1st Quarterly Progress Report
October 1, 1997 to December 31, 1997

Fundamental Neurosciences Contract N01-DC-7-2105

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

Submitted by:

Patricia A. Leake, Ph.D.
Gary T Hradek, M.S.
Stephen J. Rebscher, M.A.

Epstein Hearing Research Laboratories
Department of Otolaryngology, Room U490
University of California, San Francisco
San Francisco, Ca 94143-0526

## ABSTRACT

One key goal of our Contract research is to examine the factors and mechanisms underlying the finding that chronic stimulation in neonatally deafened cats results in increased survival of spiral ganglion neurons, at least partially preventing the slow retrograde degeneration following loss of the hair cells in the cochlea. At a recent conference Li et al. reported a study of electrical stimulation in guinea pigs in which they demonstrated a significant increase in spiral ganglion cell *density* in the stimulated ears, but no increase in absolute *number* of spiral ganglion cells. It was suggested that chronic electrical stimulation does not have a trophic effect promoting increased survival of neurons, but rather causes a "subtle narrowing of Rosenthal's canal." In view of these new data, in this Quarterly Progress Report we review our methods for documenting spiral ganglion survival and present new control data gathered in our recent temporally challenging chronic stimulation series. Direct measurements of the cross-sectional area of Rosenthal's canal in the regions of greatest difference in spiral ganglion cell density showed no significant difference between the stimulated and control sides. In addition, absolute counts of spiral ganglion cell profiles containing nuclei revealed a highly significant increase in the number of cells on the stimulated side that was proportional to the increase in density. We conclude that the large increases in cell density in the stimulated ears of our neonatally deafened chronic stimulated cats reflect actual differences in numbers of surviving neurons and that Rosenthal's canal is not altered by our electrical stimulation protocols.

In addition, in this report we evaluate the functional consequences of varying extent of spiral ganglion cell survival by examining correlation between cell survival and: i) electrically evoked auditory brainstem response (EABR) thresholds; ii) minimum neural threshold in the inferior colliculus iii) psychophysical thresholds in the various groups of experimental animals. We conclude from these data that the electrophysiological measures (EABR and minimum IC threshold) are correlated with spiral ganglion survival, at least for relatively large differences in the extent of pathology. Moreover, the two electrophysiological thresholds are correlated with each other and with psychophysical thresholds determined in the same animals. Taken together, data suggest that extent of spiral ganglion degeneration is an important factor underlying functional thresholds and intersubject variability.

## Morphometric Data Documenting Spiral Ganglion Survival: Additional Control Data

The overall goals of our Contract research at UCSF are to examine the effects of chronic electrical stimulation upon the cochlea and central auditory system and to determine the factors which contribute to neural survival and determine the viability of the central auditory pathways. One key goal of the previous Contract research was to verify initial data indicating that chronic stimulation in neonatally deafened cats results in increased survival of spiral ganglion neurons, at least partially preventing the slow retrograde degeneration that follows the loss of the hair cells in the cochlea. In the Final Report from the previous Contract (QPR #12, July 1, 1997 to September 30, 1997, Contract #N01-DC-4-2143), data were presented showing that both duration of deafness and type of stimulation (e.g., intra- vs. extracochlear; low frequency vs. temporally challenging stimuli) play key roles in the extent of increased maintenance of the primary auditory neurons, and that under our current experimental protocols highly significant increases in neuronal survival (about 20% of the normal neuronal density) are observed.

At the recent Conference on Implantable Auditory Prostheses (Asilomar, CA in August, 1997) Li, Webster and Parkins reported on a study of electrical stimulation in guinea pigs. Animals were deafened by a combination of kanamycin (400 mg/kg SC) and ethacrynic acid (40 mg/kg), implanted unilaterally and stimulated for 8 weeks (biphasic pulses, 200 μsec/ph, 100 pps, 5 hrs/day, 5 day/wk). In histological analyses, they also demonstrated a significant increase in spiral ganglion cell density in the stimulated ears, but no increase in absolute number of spiral ganglion cells. They then examined the volume of Rosenthal's canal in 5 of the stimulated animals and demonstrated a significant difference between lower values in the stimulated ears compared to the control ears. Thus, they suggested that chronic electrical stimulation did not promote increased survival of neurons, but rather caused a "subtle narrowing of Rosenthal's canal."

In light of these new data and the discussion engendered at the meeting, in this Quarterly Progress Report we review our methods for documenting spiral ganglion survival and present new control data gathered in our recent temporally challenging chronic stimulation series.

Our morphometric data on spiral ganglion survival are collected from light microscopic images of high resolution, semithin (2μm) plastic sections. Cochlear specimens that are fixed by perilymphatic perfusion *in vivo*, embedded in Epon™ or LX™, reconstructed in surface preparations, and sectioned at 2 mm intervals along the basilar membrane in the radial plane on an ultramicrotome. These methods produce very high quality histology for examination of the spiral ganglion and other areas of interest, and blocks prepared in this fashion also can be sectioned for electron microscopic analysis. Moreover, reconstruction of the cochlea in the surface preparation technique allows us to determine represented frequency at sites of interest (e.g., location of the intracochlear electrode) based on the known frequency map for the cat cochlea. To quantify the loss spiral ganglion cell somata, a point counting method modified from Weibel has been employed, as described in previous publications (Leake and Hradek, 1988, Hearing Res. 33: 11-34; Leake et al., 1991, Hearing Res. 54:251-271; Leake et al., 1992, Hearing Res. 64:99-117; Leake et al., 1995, Hearing Res. 82:65-80 ). At each of 10 -12 cochlear sites (at 2 mm intervals), sections were collected at 50 μm intervals and examined at 300 X. Rosenthal's canal was centered under a 10 mm X 10 mm counting grid (area = 90,000 $\mu m^2$). The

volume ratio or spiral ganglion cell density was determined by counting the number of grid line intersections which fell over cell somata, and dividing by the number which fell within the total area of Rosenthal's canal in a given section. It is essentially a density measurement which prevents double counting of cells and is insensitive to the enormous regional variability in the size and shape of Rosenthal's canal in the cat. Moreover, normative data for the cat spiral ganglion have been published (Leake and Hradek, 1988), so that data can be expressed as percent of normal.

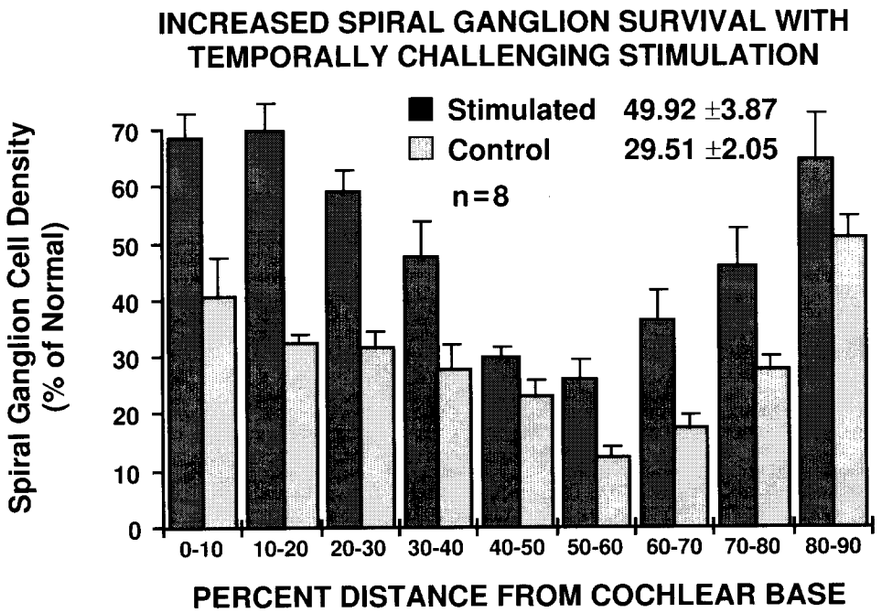



**Figure 1.** Marked increase in spiral ganglion survival induced by chronic intracochlear electrical stimulation using temporally challenging stimulation in neonatally deafened cats. Data are pooled from 8 animals. The mean stimulated less control values for spiral ganglion cell density are expressed 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% of normal, and this difference was highly significant ($P < 0.001$; Student's t-test, paired).

In order to determine whether a difference in Rosenthal's canal volume might play a role in the increased neuronal density that we have documented in neonatally deafened, chronically stimulated cats, we gathered some additional control data during the first quarter of our new Contract. These additional analyses were performed on cochleas from a group of 8 animals in the higher frequency, temporally challenging stimulation series reported previously in the Quarterly Progress Reports for our previous Contract (QPR #11, April 1, 1997 to June 30, 1997, Contract #N01-DC-4-2143 ; Final Report, July 1, 1997 to September 30, 1997, Contract #N01-DC-4-2143). Figure 1 shows summary spiral ganglion cell density data for this group. The overall neuronal density was about **49.9%** of normal in the stimulated ears and **29.5%** in the control ears in this group, a mean increase of about 20% of the normal cell population. Moreover, in the 3 most basal cochlear sectors the data show differences of 30 to 40% of the normal neural population.

For the present analysis we selected the two 10% cochlear sectors in each animal which showed the *greatest stimulated vs. control difference* in density. As shown in Figure 2a, for these selected areas the mean density was **68.3%** of normal for the stimulated ears and **31.1%** for the control data, a difference of almost 40% of the normal neuronal population. It should be noted that Li et al., and many other investigators, report such data as *percentage differences*, (i.e., density in the stimulated ears minus control density/ control X 100). Expressing the data in Figure 2a this way, we would report a **120%** increase in spiral ganglion cell density! The stimulated vs. control means for these selected sectors in the 8 individual animals are shown in Figure 2b.

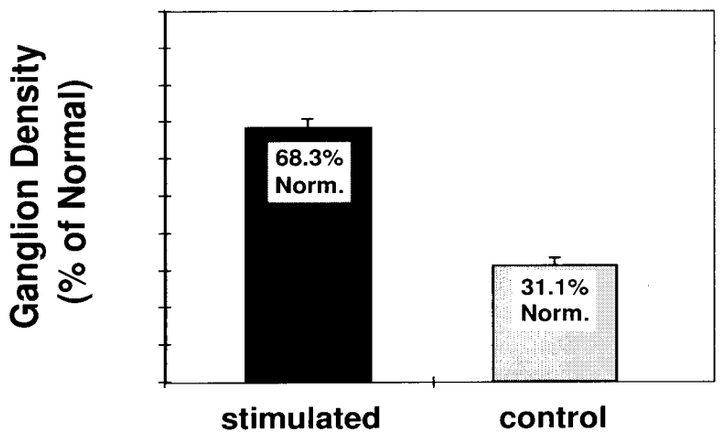
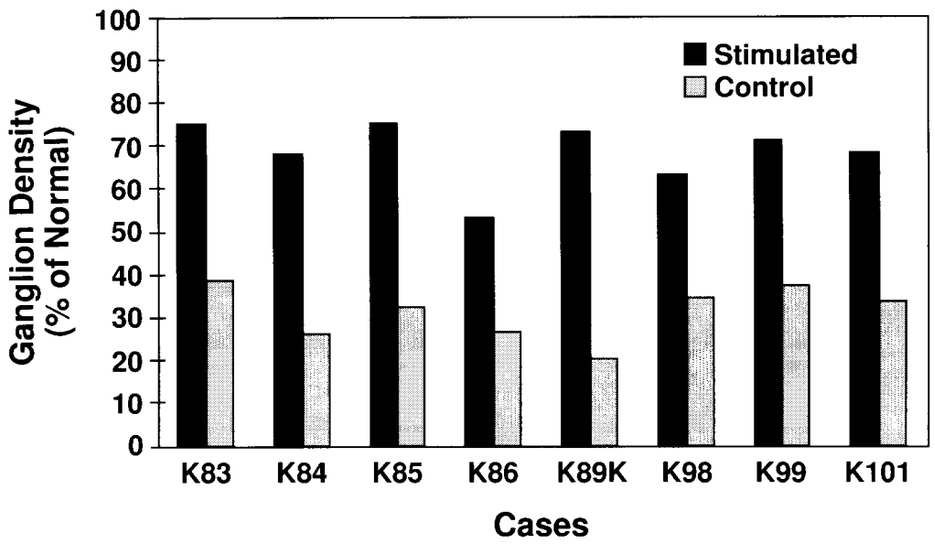



**Figure 2a**. Spiral ganglion density in the cochlear sectors with the greatest difference induced by chronic stimulation in the temporally challenging stimulation series (n=8; shown in Fig.1).

**Figure 2b. Eight Hi Frequency Cases**



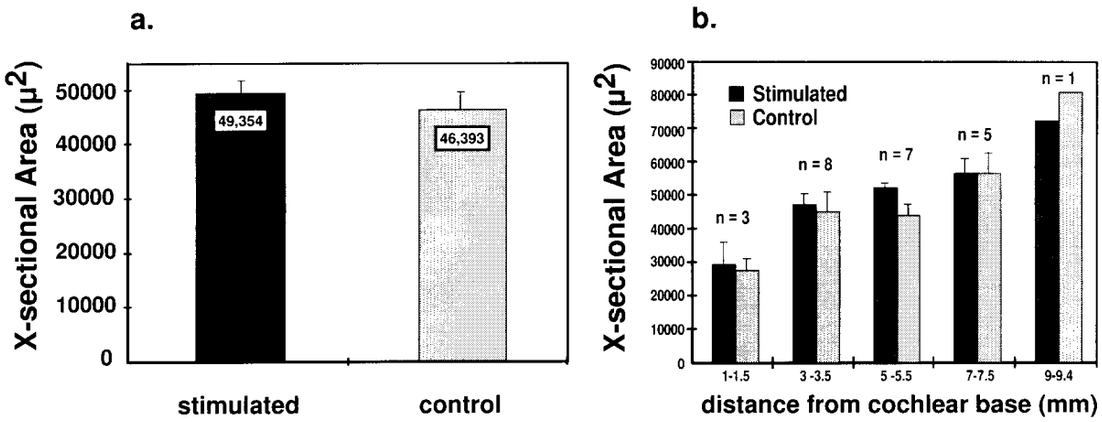



**Figure 3**. a. Mean cross-sectional area of Rosenthal's canal measured in the cochlear sections with the greatest difference in spiral ganglion density. b. Canal area for the individual cochlear sectors selected for analysis.

Figure 3 shows measurements of the cross-sectional area of Rosenthal's canal in these same cochlear sectors which showed such large differences in density. There is no significant difference between the stimulated and control sides in the pooled mean data (Fig. 3a). In fact, the mean value was actually slightly larger in the stimulated cochleas, which would tend to reduce the cell density as compared to the control data. Figure 3b presents these same data on Rosenthal's canal area again, showing data for the individual cochlear sectors selected for analysis. The difference in the absolute size of Rosenthal's canal shown in the 5 cochlear sectors emphasizes the normal regional variability in the size of the canal.

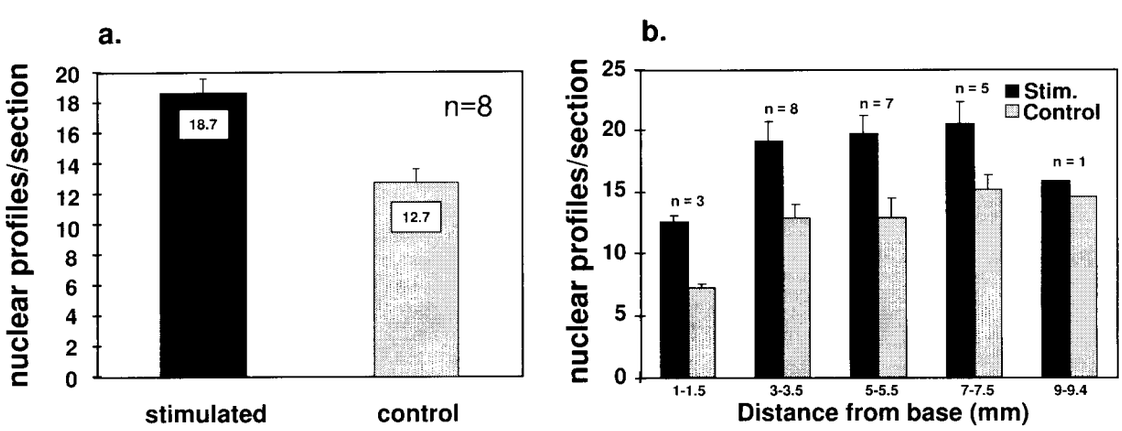



**Figure 4**. a. Mean absolute counts of spiral ganglion cells with clear nuclear profiles, again determined for regions with the greatest density difference. b. Data for individual cochlear sectors.

Figure 4a shows additional control data, comparing the absolute number of spiral ganglion cells with nuclei per section in these same cochlear sectors with the greatest

differences in neuronal density. A large, statistically significant increase in cell number is clearly demonstrated in the stimulated ears as compared to the control data. Again, the breakdown by cochlear sector is shown at the right.

We conclude from the data presented in Figures 2-4 that the cross-sectional area of Rosenthal's canal is not effected by electrical stimulation in these neonatally deafened cats. Rather, the large increases in cell density in the stimulated ears of these animals reflect actual differences in numbers of surviving neurons.

Our morphometric method for evaluating spiral ganglion density would also be sensitive to differences in cell size as well as the factors considered above (number of cells and area of Rosenthal's canal. This issue was addressed earlier (see QPR #11, April 1 to June 30, 1997, Contract N01-DC-4-2143) by measuring the diameters of spiral ganglion cells in normal, stimulated and deafened control cochleas. The data showed that the mean cell diameters (average of long and short axes) of neurons do decrease significantly in neonatally deafened animals from a mean of 22.4 μm for type I neurons in normal adult cats to 18 μm in the group of 8 animals in the temporally challenging stimulation series. Moreover, in long term deafened animals (>2.5 to 6.5 years after neonatal deafening) there is a further, significant reduction in cell diameter to a mean value of about 16 μm.

To determine if cell size differences contributed to the differences seen in spiral ganglion cell density in the temporally challenging stimulation group, we did 2 analyses. First, we measured cells throughout all cochlear sectors, and with more than 400 neurons per side, the mean for the stimulated and ears were *identical* -- 18.1 μm -- indicating that cell size does not contribute to density differences. Next, we again selected the two 10% cochlear sectors in each animal which showed the greatest stimulated vs. control difference in density. When we compared cell diameter data in those sectors, the mean value in the stimulated cochleas was 19.17 μm , was slightly larger than in controls which had a diameter of 18.93 μm. However, this small difference did not achieve statistical significance for the group comparison (P=0.227). Thus, these initial data suggest a small regional effect of stimulation upon cell size, which averages out across the entire cochlea and does not contribute significantly to the large differences seen in the spiral ganglion density data. However, this regional difference, if real, is potentially interesting, and we are currently examining the material in greater detail. Specifically, since the morphometric method for documenting cell survival is a density measure, the most appropriate comparison value is cross-sectional cell area. Therefore, instead of calculating area from diameter measurements, we are currently making direct measurements of cross-sectional areas of cell somata with clear nucleoli in digitized images of the sections in these regions, and also extending the analysis to include a larger **n** of cells. These data will be included in a future progress report.

In conclusion, the data currently available suggest that there is a highly significant decrease in spiral ganglion cell size after neonatal deafening, but only a small regional effect of electrical stimulation in reversing this shrinkage. Cell size does not appear to play a significant role in the large differences in cell density observed following chronic stimulation, although we are conducting a detailed examination of this regional effect to determine if it is significant.

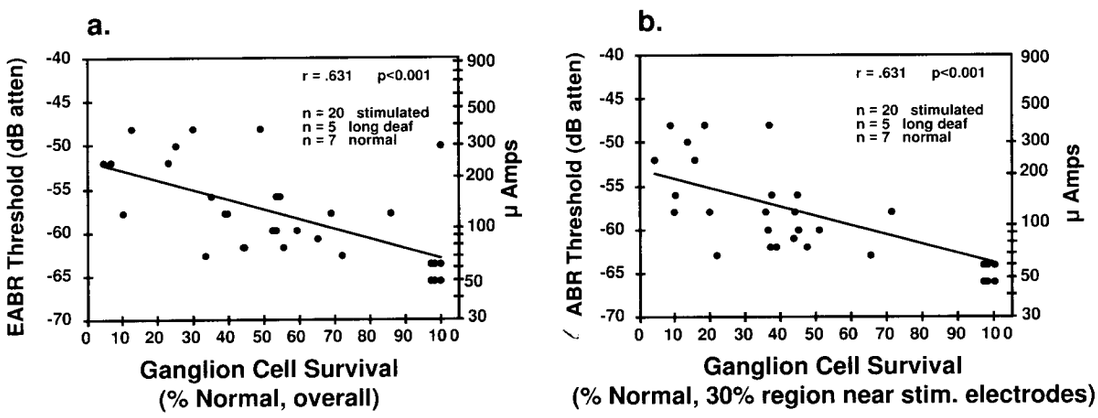
Finally, we believe that density is the most appropriate measure for evaluating regional differences in survival. The density of the spiral ganglion in normal cats is fairly uniform throughout the cochlea (unpublished data; see also Keithley, 1987, J. A.S.A. 81(4):

1036-1042) yet innervation density of IHC and the size and shape of Rosenthal's canal vary markedly. At the apex and extreme hook, there are as few as 10 spiral ganglion neurons per IHC, but in the mid-basal region where we see large differences in survival with stimulation, there are more than 30 spiral ganglion neurons per IHC (Liberman et al, 1990, J. Comp Neurol. 301: 443-460). Thus, in terms of the total absolute number of auditory neurons, our methods may well *underestimate* the impact of stimulation when by weight equally the percent survival in all cochlear sectors. However, we consider this methodology most appropriate for determining the *regional effects* of stimulation.

## Functional Correlates of Spiral Ganglion Survival: Electrophysiological and Psychophysical Data

In order to evaluate the functional consequences of varying extent of spiral ganglion cell survival we have examined correlations of cell survival and some of the electrophysiological and psychophysical measurements that have been made in the various groups of experimental animals.

**Figure 5. EABR Threshold vs. Spiral Ganglion Cell Survival**

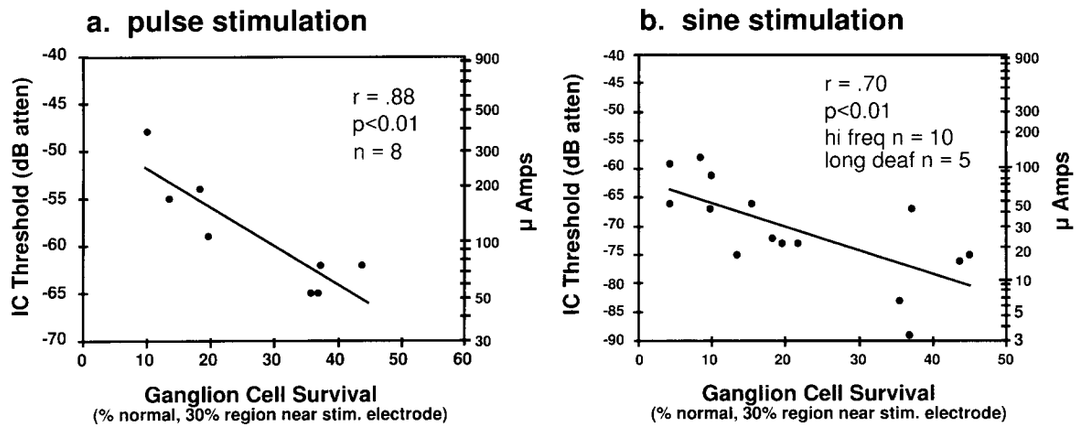



In Figure 5a the electrically evoked auditory brainstem response (EABR) threshold to 200 μsec/phase pulses is correlated to mean overall spiral ganglion cell density in 7 normal adult cats, 5 long deafened cats, and 20 chronically stimulated cats. Lower threshold is significantly correlated with better neural survival, but the variance in the data is great, and the correlation would not be significant if the extremes (normal vs. long deafened with very low survival) were excluded from the analysis. The great variability in the data for chronically stimulated animals is especially noteworthy, since many of the variables expected to contribute to intersubject variation are carefully controlled (e.g., identical intracochlear electrodes are used, insertion depths are similar, subjects are tranquilized for EABR measurements, etc.).

Figure 5b shows EABR threshold again as a function of spiral ganglion cell density, but in this case cell density is measured just in the three 10% cochlear sectors nearest the stimulating electrodes as determined for each implanted cochlea. The correlation coefficient is just the same as for overall survival (0.63). This suggests that generation of the EABR response, even at threshold, reflects rather broad neural activation.

Figure 6a correlates spiral ganglion cell density in the recent high frequency stimulation group to the minimum neural threshold in the inferior colliculus (as determined in final electrophysiological experiments in several penetrations through the IC). The signal used to determine threshold was the same 200 μsec/phase pulses used to estimate EABR thresholds. The correlation of lower neural thresholds with better spiral ganglion cell is quite good (r=.88). However, this correlation is ONLY found for the cell density value calculated for the 30% region near the stimulating electrodes, as determined for each individual implanted cochlea. This correlation breaks down completely for overall spiral ganglion cell survival averaged for all regions, suggesting that minimum neural threshold reflects local neural survival.

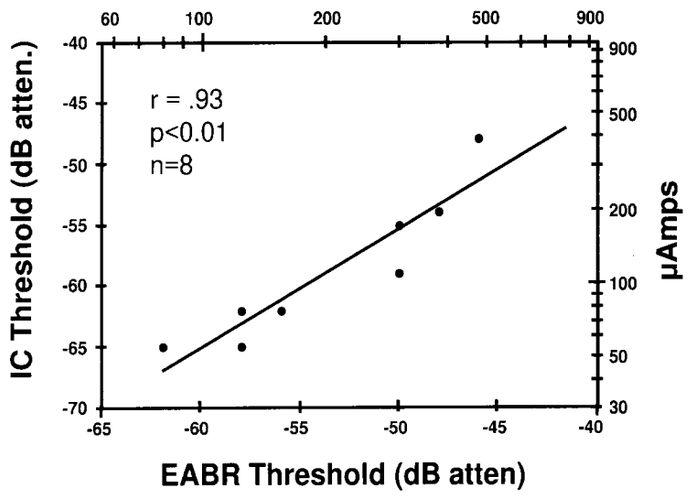
**Figure 6. IC Threshold vs. Spiral Ganglion Survival**




IC neural threshold was also measured for 100 Hz sines in most animals studied, including the long deafened cats with very poor spiral ganglion survival. Figure 6b presents data from both the long deafened and the temporally challenging stimulation groups, with sine thresholds correlated to regional spiral ganglion survival (again using just the 30% sector nearest the stimulating electrodes). The correlation (R=.70) is poorer than for pulse thresholds, but it is still significant.

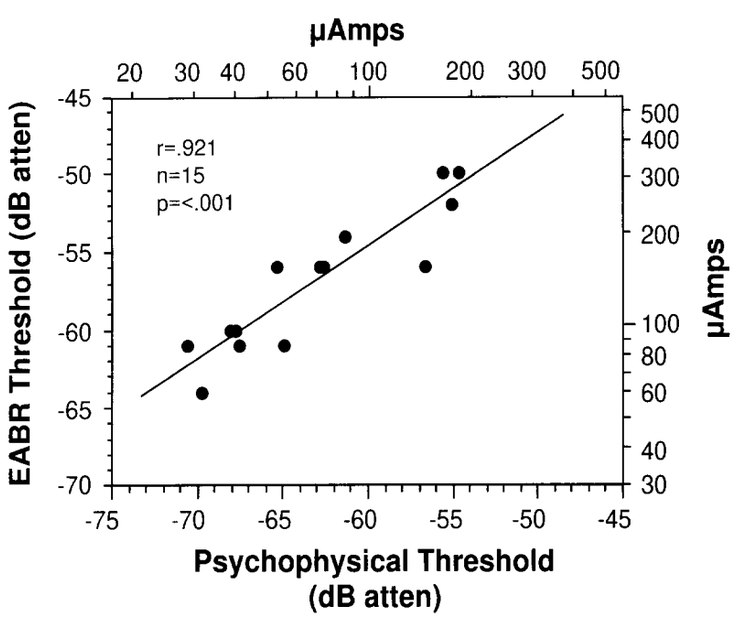
As expected based upon their correlations to ganglion survival, there is a good correlation (Figure 7) between the minimum IC neural thresholds and EABR threshold to 200 μsec/phase pulses in 8 cats for which complete pulse threshold data were collected (R= .93).

**Figure 7. IC Threshold vs. EABR Threshold**




Finally, Figure 8 relates EABR and psychophysical thresholds obtained by behaviorally training a subset of the chronically stimulated animals. The correlation coefficient. is .92, and psychophysical estimates of threshold average 6 dB lower. This may be due partly to the fact that in several cats higher frequency pulse rates were used to estimate psychophysical thresholds (e.g. 300 pps/30 Hz AM) giving slightly lower thresholds than the 20 PPS used in EABRs. But it is also likely that EABR threshold requires synchronous activation of a broader population of fibers than the population required for psychophysical detection threshold.

**Figure 8. EABR vs. Psychophysical Threshold**




We conclude from these data that these electrophysiological measures -- EABR and minimum IC threshold -- are correlated with spiral ganglion survival, at least for relatively large differences in the extent of pathology. Moreover, the two electrophysiological thresholds are correlated with each other and with psychophysical thresholds determined in the same animals. Taken together, these data suggest that extent of spiral ganglion degeneration is an important factor underlying functional thresholds and intersubject variability. Further, these findings emphasize the importance of understanding the mechanisms underlying neuronal degeneration after deafening and maintenance by electrical stimulation.

*Work Planned for the Next Quarter*

1) Two adult deafened, prior normal cats will be implanted. An acute electrophysiology experiment will be conducted during the next quarter in one animal in order to obtain additional control data for our current studies of the representation of amplitude modulated electrical stimuli in the inferior colliculus. The other animal will undergo chronic stimulation to increase the **n** in this adult deafened series. In addition, work on a manuscript documenting electrophysiological results in the temporally challenging stimulation series will continue.

2) Cochlear histopathology studies will continue, addressing the issue of whether there are significant *regional* differences in spiral ganglion cell size which contribute to stimulation induced increases in cell density. In addition, analysis of cochlear specimens from the first animals treated the GM1 ganglioside will begin. GM1 has been reported to potentiate growth factors which sustain the spiral ganglion neurons, and we hypothesize that treatment of these animals in the period after neonatal deafening and prior to cochlear implantation will further increase overall spiral ganglion survival. Additional animals in this series and one long term deafened cat will continue chronically stimulation.

3) Five members of the laboratory will attend the annual Association for Research in Otolaryngology Midwinter Meeting (February 15-19) and Dr. Leake, Dr. Vollmer and Ms. Moore will present results of this Contract research. The abstracts for these 3 presentations are appended to this Report.