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Protective Effects of Patterned Electrical Stimulation on the Deafened Auditory System

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ABSTRACT

This Progress Report presents recent results from a study of the effects of deafness and chronic electrical stimulation on spiral ganglion (SG) cell survival in cats deafened as adults. Our previous studies of neonatally deafened cats have shown that chronic electrical stimulation with a cochlear implant prevents degeneration and increases survival of SG neurons. However, other laboratories studying the effects of electrical stimulation in adult deafened animals have reported conflicting results.

The study included six normal hearing adult cats that were deafened by co-administration of kanamycin and ethacrynic acid. Cats were implanted 1-2 weeks after deafening. The stimulation protocol was identical to one used in the previous studies of neonatally deafened cats, and consisted of continuous stimulation with a 300 pps carrier sinusoidally amplitude modulated at 30 Hz (4 hr./day, 5 days/week). Current levels were set at 2 dB above individual EABR thresholds.

Morphometric methods for assessing cochlear pathology and SG cell density were identical to those used in previous studies of neonatally deafened cats. Histological data were obtained in one animal prematurely, due to anesthetic death. After 11 weeks of stimulation (13 weeks after deafening) in this cat, overall SG survival was ≅60% of normal and there was no difference between the stimulated cochlea and the unstimulated deafened ear. In two other animals, the implanted cochleas showed chronic labyrinthitis and endolymphatic hydrops, and these cases were deleted from the study of the effects of stimulation.

The remaining 3 cats were studied after 21-28 weeks of electrical stimulation. The deafened control cochleas showed progressive loss, and with the longer duration of deafness in these 3 subjects the mean overall survival was 38% of normal. The stimulated cochleas of these animals showed a mean overall SG density of ≅48% of normal, an increase of ≅10% over the density in the control deafened ears. Comparisons of neonatally- and adult-deafened cats indicate that the time course of SG degeneration is similar in the two groups.

These preliminary results suggest that chronic electrical stimulation of the cochlea can ameliorate degeneration and maintain survival of the auditory neurons in adult deafened animals, however differences are observed only following stimulation periods of 5-6 months. Moreover, the increase in SG cell density induced by electrical stimulation for a given duration may be somewhat less in adult deafened subjects than in neonatally deafened animals.

PROTECTIVE EFFECTS OF CHRONIC ELECTRICAL STIMULATION IN ADULT DEAFENED ANIMALS

INTRODUCTION

This Quarterly Progress Report presents initial results from a study of the effects of deafness and chronic electrical stimulation on spiral ganglion (SG) cell survival in cats deafened as adults, after normal development and a lifetime of normal auditory experience. Our previous studies have been conducted primarily in neonatally deafened cats that have no normal auditory experience during development and are considered to model congenital or very early acquired profound sensorineural hearing loss. Research in these neonatally deafened animals has shown that chronic electrical stimulation with a cochlear implant, even for a just a few hours per day, 5 days a week, over a period of several months will show a marked effect on neural survival, preventing degeneration and increasing survival of SG neurons (QPR #1, Contract N01-DC-7-2105, October 1, to December 31, 1997).

Other laboratories studying the effects of electrical stimulation in adult deafened animals have reported conflicting results. Three studies in guinea pigs have suggested that chronic electrical stimulation can also induce protective effects on spiral ganglion neurons in animals that have matured normally and are deafened and stimulated as young adults (Lousteau, 1987, Laryngoscope 97: 836-842; Hartshorn et al., 1991, Otolaryngol. Head Neck Surg. 104: 311-319; Miller et al., 1992, In Henderson, Salvi and Hamernik, eds., Mosby Year Book 130-145). However, other investigators, including Shepherd who studied kittens deafened at 1 month of age rather than neonatally (Shepherd et al., 1994, Hearing Res. 81: 150-166) and Li, et al. who studied guinea pigs (Li, Webster and Parkins, 1997, Abstr. ARO #314, 20:79) have found *no evidence* of a neurotrophic effect of electrical stimulation on spiral ganglion cell survival.

The goal of the present study was to determine whether these conflicting results may be related to species differences or differences in stimulation protocols or morphometry methods. Alternatively, if developmental "critical periods" play a pivotal role in the marked protective effects of electrical stimulation seen in our neonatally deafened animals, such effects may be more modest or may not occur at all in adult deafened animals. The approach described in this Report was to conduct a study in adult deafened cats, using identical stimulation and morphometry protocols as in previous studies in neonatally deafened cats and to compare results in the two groups.

METHODS

Table 1 summarizes the deafening and stimulation histories for each of the 6 animals included in this report. Normal hearing, adult animals were deafened by co-administration of kanamycin and ethacrynic acid and as described by Xu et al., (1993, Hearing Res. 71:205-215). (One animal was initially treated with kanamycin and aminooxyacetic acid. However, hearing thresholds recovered over the course of several hours of monitoring; subsequently this animal was deafened by administration of kanamycin and ethacrynic acid). Animals were first sedated with an IM injection of ketamine and acepromazine and an intravenous catheter was inserted. A baseline auditory brainstem response (ABR) intensity series was recorded and threshold determined visually. Kanamycin (300 mg/kg) was injected subcutaneously and ethacrynic acid was infused intravenously (1 mg/min.) until no ABR responses were obtained to clicks at equipment intensity limits (110 dB peak SPL). ABRs were monitored for at least 4 hours to ensure that hearing did not recover.

STIMULATION HISTORIES

ANIMALS WITH SHORTER STIMULATION PERIODS AND/OR SEVERE COCHLEAR PATHOLOGY

Animal Number	Stimulation Period (days/wks)	Level (μAmp)	Duration of Deafness (weeks)
403	*66 /11*	*50-79*	*13*
507	*70/20*	*100-200*	*22*
158	*116/22*	*100*	*24*

ANIMALS COMPLETING STIMULATION

Animal Number	Stimulation Period (days/wks)	Level (μAmp)	Duration of Deafness (weeks)
497	*133/30*	*50-126*	*32*
087	*144/32*	*100-251*	*33*
401	*105/21*	*126-200*	*23*

Table 1. *Deafening and stimulation histories for the 6 adult cats included in this study.*

Animals were implanted 1-2 weeks following deafening. Implants were the new UCSF designed "wing" scala tympani cat electrodes and consisted of an intracochlear array of four platinum-iridium wires embedded in a silastic carrier with each wire ending in a ball contact 250 μm in diameter (see 9th Quarterly Progress Report, Oct. 1, 1996-Dec. 31, 1996, Contract #N01-DC-4-2143, figure 1). Electrode contacts are numbered 1-4 with the most apical contact designated as #1.

Under sterile procedures the left auditory bulla was opened to access the round window membrane. The round window membrane was opened and the intracochlear electrode inserted into the scala tympani. The electrode was held in place with Histocryl™ tissue adhesive applied to a dacron cuff at the round window. Thresholds were determined by electrically evoked auditory brainstem responses (EABR) immediately following cochlear implantation.

Electrical stimulation was initiated at 2 dB above EABR threshold one to two days after cochlear implantation. Impedances of stimulating electrodes were recorded daily before and after stimulation. EABRs were assessed periodically and the stimulation level was adjusted, if necessary, to maintain stimulation at 2 dB above the physiological threshold. All animals were stimulated on the most apical electrode pair (1,2) by delivering a continuous electrical signal with a carrier rate of 300 pulses/second (biphasic, charge-balances pulses, 200 μsec/phase), and 100% sinusoidal amplitude modulation at 30 Hz. Stimulation was delivered 4 hours/day, 5 days/week, and the target period was 6 months (24 weeks). This stimulation protocol is one that was used in several neonatally deafened cats in recent experimental series to study the effects of "temporally challenging stimulation."

After completion of chronic stimulation periods, animals were studied in final acute electrophysiology experiments (with the exception of one animal studied histologically at 11 weeks after anesthesia death), at the conclusion of which the cochleas were preserved for histology by perfusion. Morphological procedures were identical to those described in previous reports on neonatally deafened cats. Briefly, the cochleas were perfused with an aldehyde fixative (2.5% paraformaldehyde and 1.5% glutaraldehyde in 0.1M phosphate

buffer). Non- implanted ears were perfused through the oval and round windows. In the implanted cochleas a small hole was drilled in the bone overlying the cochlear apex and perfusion was performed via this opening and the oval window with the electrode remaining in situ. Transcardiac perfusion was then performed and the cochleas were removed and immersed in fixative overnight.

The otic capsule was thinned with a dental burr until the stria vascularis was visible throughout the cochlea and several small openings into the scala vestibuli were created to facilitate embedding. The basal turn of the cochlea was opened in animals with implanted electrodes in order to visualize electrode contacts. A small dental burr approximately the size of electrode contacts was used to mark the position of each contact in the otic capsule. The electrode was then withdrawn from the scala tympani. Specimens were decalcified in EDTA (0.2 M) and embedded in Epon. Each cochlea was bisected in the mid-modiolar plane, and half-coil pieces of the organ of Corti and adjacent SG were removed and mounted in a surface preparation on a glass slide. The markers indicating electrode contact location were preserved in the surface preparation to relate electrodes to histology of the organ of Corti and morphometry of the spiral ganglion. The basilar membrane was then measured and 0.5 mm segments removed at 2 mm intervals and remounted for sectioning. Several groups of 1 to 2 μm thick sections were cut in the radial plane at 50 μm intervals from each block, stained with toluidine blue and coverslipped in immersion oil.

To quantify SG cell survival a point counting volume ratio method was employed. Rosenthal's canal was centered under a 10 X 10 counting grid in the microscope ocular. The volume ratio (density) of the spiral ganglion was determined by counting the number of grid line intersections which fell over cell somata and dividing by the number of intersections which fell within the total area of Rosenthal's canal in a given section. Five to 10 sections were analyzed in each 10% segment of basilar membrane distance. This method has been used previously to provide normative data for the cat spiral ganglion and quantitative evaluation of spiral ganglion cell degeneration in neonatally deafened, chronically stimulated cats (See Final Report, Contract #N01-DC-4-2143, September 30, 1997).

RESULTS

One of the six animals (#403) died after only 11 weeks of stimulation, when it was tranquilized to repair the implant connector. Two other animals (#507, #158) exhibited intractable infection of the percutaneous connector during chronic stimulation. Examination of their implanted cochleas revealed severe pathology related to chronic labyrinthitis and endolymphatic hydrops; and in one animal (#507) cochlear pathology was further complicated by a fracture of the round window which occurred when a second surgical procedure was conducted to replace a damaged implant after ≅12 weeks of stimulation,. The severe pathology resulting from the labyrinthitis and temporal bone fracture clearly compromised spiral ganglion survival in these ears (**Figure 1 a,b**). Thus, as indicated in **Table 1** , these first 3 animals were deleted from the study of the effects of stimulation, because of the short duration of stimulation in the first case and the severe cochlear pathology in the other 2 animals.

Figure 1a. *Section taken from the 9.5 mm region of Cat #158 showing chronic labyrinthitis and insertion trauma resulting in severe basilar membrane rupture. This cochlea was deleted from the study of the effects of stimulation because this severe pathology in the implanted cochlea was judged to have compromised SG cell survival.*

Figure 1b. *Cat #507 was excluded from the study after reimplantation surgery resulted in a fracture of the round window, rupture of the basilar membrane in the basal cochlea and severe chronic infection and inflammation.*

Table 2 presents morphometric data on SG cell density for these first 3 animals, with volume ratio values calculated as percent of normal. (Normative data were taken from Leake and Hradek, 1988 Hearing Res. 33:11-34). Data for the first cat (#403), studied after only 11 weeks of stimulation and 13 weeks after deafening, revealed that overall SG density was ≅60% of normal in both cochleas, with no difference between the stimulated side and the control, deafened ear. With the longer duration of deafness in the other 2 cats (mean of 23 weeks), the SG cells had undergone progressive degeneration in the deafened control cochleas and the mean overall survival was ≅38% of normal.

The other 3 animals successfully completed extended stimulation periods without problems. Their individual morphometric SG data are presented in **Figure 2.** SG density is again shown for 10% intervals from base to apex, with volume ratio values calculated as percent of normal for each cochlear sector. Data are compared for the stimulated and unstimulated cochleas, and the average for each cochlea is shown at the right of each graph. Careful examination of the data in the control, unstimulated cochleas reveals a variable and patchy pattern of SG loss in the different sectors of the individual cochleas. However, in almost all cochlear sectors, SG cell density was greater in the cochlea that received chronic electrical stimulation than in the paired data from the unstimulated ear (**Figure 3a-d**). A striking exception to this was seen in the 40-50% sector, where all the implanted cochleas exhibited damage (*) from the insertion trauma caused by the tip of the electrode. This trauma clearly resulted in reduced SG survival, and the extent of cell loss consistently corresponded to the degree of damage seen in histological sections in these areas (**Figure 3e**).

SPIRAL GANGLION SURVIVAL

ANIMALS WITH SHORTER STIMULATION PERIODS AND/OR SEVERE COCHLEAR PATHOLOGY

Animal Number	Stimulated (% Normal)	Unstimulated (% Normal)	Difference (Stim-Unstim)
403	*57.03*	*59.67*	*-2.64*
507	*33.16*	*32.12*	*1.04*
158	*26.99*	*41.41*	*-14.42*

ANIMALS COMPLETING STIMULATION

Animal Number	Stimulated (% Normal)	Unstimulated (% Normal)	Difference (Stim-Unstim)
497	*53.44*	*36.84*	*16.6*
087	*52.63*	*43.72*	*8.91*
401	*38.15*	*33.94*	*4.21*

Table 2. *Spiral ganglion cell survival for the stimulated and unstimulated cochlea of all animals.*

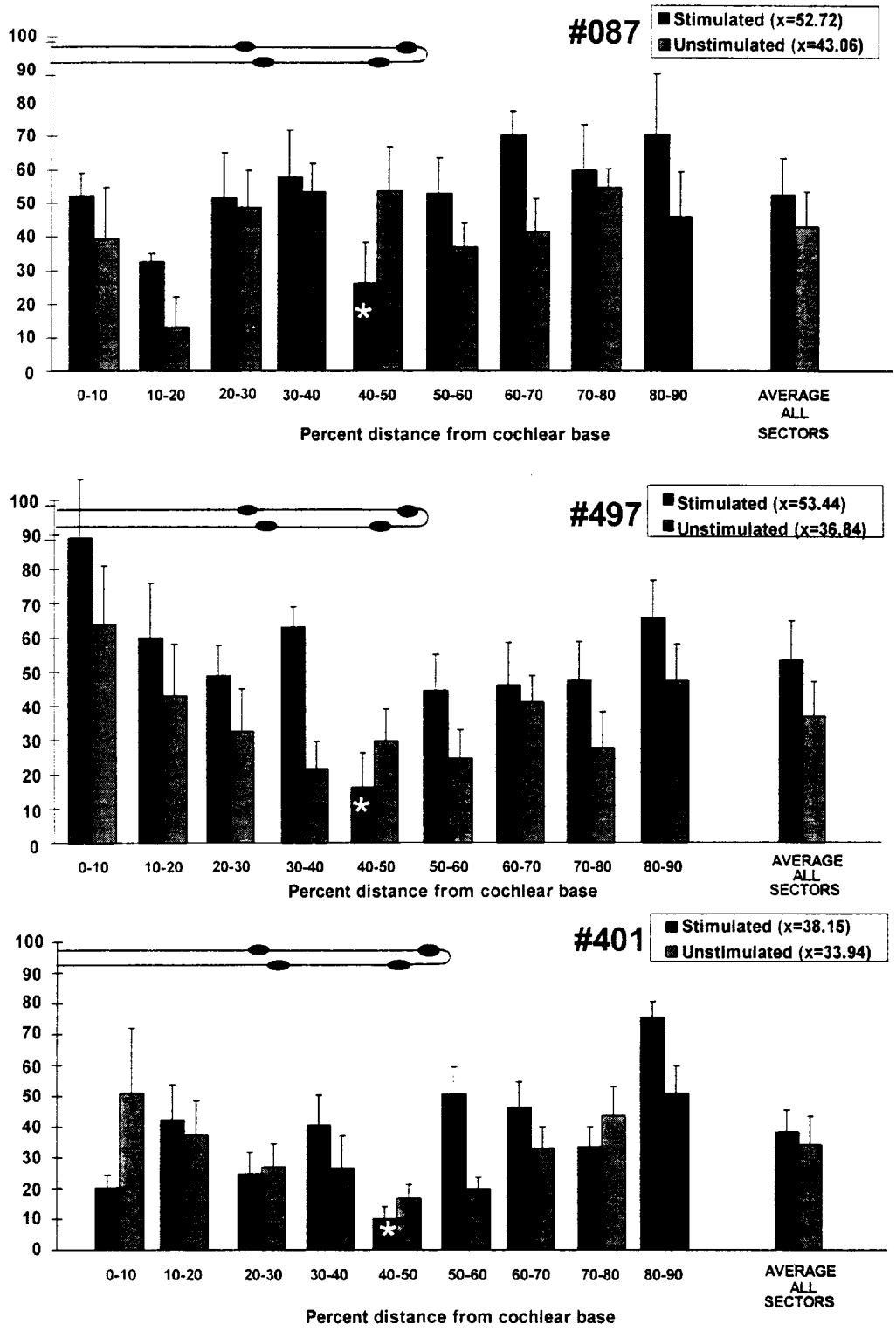

Figure 2. *Morphometric data are presented for the three cats that completed stimulation. Spiral ganglion cell density is shown for the stimulated and unstimulated ears from base to apex in 10% intervals, expressed as percent of normal and the average for each cochea is presented at the right. * Indicates electrode tip damage.*

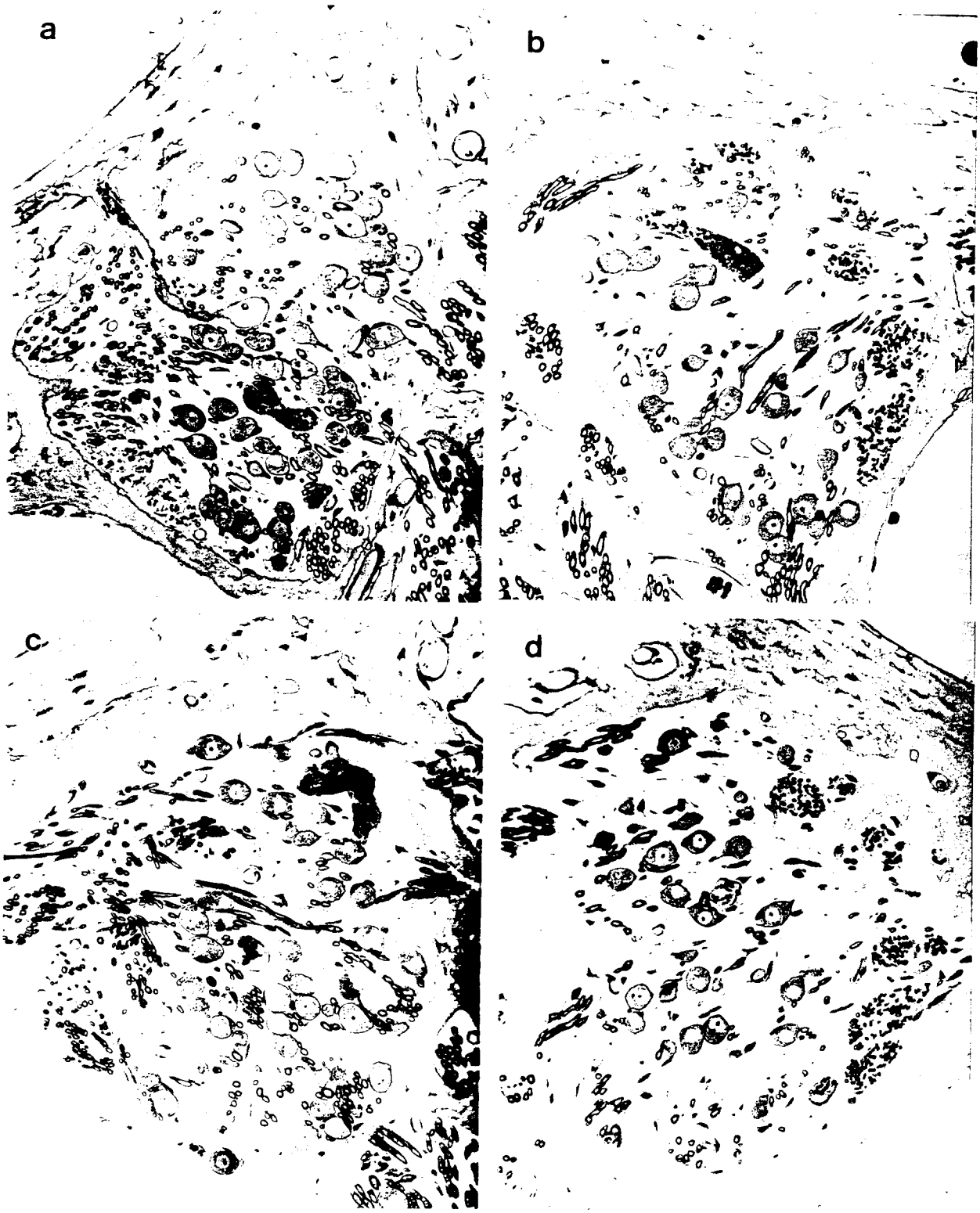

Figure 3a,b. Photomicrographs of representative histological sections from the ≅50% region of the stimulated (left) and unstimulated (right) cochleas of Cat #497 (see figure 3a). The SG cell density in this region was 44.5% of normal in the stimulated cochlea and 24.7% in the unstimulated cochlea. A higher density and increased absolute number of spiral ganglion cells is evident on the stimulated side. **3c,d.** Photomicrographs of histological sections taken from the ≅20% region of the same cat. The survival at this area was 48.78% and 32.45% for the stimulated (left) and unstimulated (right) cochlea respectively.

Figure 3e. Section from the 40-50% region showing an example of the electrode tip damage observed this region in all animals studied. An arrow indicates the damage to the osseous spiral lamina. Note the corresponding extensive loss of SG cells.

It is interesting to note that the two animals with the longest periods of electrical stimulation had the greatest increase in survival of SG cells in the stimulated ears (**Table 2**). Animal #087 was stimulated for 32 weeks and showed ≅17% increase in neuronal density; and #497 was stimulated for 30 weeks, and showed an increase of ≅9%. The survival in the stimulated cochlea was ≅53% of normal in both these animals, whereas survival in the contralateral deafened ear was 37% for the first cat and 44% for the second subject. The third cat in the group, animal #401 irreparably damaged the implant after 21 weeks of stimulation and was studied at this time. Morphometric data demonstrated only a very modest increase of ≅4% in the stimulated ear in this subject. In addition to a reduced period of chronic stimulation, it was necessary to reimplant this animal just prior to the physiology study due to device failure. Subsequently, morphological examination showed a severe, acute bacterial labyrinthitis related to the reimplantation that invaded the modiolus in some locations and also appeared to have compromised SG density (particularly in the most basal cochlear sectors; See **Figure 4**).

Figure 4. *A section from the basal sector 10-20% in Cat #401. This animal damaged its electrode after 21 weeks of electrical stimulation and was reimplanted acutely and studied in a 5-day electrophysiological experiment at that time. Although this cat was not deleted from the study, acute labyrinthitis was evident and appeared to compromise neural survival, at least in the basal cochlea.*

Figure 5 presents the average SG cell survival for this group of 3 cats, shown as % of normal for each cochlear sector in the stimulated and unstimulated ears. Each sector of the cochlea, except the 40-50% area with electrode tip damage, had higher SG cell survival in the stimulated ear as compared to the control side. SG cell density in the stimulated group, averaged over all cochlear sectors, was 10% higher in the stimulated cochleas over that in the paired, unstimulated ears. Preliminary comparison of these data to data from a group of neonatally deafened cats matched for similar periods and protocols of electrical stimulation (**Table 3**).

NEONATALLY DEAFENED
SPIRAL GANGLION SURVIVAL

ANIMAL Number	Stimulated	Unstimulated	Difference (Stim-Unstim)	Stim.Per. (wks)	Duration of Deafness (wks)
K83	*68.96*	*39.48*	*29.48*	*21*	*32*
K84	*54.41*	*29.77*	*24.64*	*35*	*45*
K89	*43.51*	*41.41*	*14.14*	*26.5*	*37*
K98	*33.67*	*21.11*	*12.56*	*32*	*39*
K101	*53.67*	*32.14*	*21.53*	*28.5*	*36.5*
K105	*37.59*	*24.8*	*12.79*	*28*	*37*
Mean	**48.64**	**31.45**	**19.19**		

Table 3. *Spiral ganglion cell survival from the stimulated and unstimulated cochlea of a group of neonatally deafened cats (mean stimulation duration = 28.5 wks.) selected to match as closely as possible the duration of stimulation in the adult deafened group (mean duration = 27.6 wks.).*

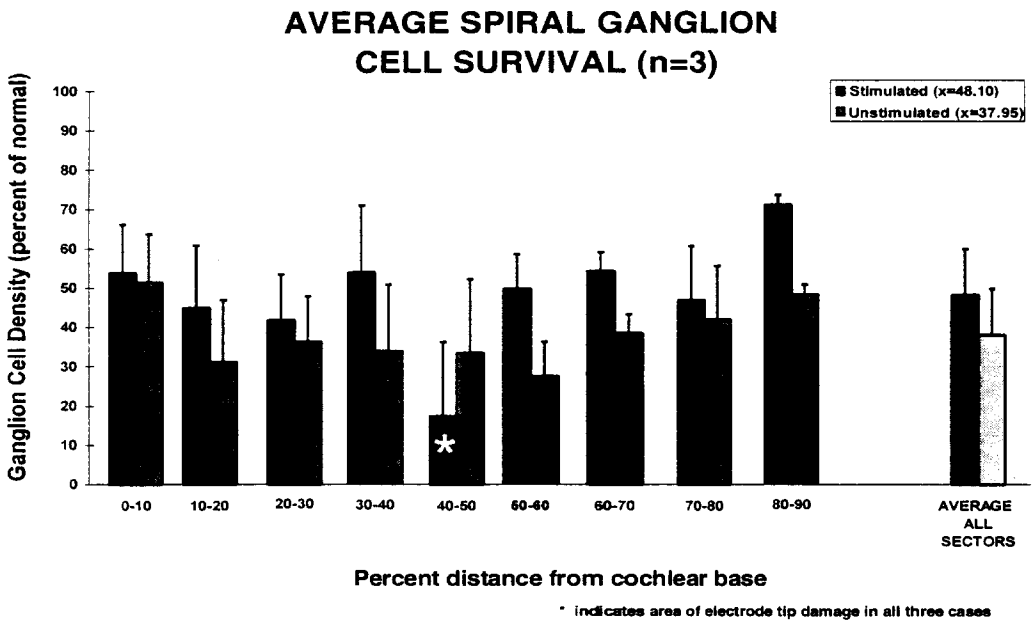

Figure 5. This summary graph shows the SG data for the group of 3 cats completing 5-6 month stimulation periods. Data are displayed as percent of normal for stimulated and unstimulated ears and for each cochlear sector from base to apex. The mean ganglion cell density for the stimulated ears was 48.10 % of normal and for the unstimulated ears 37.95.

These results suggest that the neurotrophic effect on SG cells may be greater in the early-deafened animals (mean overall increase of 17.8% of normal) than in these adult-deafened cats (mean increase of 10%). However, since the number of adult-deafened animals successfully completing stimulation is so limited at present and the results in the 3 cases were so variable, it is premature to draw any conclusion.

The *difference* in SG density between stimulated and unstimulated ears is plotted in **Figure 6**. Data are again expressed as percent of normal for each cochlear sector from base to apex. The clear effect of the insertion trauma at the tip of the electrode is shown by the negative value in the 40-50% region. All other cochlear regions showed increases in neural density as a consequence of chronic stimulation. The greatest effect was seen in the regions adjacent to the stimulating electrodes (i.e., 30-40% and 50-60%), although a comparable increase is also observed in the most apical sector. Averaged over all cochlear sectors, 10% of the normal cell density is maintained in the stimulated cochleas over that in the paired unstimulated ears. This presentation of the data gives a good representation of what the neural densities are in comparison to normal.

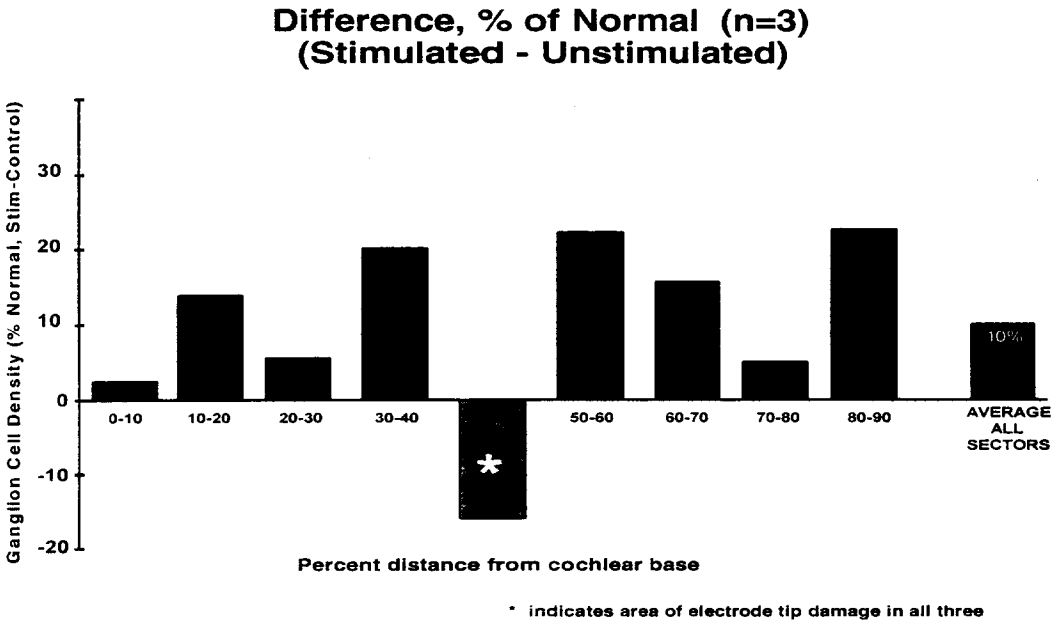

Figure 6. The SG data for the 3 cats completing 5-6 month stimulation periods are shown here as average SG cell density **difference** (stimulated - unstimulated) expressed as **% of normal**. With the exception of the electrode tip damage area (40-50% region), all sectors of the stimulated cochlea have an increase in spiral ganglion cell density. Overall ≅10% of the normal neural population was maintained in ears receiving chronic electrical stimulation as compared to paired controls.

Many investigators have reported similar SG density data in a different manner, expressed as % difference between the stimulated and unstimulated sides. These values are calculated by subtracting the control (unstimulated) value from the experimental (stimulated) value, then dividing by the control value and multiplying by 100 (**Figure 7**). When these adult SG data are shown as % difference, the greatest increases in survival again are seen in

the cochlear sectors adjacent to the stimulating electrodes. Expressed in this manner, a difference of almost 100% (a doubling of cell density) is seen in the 50-60% region, where SG survival was 27% of normal in the control ears vs. 52% in the stimulated group. However, at the apex a more modest 40% increase is calculated when survival in the control was 50% of normal vs. 70% in the stimulated ear. Averaged throughout the cochlea, SG density in the stimulated ears was increased by 27% over the paired control cochleas. This expression of the data gives an idea of the *relative* difference between the 2 sides. That is, the underlying assumption is that the control ears represent the "normal" status of the neural population after deafening, and measures how large the difference in cell density is compared to the control values.

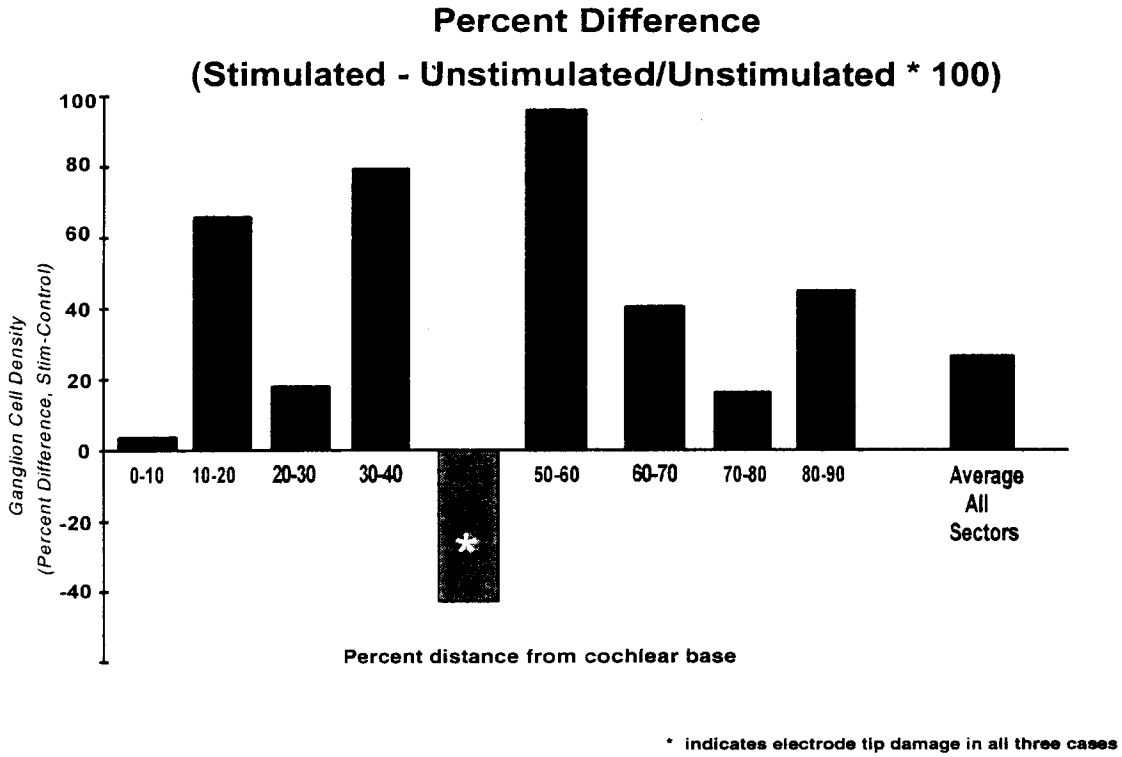

Figure 7. The SG data for the 3 adult cats completing 5-6 month stimulation periods are shown again, but expressed here as **percent difference**. In several sectors SG density in the stimulated ears is increased by more than 60%, and overall density is increased by ≅27%, expressed in this manner.

In **Figure 8**, the regional SG density data are shown for the control, unstimulated cochleas of the adult deafened group, excluding the one animal (#403) that was studied early after only 11 weeks of stimulation and at 13 weeks after deafening. These data are expressed as percent of normal. Results suggest that SG cell loss was relatively uniform throughout the cochlea, perhaps with slightly better survival at the base and apex. Mean survival averaged over all areas was 37.5% of normal for this group, which has a mean length of deafness of 27 weeks.

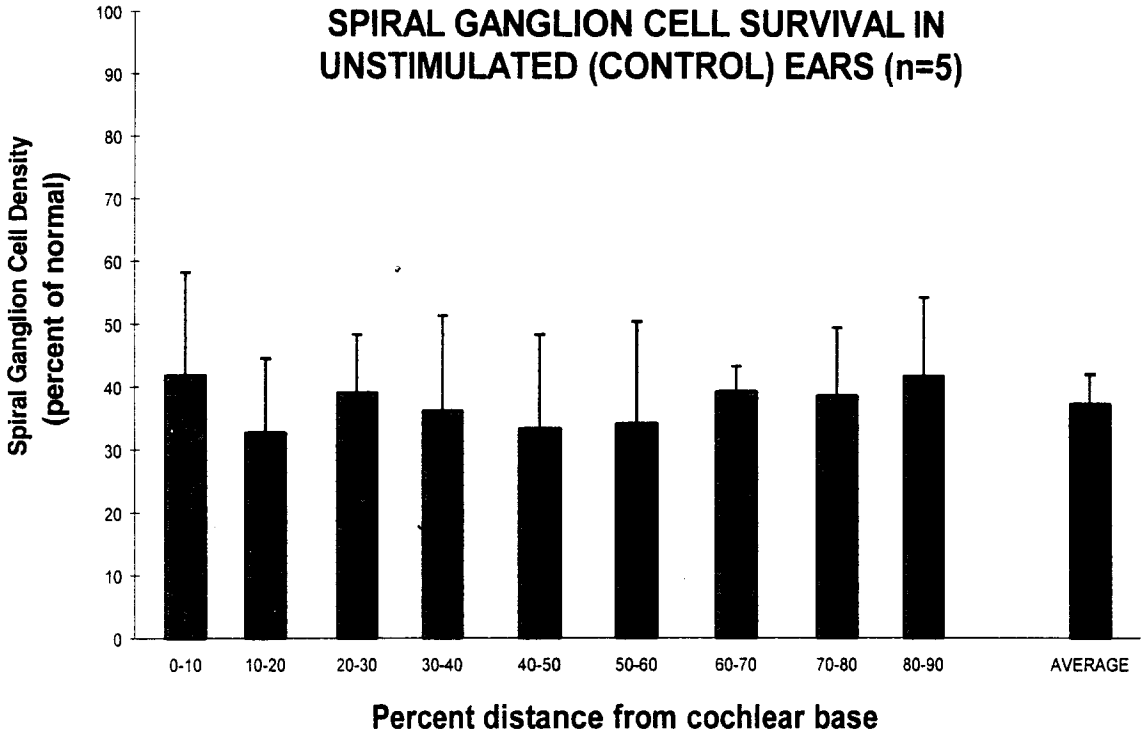

Figure 8. SG cell density data for the control (unstimulated) ears of adult deafened cats (excluding cat #403 that was studied early), showing relatively uniform survival throughout the cochlea. (bars = S.D.)

The final graph in **Figure 9** shows SG cell density in the control deafened cochleas as a function of duration of deafness for each of the individual adult cats studied. These data suggest that loss of SG cells in the adult animals was progressive over time (regression function = solid line) after deafening by co-administration of kanamycin and ethacrynic acid. The broken line in this graph shows a regression function for SG data from the control, unstimulated cochleas of a large group of cats neonatally deafened by administration of neomycin sulfate. The two functions are quite similar, suggesting that the time course and extent of cell loss for a given duration of deafness are similar in the 2 groups of animals, although substantial individual variation is noted.

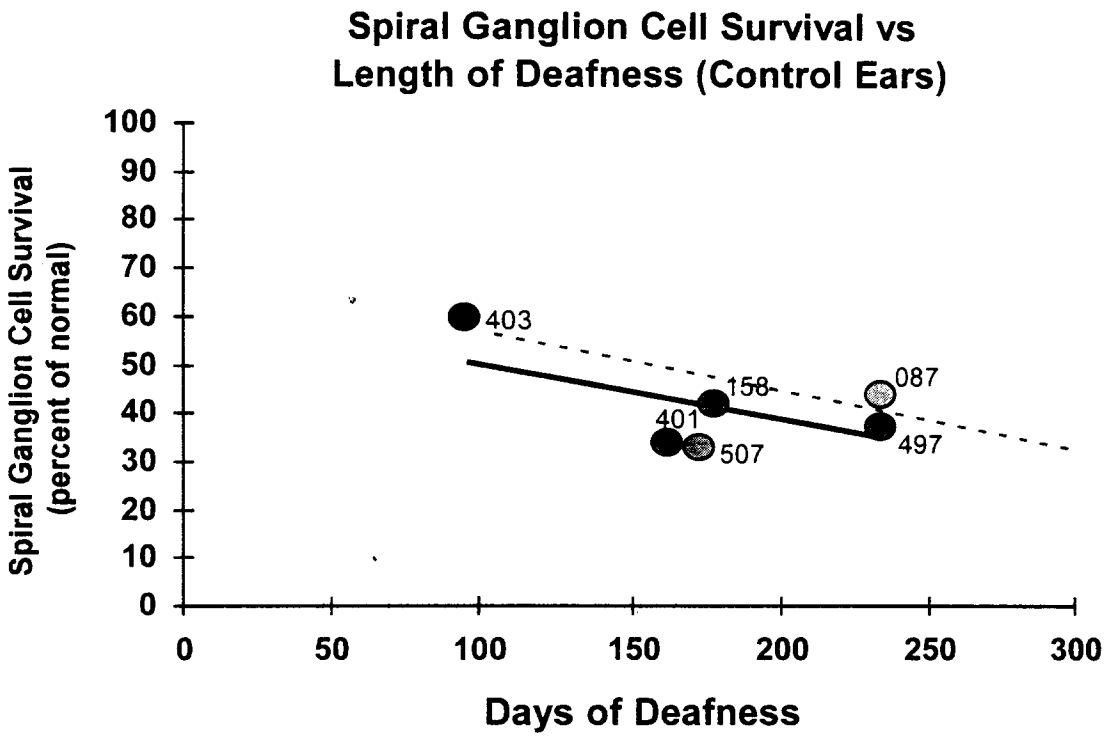

Figure 9. SG cell density data for unstimulated ears of individual adult cats are plotted as a function of length of deafness (solid regression line). The dashed line is the regression line for control (unstimulated) ears in neonatally deafened cats (n=30).

SUMMARY

1. These initial results suggest that chronic electrical stimulation of the adult-deafened cochlea, can ameliorate the degenerative effects of deafening. As in neonatally deafened, stimulated animals increased spiral ganglion cell survival is seen throughout the cochlea. The overall difference between the stimulated and unstimulated ears was **10%**.

2. Although the study is limited by the small number of animals, the results suggest that periods of stimulation of 5-6 months are required to demonstrate the trophic effects of electrical stimulation.

3. Insertion trauma and chronic infection are clearly highly detrimental to SG cell survival, causing degeneration of neurons beyond that observed with ototoxic deafening and preventing the protective effects of chronic electrical stimulation.

4. The control data demonstrate that deafening with kanamycin/ethacrynic acid results in a fairly uniform loss of ganglion cells throughout Rosenthal's canal, with somewhat reduced survival at the extreme base. Further, the data suggest that degeneration is similar to that in cats neonatally deafened by neomycin sulfate.

Work Planned for the Next Quarter

1. Analysis of temporal resolution data obtained during electrophysiological studies of these adult deafened animals will be completed and will be summarized in one of the coming QPRs. In addition, histological study of the cochlear nucleus from these animals will be carried out as time permits. An additional adult animal has been deafened and chronic stimulation is being carried out at this time.

2. Chronic stimulation of one neonatally deafened animal in the new ganglioside treatment series will continue. This animal is receiving two channel stimulation at 300pps/30 Hz AM on the apical channel and 800pps/60 Hz AM on the basal channel. Behavioral training and chronic stimulation with the analogue processor will continue in one long-deafened cat.

3. Analysis of electrophysiological data from cats in the recent high frequency stimulation and ganglioside treatment series will continue. Study of an adult-deafened, unstimulated "normal" cat is planned for the coming quarter for collection of normative data on amplitude modulated signals.