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Protective Effects of Patterned Electrical Stimulation on the Deafened Auditory System

Submitted by:

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This QPR is being sent to you before it has been reviewed by the staff of the Neural Prosthesis Program.

ABSTRACT

A current focus in chronic electrical stimulation experiments is to examine and compare the "protective" effects on the auditory nerve using electrical signals which model specific parametric attributes of stimulation delivered by CIS processors. Studies have been undertaken to compare the effects of intracochlear stimulation with higher frequency signals (designed to be temporally challenging to the central auditory system) to results obtained in previous studies using 30 pps intracochlear and extracochlear stimulation. In this Quarterly Progress Report we present additional results from morphological studies and further analyses of morphometric data.

In order to more fully characterized the animal model of congenital deafness used in our chronic stimulation studies, the time course and variability of spiral ganglion cell degeneration were evaluated in unstimulated ears following neonatal deafening. When data from control deafened cochleas are pooled for survival periods ranging from 2 weeks to one year following neonatal deafening, results show a highly significant correlation between spiral ganglion survival and duration of deafness. Data from long term deafened animals studied at prolonged intervals (2.5 to 6.5 years) show severe spiral ganglion degeneration varying from about 5 to 20% of normal density.

Data from animals in recent studies employing chronic electrical stimuli designed to be temporally challenging and stimulation periods of 5 to 10 months, showed marked increases in spiral ganglion neuronal survival. Spiral ganglion cell volume ratio (density) showed a mean value of about 56% of normal in the stimulated cochleas and 36% in the paired control (deafened but unstimulated) cochleas (n=9).

Additional analyses were performed to evaluate the relative contributions of specific stimulation parameters to the increase in spiral ganglion cell survival induced with chronic stimulation. Results clearly indicate that chronic stimulation with a round window monopolar electrode is less effective in maintaining the spiral ganglion neurons than is intracochlear bipolar stimulation using the same electrical stimulus (30 pps) and equivalent durations of stimulation. In analyses comparing intracochlear stimulation at 30 pps with higher frequency, temporally varying stimuli, results also suggest a greater effect with the latter temporally challenging stimuli. For stimulation periods of 5 to 10 months, there is no significant correlation of ganglion survival in the *stimulated cochleas* with duration of stimulation, indicating that most of the degeneration that occurs in the contralateral deafened ears is prevented by chronic stimulation with an implant. In contrast, a there is significant correlation between duration and survival in the *contralateral deafened control cochleas*, resulting in greater differences between stimulated and control cochleas with longer duration of stimulation.

Spiral ganglion cell diameters were measured to determine if differences in cell size contributed to the differences in cell density seen in stimulated animals. Although neonatal deafening results in a significant decrease in cell area, paired comparisons of the stimulated and control cochleas in the temporally challenging stimulation group showed *no difference* in cell size. Thus, the 20% increase in spiral ganglion cell density after stimulation seen in this group was due soley to an increase in the number of surviving neurons.

1. Time Course and Variability of Spiral Ganglion Cell Degeneration Following Neonatal Deafening

In order to better characterize the animal model of congenital deafness which we employ in studies of the effects of chronic electrical stimulation, the time course and variability of spiral ganglion cell degeneration were evaluated in all *unstimulated* cochleas available to date. Figure 1 shows a summary graph of spiral ganglion cell survival as a function of duration of deafness. A small group (n=4) of animals were deafened by our standard ototoxic drug protocol (neomycin sulfate, 60 mg kg I.M. SID for 16 days) and evaluated in a separate study at 16 to 24 days postnatal in order to determine the early time course and initial patterns of hair cell loss and spiral ganglion cell degeneration in these animals. Those data are included along with the contralateral control deafened cochleas from all the implanted and chronically stimulated animals studied to date (filled circles) in order to examine the variance in spiral ganglion survival as a function of duration of deafness for periods ranging from 2 weeks to one year. It should be noted that these neonatally deafened animals are weaned at 6 to 7 weeks of age, at which time they are implanted and chronic stimulation is initiated at 7 to 8 weeks postnatal. The data from the 4 youngest deafened animals in Figure 1 indicate that significant neuronal degeneration occurs in these animals *prior* to this time. We do not have direct observations at the age of initial stimulation, but extrapolating from the linear regression function suggests that survival would be about 75% of normal at 50-60 days postnatal. Although there is notable scatter in the data, the correlation between spiral ganglion survival and duration of deafness is highly significant ($r = .88$, $p<0.001$).

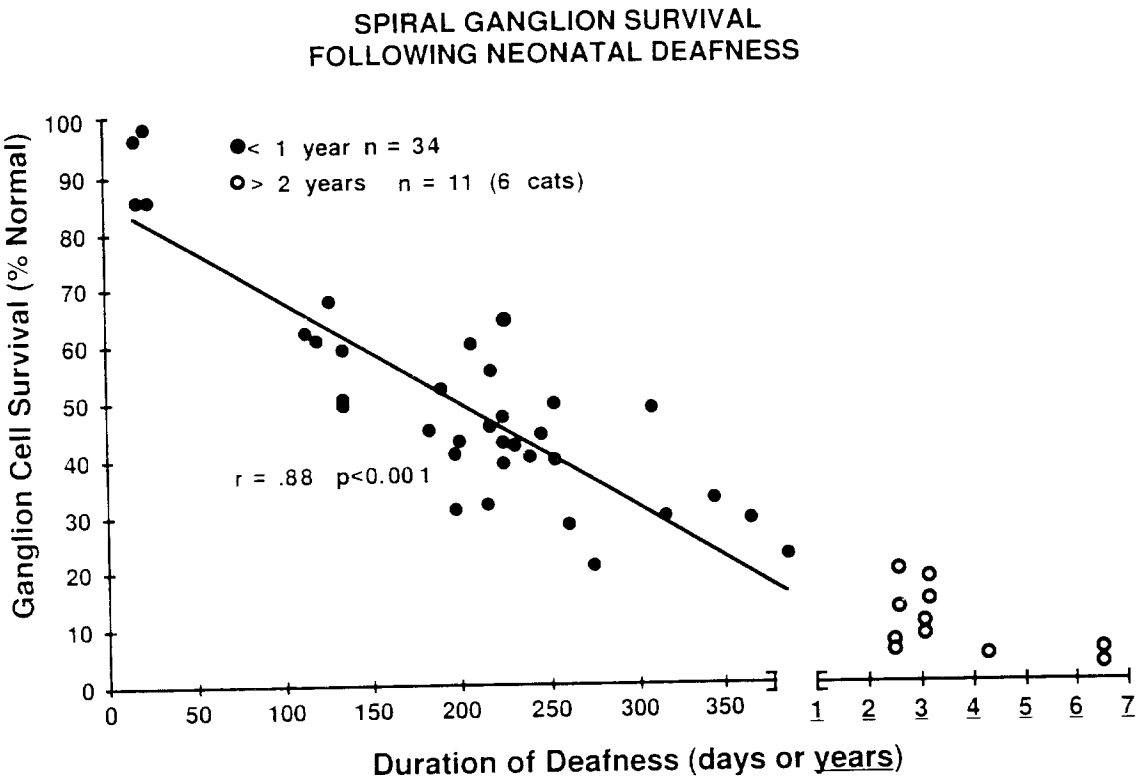

Figure 1. Spiral ganglion survival data from control, unstimulated cochleas are shown as a function of duration of deafness. Data are divide into 2 groups. In the first group animals were studied at periods of about 1 year or less (filled circles). The second group we have designated as "long term neonatally 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. Histologic and Morphometric Results with Higher Frequency/Temporally Challenging Stimulation

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 a previous Quarterly Progress Report (QPR #7, 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, there are now 9 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 and 100% amplitude modulated at 30 Hz; and 4 animals received temporally- and intensity-varying stimulation through a single channel speech processor which transduced environmental sounds into an analogue electrical signal. (3 cats 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
K62	50/16	7.5 wks	50-200 μA	22	SP	30 wks
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
			"Wing Electrode Series"			
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 9 animals in the temporally challenging stimulation groups for which spiral ganglion survival showed an increase of 20%
(*Device failed during chronic stimulation and animal was reimplantated.

Figure 2 presents summary data for this group, showing regional *increases* in spiral ganglion cell density (stimulated less control values) in sectors from base to apex of the cochlea. As a result of chronic stimulation, the mean overall spiral ganglion survival was increased by more than 20%, with a mean value of 56.3% of normal in the stimulated cochleas and 35.6% of normal in the paired control deafened ears. Mean increases of 25-35% of the normal cell density were documented over the basal one-third of the cochlea and marked increases in cell survival were observed throughout the cochlea. As reported previously, these data support our earlier preliminary conclusion that a significantly greater protective effect of chronic stimulation is seen in this higher frequency, temporally challenging stimulation group, as compared to results in previous experiments using 30 pps pulse trains with either bipolar intracochlear stimulation (13% difference in ganglion cell survival) or monopolar round window stimulation (6% difference).

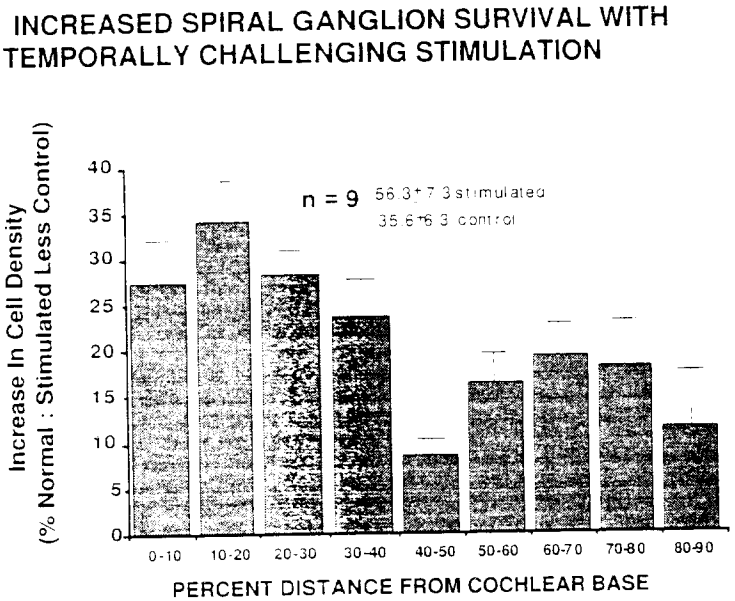

Figure 2. Marked increase in spiral ganglion survival induced by chronic intracochlear electrical stimulation using temporally challenging stimulation in neonatally deafened cats. Data are pooled from 9 animals. The mean stimulated less control values for spiral ganglion cell density are expressed as percent of normal values for each cochlear sector from base to apex and thus represent % *increases* 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. Duration of Stimulation, Temporal Characteristics of Electrical Stimuli, and Mode of Stimulation in Conservation of Spiral Ganglion Neurons

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, 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, we have

hypothesized that 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 animals 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 3 that many of the temporally challenging stimulation animals (square symbols) which exhibited greater increase in spiral ganglion cell survival, also received longer duration stimulation. (The increase in duration of chronic stimulation periods to greater than 6 months was required by the specifications of our current contract, whereas the original studies were conducted under a previous contract in which 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 with temporally challenging stimulation.

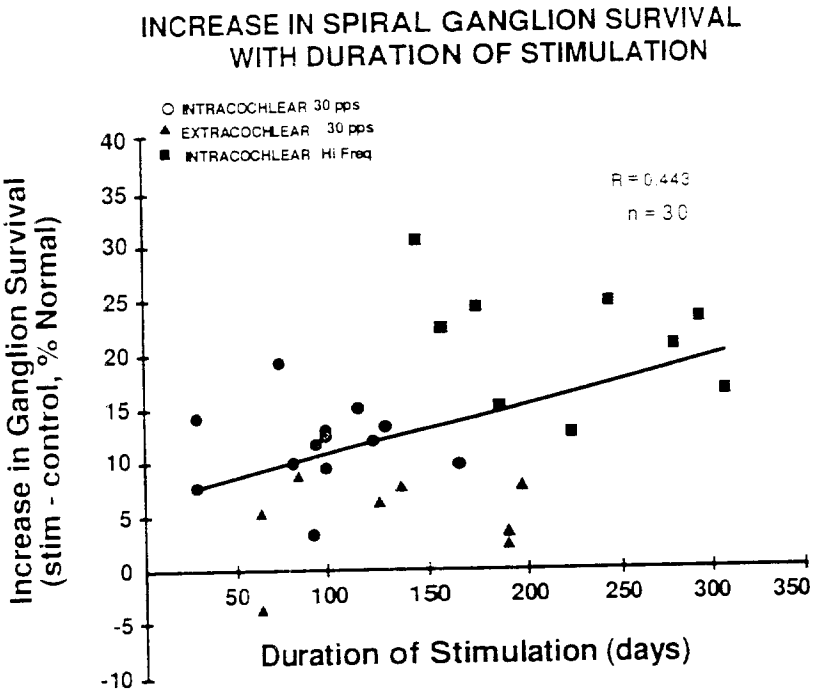

Figure 3. Increase in spiral ganglion survival induced by varying durations of chronic electrical stimulation in three groups of cats. Round window stimulation at 30 pps with a monopolar electrode (triangles) appears to be less effective in increasing spiral ganglion cell density than intracochlear bipolar 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.

To further examine the effect of duration of stimulation, Figure 4a 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. The neural survival in the stimulated cochleas (triangular symbols) also shows a significant downward trend for stimulation periods of about 1 month up to about 10 months, suggesting that there may be a significant decrement in cell density over this period that is not prevented by chronic stimulation.

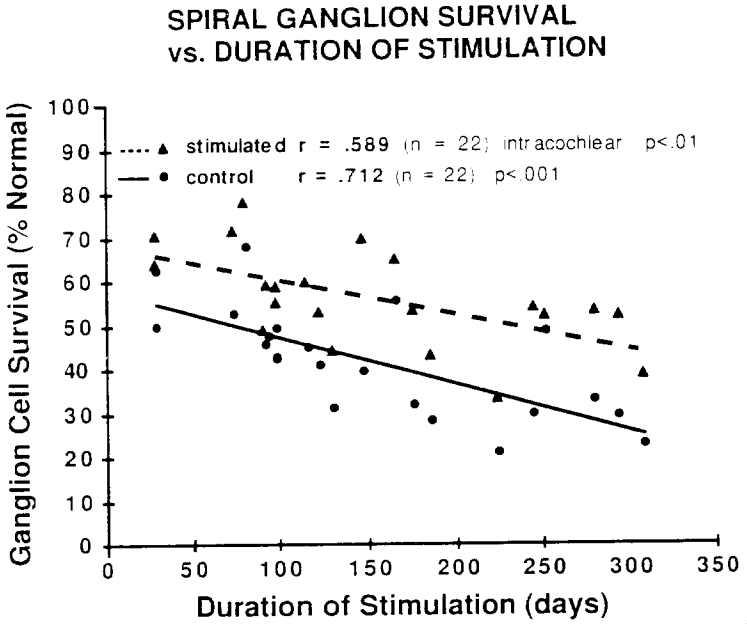

Figure 4a. Spiral ganglion survival in stimulated cochleas (triangles) and contralateral deafened control cochleas (circles) for intracochlear chronic stimulation periods ranging from 1 to 10 months.

However, it should be noted that our criterion for including animals in groups comparing the effects of stimulation is > 90 days of chronic stimulation. As shown in Figure 4b, if the linear regression analysis is performed for data restricted to animals meeting the 90 day criterion for intracochlear stimulation, the trend in the data (dashed line) is not significant ($r=.400$, NS). Thus, that for the stimulation periods of 3 to 10 months used in our chronic stimulation 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. In contrast, a highly significant correlation between lower percentage of spiral ganglion cell survival and duration of stimulation (i.e., duration of deafness) is demonstrated in the contralateral deafened control cochleas. 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.

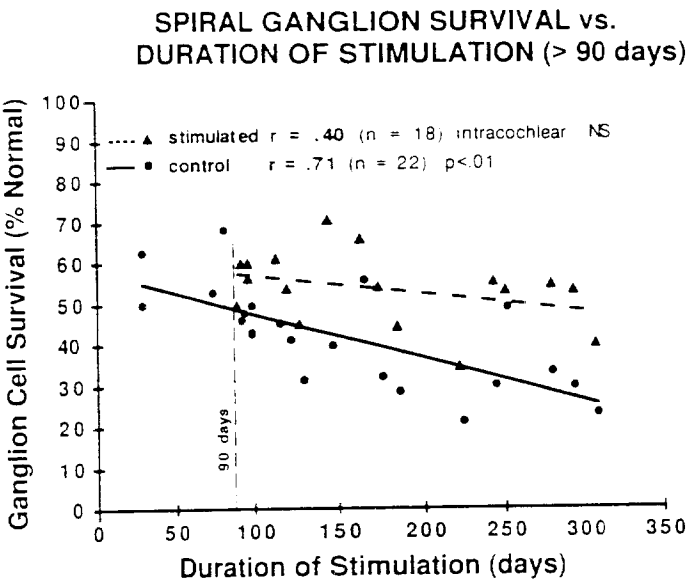

Figure 4b. Spiral ganglion survival in stimulated cochleas (triangles) with data analysis restricted to animals meeting the criterion of 90 days of chronic intracochlear stimulation. Data show little decrement in survival for stimulation periods of 3 to 10 months (r= .400; NS), suggesting that chronic stimulation over these periods tends to prevent further degeneration.

4. Spiral Ganglion Cell Diameters 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 publications reporting increased survival of spiral ganglion neurons with chronic stimulation, we evaluated ganglion cell diameters (Leake et al., Hearing Res. 1991, 54:251-271, 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, since we have now documented substantially greater effects induced in the higher frequency, temporally challenging stimulation group, it seems prudent to once again evaluate the possibility that spiral ganglion cell size might play a role in the differences observed with chronic electrical stimulation.

Figure 5 compares spiral ganglion cell diameters in normal and 2 groups of neonatally deafened cats. For this analysis, cells with clear nucleoli were selected from radial sections taken at 2 mm intervals throughout the cochlea. Five cells at each location were measured in 2 axes, across their longest and shortest dimension and the 2 measurements were averaged. In the normal cat, the great majority of spiral ganglion cells are myelinated type I neurons which receive input from the inner hair cells. Their cell somata have a mean diameter of 22.4 μm when measured in this fashion. (It should be noted, however, that there is some regional variation in this value, and cells at the base of the cochlea in the 0-20% region had a mean diameter of about 24 μm, whereas cells in the 40 to 60% sectors measured about 21 μm. Thus, in paired comparisons it is extremely important to compare cells from the same cochlear regions.) 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.

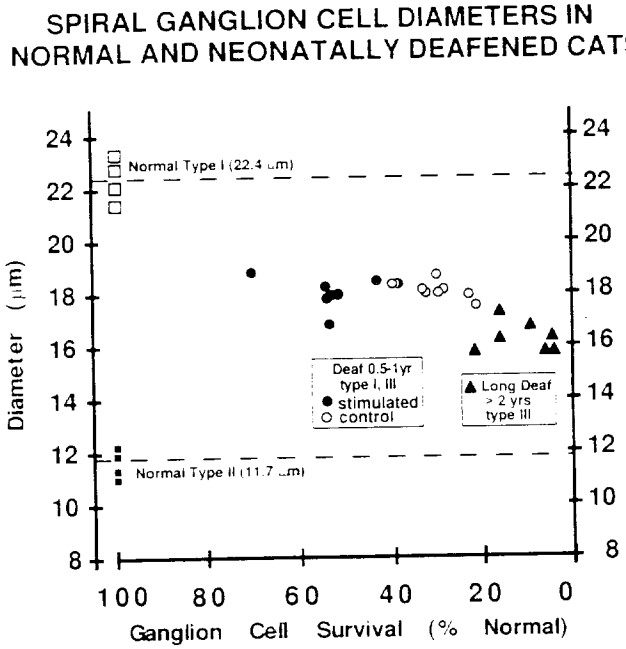

Figure 5. The mean diameter of spiral ganglion cell somata are shown for normal cats (squares), neonatally deafened cats in the temporally challenging stimulation group (circles), and long term neonatally deafened cats (triangles).

Figure 5 also shows mean cell diameters from 8 neonatally deafened animals in the temporally challenging stimulation groups described above. (One animal was deleted from this study because fixation was judged to be inadequate for these measurements.) 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) by the following criteria: shape of the nucleus (round, not irregular), the size of the cell soma and myelinated cell body (type I). It is clear that mean cell diameter is significantly reduced to about 18 µm in these 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 and are classified as type III cells, 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 a larger value was recorded in the stimulated cochleae, this difference in diameter represents an increase in cell area of only 2.60%, and it was not significant (Student's t test, paired, P=0.227) for the group. We then measured cells throughout all cochlear sectors. Figure 6 shows the results of this analysis. After measuring more than 400 neurons in each group, the mean cell size was 18.1 µm for the stimulated cochleas, and precisely the same in the unstimulated cochleas.

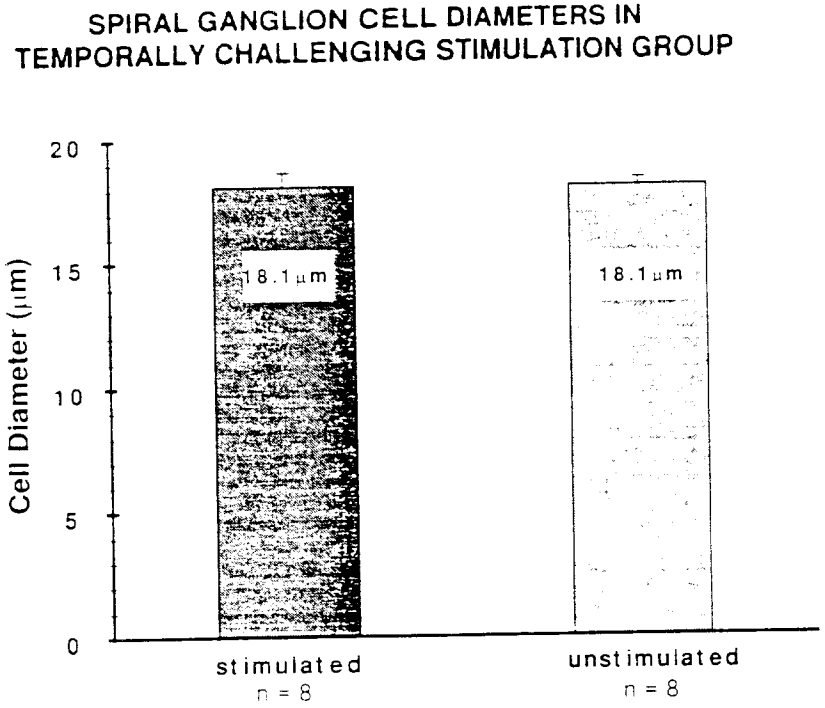

Figure 6. The mean diameter of spiral ganglion cell somata are shown for neonatally deafened cats in the temporally challenging stimulation group. There is no difference between stimulated and unstimulated cell size, indicating that the highly significant differences in density seen in the stimulated cochleas reflects solely an increase in number of surviving neurons.

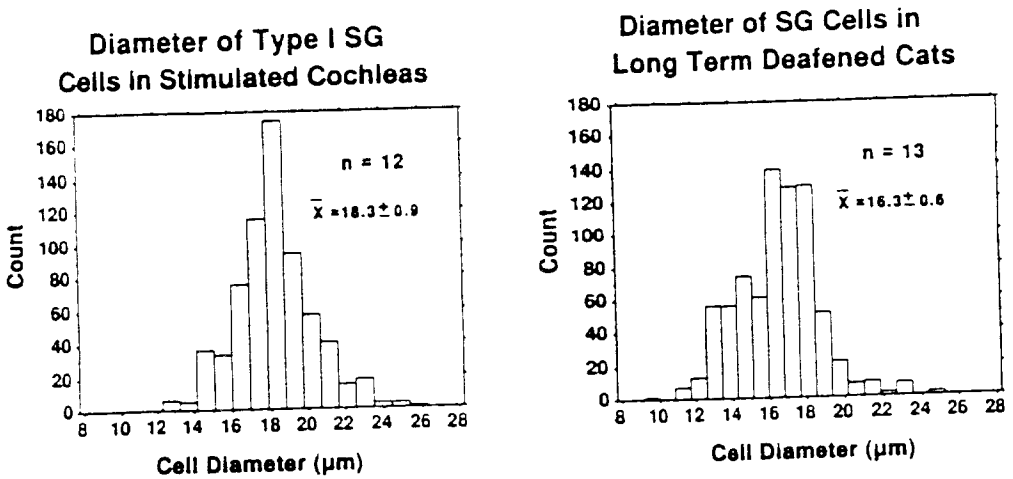

Figure 7. The histograms show the distribution of cell diameters for all neonatally deafened chronically stimulated animals (n=12) and long term neonatally deafened animals (n=7 cats, 13 cochleas) studied to date

Figure 7 compares the range and distribution (frequency of occurrence) of cell diameters measured in stimulated cochleas of animals studied at ≤ 1year of age and in a group of long term deafened cats. The left panel shows a summary distribution of cell diameters in all the stimulated cochleas in which cell diameters have been examined to date (n=12). The distribution is clearly unimodal and suggests a relatively uniform population of cells with a mean diameter of 18.3 μm. A clear bimodal distribution would have suggested that some cells were being maintained at near normal size, and a second population was undergoing rapid degeneration. However, it is clear that there are very few cells that are comparable in size to the normal type I cells which have a mean diameter of 22.4 μm (See figure 5). The right panel shows the cell diameters in long term deafened animals examined at 2.5 to 6.5 years after neonatal deafening. In this group, there is a clear shift toward smaller cell diameters (mean diameter = 16.3 μm) and a somewhat broader distribution of cell diameters in these severely degenerated cochleas with 5 to 20% neuronal density.

Taken together, these data on spiral ganglion cell diameters suggest that there is significant shrinkage in the spiral ganglion neurons after neonatal deafening which is 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 volume ratio (density) observed in the stimulated cochleas of these animals is due solely to an increase in the *number of surviving neurons*.

Work Planned for the Next Quarter

1) An acute electrophysiology experiment will be conducted during the next quarter in two chronically stimulated cats. One of these animals is currently undergoing 2 channel chronic intracochlear stimulation using temporally challenging (but passive and invariant) electrical stimuli (800 pps amplitude modulated with a 60 Hz sinusoid). This experiment is designed to better model electrical signals used in current CIS cochlear implant processors.

2) Two additional neonatally deafened kittens are currently receiving electrical stimulation on two independent bipolar intracochlear channels, and one of these animals will be studied in an acute electrophysiology experiment toward the end of the next quarter. These animals are also the first in a series examined to examine the effects of daily systemic administration of the ganglioside GM1 during the interim period between neonatal deafening (postnatal days 1 through 16) and the time of implantation and initial stimulation at about 6 weeks postnatal. GM1 has been reported to ameliorate the atrophy of spiral ganglion neurons and ventral cochlear nucleus neurons which results from neonatal conductive hearing loss (Walsh and Webster, Hearing Res. 1994; 75:54-60). It has been suggested by these authors and others ganglioside treatment potentiates growth factors which sustain spiral ganglion integrity. Two additional kittens from a litter due to be born at the beginning of August will be deafened, treated with gangliosides and implanted at 6 weeks of age and stimulation will be initiated.

3) Data analysis will continue for the group of 4 adult deafened cats that have been chronically stimulated and studied over the past year. Chronic stimulation will continue in one long term deafened cat in an experiment designed to determine whether stimulation can increase temporal resolution of central auditory neurons in animals with severe degeneration of the spiral ganglion.