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Protective Effects of Patterned Electrical Stimulation on the Deafened Auditory System

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ABSTRACT

One goal of this Contract research is to examine the mechanisms underlying the neurotrophic effects of chronic electrical stimulation of the cochlea. In neonatally deafened cats, stimulation with an implant promotes increased survival of cochlear spiral ganglion (SG) neurons and partially prevents the retrograde degeneration which otherwise results from the loss of hair cells following deafness. Recent experiments show that appropriate electrical stimulation delivered over a period of several months can maintain increased survival of about 20% of the normal neuronal population. However, stimulation only *partially* prevents the SG neural degeneration resulting from early deafening in these animals, and we are interested in developing protocols that will further ameliorate the consequences of neonatal deafness. Recent studies of cultured SG neurons by Green et al. suggest that there are multiple mechanisms underlying the neural protective effect of depolarization *in vitro*, including a cyclic-AMP pathway, autocrine neurotrophin expression, and at least one other pathway. We hypothesize that neural activity elicited by chronic electrical stimulation in our neonatally deafened animals promotes SG survival through these same mechanisms *in vivo*. Neurotrophins are of particular interest because they are involved in the development and maturation of the central nervous system and also because exogenously applied neurotrophins can promote neuronal survival following injury.

Particularly relevant to our studies is a report by Walsh and Webster (1994) that exogenous administration of GM1 ganglioside significantly ameliorated atrophy of SG neurons in mice after conductive hearing loss. GM1 ganglioside is a glycosphingolipid which has been shown to promote neuronal survival following injury and which has been the subject of a number of clinical trials in humans suggesting beneficial effects of GM1 in the treatment of stroke, spinal cord injuries and Alzheimer disease. Based upon these and other findings, we hypothesized that GM1 treatment after neonatal deafening in our animals would potentiate neurotrophins which sustain SG survival and thus ameliorate SG degeneration which occurs prior to the time when electrical stimulation can be initiated.

This Quarterly Progress Report presents preliminary results from animals that were deafened neonatally, then subsequently received daily subcutaneous injections of GM1 ganglioside during the interim period of 3-4 weeks until the time of cochlear implantation. The data collected to date show a wide range of results. Excellent survival of SG neurons was demonstrated in some subjects, but other animals showed much less modest effect on neural survival. At present it is unclear whether these intersubject differences simply reflect individual differences in the ototoxic drug effects or whether the wide range of results is related to individual differences in the efficacy of chronic electrical stimulation in activating the auditory nerve.

INTRODUCTION

An essential goal of our Contract research is to examine the factors and mechanisms underlying the neurotrophic effects of chronic electrical stimulation of the cochlea. In neonatally deafened cats, stimulation with an implant promotes increased survival of cochlear spiral ganglion neurons and at least partially prevents the retrograde degeneration which otherwise results from the loss of hair cells following deafness. Our previous studies have shown 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 increased survival of the spiral ganglion neurons, if chronic stimulation is continued for several months (Leake et al., 1999). In addition, more recent experiments have demonstrated similar results in neonatally deafened animals after chronic stimulation with higher frequency signals (e.g., 800 PPS carrier, sinusoidally amplitude modulated at 20 Hz) and/or stimulation on 2 channels of a cochlear implant (Quarterly Progress Report #6, Contract N01-DC-7-2105, Jan. 1 to March 31, 1999). With these experimental protocols, highly significant increases in SG survival of about 20% of the normal neuronal population are observed (Figure 1).

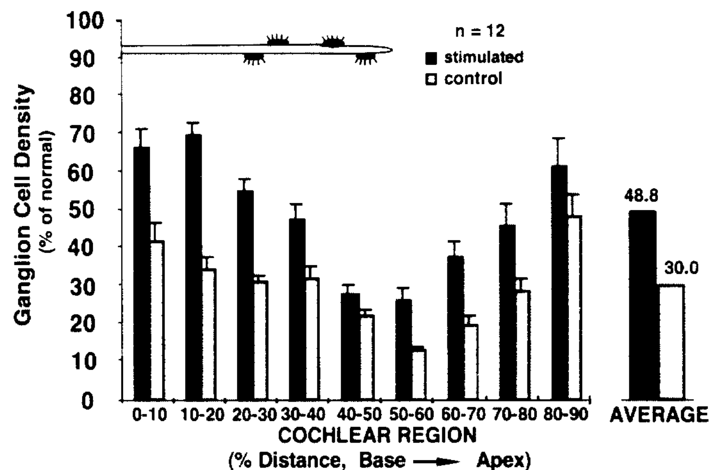

Figure 1. Summary figure showing pooled SG morphometric data for neonatally deafened cats that received several months of chronic electrical stimulation using temporally challenging signals (n=8) and higher frequency or 2-channel stimulation (n=4). Data are expressed as percent of normal density for 10% sectors of the cochlea from base to apex. Mean SG cell density was about 49% of normal in the stimulated cochleae and about 30% of normal in the contralateral control, deafened ear.

It is clear from these data, however, that the electrical stimulation only *partially* prevents the SG neural degeneration resulting from early deafening in these animals. Averaged over all cochlear sectors, SG survival is about 50% of normal in the stimulated cochleae. Thus, we are interested in developing protocols that will further ameliorate the consequences of neonatal deafness in these animals. Recent studies of cultured SG neurons by Green and colleagues at Iowa (Hegarty et al., 1997; Hanson et al., In press), suggest that there are multiple mechanisms underlying the neural protective effect of depolarization *in vitro*, including a cyclic-AMP pathway, autocrine neurotrophin expression, and at least one other pathway. We hypothesize that neural activity elicited by chronic electrical stimulation in our neonatally deafened animals is effective in engaging and driving these same mechanisms *in vivo*. Neurotrophins are of particular interest because they are involved in the development and maturation of the central nervous system (for review, see Fritzsch et al., 1997).

Moreover, numerous studies have shown that various exogenously applied neurotrophins can promote neuronal survival following injury (e.g., Keithley et al, 1998; Miller et al., 1997; Zheng et al., 1997).

Two studies are particularly relevant to our experiments. First, is a study by Walsh and Webster (1994) showing that administration of GM1 ameliorated atrophy of SG neurons in mice with conductive hearing loss. These authors suggested that GM1 potentiates expression of neurotrophins, which sustain SG neural integrity. In addition, Parkins et al. (1999) recently reported that GM1 administration significantly ameliorated SG degeneration after deafness induced by the co-administration of the ototoxic drugs kanamycin and ethacrynic acid in guinea pigs. GM1 ganglioside is a glycosphingolipid with an attached monosialic acid moiety which clearly has been shown to promote neuronal survival following injury and which has been the subject of a number of clinical trials in humans. Beneficial effects of GM1 in the treatment of spinal cord injuries (Geisler et al., 1991), stroke and Alzheimer disease of early onset have been reported (Svennerholm, 1994).

Based upon these various findings, we hypothesize that GM1 treatment after neonatal deafening in our animals will potentiate neurotrophins, which sustain SG survival and thus ameliorate SG degeneration that occurs prior to the time when electrical stimulation can be initiated. This Quarterly Progress Report presents preliminary results of a study in which GM1 was administered in neonatally deafened cats.

METHODS

a. Neonatal Deafening and GM1 Treatment.

Table 1 presents deafening and chronic stimulation history data for the animals in the GM1 treatment group and for a ***comparison*** group selected to match the GM1 subjects as closely as possible with regard to stimulation history and duration of deafness.

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 one day after birth and continued for 16 days postnatal. 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) the 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 the period of neomycin administration in these experimental groups ranged from 16 to 21 days.

In the GM1 group, GM1 administration was initiated on the day that the profound hearing loss was documented. The GM1 (Monosialotetrahexosyl-ganglioside sodium salt, 99%) was supplied by FIDIA, Abano Terme, Italy. Kittens received daily subcutaneous injections (30 mg/kg) of GM1 dissolved in sterile saline. Injections were continued throughout the period prior to cochlear implant surgery and until the initial day of chronic electrical stimulation. This period ranged from a minimum of 28 days in K125 to a maximum of 38 days in K117. *However, it should be noted that in subject (K133), GM1 treatment had to be discontinued after 24 days, and there was a delay of 12 days before chronic stimulation could be initiated.* (Difficulties in importing GM1 at that time had resulted in a shortage. GM1 is derived from bovine brain, and the USDA had just placed severe restrictions on importation of all such substances from Europe due to concerns about bovine spongioform encephalopathy or "mad cow disease." It was a lengthy process to obtain licensure from the USDA for continued importation of GM1 from FIDIA in Italy.)

b. Chronic Stimulation.

Kittens in both groups underwent unilateral cochlear implantation of a scala tympani electrode at 6 to 9 weeks postnatal, and chronic electrical stimulation was initiated at 7 to 10 weeks postnatal (Table 1).

Table 1. STIMULATION HISTORIES

Cat #	Neomycin mg/kg/days	Age at Initial Stimulation (weeks)	Stim. Intensity electrode pair/μA	Stim. Period (weeks)	Stim. Frequency	Age at Study (weeks)
			GM1 Ganglioside and Two Channel Stimulation			
K109	60/19	7	**1,2:** 50-71 **3,4:** 12-158	25	300pps/30Hz 900pps/50Hz	32
K117	60/18	8	**1,2:** max=45-71* **3,4:** max=36*	28	SP	36
K119	60/17	7	**1,2:** 32-126 **3,4:** 100-141	34	300pps/30Hz 900pps/50Hz	41
K125	60/21	7	**1,2:** 40-158 **3,4:** 63-71	30	100-800pps/50Hz	37
K127*	60/17	9*	**1,2:** 79-71 **3,4:** 71	15*	100-800pps/50 Hz	24*
K129*	60/17	7*	**1,2:** 126 **3,4:** 100	3*	100-800pps/50Hz	10*
K133	60/21	8	**1,2:** 36-112 **3,4:** 224-31	36	100-800pps/50Hz	44
Means, (n=5*):		**7.4 wks**		**31 wks**		**38 wks**
			Comparison Group: Matched for Age and Stimulation History			
K83	60/19	10	125 μA	22 wks	80 pps	32
K89	60/19	10.5	80-100 μA	27.5 wks	300pps/30Hz	38
K98	60/20	7	50-100 μA	33 wks	SP/beh	40
K105	60/20	9	63-355 μA	33.5	800pps/20Hz	38
K106	60/20	9	80-400 μA	33	800pps /20Hz	42
K101	60/18	8	**1,2:** 2/79-200 **3,4:** 2/100-316	29	300pps/30Hz/beh 300pps/30Hz	37
K107	60/16	9	**1,2:** 2/100-200 **3,4:** 2/71-100	22	800pps/60Hz	31
Means, (n=7):		**8.9 wks**		**29 wks**		**37 wks**

* For the subject stimulated with the analogue processor, the maximum amplitude is indicated
** Two animals, K127 and K129, were eliminated from the study (see text) and, therefore, are not included in the calculations of mean duration of stimulation and age at study for the group or in the summary figure for the GM1 SG morphometry data shown in Figure 7a.

All animals in both groups received chronic stimulation using signals that were considered to be "temporally challenging" to the central auditory system. Stimulation periods were 4 hours/day, 5 days/week for a minimum of 6 months, as required by the technical specifications of this Contract. In most animals, electrical signals were computer-generated trains of biphasic pulses (200 μsec/phase), and stimulation intensities were set at 2 dB above EABR threshold, as determined (using 200 μsec pulses) for each individual stimulated channel. Carrier rates for these pulsed signals ranged from 80 to 900 pps, and the higher frequency carriers (≥ 300 pps) were also sinusoidally amplitude modulated (modulation depth of 100%). One subject in each group received stimulation from a functional analog cochlear implant processor set at maximum stimulus amplitude of 6 dB above EABR threshold.

All animals in the GM1 group received chronic stimulation on 2 channels of their cochlear implants, using both the apical and basal bipolar pairs (electrodes 1,2 and 3,4, respectively). The first 3 GM1 subjects received ***concurrent*** stimulation of the 2 channels. K109 and K119 were stimulated on the apical channel a carrier rate of 300 pps that was sinusoidally amplitude modulated at 30 Hz, and on the basal channel the electrical signal was 900 pps, modulated at 50 Hz. The signals delivered on the 2 channels were offset in time such that pulses were "interleaved" and did not occur simultaneously, modeling current clinical "CIS" processors. The last four subjects in the GM1 group were stimulated in an alternating fashion with stimulation for 2 hours on one channel followed by 2 hours on the other channel. In these four subjects we applied a repertoire of 4 temporally challenging signals that were ***varied sequentially*** throughout the chronic stimulation period according to the following schedule: 1) For the first week, each channel delivered a simple, unmodulated 100 pps signal. 2) During the second week, stimulation continued using a carrier rate of 300 pps, 100% sinusoidally amplitude modulated at 30Hz. 3) In the third week, the signal was 500 pps/40 Hz AM. 4) In the fourth week, the signal was 800 pps/50 Hz. This sequence was then repeated over 4-week intervals until the chronic stimulation period is completed.

As mentioned previously, the comparison group is a subset of subjects in the temporally challenging/high frequency stimulation groups which have been described in detail in previous publications (Leake et al., 1999,2000) and in a previous Quarterly Progress Report for this Contract (QPR #6, January 1 to March 31, 1999). The group consists of 7 neonatally deafened subjects that did ***not*** receive GM1, and were selected from the previous chronic stimulation experimental group (Figure 1) because they had stimulation histories and durations of deafness at study that were matched as closely as possible to the GM1 subjects. (See Table 1 for individual stimulation histories.)

EABR thresholds were determined monthly throughout chronic stimulation periods, and stimulators were adjusted as necessary to maintain the appropriate current levels relative to EABR thresholds. Two of the 7 animals in the GM1 group are presented in this report, but have been deleted from the pooled data and statistical comparisons because we were unable to complete the protocol specifications. One cat, K129, damaged its implant after only 3 weeks of chronic stimulation, and the animal died when anesthetized to repair the device. A littermate, K127, died similarly after about 15 weeks of stimulation when it was anesthetized for EABR measurements. It should be noted that we eventually lost all the kittens in this particular litter which showed clinical signs of a severe feline herpesvirus (FHV) that was active in our breeding colony at that time. FHV has a high mortality rate in young kittens (up to 70% even without the stress of anesthesia).

Chronic stimulation periods in the remaining 5 GM1 subjects ranged from 22 to 36 weeks, with a mean of 31 weeks, and the comparison group had a similar range (22 to 33.5 weeks) and mean stimulation period (29 weeks). All the animals were then studied in terminal acute electrophysiology experiments, and tissues were harvested for histopathological and morphological studies. The total

duration of deafness at the time of study ranged from 32 to 44 weeks for the GM1 group (mean = 38 weeks) and the comparison group was very similar.

RESULTS and DISCUSSION

a. Initial Effects of Neonatal Deafness and GM1 Treatment on SG Survival.

We have previously published data showing that the schedule of ototoxic drug (neomycin) administration applied in these kittens causes precipitous degeneration of the cochlear hair cells (Leake et al., 1997). The profound hearing loss documented by the absence of a click-evoked ABR response at 110 dB SPL after 2 to 3 weeks of drug administration is associated with virtually complete hair cell degeneration throughout the cochlea. Figure 2a illustrates SG density data from a group of animals studied immediately after 16- 24 days of ototoxic drug treatment. Degeneration of SG neurons apparently occurs concomitant with the degeneration of the sensory hair cells, and survival is already reduced to about 82% by the time that ototoxic drug administration is completed. It is clear that significant neural degeneration occurs rapidly in these animals.

Figure 2b presents preliminary data from 2 subjects that were deafened by the identical ototoxic drug protocol and then received daily GM1 ganglioside injections (30 mg/kg, SQ) after profound hearing loss was confirmed. Injections were continued until these animals were studied at 6-7 weeks of age, at the time that chronic electrical stimulation was initiated in their littermates that had received a cochlear implant (K117 and K119). These SG morphometry data also show a significant loss of SG neurons, but the survival in these older kittens is 83% of normal and is equivalent to that seen in the younger group immediately after ototoxic drug treatment. These preliminary data suggest that all the SG neurons that survived after the deafening procedure may have been maintained until the time these animals would have undergone cochlear implantation.

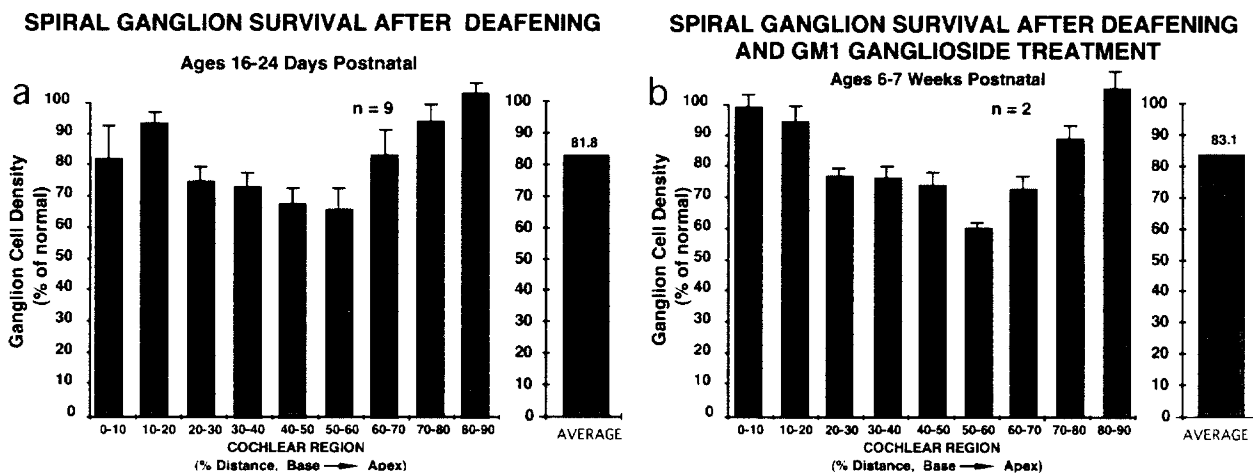

FIGURE 2. a. SG cell density (% of normal) in animals studied at 2 to 3 weeks of age immediately after documentation of profound deafness. **b.** SG cell density in neonatally deafened animals after GM1 treatment at 6-7 weeks of age, at the time chronic electrical stimulation was initiated in their littermates that received a cochlear implant. Data suggest that GM1 may maintain the neural population and ameliorate progressive degeneration after deafening.

One interesting finding in the histological examination of these GM1 treated kittens was the observation that SG cell loss seemed to occur predominantly in the upper portion of Rosenthal's canal, closer to the scala vestibuli. Figure 3 illustrates this point, showing a histological section in which the lower part of ganglion (which is nearer the scala tympani) appears to have near-normal cell survival, but there is substantial loss in the upper aspect of Rosenthal's canal. This result is of interest because previous studies have suggested that there is a differential distribution of high- and low-spontaneous rate neurons within the ganglion (Leake et al., 1993). Specifically, low-SR neurons are located primarily in the scala vestibuli sector of the ganglion, which predominantly showed cell loss in these GM1 specimens. The scala tympani sector of the ganglion contains primarily high-SR neurons, which appeared to exhibit better survival with GM1 administration. This unusual pattern of degeneration suggests that GM1 may be more effective in preventing degeneration of high SR neurons. Ferrari et al. (1995) have shown that GM1 acts via trkA. TrkA expression and activity are necessary for the survival-promoting function of GM1, and GM1 stimulates trkA activity in the absence of nerve growth factor. One possibility is that neurons with higher spontaneous activity might have higher trkA expression, which could cause them to be preferentially effected by GM1 and to survive longer.

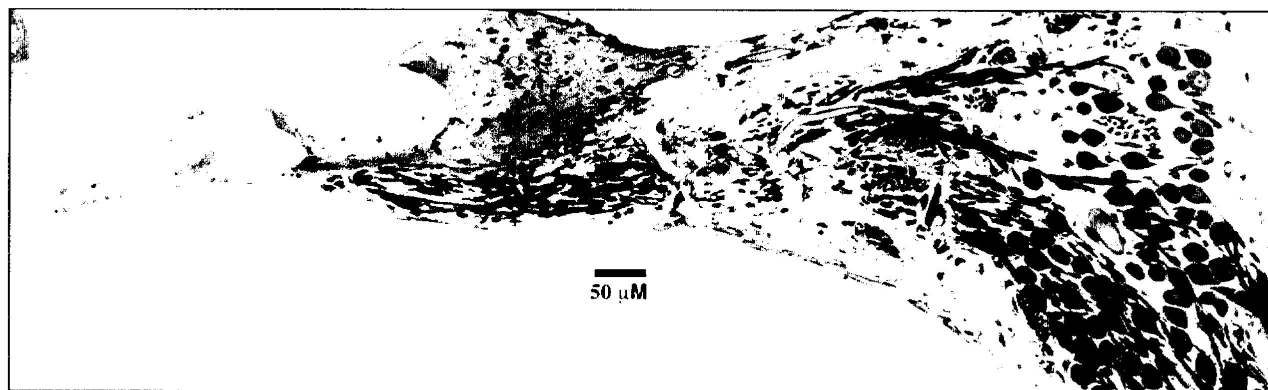

FIGURE 3. Histological section taken from the upper basal turn of the cochlea, about 11 mm from the base (40-50% sector), in a neonatally deafened, GM1 treated animal studied at 7 weeks of age. Note that substantial loss of SG cells is evident in the upper portion of the ganglion, whereas the lower aspect of the cell cluster appears to be nearly normal.

b. Data from Individual Subjects in the GM1 Group

1. GM1 Subjects with Shortened Chronic Stimulation Periods.

Figure 4 shows morphometric SG density data from the 2 cats in the GM1 experimental group that died before completion of chronic stimulation protocols. K129 damaged its implant after only 3 weeks of chronic stimulation and was studied at 10 weeks of age. Two points are interesting in the data from this individual subject (Fig. 4a). First, after only 3 weeks of electrical stimulation, a trend toward increased survival in the stimulated cochlea is suggested by the finding that mean SG cell density is 5% higher in the stimulated cochlea. Secondly, while excellent SG survival was recorded in the stimulated ear of this subject, the overall mean (72% of normal) is reduced by about 10% compared to the 2 subjects studied immediately after deafening as described above. A second animal, K127, that died after 15 weeks of stimulation and was studied at 24 weeks of age. The SG data from this subject (Fig. 4b) are remarkable for the poor neural survival recorded, especially in consideration of the relatively short duration of deafness in this subject. As mentioned previously,

both these animals were members of a litter that exhibited clinical signs of FHV infection and all 5 kittens in that litter died early in life. It should be noted that K127 had a very low birth weight 94 g at birth, was too small to undergo implant surgery until 9 weeks of age (it weighed only about 500 g, although we usually wait until kittens are 600 g to implant), and at the time of death was about the same size as kittens belonging to a litter born almost *2 months* later! Thus, it seems likely that the results in this animal may have been effected by its compromised health and the FHV infection.

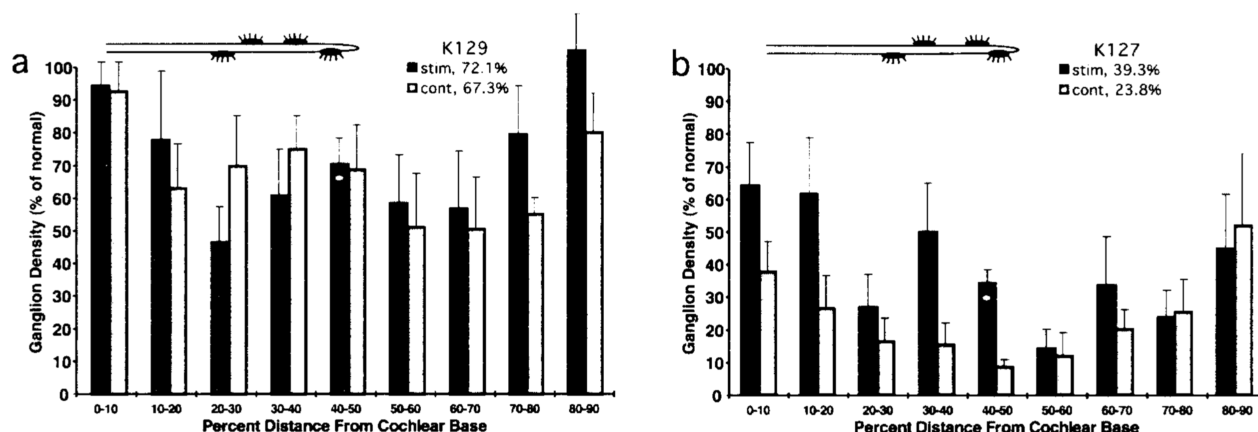

FIGURE 4. Morphometric SG data from 2 individual animals in the GM1 experimental group that did not complete their chronic electrical stimulation protocols and were studied prematurely at 10 weeks (K129) and 24 weeks (K127) of age.

2. GM1 Subjects that Completed Chronic Stimulation Protocols.

The morphometric SG density data for the remaining 5 subjects in the GM1 experimental group that did complete the electrical stimulation protocol are illustrated in Figure 5. The individual subjects all show markedly higher SG density in the cochlea that underwent implantation and chronic electrical stimulation. However, there is a substantial variation in the absolute cell densities and their distribution in the stimulated cochleae of the individual subjects. In the first two subjects in the GM1 group , K 109 and K117, excellent neural survival is maintained in the stimulated cochleae, with a mean overall SG density that is about 66% of normal. SG survival is particularly remarkable in the basal one-third of the cochlea where mean SG cell density is maintained at >85% of normal. This is markedly better survival than that seen in the previous temporally challenging stimulation group shown in Figure 1, in which SG density was less than 50% of normal overall and about 62% of normal in the basal one-third of the cochlea. Figure 6 illustrates the excellent SG neural survival seen in histological sections from the basal cochlea in one of these subjects.

The highly promising results demonstrated in these first two subjects in the GM1 treatment group contrast sharply with the data from two other subjects, K119 and K133. Both showed substantially poorer neural survival, with overall mean SG densities of 39% and 46% of normal, respectively, in the stimulated cochleae. The final subject in the GM1 group, K 125, showed SG data that were intermediate between the excellent results in the first 2 subjects and the lower overall SG densities in K119 and K133. Averaged over all cochlear sectors, SG density in K125 was 54% of normal, and again there was a pattern of particularly good neural survival in the basal cochlea, where SG density was more than 67% of normal.

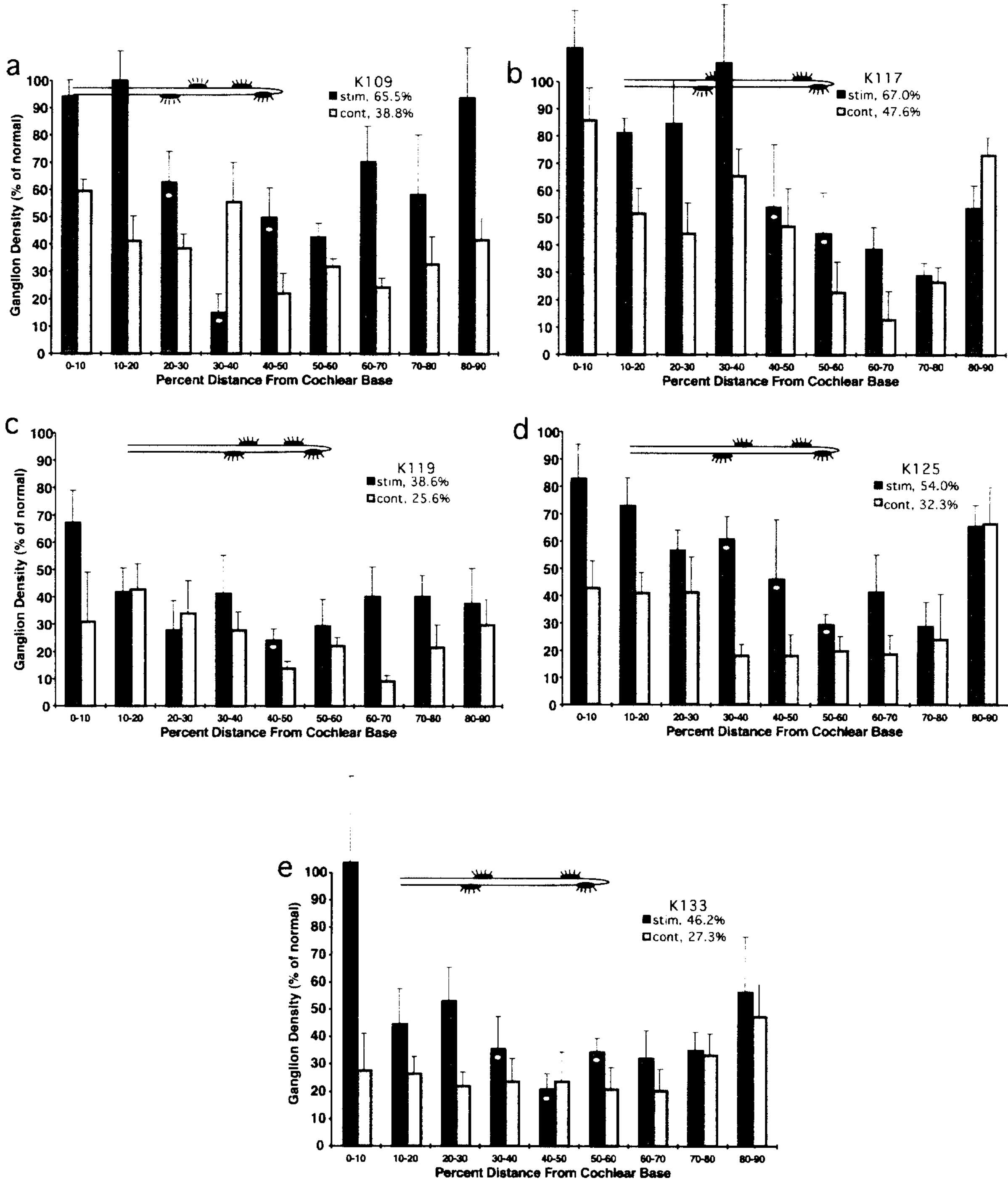

FIGURE 5. SG data from the 5 individual animals in the GM1 experimental group that completed the chronic electrical stimulation periods of 6 to 9 months and were studied at 8 to 10 months of age. All subjects exhibit significantly higher SG cell density in the stimulated cochlea, but there is tremendous individual variation in the absolute values of SG cell densities maintained in the stimulated ears.

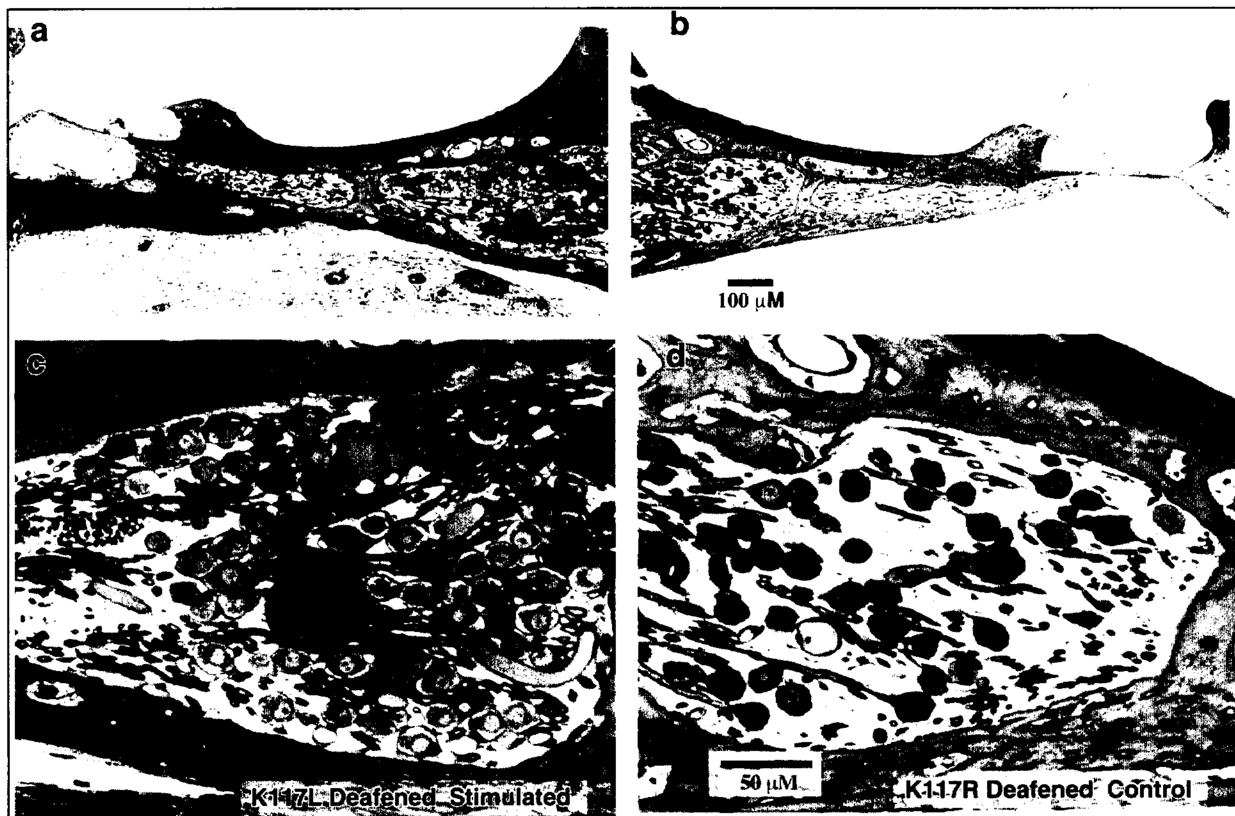

FIGURE 6. Histological sections taken from the cochleae of subject K117, illustrating SG neural survival in the region 20-30% from the base. In the stimulated cochlea (left, panels **a, c**) mean SG density is about 84% of normal, and in and the contralateral deafened, unstimulated ear (right) SG density is about 44% of normal. The low magnification photomicrographs above also illustrate the finding that many more of the myelinated radial nerve fibers (peripheral processes of SG neurons) are maintained within the osseous spiral lamina in the stimulated cochlea, as compared to the other side.

Careful review of the individual histories of these 5 subjects suggests that the differences in neural survival may be related to the individual differences in the efficacy of applied electrical stimulation, especially differences in the efficacy of *initial* stimulation period immediately following implantation surgery:

Subject K109: K109 had initial EABR thresholds for chronic stimulation that were quite low on both the apical channel (40 μA) and basal channel (100 μA). Stimulation was initially set 2 dB *below* (lower intensity than) EABR threshold to allow the kitten to become accustomed to "hearing" the electrical signals. The electrical signal intensity was sequentially increased over the next few days, and by the fifth day of stimulation, the specified level of 2 dB above EABR threshold in both channels was attained (50 μA for the apical channel and 126 μA on the basal channel), with no adverse response from the animal. In addition, EABR thresholds were very stable in this animal: thresholds were re-evaluated after 4 weeks of stimulation and were identical to initial values, and by the end of the > 6 months of chronic stimulation the threshold for the basal channel was *improved* by 1 dB and the threshold for the apical channel was elevated just slightly to 56 μA. Consequently, this animal had a nearly ideal stimulation history, with electrical signal levels on both channels clearly being adjusted to evoke activation of the auditory system sufficient to elicit an evoked brainstem response over the entire period.

Subject K 117: K117 was the second subject that showed superior neural survival. This animal had also had low initial thresholds (apical channel, 50 μA; basal channel, 56 μA). Again, thresholds were very stable throughout chronic stimulation (within 2 dB of initial values on apical channel, and the threshold actually *decreased* on the basal channel). This animal was stimulated concurrently on 2 channels of an analog cochlear implant processor that transduced environmental sounds. The dynamic range for each channel was set at 6 dB, and the maximum amplitude of the analogue stimulus waveform was set relative to the EABR threshold (determined to 200 μsec/phase pulses). Specifically, the basal channel had a bandwidth of 850-1450 Hz, so its maximum amplitude was adjusted to a current level *equal* to the EABR threshold in order to adjust for the longer phase duration of the analogue signals delivered by the processor (i.e., the phase duration of the 200 μsec/phase pulse used to measure EABR threshold would be approximately equivalent to 1600 Hz.) The apical channel had a bandwidth of 250-850 Hz, and the maximum amplitude of the stimulus was set at 6 dB below (subthreshold) to the EABR threshold. (The phase duration at the middle of this lower frequency band for the apical channel was 550 Hz, about double that of the basal channel which is about 1150 Hz. Thus, the maximum amplitude of the apical channel was reduced by 6 dB, or about half, to equalize the loudness of the two channels.) Thus, the 6-dB dynamic range and the varying phase duration in the electrical signals generated by the analogue processor ensured that highly effective stimulation was delivered on both channels throughout the chronic stimulation period.

Subject K119: In contrast to the clearly effective stimulation histories in the first two subjects, chronic stimulation in K119 was more problematic. K119 had initial thresholds that were very similar to K109 (apical channel = 32 μA; basal channel=100 μA). As in the previous subjects, stimulation was initially set at a lower intensity (in this case, initial stimulation was introduced *at* EABR threshold, first on the individual channels and after a couple of days on both channels concurrently) to allow the kitten to become accustomed to "hearing" the electrical signals. However, in this subject, stimulation was erroneously continued at this level for several weeks. Moreover, subsequent EABR testing was postponed, first because the animal was ill and had a high temperature, then because of equipment problems. When thresholds finally were evaluated, a significant elevation had occurred on both channels (apical channel = 79 μA; basal channel=158 μA). At this time, almost 3 months into the chronic stimulation period, the signal intensity was cautiously adjusted on both channels, but the animal reacted adversely (perhaps because the signals had been inaudible over much of the initial stimulation period), so the intensity was adjusted to a level 1-2 dB *lower* than the EABR threshold for a period of adaptation and finally set *equal to* EABR threshold for the remainder of the chronic stimulation period. Thus, although this subject clearly heard and responded to the chronically applied stimulus, the signal intensities used for most of the stimulation period may have been inadequate to excite the auditory nerve sufficiently to elicit an evoked brainstem response. Out of concern for the humane treatment of these animals, we must be conservative about setting stimulus levels in occasional timid subjects. It seems likely that the lack of effective stimulation (due to the very conservative initial levels of stimulation) led to an exaggerated reaction when the stimulus was later adjusted to a perceptible level and may also account for the relatively poor maintenance of neural survival in this subject.

Subject K 133: There were also problems with the initial electrical stimulation in this subject. The initial EABR threshold on the apical channel was very low (36 μA), while the basal channel threshold was much higher than usual (200 μA). Since the animal reacted strongly to the stimulus, despite the fact that stimulation was delivered in the ***alternating*** mode so that only a single channel was activated at a time (2 hours on one channel, then 2 hours on the other). Consequently, stimulation was again delivered at subthreshold levels for the first 3 weeks until the

animal recovered fully from surgery and the EABR threshold could be re-checked. (We suspected that there might be air trapped around the basal electrodes, which would elevate impedances and result in a spuriously high threshold.) When EABR testing again showed this discrepancy between channels, the intensity was sequentially adjusted to the specified level of 2 dB above EABR threshold and effective stimulation was maintained over the remainder of the chronic stimulation period. However, the initial month of stimulation was clearly subthreshold in this subject. Moreover, as noted previously, K 133 was the animal in which GM1 treatment had to be discontinued after 24 days (due to our inability to obtain GM1 at that time), and there was a delay of almost 2 weeks before chronic stimulation could be initiated. Thus, there was an interval of about 6 weeks after GM1 treatment until the auditory system was effectively stimulated electrically.

Subject K125: The initial stimulation in this subject was adjusted to the specified levels by the middle of the first week of stimulation with no problems. Also, the monthly EABR testing was carried out on schedule, and stimulation levels adjust appropriately to maintain intensity on each channel at 2-dB suprathreshold relative to the EABR threshold. However, after the first 4 months of stimulation, the animal damaged its implant and the basal channel was no longer functional. The breakage was too far proximal to be repaired for chronic stimulation, so only the apical electrode pair was stimulated for the remainder of the chronic stimulation period.

Thus, the two subjects that had ideal chronic stimulation histories (K109 and K117) also showed excellent SG survival, the two cats that had severely compromised chronic stimulation histories (K119, K133) had relatively poor neural survival, and the subject that had a slightly compromised chronic stimulation history (K125) showed intermediate SG survival. Based upon these findings, we suggest that that the differences in neural survival may be related to the individual differences in the efficacy of applied electrical stimulation. Further, the efficacy of stimulation during the initial period immediately following implantation surgery may be particularly important in maintaining a possible survival-promoting effect of GM1.

However, it is also possible that the differences in SG survival among subjects is due simply to individual variation in the response to the ototoxic drugs used to induce deafness in these young animals. Thus, we consider the results in this GM1 experiment as preliminary due to the small *n* of animals completing the specified chronic stimulation period and the potentially important differences in efficacy of stimulation among subjects.

c. GM1 treatment and Chronic Electrical Stimulation: Group Comparisons

The pooled SG data from the 5 subjects in the GM1 experimental group are illustrated in Figure 7a. The mean SG density in the stimulated cochleae is about 54% of normal, with survival on the control side of about 34% or normal. Particularly interesting is the survival in the extreme basal sectors of the cochlea, with SG density of >90% in the 0-10% sector and a mean of >70% of normal for the basal one-third (0-30%). Given the questions about the efficacy of stimulation in two of these subjects, these data seem promising. (It may be worthwhile to note that if we delete the 2 subjects with the major stimulation problems, the SG densities for the remaining 3 subjects were: 62% of normal in the stimulated ear and 40% of normal on the control side.)

Figure 7b illustrates SG data from the comparison group of 6 neonatally deafened, chronically stimulated cats that did not receive GM1. These subjects were specifically chosen to match the GM1 group for chronic stimulation parameters, duration of stimulation and age at study (Table 1). The GM1 group shows a slightly higher SG density in the stimulated ears, but this difference is only about 5% of normal. Obviously, a final conclusion regarding the possible effects of GM1 in this animal model must await additional data.

INCREASED SPIRAL GANGLION CELL SURVIVAL

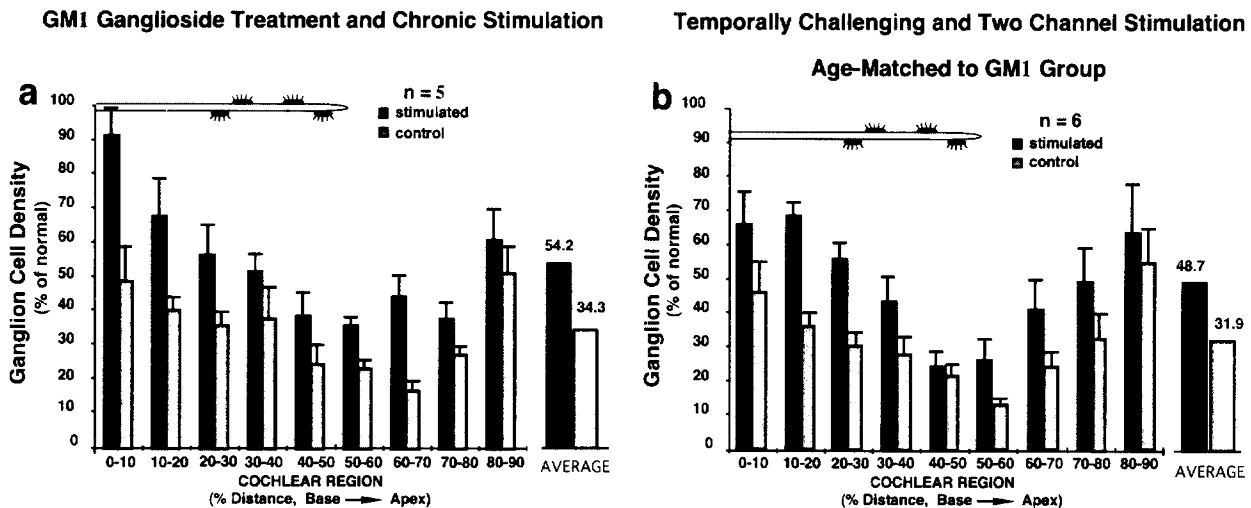

FIGURE 7. **a.** Pooled SG data from the 5 subjects in the GM1 experimental group that completed the chronic electrical stimulation periods of 6 to 9 months. Significantly higher SG cell density in the stimulated cochleae than in the control deafened ears. However, neural survival is only slightly higher in this group than in the comparison group of cats shown in the right panel. These subjects were selected to *match* the GM1 group, both in the applied chronic electrical stimulation protocols and in the duration of stimulation periods and age at study.

It should be noted that the preliminary report of the study conducted by Parkins et al., (1999) indicated that GM1 treatment alone produced a highly significant increase of 77% in SG survival in guinea pigs deafened acutely by co-administration of kanamycin and ethacrynic acid. Since results are expressed in percent increase, it is unclear what extent of SG neural survival was actually seen in these animals. (For example, if SG survival in deafened untreated guinea pigs was 50% of normal, than survival in the GM1 treated animals would be 85% of normal; but if survival in the deafened animals was only 20% of normal, then survival in the GM1-treated group would be about 35% of normal.) However, it is clear that either GM1 does not have such a marked effect in our kitten model, or else, a putative initial survival-promoting effect is not maintained over the months of chronic electrical stimulation with a cochlear implant.

There are several obvious differences between our study and the guinea pig study that are of interest with regard to the possible mechanisms underlying these disparate results. First, there are species and age differences. The Parkins study was of young adult guinea pigs, whereas our subjects are neonatal kittens. It seems unlikely, however, that species or developmental differences account for the disparity between the studies, because neurotrophins appear to be highly conserved across mammalian species and are well known to be involved in development. (GM1 actually is present in higher concentrations during development). Secondly, there is a difference in the drugs used to deafen the animals: The guinea pigs were deafened acutely (over a 1-2 hr period) by subcutaneous injection of kanamycin followed by infusion of ethacrynic acid, whereas our kittens received daily injections of neomycin that induced hearing loss over periods of 17-21 days. However, it is unclear why GM1 would be more effective in preventing an acute ototoxic insult than one of more gradual onset. Finally, there is a difference in the timing of the GM1 administration in the 2 studies: In the guinea pig study, GM1 was administered *prior to* the deafening procedure and daily thereafter. In our kittens, GM1 treatment was delayed by 17-21

days, until *after* the hearing loss was profound. It does seem plausible that the GM1 would be more effective if it were already present in the tissues at the time of the ototoxic drug insult. Thus, it would be extremely interesting to initiate GM1 injections *earlier* in a group of our neonatally deafened cats, administering the GM1 concomitant with neomycin injections and then continuing GM1 injections until chronic electrical stimulation is initiated. While the guinea pig data suggest that GM1 ganglioside can ameliorate the initial SG degeneration consequent from ototoxic drug insult, it is imperative to determine if such survival-promoting effects can be maintained over the long-term by stimulation with a cochlear implant. Otherwise, even if we can develop GM1 protocols that ameliorate SG degeneration, they may be of little practical value clinically if the "rescued" neurons are not viable over the long term.

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Work Planned for the Next Quarter

1) Two neonatally deafened animals in the GM1 ganglioside/2-channel stimulation group will continue chronic electrical stimulation throughout the next quarter. One of these subjects is receiving ***simultaneous*** 2- channel stimulation with the analog processor; the second receiving ***alternating*** AM pulsatile 2- channel stimulation.

2) Two additional neonatally deafened animals that are NOT part of the GM1 ganglioside series will also continue chronic electrical stimulation throughout the next quarter. One of these subjects is receiving ***simultaneous*** 2- channel stimulation with the analog processor; the second receiving ***alternating*** 2- channel stimulation using computer-generated AM pulse trains.

3) Evaluation of spiral ganglion survival will be completed in 2 additional animals from the GM1-treatment group that were studied in final experiments last quarter. Given the difficulties with chronic stimulation protocols in 2 of the initial subjects in this series, our working hypothesis is still that treatment of these animals in the period after neonatal deafening and prior to cochlear implantation will further increase overall spiral ganglion survival. Thus, we are very interested in examining the cochlear histology and SG morphometric data in these additional subjects.

4) Drs. Leake, Snyder, Vollmer, Moore and Beitel will attend the annual ARO Midwinter Meeting, which will be February 20-24 in St. Petersburg Beach, FL.