3rd Quarterly Progress Report
April 1, 1995 to June 30, 1995

Fundamental Neurosciences Contract N01-DC-4-2143

Protective Effects of Patterned Electrical Stimulation on the Deafened Auditory System

Submitted by:

Patricia A. Leake, Ph.D.
Russell L. Snyder, Ph.D.
Ralph Beitel, 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 current focus in chronic electrical stimulation experiments has been to systematically vary specific stimulation parameters in order to define features that are critical in maximizing the "protective" effects on the auditory nerve. Specifically, studies have been initiated to evaluate effects of intracochlear stimulation with higher frequency stimuli that are designed to be temporally challenging to the central auditory system. In a previous Quarterly Progress Report (QPR #10 October 1-December 31, 1993; Contract N01-DC-2400), the spiral ganglion morphometric data were presented for the first four neonatally deafened kittens in this new intracochlear series. The data showed striking protective effects on spiral ganglion cell survival, with an overall mean survival of about 54% in the stimulated cochleas as compared to 30% in the control deafened but unstimulated cochleas -- an increase of about 24% as a consequence of stimulation.

This Quarterly Progress Report presents a summary of histological findings and morphometric analyses of spiral ganglion cell survival in cochlear specimens from 5 additional neonatally deafened cats in this new chronic stimulation series. Three of these cats received passive, invariant stimulation with continuous trains of biphasic pulses (200 μsec/phase) delivered at 800 pps and sinusoidally amplitude modulated at 30 Hz. The other 2 animals were stimulated with a processor that transduced environmental sounds detected by a portable microphone. The latter 2 cats also received extensive behavioral training to determine psychophysical thresholds to specific electrical stimuli.

During the course of chronic stimulation, 3 of these animals showed markedly elevated EABR thresholds, and the two behaviorally trained cats showed parallel elevations in psychophysical thresholds. Histological studies documented profound cochlear pathology and severe spiral ganglion degeneration correlated with these threshold elevations. Morphometric studies comparing regional spiral ganglion cell density in the stimulated cochleas with that in the contralateral control ears showed an increase of about 11% in neuronal survival as a consequence of electrical stimulation. However, the mean cell survival averaged over all sectors in the stimulated cochleas was only 34.8% of normal, while the mean for the contralateral control deafened cochleas was only 23.4%. These results present a sharp contrast to the findings from the previous group of experimental animals that received very similar schedules of chronic stimulation. Some of the possible specific causes of the pronounced differences between these experimental groups are discussed.

Initial 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 80 pps stimuli induced a significant "protective effect," partially preventing the degeneration of spiral ganglion cells in neonatally deafened animals.

Subsequently, a new intracochlear experimental series was initiated in which various aspects of the electrical stimulation have been varied, in order to define specific parameters that are critical in maximizing the "protective" effects on the auditory nerve. In a previous Quarterly Progress Report (QPR #11: October 1-December 31, 1993; Contract N01-DC-2400), spiral ganglion morphometric data analyses were reported for the first four neonatally deafened kittens from this new intracochlear series. This group included 2 animals (K83, K85) that received chronic passive intracochlear stimulation using 80 pps (200 μsec/phase) and two additional cats (K84, K86) that received temporally and intensity varying stimulation though a single channel operational prosthesis. These latter animals also received extensive behavioral training to determine psychophysical thresholds for selected electrical stimuli. Table 1 shows the stimulation histories for these animals. Stimulation sessions were 4 hours daily, 5 days a week, continuing for periods of 5-11 months. In all cases, the bipolar intracochlear electrodes (apical pair 1,2) were positioned in the cochlear location corresponding to about 3-7 kHz (about 45% from the cochlear base).

Since morphometric data documenting spiral ganglion cell survival in these four cats were reported in detail previously, results are only summarized here for direct comparison to data obtained during this past quarter from 5 additional cats in this new intracochlear stimulation series. The volume ratio (density) for each cochlear segment (from base to apex) in the left chronically stimulated cochlea was compared with the paired data from the contralateral deafened, unstimulated ear and expressed as percentage of normal for animal. The results showed striking increases in neuronal survival as a consequence of chronic stimulation. Regional increases in spiral ganglion cell survival of 40% or more were seen in each stimulated cochlea as compared to data from the contralateral deafened ear.

Table 1. Higher Frequency/Temporally Challenging Stimulation

Cat #	Age at Surgery	Age at Initial Stimulation	Stim. Current	Stim. Period	Stim. Frequency	Age at Sacrifice
K83	6 wks	10.5 wks	125 μA	21 wks*	80 Hz	32 wks
K84	7.5 wks	10 wks	200-400 μA	35 wks	SP/ beh.	45 wks
K85	7 wks	10 wks	125 μA	42 wks	80 Hz	52 wks
K86	6 wks	9 wks	30-160 μA	44 wks*	SP/ beh.	55 wks
			*******			
K89	6.5 wks	10.5 wks	80-100 μA	26.5wks	300/ 30 Hz	37wks
K91	6 wks	6.4 wks	100-400 μA	31.5 wks	300/ 30 Hz	38.5 wks
K92	6 wks	6.4 wks	150 μA	23 wks*	300/ 30 Hz	31 wks
K93	7 wks	8 wks	40-400 μA	36 wks	SP/ beh.	44 wks
K94	7 wks	8 wks	40-500 μA	34 wks	SP/ beh.	42 wks

*indicates that the electrode was dislodged by the animal and had to be replaced in order to complete the chronic stimulation period

Figure 1 summarizes the morphometric data for the four cats, K83-K86, showing the mean stimulated less control difference in spiral ganglion cell density. Averaged over all cochlear sectors, the mean difference in neuronal density was 24% -- a much greater difference than that seen in previous experiments using 30 Hz pulsatile stimuli with either intracochlear stimulation (13% difference in ganglion cell survival) or extracochlear stimulation (6% difference). The specific parameter(s) of stimulation responsible for the striking "protective effect" in this experimental group are not clear. In addition to using the temporally challenging stimulation in these experiments, it was possible to maintain implanted animals for longer stimulation periods (up to 10 and 11 months in K85 and K 86). This may account in part for the large differences in neuronal survival-- since there was more time for the contralateral spiral ganglion to degenerate. However, case studies and comparisons between the data from individual animals in the different experimental groups suggest that other parameters of stimulation (e.g. frequency of stimulus, intra- vs. extracochlear mode) may also be critically important in maximizing the "protective effect" of electrical stimulation on the auditory nerve.

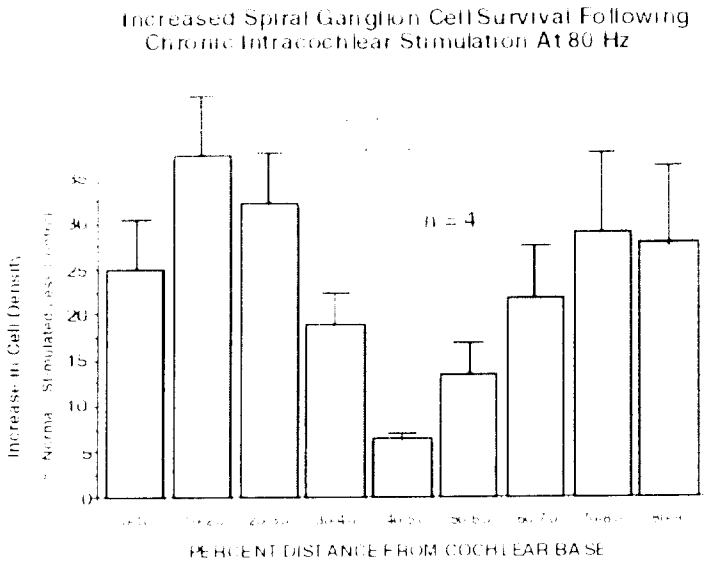

Figure 1 Striking increase in spiral ganglion survival induced by chronic intracochlear electrical stimulation in the new intracochlear experimental series of neonatally deafened cats. The data are pooled from 2 animals chronically stimulated with 0.2 ms/phase pulses at 80 Hz (K83, K85) and 2 animals stimulated via a single channel cochlear prosthesis (K84, K86) processor that transduced environmental sounds. Data are shown as mean stimulated less control data for spiral ganglion cell density, expressed as percent of normal for each cochlear sector 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 four cases. Overall spiral ganglion cell density was increased by approximately 24%, and this difference was highly significant ($P < 0.001$, Student's paired t-test).

New Data from Higher Frequency/Temporally Challenging Intracochlear Stimulation Series

In order to confirm the above results and to further explore the effects of modulating specific parameters of electrical stimuli, additional animals were implanted and chronically stimulated. During the past quarter morphometric analyses of spiral ganglion cell survival were completed in 5 more cats in this higher frequency/temporally challenging chronic stimulation group (Table 1). Three of these neonatally deafened cats (K89, K91, K92) were chronically stimulated with pulses (biphasic, 200 usec/phase) delivered in continuous trains at 300 pps, and 100% amplitude modulated at 30 Hz. Two additional 2 cats (K93, K94) were stimulated with an operational speech processor which transduced environmental sounds to an analogue electrical signal; these animals also received extensive behavioral training to determine thresholds to selected electrical stimuli. The daily stimulation periods (4 hrs./day, 5 days/week), intensity of stimulation (2 dB above EABR threshold for 300 Hz/30 Hz stimulation and a

6 dB dynamic range: 0 to 6 dB above EABR threshold for the processors) and duration of stimulation were matched as closely as possible to the previous series (see Table 1). The mean stimulation period for the K89-K94 group was 30.2 weeks, whereas for the earlier experimental group, K83-K86, this value was 35.5 weeks. All animals (in both groups) were stimulated with the apical bipolar pair positioned in the scala tympani at ~35-45% from the base of the cochlea.

It should be noted that 3 of the cats in this group exhibited elevations in thresholds during their chronic stimulation periods, as reflected by the final stimulation levels shown in Table 1. Chronic stimulation levels are always adjusted relative to EABR thresholds, which are determined twice a month. Two animals (K91 and K93) had final chronic stimulation levels of 400 μA and one animal (K94) had a final chronic stimulation level of 500 μA. In K92, although the final stimulation level was 150 μA, at the time of the final electrophysiology experiment in this animal the EABR threshold had shifted up to 310μA. Behavioral thresholds to pulses were also determined in three of these animals (K91, K92, and K93) during chronic stimulation periods. In all cases, behavioral thresholds were very close to EABR thresholds and were also unusually high in these animals compared to the previous intracochlear experimental series.

Histological evaluations of the implanted and stimulated cochleas from this group of animals presented some highly unusual findings. Figure 2 illustrates the histopathology observed in K 91, one of these animals with elevated thresholds. The section was taken from the region 8.5-9 mm from the cochlear base, adjacent to the most apical electrode, contact #1, which was one of the stimulating bipolar pair (1,2). Severe hemorrhage was observed in the modiolus and total degeneration of spiral ganglion cells in Rosenthal's canal over a 1.5 mm region directly adjacent to the stimulating electrodes. Extensive ectopic bone formation was also observed at more basal locations along the electrode carrier (Figure 3), although neuronal survival was greatly improved in this area.

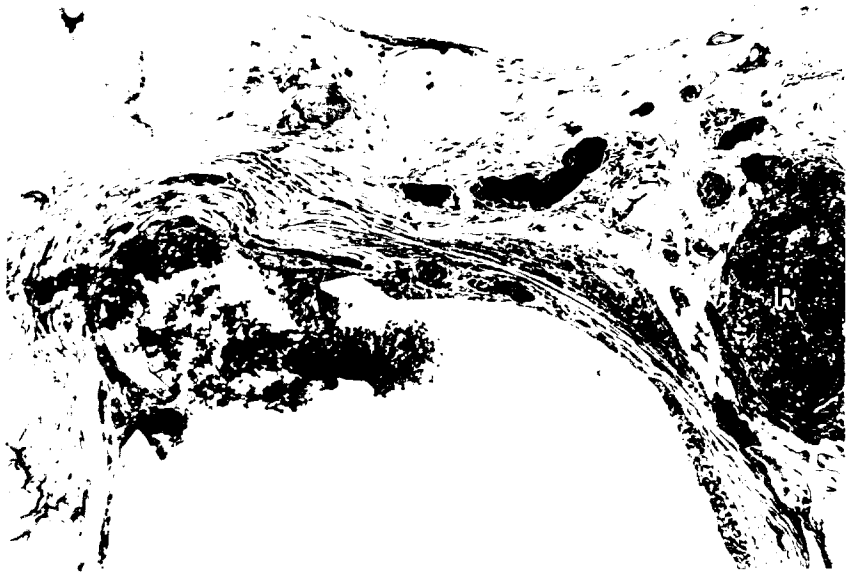

Figure 2. Striking histopathology observed in K91, one of the neonatally deafened, chronically stimulated cats in the new intracochlear experimental series. This section was taken at a site approximatedly 8.7 mm from the basal extreme of the cochlea, adjacent to electrode #1 which was one of the bipolar pair of electrodes used for chronic stimulation. The micrograph shows the severe hemorrhage in the modiolus, which resulted in complete degeneration of the spiral ganglion neurons in Rosenthal's canal (R). This extreme pathology extended over a 1.5 mm region subtending the stimulating electrodes.

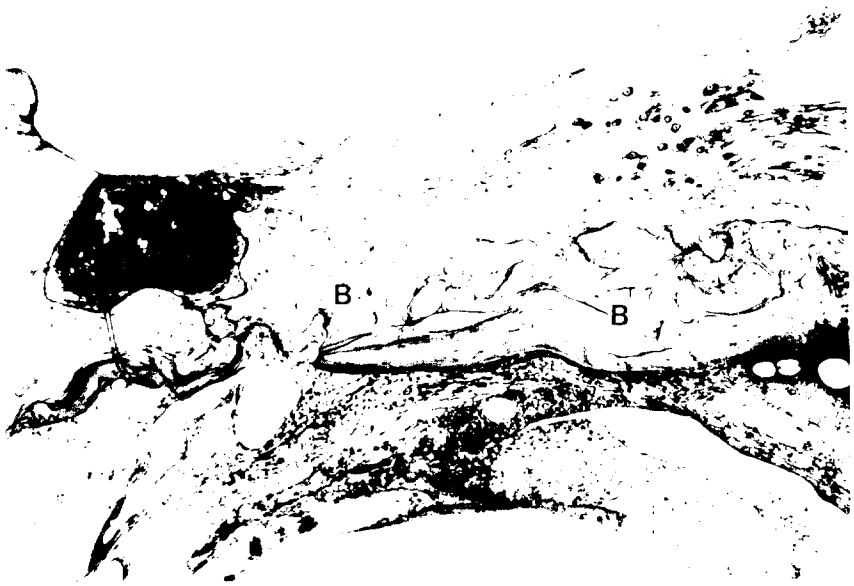

Figure 3. At a more basal region in K91, extensive new bone (B)growth was noted between the electrode and all the overlying structures of the scala tympani. It is clear that this degree of osteoneogenesis would displace the electrodes away from the excitable neuronal elements and tend to insulate them. The section was taken near the round window at a location about 3.5 mm from the base.

Extensive ectopic bone formation was also observed in two other cats in the group. In K92 new bone formation was observed over a region of approximately 7 mm in relation to the electrode carrier and primarily under the spiral ligament and adjacent to the modiolus (Figure 4). In K93, massive new bone was noted during the initial dissection of the cochlea. This dissection is done in order to visualize and record the placement of the stimulating electrodes in the scala tympani. In this cochlea, ectopic bone partly occluded the scala vestibuli in the region of the stimulating electrodes, and their location had to be determined by opening the scala tympani from below and from the positions of the more basal electrodes which could be visualized from the scala vestibuli through the basilar membrane. Figures 5 and 6 show histological sections illustrating the extensive bone formation throughout the region occupied by the electrode carrier in this cochlea. Sections through the region showed that new bone completely surrounded the electrode carrier from 7 to 10.5 mm. In the region overlying the stimulating electrodes, the scala media was completely ossified and ectopic bone extended far into the scala vestibuli.

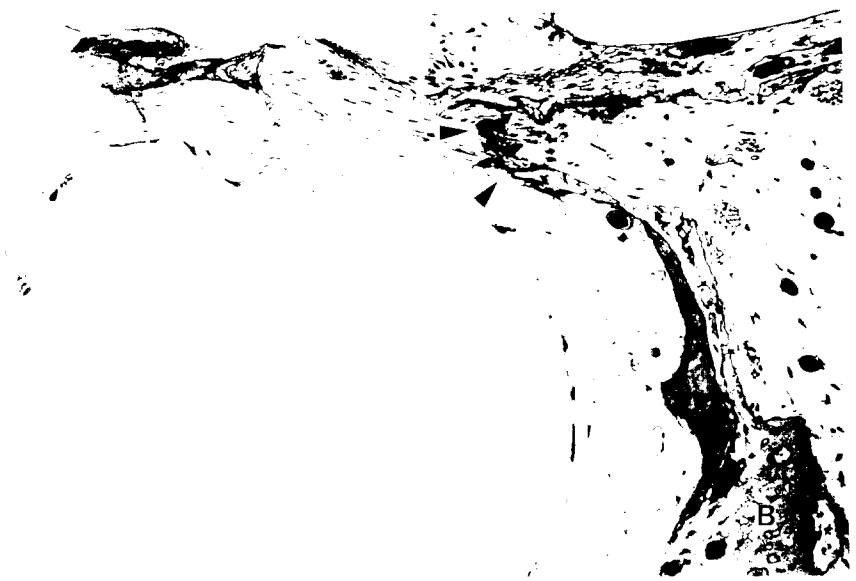

Figure 4 Osteoneogenesis was observed in K92 over most of the region occupied by the electrode carrier within the scala tympani. This section was taken directly adjacent to electrode #1, one of the chronically activated electrodes. Arrows indicate ectopic bone adjacent to the modiolus and Rosenthal's canal and within the scala media. The spiral ligament was also ossified throughout much of the region. Moderate insertion damage is also evident, with a small fracture of the osseous spiral lamina (arrowheads). As a consequence of this widespread pathology, spiral ganglion degeneration is very severe in this region.

Figure 5 Very extensive osteoneogenesis was observed in K93. New bone surrounded the electrode carrier within the scala tympani over the region 7-10 mm from the base. This section taken 7 mm from the base shows new bone (B) separating the electrode from the basilar membrane, the osseous spiral lamina, the spiral ligament, and the lateral cochlear wall. Only a few spiral ganglion cells (G) have survived.

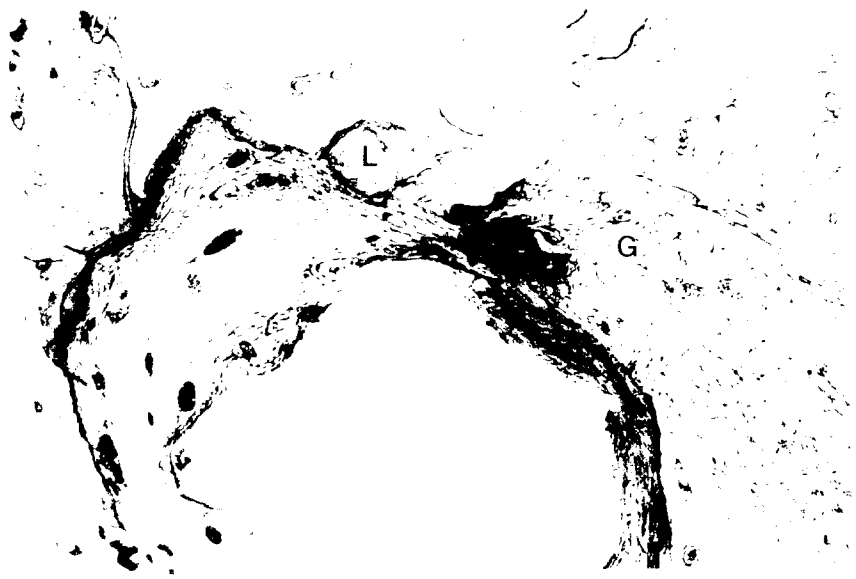

Figure 6 Another example of the histopathology observed in K93. This section was taken directly adjacent to chronically stimulated electrode #1 at approximately 9.7 mm from the base. The spiral limbus (L) and scala media have become completely ossified and new bone extends far into the scala vestibuli. Degeneration of spiral ganglion cells (G) is very severe in this region.

In the other two cats in the series (K89 and K94), the cochlear histopathological findings also included some osteoneogenesis, but it was less extensive and did not appear to displace or insulate the stimulating electrodes from the spiral ganglion cells in Rosenthal's canal (or from the peripheral fibers, if present, in the osseous spiral lamina). In K 89, there was significant insertion trauma near the tip of the electrode, and also more basally near electrode #3. The primary pathology in K94 also appeared to be related to insertion trauma, with a fracture of the osseous spiral lamina in the region adjacent to the apical electrode pair (1,2).

Morphometric data documenting the volume ratio (density) of spiral ganglion cells in Rosenthal's in these five individual cases are shown in Figure 7. The neuronal survival in the chronically stimulated cochleas are shown for each cochlear segment (from base to apex) by the black data bars, and the paired data from the contralateral deafened, unstimulated ears are shown in the striped data bars. As in the previous experimental group, a few regions in several of these cases showed very large differences in neuronal survival as a consequence of chronic stimulation. For example, the 10-20% sector of K93 shows an increase of over 50% in neuronal survival in the stimulated cochlea as compared to the same region in the control deafened cochlea; and K89 shows an increase of more than 60% in the 0-10% region and an increase of about 45% in neuronal survival in the 10-20% sector.

Two of the most striking features of these data are the great variability among individual animals and the very low survival in the middle cochlear sectors (30-60%) near the stimulating electrodes (which were always located 35-55% from the basal extreme of the cochlea). The pattern and degree of ototoxic drug-induced ganglion cell degeneration can be inferred from the data in the contralateral deafened, unstimulated cochleas. It is known that spiral ganglion degeneration is progressive over time after deafening (which took place at birth in these animals). Thus, considering the extended periods of implantation and chronic stimulation in these animals, it is not surprising that relatively severe degeneration has occurred. However, the degeneration seen in K93 (< 20% survival all but the basal 10% sector) and K91 (<10% survival over the sectors 20-80%) is much more severe than that noted in any specimens in the previous series, which included cats with even longer survival periods (e.g., K86 ad K85). In all five animals the intracochlear electrode caused a variable amount of mechanical damage to

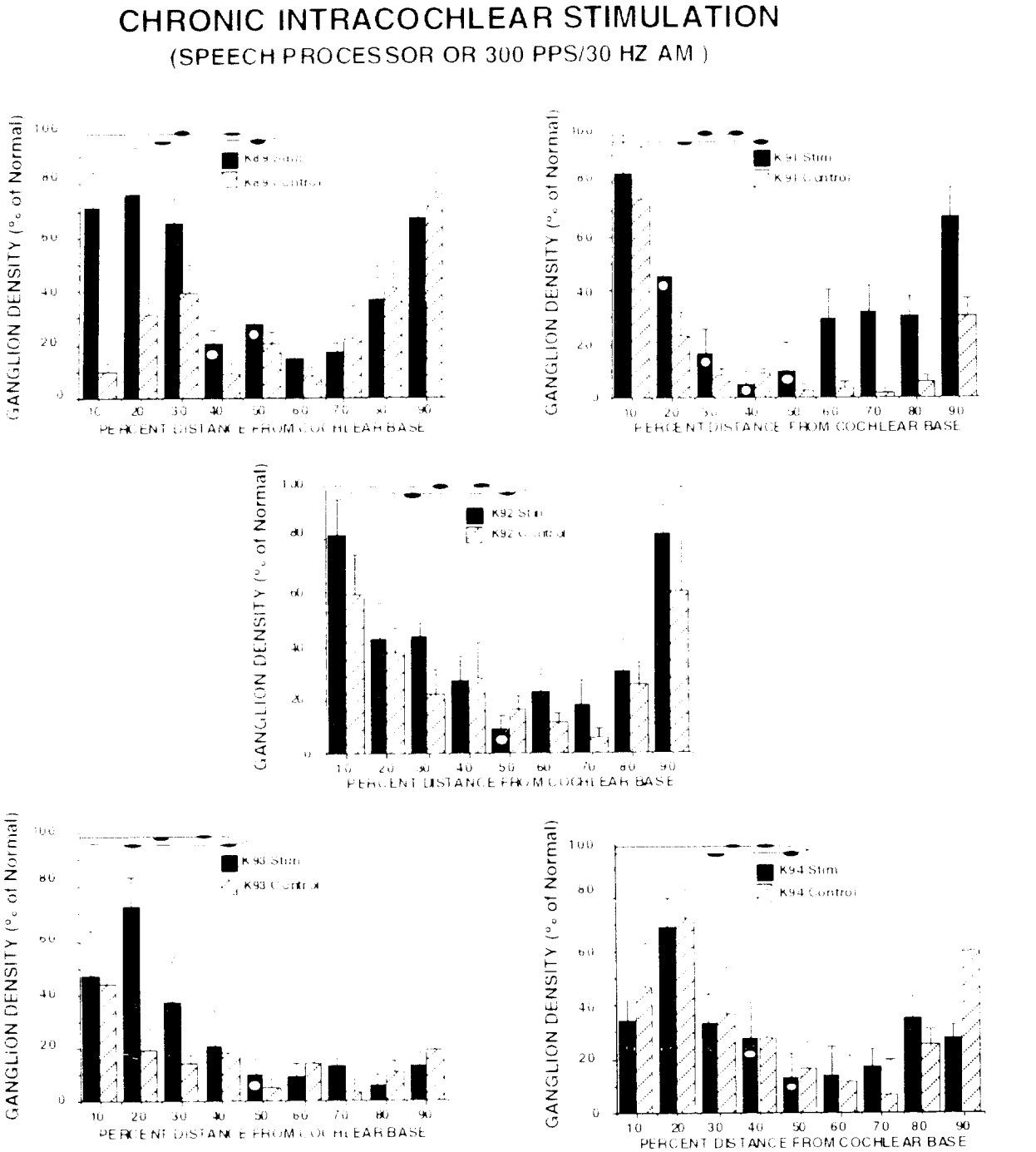

Figure 7. Individual data from 5 neonatally deafened cats in the new higher frequency/temporally challenging intracochlear stimulation series. The first 3 cats (K89, K91 and K92) were stimulated with the 300 pps/ 30 Hz paradigm. K93 and K94 were stimulated with the speech processors and received behavioral training. Morphometric data show spiral ganglion volume density in stimulated ears (black data bars) compared to data from contralateral deafened control ears (striped bars) for cochlear segments from base to apex. White markers indicate sectors in which mechanical damage from insertion of the cochlear implant was observed.

the osseous spiral lamina and/or basilar membrane in the 40-50% region, and in 3 out of 5 cases, damage was also seen in the 30-40% region (as indicated by white dots on the data bars in Figure 2). This damage probably accounts for part for the drop in ganglion cell density in those sectors of the stimulated cochleas.

Figure 8 summarizes the morphometric data in this new experimental group, showing the mean stimulated less control increases in spiral ganglion cell density. The mean difference in neuronal density, averaged over all cochlear sectors, is 11%. Given the unusual and severe nature of the histopathology observed in the stimulated cochleas and the obvious drop in spiral ganglion survival in the 30-50% region, we believe that these findings are strongly indicative of stimulation induced damage to the cochlear neurons. As in the previous experimental series, these animals were maintained in chronic stimulation for extended periods (up to 8-9 months in K93 and K94). However, in comparison to the data shown in Figure 1, these data provide a striking contrast to earlier results, again clearly suggesting that severe damage was induced in the regions nearest to the stimulating electrodes.

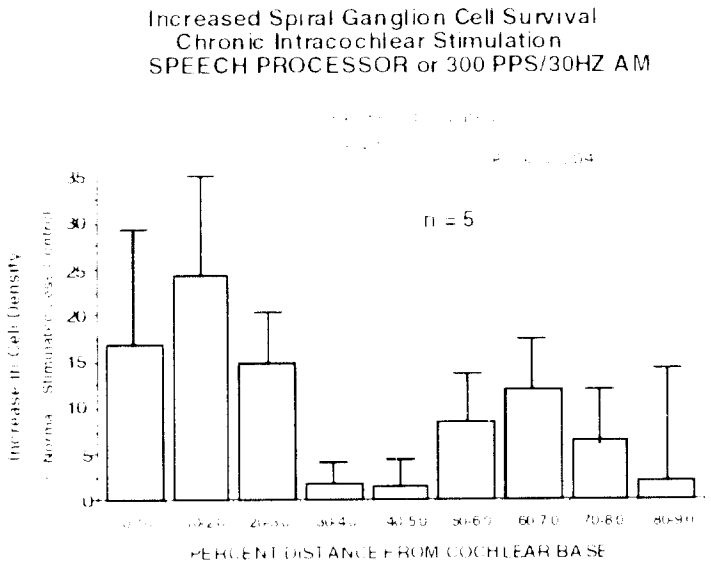

Figure 8. Difference in spiral ganglion survival induced by higher frequency chronic intracochlear electrical stimulation in the new experimental series. The data are pooled from the 5 neonatally deafened animals shown in Figure 7. Mean stimulated less control data for spiral ganglion cell density are expressed as percent of normal for each cochlear sector (i.e. increases in neuronal survival). The prominent drop in neuronal survival seen in the regions 30-50%, from the base are interpreted as stimulation induced damage.

The degree to which these results relate to the various specific parameters of stimulation is not yet clear. We believe the damage may have been cause by a few episodes when a stimulator went into oscillation during EABR testing in these animals. However, there was also one feature of the electrodes that was different in this group as compared to the previous series: slightly smaller electrode contacts (~175 µm - 200 µm) were used to allow the fabrication of more sophisticated, multielectrode arrays. These smaller contacts exhibited somewhat higher impedances initially and further increases over time, so that with the elevated thresholds in some cases the compliance voltage of the battery driven stimulators used for chronic stimulation in K93 and K94 may have been exceeded. Finally, at present we cannot completely rule out the possibility that the continuous, higher frequency stimulation may have been damaging in the animals in which the 300 pps / 30 Hz paradigm was employed (K89, K91, K92). All animals subsequent to this series have been implanted and chronically stimulated with completely newly designed electrodes, incorporating larger contacts (~250µm). Initial thresholds have been very low in this group and so far are quite stable. Therefore, our strategy will be to treat the K89-94 group as exceptional and to try to replicate the striking protective effects seen in the original K80 series animals, using the same stimulation strategies (speech processors and 300 pps / 30 Hz paradigm).

This stimulation damage group comprises a very interesting group for contrasting with previous series in which stimulation had a more robust effect in maintaining the auditory neurons. It provides the opportunity to compare EABR, electrophysiological and in some cases behavioral data from a group of neonatally deafened, chronically stimulated animals with very poor spiral ganglion survival in the immediate region of the stimulating electrodes. For example, one very important question is the degree to which spiral ganglion cell survival determines various parameters of implant function? Figure 9 illustrates that for these cat studies, EABR threshold *was correlated* with the % survival of auditory neurons. In fact, with our bipolar intracochlear electrodes, the correlation was better if it was made using just the regional neuronal survival, calculated for the 30° cochlear sector adjacent to the stimulating electrodes, as opposed to overall neural survival throughout the cochlea. These data are widely scattered, and it seems clear

that a number of other factors such as exact electrode location, bone formation, etc. clearly are significant determinants of threshold. In fact, it appears that only relatively large differences in neural survival are correlated with absolute threshold measures.

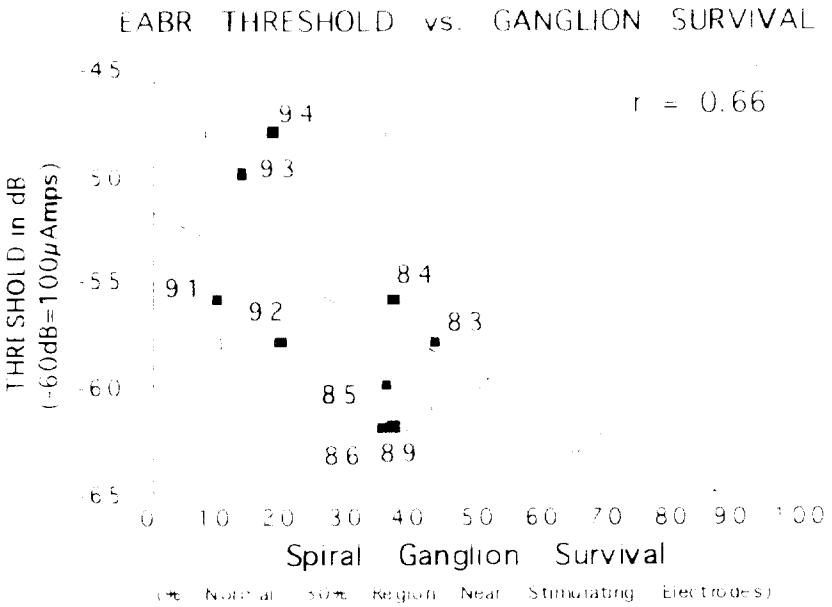

Figure 9. Final EABR threshold is correlated (r = 0.66) with spiral ganglion survival in neonatally deafened, chronically stimulated cats. The spiral ganglion survival was calculated for the 30% sector closest to the stimulating electrodes in each cat. The K83-86 and K89-94 series animals discussed above are indicated by the filled symbols.

Figure 10 shows the subset of animals from these groups for which behavioral thresholds have been determined, including the 4 cases discussed above with the large stimulation induced increase in spiral ganglion survival and 3 of the recent cases with stimulation related damage cases. These animals form two quite separate groups and thus may give a spuriously high correlation. However, we would assume that this degree of difference in both physiological (EABR) and behavioral thresholds probably would have an effect on channel interaction in a multichannel implant and thus effect implant performance.

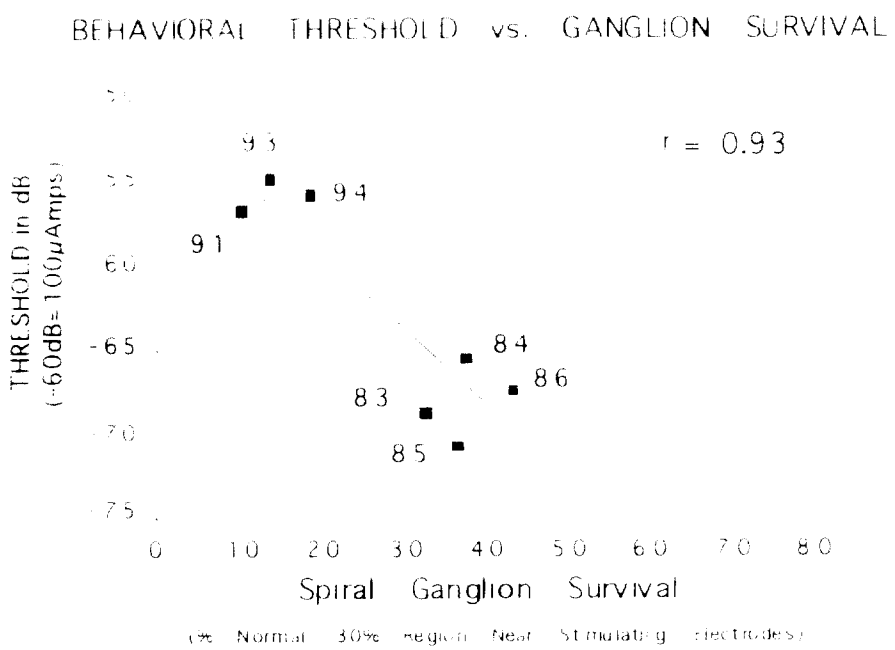

Figure 10. Behavioral thresholds (200 μsec/phase pulses) are highly correlated (r=0.93) with spiral ganglion cell survival in the 30% sector nearest the intracochlear stimulating electrodes. The subset of animals in which these data are available includes two clearly distinct groups: 1) the four cats in which the largest increase in spiral ganglion survival was seen after chronic stimulation (K83-K86) and 2) three of the stimulation-damage cases (K91-K93).

Work Planned for the Next Quarter

1) Two adult-deafened cats have recently been implanted with the new model UCSF cat electrode and EABR thresholds have now stabilized at acceptable levels. During the next quarter, these animals will be chronically stimulated using a temporally challenging (but passive and invariant) electrical stimulus (300 pps amplitude modulated with a 30 Hz sinusoid). These animals will eventually be studied in acute electrophysiological experiments to evaluate spatial selectivity (STC widths) in the inferior colliculus and AI and thus to determine if functional alterations observed in neonatally deafened cats after chronic stimulation are also observed in adult-deafened subjects. Moreover, studies of spiral ganglion cell survival and cochlear nucleus morphology should allow us to determine whether the protective effects of chronic electrical stimulation previously observed in neonatally deafened cats are dependent upon critical periods of development or, alternatively, can also be induced in animals deafened as adults.

2) Four neonatally deafened kittens have been implanted and are currently undergoing chronic electrical stimulation. Chronic stimulation in the first two kittens has been initiated using an operational prosthesis which consists of a single channel speech processor that responds to sounds in the animals' environment and generates an analogue electrical signal delivered to the intracochlear electrode. Behavioral training has also been initiated in these animals. The other two kittens are receiving passive chronic stimulation with temporally challenging electrical stimulus (300 pps, amplitude modulated with 30 Hz sinusoid).

3) Histological processing of the cochlear nucleus specimens has been completed for several chronically stimulated cats in a group that showed marked protection of the spiral ganglion as a consequence of chronic electrical stimulation. Morphometric analyses including volume of individual subdivisions of the cochlear nucleus, neuronal cell density and cross-sectional area of large spherical cells in the AVCN has been initiated and will be continued throughout the next quarter.

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

BERKELEY • DAVIS • IRVINE • LOS ANGELES • RIVERSIDE • SAN DIEGO • SAN FRANCISCO • SANTA BARBARA • SANTA CRUZ

PATRICIA A. LEAKE, PH.D.
DEPARTMENT OF OTOLARYNGOLOGY - U-0901
UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
SAN FRANCISCO, CA 94143-0526

Saul and Ida Epstein
Hearing Research Laboratory
Telephone (415) 476-5958
Fax (415) 476-1941

July 31, 1995

Dr. F. Terry Hambrecht
Head, Neural Prosthesis Program
Division of Fundamental Neurosciences
NINDS, National Institutes of Health
Federal Building, Room 916
7550 Wisconsin Avenue
Bethesda, Maryland, 20892

Dear Terry:

Please find enclosed the third Quarterly Progress Report for our Contract entitled "Protective Effects of Patterned Electrical Stimulation on the Deafened Auditory System." In this report we have presented a summary of cochlear histopathology and morphometric evaluation of spiral ganglion survival in neonatally deafened animals undergoing chronic stimulation designed to be temporally challenging to the central auditory system. The big difference between the stimulation damage cases described in this Report and the initial animals in this experimental series is really very intriguing, but I wish I had a definite answer about the cause of the damage.

Alena and I hope to have the Asilomar conference program and abstracts book be ready to go off to the printer for copying and binding on August 1, and I am still hoping to mail it out to moderators so they will have the abstracts a couple of weeks before the meeting. I'm looking forward to seeing you at Asilomar and hope we have a chance to visit some there.

Best wishes,

Patricia A. Leake, Ph.D.
Professor in Residence
Research Director, Epstein Lab