) are critically important in maximizing the protective effect of electrical stimulation on the auditory nerve. However, as indicated by Figure 1, another important variable which may contribute to the relative effects induced by stimulation is *duration of stimulation*. Since the spiral ganglion cells in the contralateral deafened but unstimulated cochlea continue to degenerate over time, longer duration of stimulation should result in greater differences in survival between stimulated and control cochleae.

Figure 3 shows the *% increase* in spiral ganglion cell survival in individual subjects from 3 chronic stimulation groups, as a function of duration of electrical stimulation. It is clear that the "extracochlear" group (triangles) which received 30 pps stimulation through a large monopolar electrode positioned near the round window, forms a relatively distinct data set with *less increase* in spiral ganglion cell survival than the intracochlear 30 pps group (circles) which had equivalent or shorter durations of stimulation. In fact, the data for the two groups are virtually non-overlapping for increase in cell survival.

It is also clear in Figure 3a that many of the animals in the temporally challenging stimulation group (square symbols) which exhibited greater increase in spiral ganglion cell survival, also received longer periods of stimulation. (The increase in duration of chronic stimulation periods of at least 6 months was required by the specifications of our current contract,

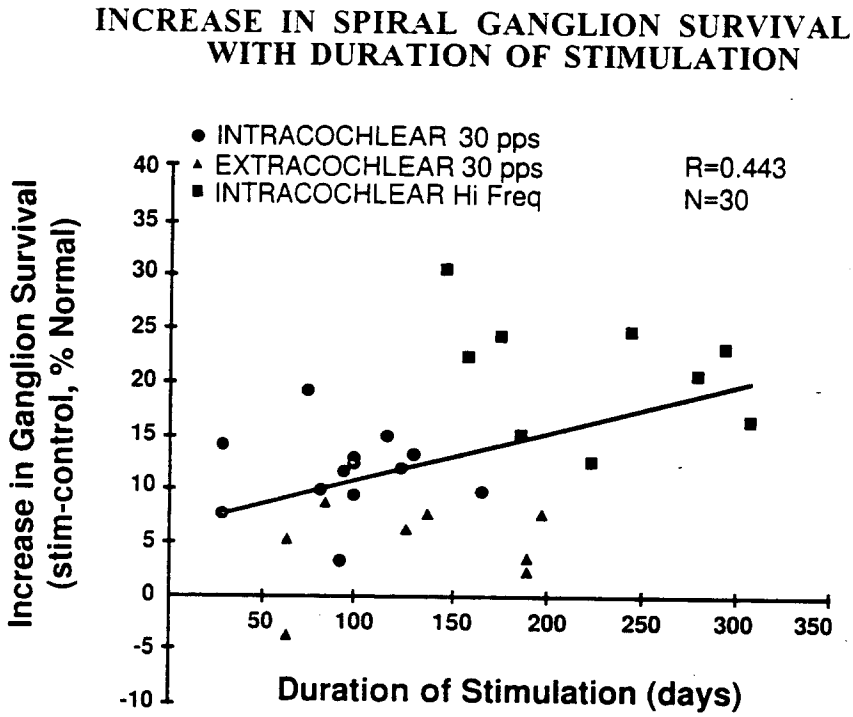

Figure 3a. Increase in spiral ganglion survival induced by varying durations of chronic electrical stimulation in three groups of cats. Round window stimulation at 30 pps with a monopolar electrode (triangles) appears to be less effective in increasing spiral ganglion cell density than intracochlear bipolar stimulation at 30 pps for equivalent durations (circles). Temporally challenging stimulation (see text) using bipolar intracochlear electrodes and longer durations of stimulation (squares) is more effective than 30 pps in maintaining neural survival.

whereas earlier studies were conducted under a previous contract and the criterion was stimulation for periods of 3-4 months.) However, although there is a clear trend in the data, the regression statistics do not show a significant correlation ($r=0.443$; $p> 0.03$) between increase in spiral ganglion survival and duration of stimulation over the periods of chronic stimulation employed. Moreover, comparison of individuals with stimulation periods of 125 to 170 days suggests a greater effect on survival with temporally challenging intracochlear stimulation.

To further examine the effect of duration of stimulation, Figure 3b compares ganglion cell survival in the stimulated cochleas and in the paired control, unstimulated cochleae as a function of duration of stimulation. For this comparison, data are limited to the 2 intracochlear stimulation groups, since the extracochlear group appears to form a separate data set, indicating that extracochlear stimulation is less effective in maintaining spiral ganglion neurons for a given duration of stimulation. The ganglion cell survival in the control deafened cochleas (round symbols) shows good correlation ($r=.712$; $p<0.001$) with duration of stimulation (deafness), indicating that although there is some variability in these animals deafened by ototoxic drugs at birth, cell degeneration is clearly progressive over these time periods.

It should be noted that our criterion for including animals in groups comparing the effects of stimulation is > 90 days of chronic stimulation. If a linear regression analysis is performed for subjects meeting this 90 day criterion, the trend in the data (dashed line) is not significant ($r=.400$, NS). Thus, for stimulation periods of 3 to 10 months used in our experiments, there is no significant correlation between neuronal survival in the stimulated cochleas and duration of stimulation. This suggests that chronic stimulation prevents most, if not all, of the degeneration that otherwise would have occurred over this time period. Although a significant effect of stimulation is clearly evident at our criterion benchmark of 90 days of stimulation, the divergence of the linear regression lines for the stimulated and unstimulated data indicates that longer duration of stimulation is correlated with greater differences in neuronal survival, as predicted.

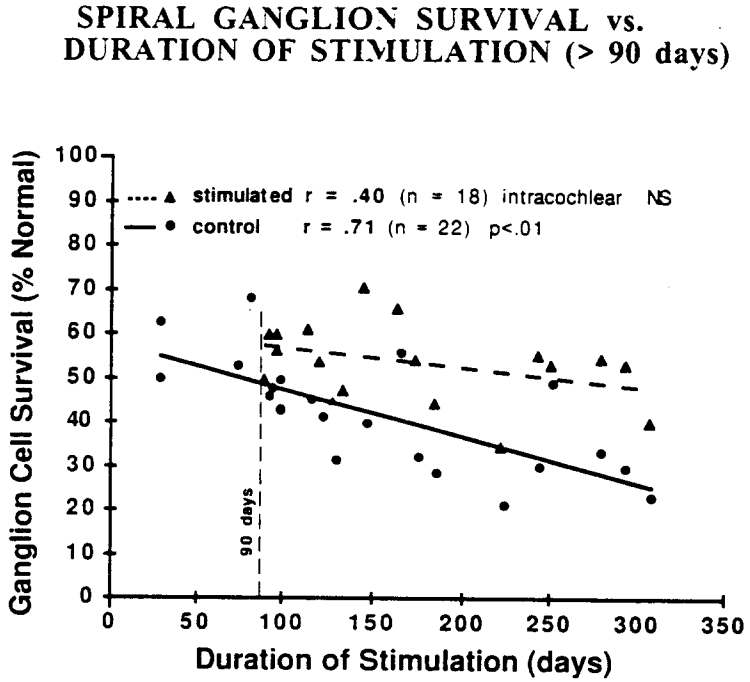

Figure 3b. Spiral ganglion survival in stimulated cochleas (triangles) with data analysis restricted to animals meeting the criterion of 90 days of chronic intracochlear stimulation. Data show little decrement in survival for stimulation periods of 3 to 10 months ($r= .400$; NS), suggesting that chronic stimulation over these periods tends to prevent further degeneration.

4. Spiral ganglion cell size following neonatal deafening and chronic stimulation

Our morphological data examining the effects of chronic electrical stimulation employs a point-counting *volume ratio* measure, which is essentially a measure of density that (by the size of the grid squares) prevents double counting of cells and is not influenced by the substantial regional variations in the size and shape of Rosenthal's canal. Any measure of density would be influenced by differences in cell size as well as cell number. In our initial study reporting increased survival of spiral ganglion neurons with chronic stimulation, we evaluated ganglion cell diameters (Leake et al., '91, figure 7), and there was no significant difference between stimulated and control even in the cochlear regions with the greatest differences in neuronal density. However, with the substantially greater effects induced in the higher frequency, temporally challenging stimulation group, it seems worthwhile to once again evaluate the whether spiral ganglion cell size plays a role in the differences observed with chronic electrical stimulation.

Figure 4 compares spiral ganglion cell diameters (mean of long and short axes) in normal and 2 groups of neonatally deafened cats. In the normal cat, the great majority of spiral ganglion cells are myelinated type I neurons which receive input from the inner hair cells and provide the main afferent auditory information pathway to the brain. Their cell somata have a mean diameter of 22.4 μm. A small percentage (7%) of ganglion cells in the cat are smaller, unmyelinated type II neurons which receive their input from the outer hair cells. The mean diameter of the type II cells was 11.7 μm.

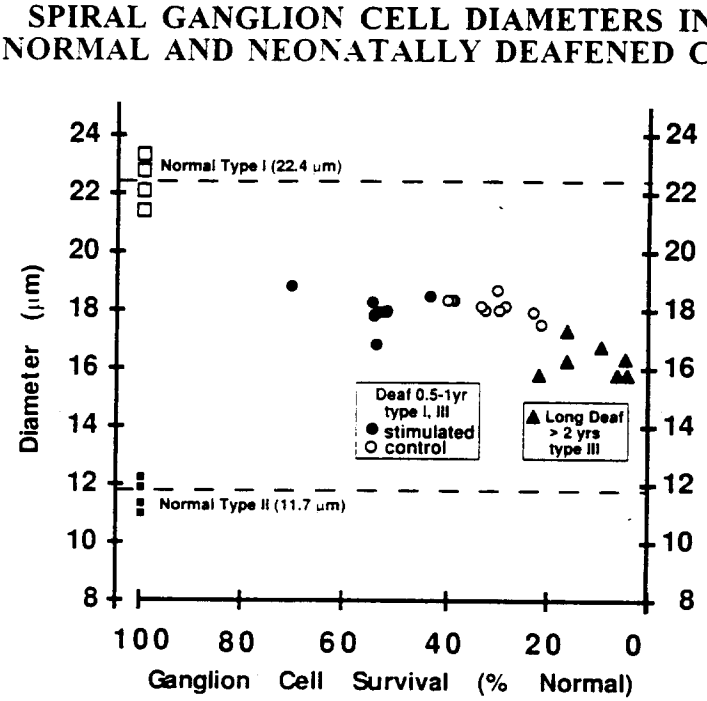

Figure 4. The mean diameter of spiral ganglion cell somata are shown for normal cats (squares), neonatally deafened cats in the temporally challenging stimulation group (circles), and long term neonatally deafened cats (triangles).

Figure 4 also shows mean cell diameters from 8 neonatally deafened animals in the temporally challenging stimulation groups described above. The filled circles show data from the chronically stimulated cochleas and the open circles show the contralateral control deafened cochleas. Measurements were made in the same manner as for normal cats, and all neurons selected were identified as either type I or type III (degenerating type I neurons with thinned or no myelin around the cell perikaryon). It is clear that mean cell diameter is significantly reduced to

about 18 μm in the neonatally deafened animals. Moreover, in the long term deafened animals (>2.5 to 6.5 years after neonatal deafening) where virtually all remaining neurons have undergone demyelination (type III), there is a further reduction in cell diameter to a value of about 16 μm.

To determine whether maintenance of cell size contributed to the difference recorded in volume ratio of the spiral ganglion neurons, we did 2 analyses. First, we selected the two 10 % cochlear sectors in each animal which showed the *greatest stimulated vs. control difference* in density and compared the cell diameters for the 8 animals. The mean cell diameter in the stimulated cochleas was 19.17 μm and the control value was 18.93 μm. Although the value was larger in the stimulated cochleae, the difference in diameter represents an increase in cell area of only 2.60%, which was not significant for the group (Student's t test, paired, P=0.227). We then measured cells throughout all cochlear sectors. The small regional difference averaged out in the overall analysis, and the mean cell size was 18.1 μm for both the stimulated cochleas, and the unstimulated cochleas.

Taken together, these data on spiral ganglion cell size suggest that there is significant shrinkage in the spiral ganglion neurons after neonatal deafening which is largely irreversible with subsequent chronic electrical stimulation, at least over periods of 5 to 10 months as applied in our studies. Thus, the highly significant increases in spiral ganglion density observed in the stimulated cochleas of these animals is due mainly to an increase in the *number of surviving neurons*.

5. Neonatal deafness causes profound degenerative alterations in the cochlear nuclei; only a modest prevention or reversal of these changes is observed, even when chronic electrical stimulation induces marked increases in spiral ganglion cell survival. Histological studies of the cochlear nuclear complex (CN) of these same neonatally deafened, chronically stimulated cats have demonstrated profound degenerative changes in the cochlear nuclei -- changes that are progressive for many months after deafening (Hultcrantz et al., '91; Lustig et al., '94). As compared to measurements in normal adult cats, the cochlear nuclei of neonatally deafened cats showed: i) marked shrinkage in the volume of the CN, with the total volume reduced by about 35%; ii) a striking reduction of approximately 40% in the density of spherical cells within the anteroventral cochlear nucleus (AVCN); and iii) a highly significant reduction of approximately 20% in the mean cross-sectional area of AVCN spherical cells. These adverse effects are completely consistent with many previous studies showing that neonatal sound deprivation or deafening results in profound adverse effects within the cochlear nucleus (Coleman and O'Connor, '79; Coleman et al., '82; Trune, '82; Webster, '83, Webster, '88; Webster and Webster, '77, '79).

Comparisons between stimulated and control CN in our neonatally deafened animals revealed no significant differences in either nuclear volume or spherical cell density. However, for cross-sectional areas of spherical cell somata in the AVCN, a modest but significant difference was observed: cells in the stimulated CN were 6% larger than controls (Hultcrantz et al., '91; Lustig et al., '94). It should be emphasized that the animals included in these studies of the cochlear nucleus were the same animals in which significant regional conservation of the spiral ganglion was induced by electrical stimulation at 30 pps. It is thus intriguing that relatively little effect on the pronounced morphological changes after deafening was observed in the CN.

Even more striking are the CN data from 4 cats in the recent temporally challenging stimulation group (with a mean increase in spiral ganglion survival of 21%) which showed ***identical*** results (QPR #4, July 1-Sept. 30, 1995)). Figure 5 illustrates the lack of stimulation effects in preventing shrinkage of the CN in this group of neonatally deafened cats, and Figure 6 shows the modest (again, mean 6%) increase in spherical cell area observed after chronic stimulation. It is interesting that relatively little prevention of the pronounced morphological consequences of deafening was observed in the cochlear nucleus, given the substantially greater population of spiral ganglion neurons surviving and chronically activated in the stimulated cochlea. One possible explanation for the modest effect of stimulation in reversing the degenerative CN changes in these animals is the delay that occurred before intervention with electrical stimulation was possible in our earlier experiments.

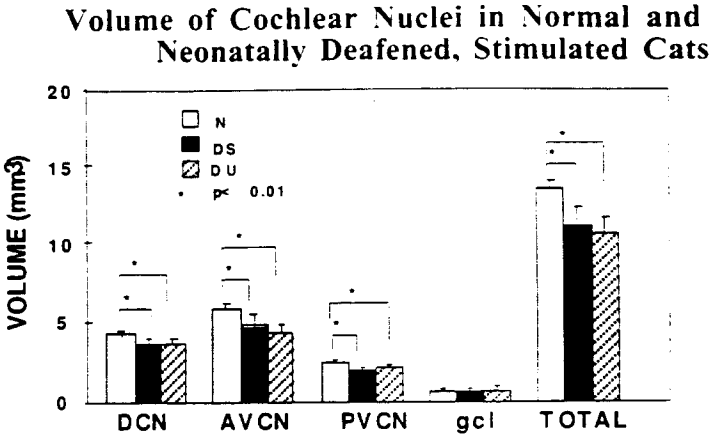

Figure 5. Volumes of the cochlear nucleus and it's component subdivisions in Normal (N) and in a group of 4 neonatally deafened, chronically stimulated cats. Both deafened, stimulated (DS, black bars) and unstimulated (DU, striped bars) cochlear nuclei are significantly decreased in volume from normal animals. This difference is due mainly to reduction in volumen of the anteroventral (AVCN) and posteroventral (PVCN) subdivisions. The effect of deafening is not prevented or reversed by stimulation. DCN, dorsal cochlear nucleus; GCL: granular cell layer.

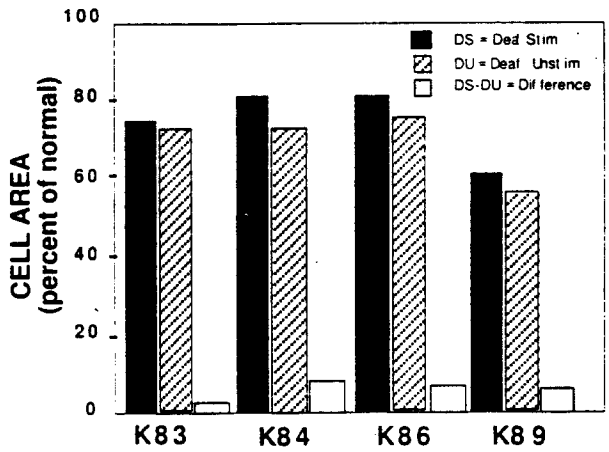

Figure 6. Cross-sectional areas of spherical cells in the rostral AVCN in 4 neonatally deafened, chronically stimulated cats. Data are shown as percent of normal, and there is a clear reduction in cell size as a consequence of deafening. Only a modest, although significant increase in spherical cell size was measured in the stimulated AVCN as compared to the contralateral deafened, unstimulated side (mean increase,6%). This finding is particularly interesting since these animals were in the group in which large increases in spiral ganglion cell survival (>20%) were seen in the stimulated cochleae as compared to the contralateral side (see Figure 3). Thus, the CN changes do not appear to parallel those degree of auditory nerve maintenance after chronic stimulation.

According to Larsen ('84), during the development of the AVCN there is an early growth phase with rapid increase in nuclear and cytoplasmic cross-sectional areas and rapid decrease in cell density during the first month of postnatal life. This is followed by a second, longer period of development during which the neurons gradually reach mature size, by about 12 weeks postpartum. In our neonatally deafened cats, ototoxic drugs are administered during the period when this early, rapid development normally occurs. Intracochlear electrical stimulation was initiated at an average age of 9-10 weeks in these earlier experiments, well after the first postnatal month rapid growth. Thus, it is possible that intervention with electrical stimulation in these kittens took place too late in development to reverse the profound consequences of early deafness. A preliminary report of these findings was presented at the 1995 Conference on Implantable Auditory Prostheses (Moore et al., '95, See Appendix); a complete report will be published as soon as data analysis can be completed for all cats in this temporally challenging stimulation series.

These findings suggest that some of the cochlear nucleus changes induced by deafness are irreversible at the time when electrical stimulation was initiated in these animals -- that there may be a critical period after which these changes are largely irreversible. Further studies to be conducted under our new Contract will directly address these important issues by examining CN data from adult-deafened animals and kittens deafened at later ages after initial normal development. These data will be extremely interesting because they will indicate whether earlier stimulation is more effective in

thwarting the degenerative changes in the cochlear nucleus caused by early deafening, and they also should help to define critical period effects.

In this context, it should be noted that the cochlear implant research group at Melbourne University, Australia has reported data from a similar study of 4 chronically stimulated cats (Matshushima, '91). Their animals were deafened at 1 month of age (rather than neonatally), and their results on cochlear nucleus cell density suggested that similar periods of chronic electrical stimulation initiated at similar ages were more effective in preventing degenerative changes in the CN; however, they did not see differences in spiral ganglion survival. This suggests that the age at time of deafening may be a critical parameter in determining whether the CN will be sensitive to stimulation-induced "protective" effects. However, given the other differences between the two sets of results, this is clearly an area requiring additional study.

6. Chronic electrical stimulation in neonatally deafened cats alters spatial selectivity (i.e., cochleotopic maps) in the auditory midbrain. An extensive series of acute electrophysiological experiments conducted for this Contract research has examined the topographic organization and the temporal patterns of neuronal responses evoked by cochlear electrical stimulation within the auditory midbrain or inferior colliculus (IC) Studies have been conducted in: a) animals that were deafened, implanted as adults and studied acutely ("normal"); b) neonatally deafened, chronically stimulated cats (including both initial experimental groups stimulated at 30 pps and more recent animals stimulated with more temporally challenging paradigms as described above; and c) neonatally deafened but unstimulated controls examined at the same ages as the stimulated group. Data from the neonatally deafened/unstimulated group suggest that the connectional specificity normally accounting for the frequency representation within the principal midbrain auditory nucleus, the central nucleus of the inferior colliculus (ICC), develops normally (or nearly so) in neonatally deafened, unstimulated cats. That is, the spatial selectivity elicited with our standard bipolar intracochlear electrodes is normal in this group.

On the other hand, when neonatally deafened animals are chronically stimulated at a young age, even at very low stimulus intensities, spatial selectivity assessed at the midbrain level was greatly altered. Specifically, the central representation of cochlear location - the area within the ICC activated by a single bipolar electrode pair - was significantly expanded. In such chronically stimulated cats, the midbrain representations of chronically activated electrodes were on average 2 times greater in extent than those of identical electrodes implanted in either control neonatally deafened cats, or in acutely deafened adults (Snyder et al., '90; Leake et al., '95) (figure 7). Chronic stimulation, using either a single bipolar intracochlear channel or broad-band extracochlear stimulation, results in significant expansion of the central representation of the chronically activated electrode(s). These findings suggest that electrical stimulation with a highly stereotyped, synchronous stimulus results in significant distortion, perhaps best viewed as degradation, of the cochleotopic organization of the central auditory system, at least to the level of the midbrain.

We conclude that while early chronic stimulation may result in positive conservation of the auditory nerve in children, *the wrong type of stimulation can also have negative consequences for the functional organization of the auditory nervous system.* Thus, the evaluation of 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 an evaluation of the functional consequences of such stimulation.

Spatial Selectivity is Evaluated by Threshold Distributions in the Inferior Colliculus

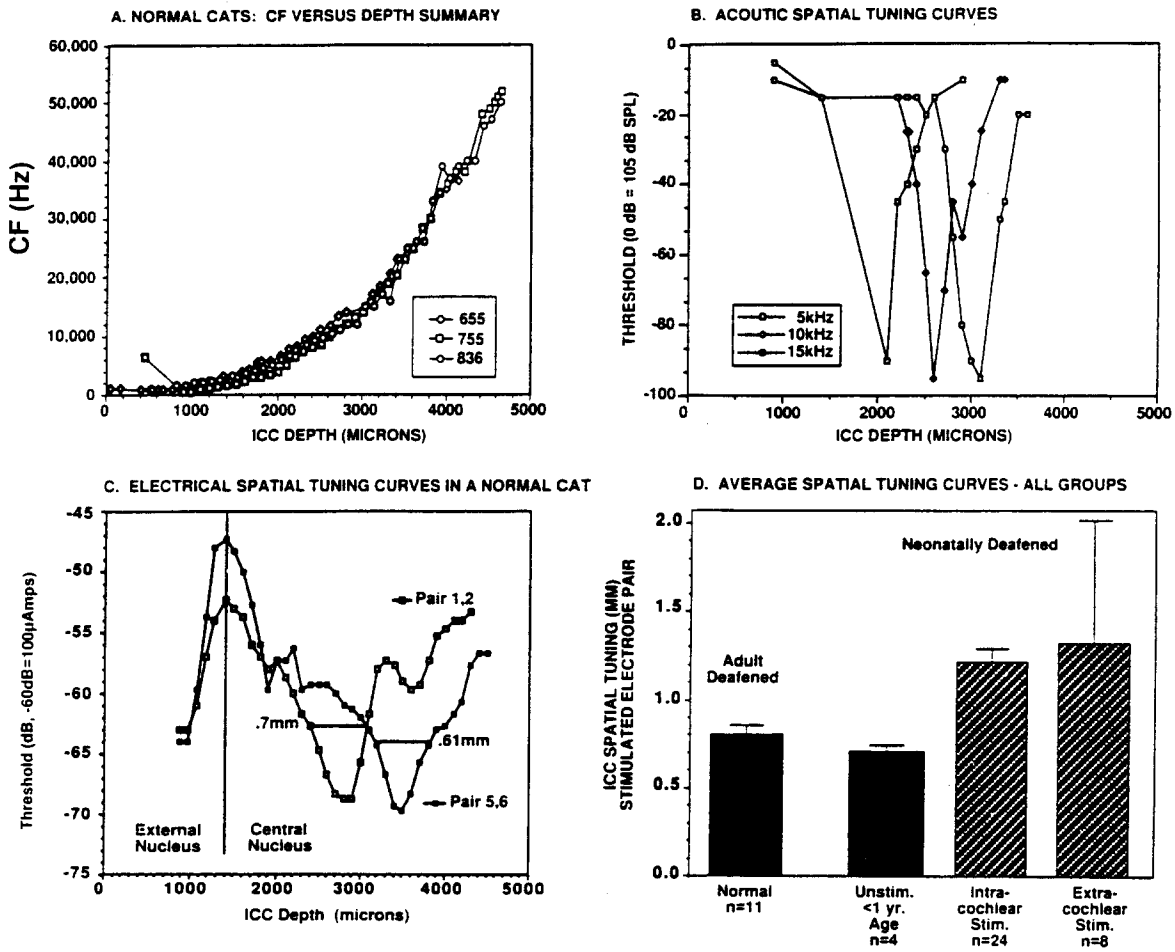

Figure 7. **A.** Plots of the frequency gradient across the central nucleus of the inferior colliculus [ICC] in three normal cats. The characteristic frequency for each location [intermingled single unit and multiple unit clusters] is plotted as a function of penetration depth for a single illustrative penetration in each of these cats. The curves are superimposed by shifting two of them along the abscissa. As has been demonstrated in earlier studies, cochlear position [best frequency] is represented in a highly ordered and predictable way in the ICC. This uniform topographic organization of the IC has been exploited as a basis of 'mapping' the relative selectivity of excitation of the cochlea evoked by stimulation with intracochlear electrodes in deaf cats. **B.** Plots of threshold as a function of depth across the cat inferior colliculus using three 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. The frequencies correspond to about the same cochlear locations that would be excited by our intracochlear electrodes. (The 5 kHz and 15 kHz functions roughly correspond to the electrical stimulation functions in figure C.) **C.** Electrical spatial tuning curves in a prior-normal cat, acutely deafened and implanted as an adult. Plots of stimulus threshold for two electrode pairs (an apical and a basal pair) as a function of depth for a single penetration through the ICC are shown. The apical electrode pair (1,2) has a slightly lower absolute threshold and the most superficially located threshold minimum (corresponding to the lower frequency, more apical location in the cochlea). Both these electrodes were off-radial pairs separated by 1 mm, and they have 6 dB spatial tuning curve bandwidths of about .6 -.7 mm. That corresponds with the ICC spatial tuning bandwidth evoked by a stimulus tone delivered at roughly 50-60 dB SPL. **D.** Mean and standard deviation of electrical spatial tuning curve widths in normal, neonatally deafened/unstimulated, and neonatally deafened/chronically stimulated cats. The STC widths for the apical electrode pair (1,2), like that seen in C are measured at 6 dB above minimum threshold and averaged for all penetrations in each cat. The mean 6 dB width for STCs in 11 normal cats was 0.80 mm; the mean for neonatally deafened, unstimulated cats was 0.71 mm; the overall mean for the 30 pps intracochlear stimulation group (12 cats) and the recent higher frequency stimulation group (12 cats) was 1.21 mm; and for the extracochlear stimulation group (8 cats) the mean was 1.32 mm. *Thus the average STC width of chronically stimulated animals was increased by more than 50% as compared to prior normal animals and neonatally deafened, unstimulated animals.*

7. **Experiments conducted in primary auditory cortex (A1) indicate that alterations in the spatial input selectivity also occur at the cortical level in these neonatally deafened and chronically stimulated 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 cats described above. 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 most 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 crest of the middle ectosylvian sulcus), and a series of penetrations made across the isofrequency gradient of AI (across the ventral-to-dorsal axis) (Raggio and Schreiner, '94; Schreiner and Raggio, '96).

Results in normal cats (deafened and implanted as adults) show that stimulation of an individual intracochlear bipolar electrode pair produces a dorsal to ventral "ridge" of higher sensitivity, lower threshold in AI. This ridge is located caudally for apical electrodes, and it shifts progressively more rostral with excitation of more basal electrode pairs on the cochlear implant. These preferential threshold locations for different intracochlear electrodes are consistent with the known tonotopic organization of AI demonstrated by acoustic stimulation, indicating that tonotopic organization, or "tuning" also occurs with electrical stimulation.

In contrast to normal animals, neonatally deafened/chronically stimulated cats exhibit much poorer electrode specificity and degraded tuning (Figure 8). For analyses of cortical data, the animals are divided into "passively stimulated" cats that were neonatally deafened and received chronic stimulation with a meaningless, stereotyped electrical stimulus (30 pps, 80 pps or 300 pps/30 Hz AM); and "behaviorally trained" cats that received initial stimulation through an analogue speech processor, and when mature enough, were behaviorally trained to detect and respond to a variety of electrical signals. In the passively stimulated group, electrode specificity appears to be somewhat poorer than normal, and although tuning may be present, it appears to be broader than normal. Results in behaviorally trained animals were even more markedly different from normals, with many of the response threshold distributions being nearly flat and lacking any spatial selectivity whatsoever. These results indicate that electrical stimulation of a bipolar intracochlear electrode pair initially produces a preferential, spatially selective response in primary auditory cortex, but after chronic stimulation with a single electrode pair, this selectivity or tuning is lost. This equalization of thresholds across a broad region of AI is particularly impressive because absolute threshold is typically very low in these behaviorally trained animals, suggesting *an increase in sensitivity* of neurons throughout the cortex or a *broadening of the spectral/place representation*. These findings are interpreted as suggesting that chronic stimulation, particularly with behaviorally relevant signals, can result in profound functional alterations or reorganization of auditory cortex. It would be extremely valuable in future experiments to determine the effects of chronic stimulation like that used in current CIS processors, and to explore the central representations of such signals by defining physiological thresholds for such higher frequency and more complex stimuli.

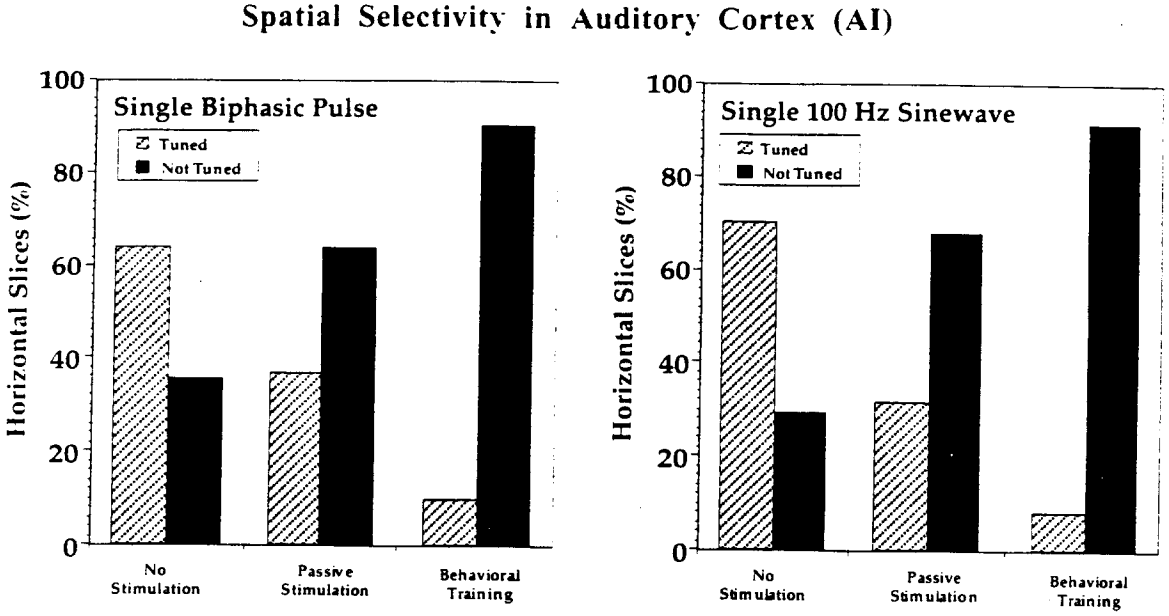

Figure 8. To evaluate cortical "tuning," thresholds from a single line of data points across the caudal-to-rostral extent of AI (across the frequency gradient) are plotted, and each such "slice" of data points is analyzed. Criteria for tuning are: 1) a preferential spatial locus of best response for different intracochlear stimulating electrodes, and 2) threshold profile (STC) with a V- or U-shaped configuration. Slices classified as "tuned" must have STC widths of less than 3 mm (at 10 dB above threshold). The graphs show slice data for 200 μSec biphasic pulses (left) and single 100 Hz sines (right). More than 60% of slices in normal cats exhibit tuning for both signals. After "passive" chronic electrical stimulation, these values are essentially reversed and the incidence of tuned slices is significantly decreased (pulses: 37%; sines: 32%). In behaviorally trained cats there is a striking loss of the spatial selectivity (frequency representation) of bipolar intracochlear electrodes, with less than 10% of slices meeting the criteria for tuning.

8. Chronic electrical stimulation in neonatally deafened cats profoundly alters temporal response properties of neurons in the auditory midbrain. In addition to the above studies of spatial representations, extensive electrophysiological studies have analyzed temporal response properties of single neurons in the IC, elicited by electrical stimuli. Previously published data showed that many temporal features of IC unit responses are very similar to those seen with acoustic stimulation. All major response types are identified, first spike latencies and phase-locking capacities appear to be very similar. However, quantitative analysis of response patterns (PSTHs or per stimulus time histograms) in neonatally deafened cats that were chronically stimulated at a young age revealed apparent stimulus-induced alterations in the temporal response properties of midbrain neurons (Snyder et al., '91). For example, neurons in chronically stimulated cats had significantly increased occurrence of inhibitory and late responses. Further, recently published studies conducted under our current Contract, indicate that 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 this controlled, temporally-stereotyped electrical stimulation (Snyder et al., '95). When modulation transfer functions (MTFs) for all IC neurons were analyzed quantitatively for adult deafened "normal" control animals, the average maximum following (phase locking) frequency is 99 pps. A group neonatally deafened, unstimulated animals, studied at prolonged intervals after deafening demonstrated a decrease in the temporal resolution of IC neurons to an average of 81 pps. In contrast, chronically stimulated cats showed an *increase* in temporal resolution to an average of 113 pps, significantly higher than deafened/ unstimulated or normal cats (Snyder et al., '95).

This initial finding was remarkable because most of the chronically stimulated animals studied at the time of our initial report received only low frequency pulsatile stimulation at 30 pps, a stimulus that is not temporally challenging. Figure 9 presents an update of these temporal resolution data, including animals from more recent experiments using higher frequency chronic stimulation. The data show that animals stimulated exclusively with simple 30 pps pulse trains exhibit only a slight increase in temporal resolution (mean maximum following frequency of 106 pps), indicating a maintenance of normal temporal resolution, but not a significant increase above normal. However, higher frequency, modulated, and in behaviorally-relevant electrical stimulation resulted in a highly significant *increase* in temporal resolution with a mean maximum following frequency of 151 pps.

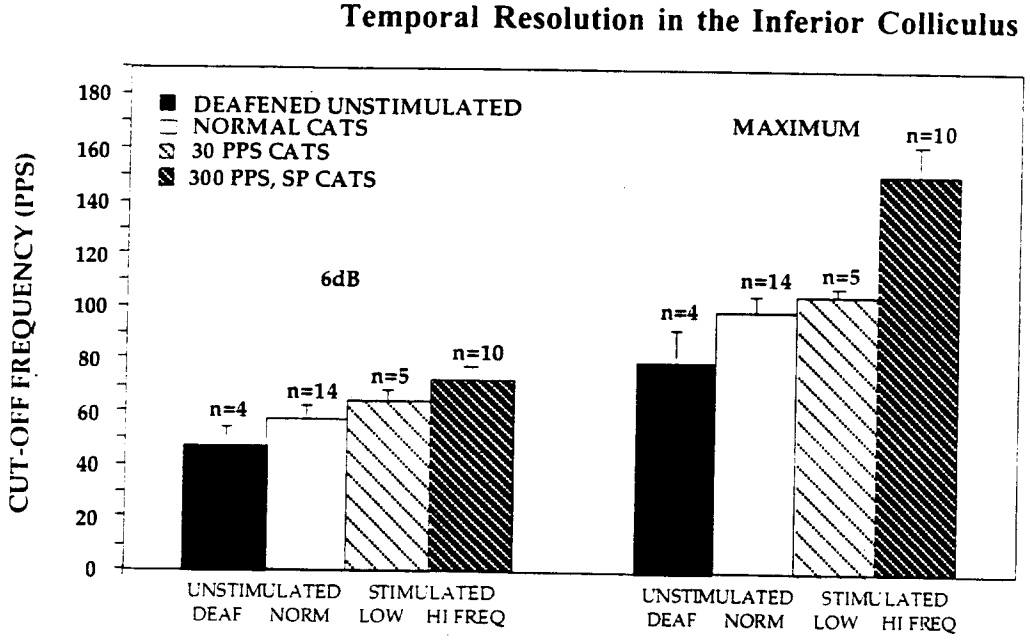

Figure 9. The mean 6 dB and mean maximum following (phase-locking) frequencies for IC neurons (single unit recording) in four groups of cats: i) neonatally-deafened, unstimulated cats, ii) acutely-deafened prior normal cats, iii) neonatally-deafened cats chronically stimulated with 30 pps pulses and iv) neonatally-deafened cats stimulated chronically at 80 pps, with a 300 pps carrier amplitude modulated at 30 pps, or with an operational speech 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 used for chronic stimulation. Additional progress during the current Contract was to demonstrate that neurons in the external and central nuclei of the IC show different effects. Temporal resolution is significantly poorer for units in the external nucleus than in the ICC, and temporal resolution in the external nucleus is not significantly different after chronic stimulation whereas the ICC shows marked increase in 6-dB and maximum following frequencies (Vollmer et al., 1997-Appendix).

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 patients improve over time. We hypothesize that this ability to follow electrical pulse trains at extremely high frequencies may account for the success of recent sophisticated CIS speech processor designs which utilize amplitude modulation of high frequency (≥1000 pps) pulse trains. It is possible that poorer speech recognition performances of some subjects may be accounted for by an inability of their central auditory systems to entrain to higher frequencies (e.g., due to specific deafness pathologies). These issues can only be addressed by systematic examination of the functional consequences of various parameters of chronic electrical stimulation in appropriate deaf animal models.

9. Psychophysical thresholds for higher frequency, modulated stimuli can be significantly lower than EABR thresholds; this difference varies in individual animals. 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 was 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. This work represents a milestone, because this is, to our knowledge, the first time that psychophysical data and single- and multi-unit electrophysiological data have been derived and directly compared *in the same animals*. Initial experiments showed that EABR, psychophysical and IC single unit thresholds measured in the same cat were always nearly identical. 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 for low frequency signals (e.g., at 2 dB above EABR threshold.)

One of the objectives of future work is to apply chronic stimulation in animal models at *higher frequencies* that more closely model signals used in current CIS human cochlear implant processors (which use amplitude modulation of carrier rates up to 1600 pps). However, since studies in both human cochlear implant subjects and in animals have demonstrated that perceptual thresholds become somewhat lower with increasing stimulus frequency (Pfingst and Morris, '93; Shannon, '85). Thus, for higher rates of stimulation, psychophysical thresholds would be expected to be somewhat lower than EABR thresholds (which are determined with low frequency pulse rates). Figure 10 shows threshold data for 4 behaviorally trained, neonatally deafened cats. 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 were within a few dB of EABR thresholds But the magnitude of this difference varies from 0 to 6 dB in individual cats. 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, particularly in experiments in which 2 or more channels will be stimulated. 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.

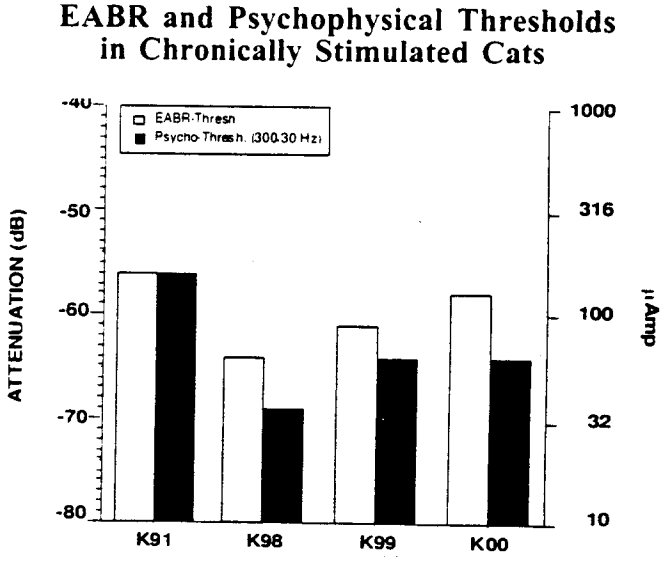

Figure 10 EABR thresholds (shaded data bars), and psychophysical thresholds to the 300 pps/30 Hz stimulus used for chronic stimulation (black bars) for 4 individual behaviorally trained cats. Differences between EABR and behavioral thresholds vary from 0 to 6 dB, presumably reflecting the individual dynamic range.

A number of behavioral studies have been conducted to study cochlear implant stimulation in animals, primarily by Pfingst and colleagues in the monkey, and disparities between behavioral and physiological thresholds have long been reported (Javel et al '87, '89; Parkins, '89, Pfingst et al., '81; Pfingst '88; van den Honert and Stypulkowski, '87a,b). However, direct comparisons of behavioral, single unit and EABR thresholds have not previously 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. Therefore, we recommend that future research should 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 subjects.

10. <u>It is not clear whether developmental "critical periods" play a role in the stimulation-induced effects documented in these studies.</u> There is considerable 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 the refined connections in the visual system is believed to be dependent upon correlated activity from local retinal locations (see Miller et al., '89; Schmidt and Tieman '85, '89; Singer, '87 for reviews). 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 (Stryker and Strickland, '84), or by stroboscopic illumination that results in nearly synchronous inputs from both eyes (Cremieux et al., '87; Eisele and Schmidt, '88; Kennedy et al., '80). Stroboscopic illumination during development 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 important for the design of 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 over one eye (Tumosa et al., '80; van Sluyters and Livitt, '80) or by alternate monocular deprivation (Tumosa et al., '80; Altman et al., '87; Hubel and Wiesel, '65). These results are interpreted as reflecting the effects of competitive processes which act to segregate different input populations driven by uncorrelated inputs.

It might be noted that while there is a normal 'critical period' for these coincidence-based developmental effects in the visual system, this period can be extended substantially in animals that are profoundly deprived of normal sensory inputs (Cnyader and Mitchell, '80; Mower et al., '81; Mower and Cristen '85). Once inputs are reintroduced, a critical period is initiated and results in reorganization that stabilizes over a period of 6 to 8 weeks. *If the central auditory system is governed by similar developmental mechanisms, 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.*

It is well-known that early sound exposure is important for development and maturation of the auditory pathways in mammals and that neonatal sound deprivation results in profound adverse effects on the central auditory system (Eggermont and Bock, '86; Rubel et al., '84; Rubel et al., '90; Rubens and Rapin, '80). After neonatal deafening or conductive hearing loss, animals show severe atrophy of the cells in the cochlear nucleus (CN) (Coleman and O'Connor, '79; Coleman et al., '82; Trune, '82; Webster and Webster, '77, '79), decrease in the volume of the CN (Coleman et al., '82; Trune, '82; Webster, '88;), physiological changes (e.g., Evans et al., '83), as well as transneuronal changes at higher levels of the auditory system (Feng and Rogowski, '80; Jean-Baptist and Morest, '75; Powell and Erulkar, '62). Other research has shown that neonatal cochlear lesions can result in substantial modification in the anatomical projections from the contralateral CN to the inferior colliculus (Moore and Kitzes, '85; Moore and Kowalchuk, '88; Nordeen et al., '83). Further, many studies suggest that deprivation later during development does not have the same profound impact on the central auditory system (Blatchley et al., '83; Webster, '83). Thus, deprivation during early development clearly produces profound changes, and there is evidence for the existence of critical periods. 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