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Protective Effects of Patterned Electrical Stimulation on the Deafened Auditory System

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ABSTRACT

One key goal of this Contract research is to examine the factors and mechanisms underlying the neurotrophic effects of chronic electrical stimulation of the cochlea in neonatally deafened cats which promotes increased survival of spiral ganglion neurons, at least partially preventing the retrograde degeneration which otherwise results from the loss of hair cells following deafness. Previous results showed that "temporally challenging" stimulation (e.g., amplitude modulated pulse trains with a carrier rate of 300 pps and 100% sinusoidal AM at 30 Hz or stimulation from a single channel analogue cochlear implant processor) can be highly effective in maintaining survival of the spiral ganglion neurons, if chronic stimulation is continued for several months. This Quarterly Progress Report presents data from a new series of deafened cats that received chronic high frequency stimulation (e.g., 800 pps carrier sinusoidally amplitude modulated at 20 Hz) and/or stimulation by 2 channels of the cochlear implant.

Results indicate that chronic stimulation with these higher frequency pulse rate signals and/or 2 channels of the cochlear implant induce significant effects in promoting survival of spiral ganglion neurons with results similar to those seen in previous studies using "temporally challenging" stimulation. Moreover, even when chronic stimulation is delivered at an intensity level equal to EABR threshold, significant effects of the higher frequency electrical pulse trains in promoting survival of SG neurons were demonstrated throughout most regions of the deafened cochleas.

INTRODUCTION

The overall goals of our Contract research at UCSF are to examine the effects of chronic electrical stimulation on the cochlea and central auditory system and to examine the factors which contribute to neural survival and which determine the viability of the central auditory pathways. One key goal of the previous Contract research was to verify initial results indicating that chronic stimulation in neonatally deafened cats promotes increased survival of spiral ganglion (SG) neurons, at least partially preventing the slow retrograde degeneration that otherwise follows the loss of 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) appear to play key roles in the extent of neurotrophic effect in maintaining the auditory neurons, and that under our current experimental protocols highly significant increases in neuronal survival (about 20% of the normal neuronal density) are observed.

These data suggested that "temporally challenging stimulation" may be more effective in inducing neurotrophic effects than a simple low frequency stimulus of 30 pps. In the temporally challenging stimulation experiments, animals were stimulated either with a functioning analogue cochlear implant processor, or with a computer-generated signal consisting of a continuous train of electrical pulses (200 μsec/phase) delivered at a rate of 300 pps and sinusoidally amplitude modulated at 30 Hz.

This latter signal was specifically designed based upon results of electrophysiology studies which demonstrated that neurons in the auditory midbrain have a maximum cut-off frequency of approximately 300 pps and a mean following frequency of about 100 pps. That is, only a few units in the inferior colliculus can follow or respond to individual pulses delivered at rates of 300 pps or higher, and the average for all units is about 100 pps. Further, neurons in auditory cortex (AI) respond best to AM signals of about 8-12 HZ. Thus, the 300/30 Hz signal was chosen for chronic stimulation with the hypothesis that it would extend the upper limit of frequencies that these central auditory neurons normally follow. In fact, subsequent electrophysiological studies did demonstrate marked effects of chronic stimulation using this signal in increasing temporal resolution of central auditory neurons. But in terms of modern CIS processors for clinical cochlear implants, this signal is still relatively low frequency. Therefore, in more recent experiments we have used various additional signals for chronic stimulation, specifically selecting signal parameters which model

specific attributes of signals used in clinical cochlear implants. In this report we present SG density data from experiments using some higher frequency signals for chronic stimulation (i.e., 800-900 pps pulse trains, sinudoidally amplitude modulated at 20-60 Hz). Another goal has been to examine the results of multichannel chronic stimulation and the effects on the developing auditory system of competing inputs delivered by at least two adjacent cochlear implant channels. SG density data also are presented from initial efforts to implement chronic 2 channel stimulation in 4 subjects.

METHODS

Table 1 presents deafening and chronic stimulation history data for the previous temporally challenging stimulation experimental group, in comparison to histories of individual subjects in the new higher frequency and/or 2 channel stimulation group.

Table 1. Higher Frequency, Temporally Challenging Stimulation Histories

Cat #	Neomycin mg/kg/days	Age at Initial Stimulation	Stim. Current	Stim. Period	Stim. Frequency	Age at Study
			Temporally Challenging Stimulation			
K83	60/19	10 wks	125 μA	22 wks*	80 Hz	32 wks
K84	60/19	10 wks	200-400 μA	34 wks	SP/beh.	44 wks
K85	60/19	10 wks	125 μA	41 wks	80 Hz	51 wks
K86	60/19	9 wks	30-160 μA	47 wks*	SP/beh.	56 wks
K89	50-60/19	10.5 wks	80-100 μA	27.5wks	300/30 Hz	38 wks
K98	60/20	7 wks	50-100 μA	33 wks	SP/beh	40 wks
K99	60-70/25	8 wks	32-100 μA	38 wks	300/30 Hz/beh	46 wks
K102	60/18	8 wks	80-160 μA	39 wks	300/30 Hz/beh	47 wks
			Means (n=8):	**35 wks**		**44.5 wks**
			High Frequency and 2 Channel Stimulation			
K101	60/18	7.5 wks	1,2=79-200 μA	29.5 wks	300/30Hz /beh	37 wks
			3,4=100-316μA	**2ch-s**		
K104*	60/19	7 wks	25-180 μA	19 wks:**2ch-s**	300/30 Hz	42 wks
				11wks:beh; 5 wks:**1ch**		
K105	60/20	9 wks	63-355 μA	29 wks	800/20 Hz	38 wks
K106	60/20	9 wks	80-400 μA	33.5 wks	800/20 Hz	43 wks
K107	60/16	9 wks	1,2 = 100-200 μA	22 wks	800/60 Hz	31 wks
			3,4 = 71-100 μA	**(2 ch-alt)**		
			***Means, n=4:**	**28.5 wks**		**37.5 wks**

* K104 was subsequently eliminated from the study because chronic stimulation intensity did not meet protocol specifications. This animal, therefore, is not included in the calculations of mean duration of stimulation and age at study for the group.

All animals were deafened neonatally by daily administration of the ototoxic drug neomycin sulfate at a dosage of 60 mg/kg of body weight. Drug administration was initiated the day after birth and continued for 16 days postnatal (P16). At this time ABR testing was done, and if a profound hearing loss was demonstrated (absence of click-evoked ABR at the maximum output of our system, 110 dB peak SPL) ototoxic drug injections were discontinued. If residual hearing was observed, drug administration was continued in increments of 2 to 3 days until the hearing loss was profound. As shown in Table 1, drug treatment in the new experimental group ranged from 16 to 20 days. Kittens were implanted unilaterally with scala tympani electrodes at 6 to 8 weeks postnatal and chronic stimulation was initiated at ages ranging from 7.5 to 9 weeks postnatal. Stimulation periods were 4 hours/day and 5 days/week.

Two animals (K105 and K106) were stimulated on a single channel of their cochlear implant, using a "high frequency signal" consisting of biphasic pulses (200 μsec/phase) delivered at a rate of 800 pps and 100% sinusoidally amplitude modulated at 20 Hz. In these cases, single channel stimulation was delivered by the apical bipolar electrode pair (electrodes 1,2) of our current feline electrodes. The other 3 subjects received chronic stimulation on 2 channels, using both the apical and basal bipolar pairs (electrodes 1,2 and 3,4, respectively) of their cochlear implants. Up to now, our protocol has specified a stimulation intensity of 2 dB above EABR threshold, as evaluated individually for each stimulated channel. However, due to concerns about 1) possible overstimulation with 4 hours at these higher frequency pulse rates and 2) possible channel interaction or summation in 2-channel experiments, in some animals stimulation was delivered *at* EABR threshold (see below for individual histories). EABR thresholds were measured monthly and stimulators adjusted as necessary to maintain the appropriate current levels re: EABR thresholds. Chronic stimulation periods ranged from 22 to 35 weeks. Animals were then studied in terminal acute electrophysiology experiments, and tissues harvested for histopathological and morphological studies. The total duration of deafness at the time of study ranged from 32 to 44 weeks.

RESULTS AND DISCUSSION

Chronic Stimulation .

All animals in the high frequency/2-channel group exhibited some degree of threshold elevation over the many months of chronic stimulation. The first 2-channel cat, **K101**, was stimulated at full intensity (EABR threshold + 2 dB) using 300 pps/30 Hz on both channels. This subject had relatively stable thresholds on the apical channel (pair 1,2; shift < 150 μA) with a slightly greater threshold elevation (216 μA) on the basal channel (pair 3,4.)

The second animal in the 2-channel group (**K104**) initially exhibited an aversive reaction to electrical stimulation and as a consequence of a misunderstanding was stimulated at EABR threshold level, instead of 2 dB suprathreshold for the initial 11 weeks of chronic stimulation. The intended protocol was to initiate stimulation at a lower lever, but to increase the intensity as the animals became accustomed to the stimulus. This animal was subsequently assigned to a behavioral training experiment for 19 weeks, during which time it received only the very limited stimulation delivered for training (approximately 45 minutes/day, during which the animal ran about 60 trials and received approximately 30 electrical stimuli, 1 second in duration, 300 pps/30 Hz). Then one of the electrode lead wires was broken and the final 5 weeks stimulation was delivered only on the apical channel. Since this animal did not meet the original specifications of our chronic stimulation protocol (EABR threshold +2dB for 300 pps/30 Hz), it has been deleted from the pooled data analyses with regard to the effects of chronic stimulation on spiral ganglion survival. This subject showed relatively low, stable thresholds throughout chronic stimulation.

Two cats (**K105** and **K106**) were stimulated on a single channel (apical bipolar channel using electrodes #1 and 2) with the higher frequency pulsed stimulus of 800 pps/20 Hz. Since higher pulse rates produce slightly lower psychophysical thresholds and because of concern that 4 hours of continuous stimulation at these higher frequencies might produce overstimulation/ neural

damage, these animals were stimulated at EABR threshold level, rather than 2 dB suprathreshold. Despite this conservative measure, both these animals showed substantial elevations of threshold on the stimulated channel during the chronic stimulation periods of 9-10 months. Their thresholds shifted from initial values of 63 (K105) and 80 μA (K106), to final stimulation levels of 355 and 400 μA, respectively.

The final subject (**K107**) was the first animal in which high frequency stimulation was combined with stimulation of both channels of the cochlear implant. In this animal, stimulus intensity again was set at 2 dB above EABR threshold. However, because of the concern that the continuous high frequency stimulation in the new experimental protocol might possibly cause neural damage and because of the possibility of channel interaction or summation, stimulation in this animal was *alternated* between the apical and basal channel on consecutive days, instead of simultaneously activating both channels. EABR thresholds in this subject remained quite stable on both channels, with relatively low final stimulating levels of 200 and 100 μA on the apical and basal channel, respectively.

Locations of Intracochlear Electrodes

After completion of chronic stimulation periods and final electrophysiology experiments, animals were euthanized and the temporal bones and brain of each animal was preserved for histological studies. Prior to histological processing, each implanted cochlea was dissected, and the scala vestibuli of the basal turn was opened to visualize the actual positions of the hemispherical platinum-iridium electrode contacts within the scala tympani underneath the basilar partition. Once located, the positions of electrodes were marked in the adjacent bone using a small diamond burr (300μm) that was similar in diameter to the electrodes. The implant was then removed from the cochlea, and the specimens were post-fixed in osmium tetroxide, decalcified briefly (48 hr.), dehydrated in ethanol and embedded in plastic (Epon™ or LX™). Cochlear specimens were prepared as thick surface preparations including the spiral ganglion, which allows us to reconstruct the cochlear spiral, measure total basilar membrane length and accurately relate electrode locations, observed pathology and spiral ganglion cell survival to the known frequency map of the cat cochlea (Liberman, 1982; J.A.S.A. 72:1441-1449).

Table 2 shows the total basilar membrane length and electrode contact locations in the new high frequency/2-channel stimulation group. Mean basilar membrane length was about 24 mm, which is very similar to the mean value of 23.9 mm for the temporally challenging stimulation group (Vollmer et al, In Press, J. Neurophysiol.). The position of the most apical contact ranged from 43 to 51% from the basal end of the cochlea, with a mean of 47.26%. This mean location has a represented frequency of about 5.5 kHz, as calculated by the Greenwood frequency/position function (Greenwood, D.D. 1990, J.Acoust. Soc. Am. 87, 2592-2605) and using the revised constants for the cat cochlea provided by Liberman (Liberman, M.D. 1982, J. Acoust. Soc. Am. 72, 1441-1449). Electrode contact #2 had a mean position of 43.7% equivalent to a represented frequency of about 6.5 kHz. Thus the apical bipolar channel (1,2) was centered around 6 kHz in this experimental group. The electrodes comprising the basal channel, contacts 3 and 4, had mean locations of about 13 and 15 kHz, respectively and thus were centered at 14 kHz.

TABLE 2. Locations of Individual Intracochlear Electrodes

Animal #	BASILAR MEMBRANE (Length in mm)	ELECTRODE CONTACT LOCATIONS (% Distance from Cochlear Base) E1	E2	E3	E4
K101	23.2	43.1	39.7	- -	- -
K104	23.0	46.5	44.3	- -	- -
K105	24.3	51.4	46.1	30.9	28.8
K106	26.6	49.6	46.2	29.3	25.6
K107	23.0	45.7	42.2	29.6	- -
Mean	24.02 mm	47.26%	43.7%	29.93%	27.2%
Frequency:		5.5 kHz	6.55 kHz	13.1 kHz	15.0 kHz
mm Position: (24 mm cochlea)		11.3 mm	10.5 mm	7.2 mm	6.5 mm

Cochlear Histopathology and Spiral Ganglion Survival.

Graphs illustrating SG density data in the 5 individual animals are presented in **Figure 1**. Each graph compares regional spiral ganglion cell density in the stimulated ear with the paired data from the control, unimplanted ear. Data are expressed as percent of normal ganglion cell density for 10% sectors of the cochlea from base to apex.

Data in the control, unstimulated cochleas presumably reflect solely the effect of the neonatal deafening. It is interesting to note that substantially better neural survival was maintained in the control ear of K107 (45.7% of normal) as compared to the rest of the group, with survival in the other 4 animals ranging from 24.8% to 34% of normal. It seems likely that this finding relates to the shorter duration of neomycin treatment required to induce a profound hearing loss in this animal (16 days) and the shorter duration of deafness (31 weeks) at time of study. In contrast, SG cell survival was markedly reduced in the control ears of K105 and K106, and both these animals had 20 days of neomycin injections and had longer duration of deafness (K105= 38 weeks; K106=43 weeks).

In all but one of the cases, neural survival in the stimulated ear was higher than that in the paired control data in most cochlear regions. A single exception to the finding of better survival in the stimulated ear was seen in the data for K104. This is the animal in which the chronic stimulation intensity for the 300 pps/30 Hz signal was erroneously set at EABR threshold level for the first 11 weeks of stimulation, rather than at 2 dB suprathreshold as specified by the protocol for this stimulus. This animal then received very limited stimulation, consisting of approximately 30 suprathreshold stimuli of 1 sec duration, during less than an hour of daily behavioral training for a subsequent period of 19 weeks. Mean neural survival is approximately equal in the 2 ears of this subject. It is interesting to note, however, that there is a suggestion of an effect of stimulation over the basal 30% of the cochlea, where SG density is uniformly about 20% higher in the stimulated ear. Neural density is about the same for the paired data sets throughout most of the rest of the cochlea, and the increased survival in the base of the stimulated ear is offset only by the unusual finding of nearly normal survival in the control ear at 80-90%. Our interpretation of these data is that the initial low intensity of stimulation and the limited duration of stimulation delivered during the extended behavioral training period were insufficient to promote a significant increase in neural survival in this animal. This animal was therefore deleted from the study and is not included in the pooled data analysis for the group.

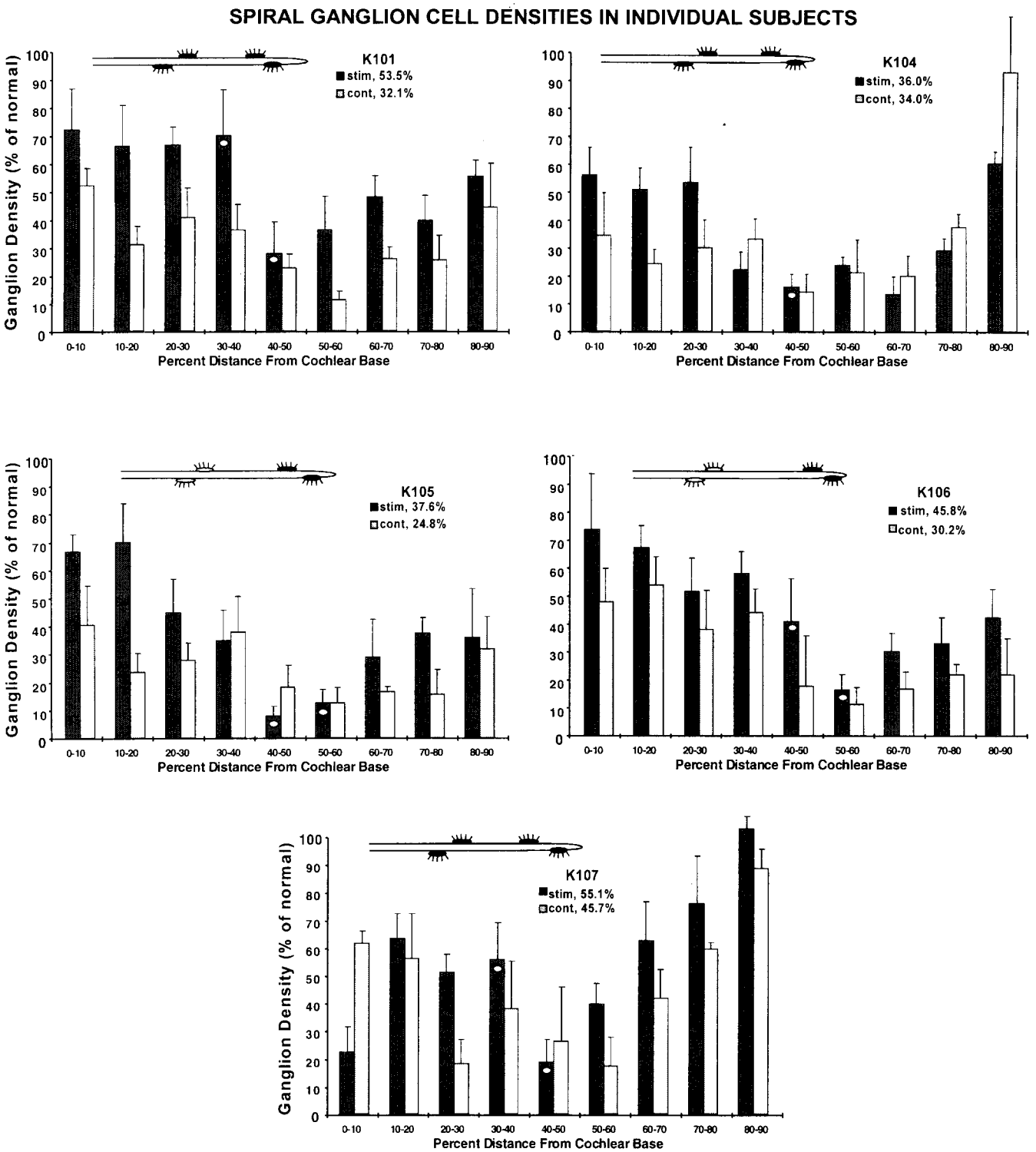

Figure 1. Individual spiral ganglion cell morphometric data for the 5 neonatally deafened cats in the high frequency, 2-channel chronic stimulation group. Spiral ganglion volume ratio is expressed as percentage of normal for cochlear regions from base to apex. Data in the stimulated ears are shown by the darker bars and paired data for the control deafened, unstimulated ear are shown by the lighter data bars. Small diagrams at the upper left of each graph show the approximate locations of the cochlear implant and the stimulating electrode contacts.

An interesting contrast to these morphometric results in K104 is seen in K101, which was the animal that also was stimulated on 2 channels @ 300 pps/30 Hz -- but in this case stimulus intensity was set at 2 dB above EABR threshold. The data from K101 showed the greatest difference in neural survival recorded in this group, with SG density in the stimulated cochlea 21% higher than in the control deafened ear. Increased spiral ganglion cell density is demonstrated throughout the cochlea, but the greatest difference is observed in the basal turn (10-40% or about 2.5 to 10 mm from the base). Figure 2 shows histological sections illustrating examples of one of the regions with maximum differences in spiral ganglion density seen in this cat (**Figure 2a,b**).

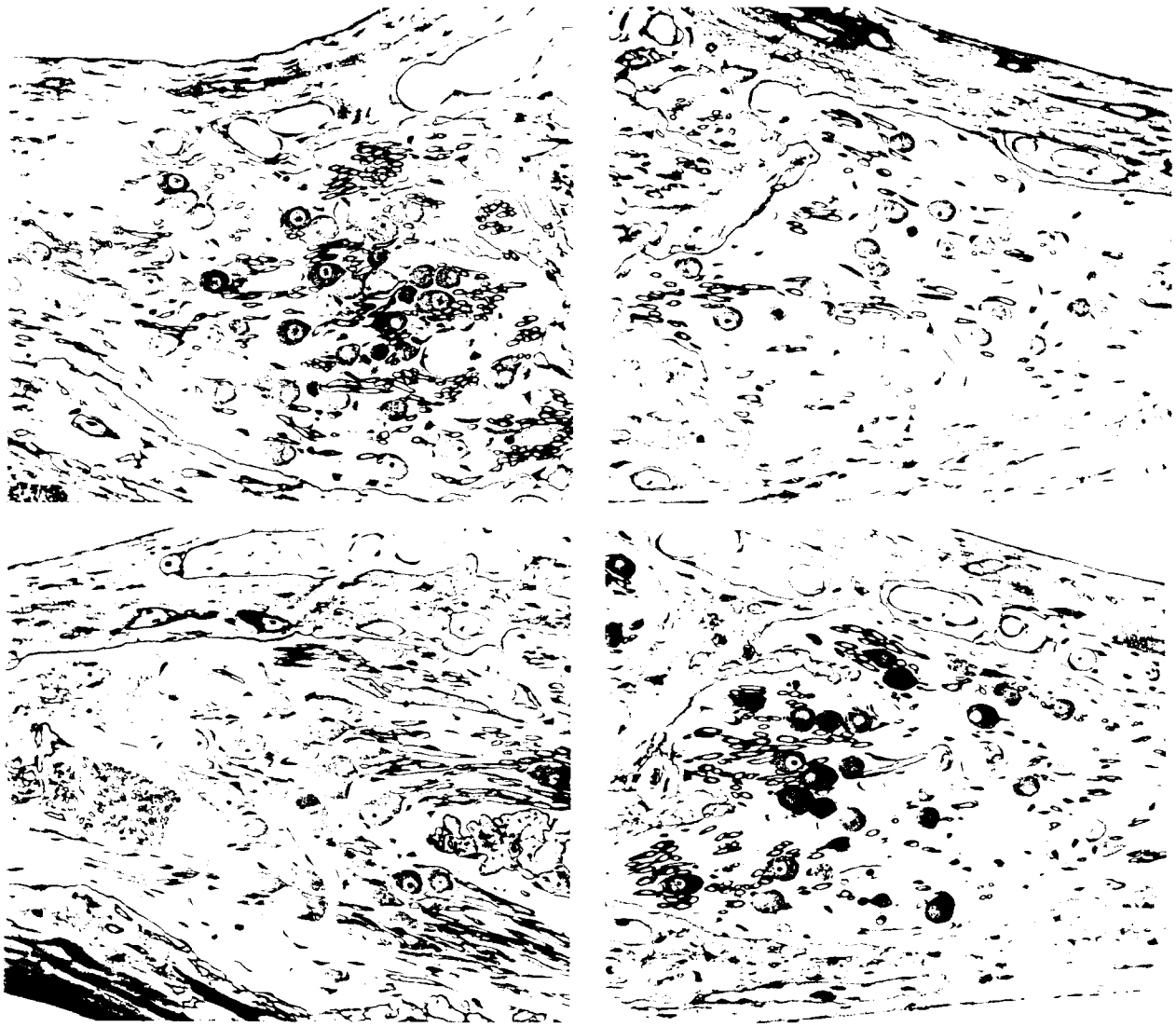

Figure 2. Photomicrographs illustrating regions of maximum increases in SG density following chronic electrical stimulation. Histological sections of the SG in **K101** illustrate markedly better cell survival in the stimulated cochlea (**a**) in the region 3 mm from the base (10-20%) which had a mean SG density of 66% of normal, as compared to the paired region from the opposite ear (**b**) where SG density was 31% of normal. In **K107**, the maximum difference in SG survival was seen in the region 5 mm from the cochlear base (20-30%) where cell density in the stimulated ear (c) was about 50% of normal, as compared to the same region in the control ear (d) where SG density was about 20% of normal.

The first two animals stimulated with the higher frequency stimulus of 800 pps/20 Hz also showed improved SG survival in the stimulated ear. An overall increase of 13% of the normal neural population was maintained by chronic electrical stimulation in K105 and in K106 an increase of 16% was observed. It should be noted that these animals received stimulation at EABR threshold (rather than at 2 dB suprathreshold). The marked threshold shifts observed in both these first 2 cats stimulated at this higher pulse rate led to concern that the continuous high frequency stimulation might be inducing damage to the local spiral ganglion neural population near the stimulating electrodes, despite the lower stimulus intensity used for chronic stimulation. Histological data in these animals, in fact, clearly show markedly poorer neural survival in the region near the stimulating electrodes pair (e.g., 40-60% in K105; 50-60% in K106). However, detailed study suggests that this loss is more likely related to 2 other factors: 1) Observation of data in the control ears shows great regional variation in the ototoxic drug effect with the greatest SG cell loss occurring in this region in the unstimulated ears as well. 2) Both implanted cochleas had mechanical trauma from insertion of the cochlear implant electrode array in the 40-50 and 50-60% regions, and this damage was particularly severe in K105 (Figure 3a). Study of serial sections through these regions suggest that the neural loss is proportionate to the extent of damage to the basilar partition. Given the similarity of the patterns of neural survival in K101

Figure 3a. Severe trauma near the tip of the cochlear implant in **K105** caused marked neural loss (SG) in the cochlear region about 11 mm (50%) from the cochlear base. The electrode (E) has ruptured the basilar membrane and eroded the osseous spiral lamina (arrows). Marked chronic foreign body reaction and inflammation are evident in the Scala Vestibuli. L, spiral limbus.

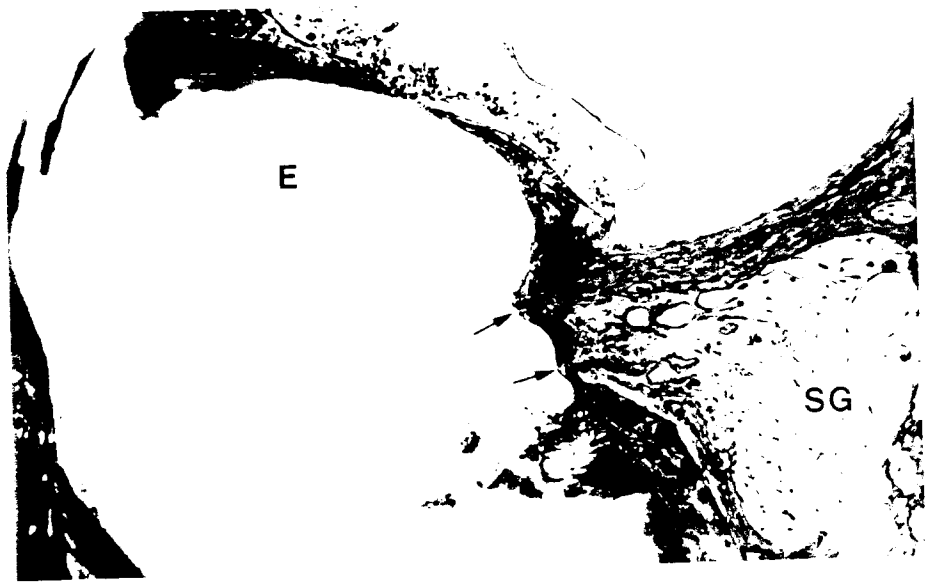

Figure 3b. The cochlear implant electrode in **K107** also caused severe trauma, rupturing through the basilar partition and the osseous spiral lamina (arrows) in this region 9.5 mm or 40% from the base. The tip of the electrode (E) is lodged primarily in the scala vestibuli rather than in the scala tympani. SG cell loss is very severe.

(300 pps/30 Hz stimulation) and these higher frequency stimulation cases, there is no evidence that the higher frequency stimulus was more damaging in itself. However, we cannot rule out an interaction of the effects of mechanical trauma and direct electrical stimulation in inducing the marked local neural loss seen in these regions where the tip of the electrode always causes mechanical trauma.

The overall means of the morphometric data in the final subject K107 showed an improvement in SG cell density of only about 10% in the stimulated ear. However, during chronic stimulation this animal was treated for a long-standing, intractable infection of the percutaneous connector which involved the middle ear and tracked into the most basal region of the cochlear "hook," resulting in extensive ectopic bone formation and inflammation as illustrated in **Figure 4**. In fact, if this basal 10% sector of the cochlea is deleted from the data, the survival would be 59.1% in the stimulated cochlea and 43.6% in the control cochlea, thus bringing the difference up to 15.5%. As mentioned previously, another factor which can have a major impact on spiral ganglion survival in the implanted ears is mechanical trauma to the basilar partition or insertion trauma. Particularly severe trauma was observed in the 40-50% region of K107. As illustrated in **Figure 3b**, the tip of the implant ruptured of the basilar membrane and fractured the osseous spiral lamina and actually was positioned in the scala vestibuli in this region. This mechanical damage clearly offset the trophic effects of stimulation and resulted in neural degeneration that was proportionate to the extent of damage observed in sections through the region.

Figure 4.
Cochlear histopathology in the most basal "hook" region of the implanted cochlea of **K107** resulting intractable infection of the implant. Severe inflammation and new bone formation is seen in the scala tympani and infiltrating the osseous spiral lamina.

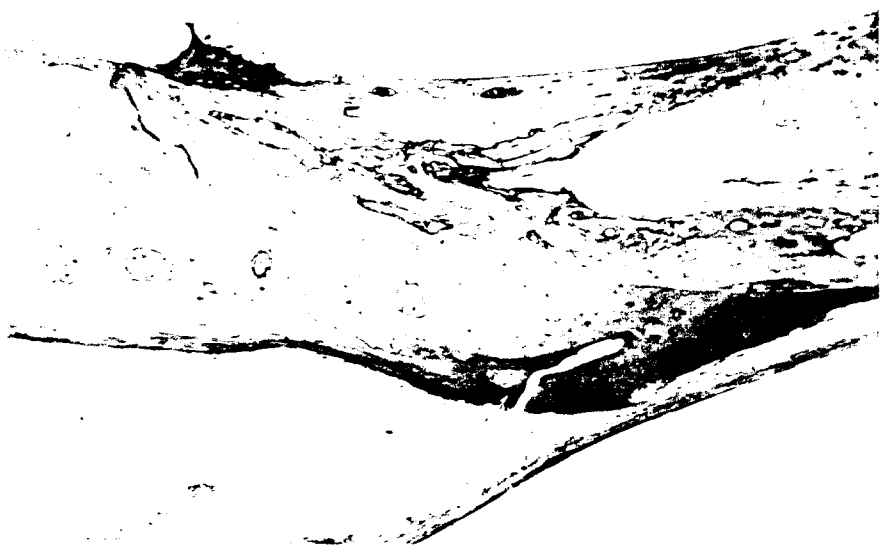

Figure 5 presents the pooled spiral ganglion data for the 4 animals in this new high frequency and 2-channel stimulation series (excluding K104). On average, higher neural density is observed throughout the entire cochlea, with a mean of 48% of normal in the stimulated ears and 33% in the controls. This 15% difference in neural survival is highly significant for the group ($P<0.01$; Student's t-test, paired; overall means stimulated vs. control SG data). It should be noted that a somewhat greater protective effect on neural survival was recorded in the previous "temporally challenging" chronic stimulation group, where the increase in neural survival was about 21%. However, several considerations argue that these initial results in the high frequency and 2-channel stimulation group actually should be viewed as fairly comparable to the previous series. First, mean stimulation period was somewhat shorter in the high frequency/2-channel group (mean, 28.5 weeks) than in the temporally challenging stimulation group (mean, 35 weeks). Another compromising factor in the high frequency/2-channel stimulation group was the finding

of especially severe insertion trauma in two of the implanted ears, K105 and K107. In addition, stimulation was set at an intensity equal to EABR threshold in K105 and K106 rather that at 2 dB suprathreshold, possibly reducing the extent of the trophic effects of electrical stimulation in these subjects. Finally, the severe infection which spread into the basal hook region of the cochlea in K107 clearly compromised survival in the implanted ear of this subject. In fact, considering this list of extenuating circumstances, it is, quite encouraging that the trophic effects of stimulation were significant in this initial group of only 4 subjects.

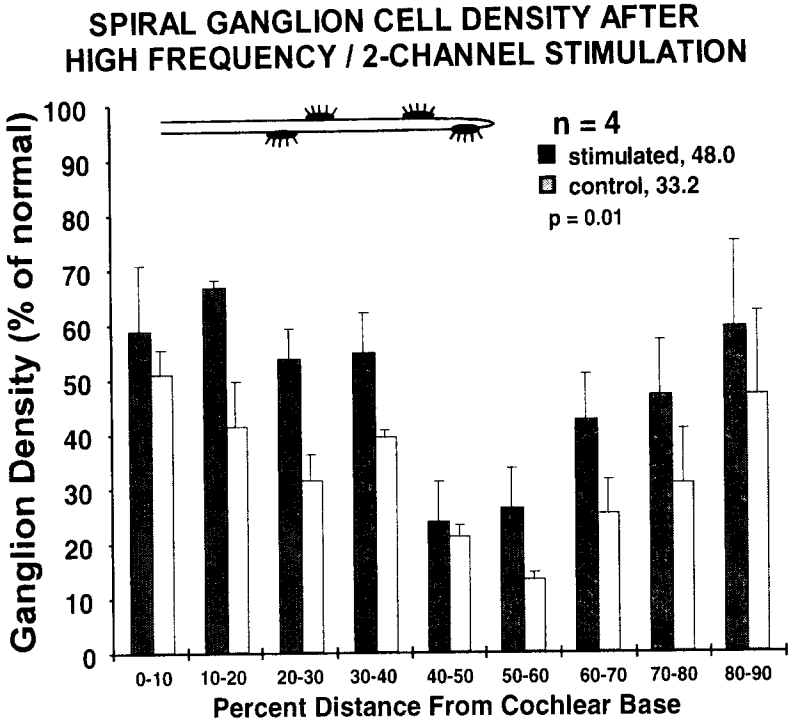

Figure 5. Pooled SG morphometric data for the the high frequency /2-channel stimulation group (n=4) presented in Figure 1.

The pooled SG data are shown again, expressed as the *difference* between stimulated and control ears in **Figure 6**. This presentation of the data emphasizes the regional variation in the stimulation-induced maintenance of the spiral ganglion neurons. The greatest effect of stimulation is seen in the basal turn, in the cochlear region 10-40% from the base. Significant differences also are seen throughout the cochlea with the exception of the most basal "hook" region (0-10%) and the 40-50% region where insertion trauma compromised survival in every implanted cochlea.

Finally, **Figure 7** shows the SG density data expressed as % difference between the stimulated and unstimulated sides. These values are calculated by subtracting regional SG density values for the unstimulatedears from the paired stimulated values, then dividing by the control value and multiplying by 100. Expressed in this manner an greatest increase (100% increase) is recorded in the 50-60% region region, where SG survival was 13% of normal in the control ears vs. 26% of normal in the stimulated group. However, at the apex, a more modest 27% increase is calculated when survival in the controls was about 47% of normal vs. about 60% in the stimulated ears. Averaged throughout the cochlea, SG density in the stimulated ears was increased by 44% 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" regional status of the neural population after deafening, and measures how large the difference in cell density is relative to these control values.

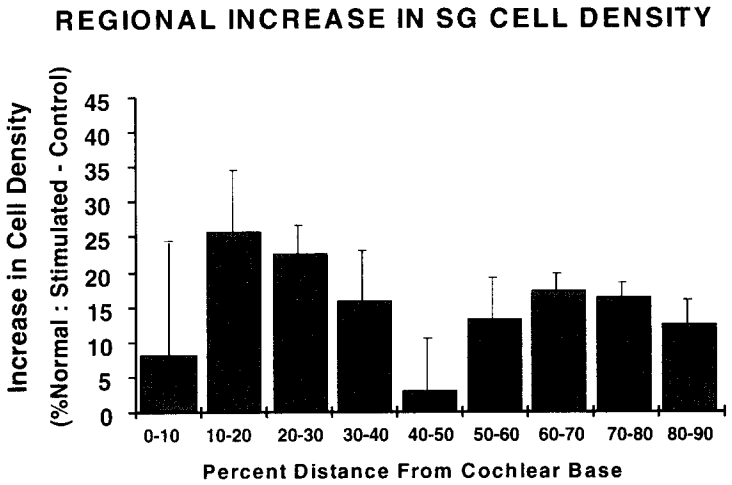

Figure 6. Mean *difference* in SG cell density (stimulated less control data) for 10% cochlear sectors from base to apex.

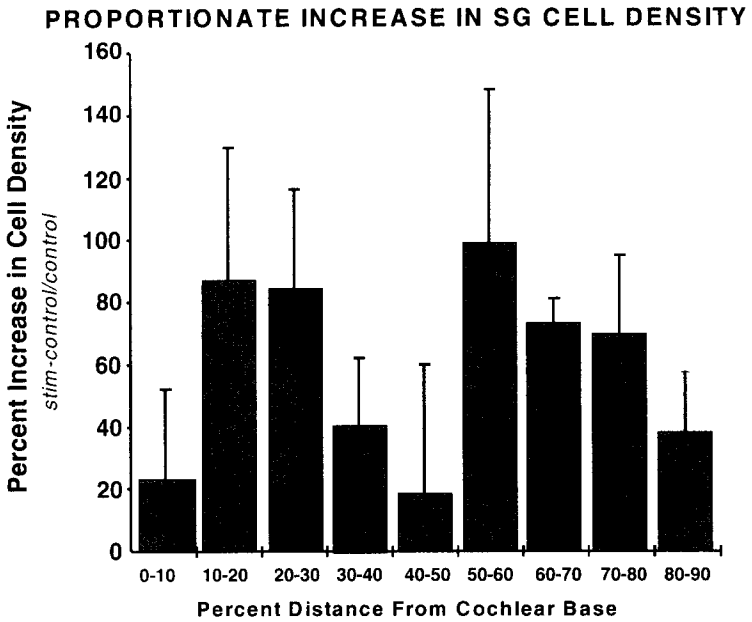

Figure 7. Pooled SG cell density data expressed as % increase. (See Text.)

Our working conclusions from these data include the following:

1) The higher frequency pulsed carrier rates (800 and 900 pps, 100% sinusoidal amplitude modulation) used in these protocols appear to be safe for chronic stimulation as applied in these studies, although we cannot rule out the possibility that electrical stimulation may exacerbate the neural loss in regions of mechanical trauma.

2) Even when chronic stimulation is delivered at an intensity level equal to EABR threshold, significant effects of these higher frequency electrical pulse trains in promoting survival of SG neurons were demonstrated throughout most regions of the deafened cochleas.

Work Planned for the Next Quarter

1) Two adult deafened, prior normal cats will be implanted. Two to 3 acute electrophysiology experiments will be conducted during the next quarter to study chronically stimulated cats. One adult-deafened, stimulated cat will be studied in the final acute electrophysiological experiment, completing a series of 6 animals in Dr. Charlotte Moore's study. Two neonatally deafened animals in the GM1 ganglioside/2-channel stimulation group also will be studied in terminal experiments.

2) Cochlear histopathology studies will continue. A recent outbreak in our cat colony of feline herpesvirus (FHV), which has a mortality rate of up to 70% in newborns, has resulted in the loss of 2 neonatally deafened kittens in our GM1 ganglioside /2- channel stimulation group. One kitten did not survive the implantation surgery, another died within 2 weeks of implantation and a third animal died after 10 weeks of chronic stimulation when it was anesthetized for EABRs. (All of these animals were from the same litter that showed early clinical signs of FHV infection.) Evaluation of spiral ganglion survival will be completed in these animals and data will be presented in the next Quarterly Progress Report along with data from 3 GM1 animals that completed chronic stimulation. GM1 has been reported to potentiate growth factors which sustain the spiral ganglion neurons, and our hypothesis is that treatment of these animals in the period after neonatal deafening and prior to cochlear implantation will further increase overall spiral ganglion survival. Three additional animals in the GM1 series will continue chronic stimulation throughout the next quarter in addition to the 2 that will be studied in terminal experiments.

3) Three members of our group will attend and present data at the 2nd Symposium on Molecular Mechanisms in Central Auditory Function and Plasticity to be held in Park City, Utah June 25 through June 27.