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Protective Effects of Patterned Electrical Stimulation on the Deafened Auditory System

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ABSTRACT

One goal of our 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. Previously published findings (Leake et al., 1999) show that appropriate electrical stimulation delivered over a period of several months promotes increased survival of about 20% of the normal neuronal population. However, the applied 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. (Hegarty et al., 1997) 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 study by Walsh and Webster (1994) suggesting that exogenous administration of GM1 ganglioside significantly ameliorated atrophy of SG neurons in mice after conductive hearing loss. Further, Parkins et al., (1999) reported that GM1 treatment produced a highly significant increase of 77% in SG survival in guinea pigs deafened acutely by ototoxic drugs. GM1 ganglioside is a glycosphingolipid that has been shown to promote neuronal survival following injury. GM1 has been the subject of a number of clinical trials in humans suggesting that it has beneficial effects 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 summarizes results from 2 experimental series in which animals were deafened neonatally, and received daily subcutaneous injections of GM1 ganglioside either concomitant with ototoxic drug administration and/or during the subsequent period of 3-4 weeks until the time of cochlear implantation. Animals then received a minimum of 6 months of chronic electrical stimulation via a cochlear implant. The data suggest a significant but modest improvement in neural survival with GM1 treatment, as compared to electrical stimulation alone.

INTRODUCTION

One important 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 (SG) 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., applied stimuli were amplitude modulated pulse trains with a carrier rate of 300 pps and 100% sinusoidal AM at 30 Hz or stimulation was delivered through an analogue cochlear implant signal processor) can be highly effective in maintaining increased survival of the spiral ganglion neurons, if stimulation is continued for several months (Leake et al., 1999). In addition, subsequent experiments 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).

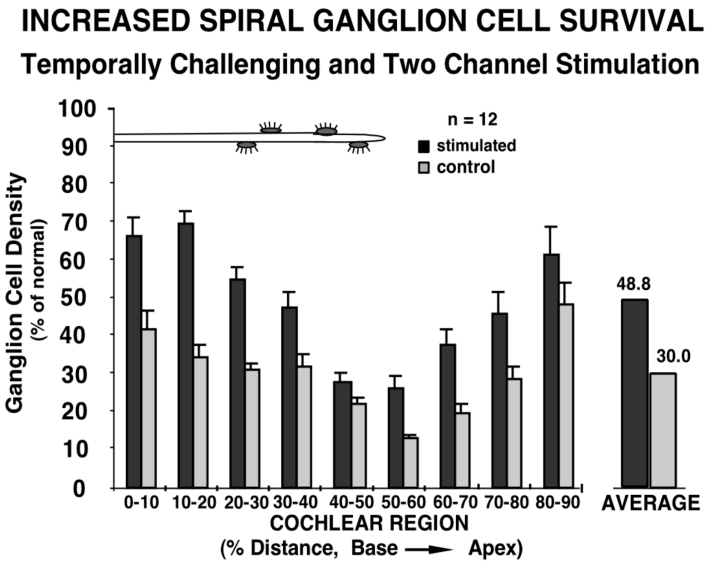

Figure 1. Summary figure showing SG morphometric data for neonatally deafened cats that received several months of chronic electrical stimulation, using temporally challenging signals (n=8) and higher frequency or 2-channel stimulation (n=4). Data are expressed as percent of normal SG cell density for10% sectors of the cochlea from base to apex. Mean density was about 49% of normal in the stimulated cochleae and about 30% of normal in the contralateral control, deafened ear (P<.001).

Although a pronounced neurotrophic effect is clearly evident in the stimulated ears, it is equally clear 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 only about 50% of normal in the stimulated cochleae. Thus, we are interested in investigating 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., 2001), 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). Further, a number of recent studies have reported that exogenous administration of neurotrophins (Staecker et al. 1996, 1998; Miller et al. 1997; Ernfors et al. 1996; Schindler et al. 1995; Shah et al. 1995; Zheng et al. 1995) and other neurotrophic factors such as glial cell line-derived neurotrophic factor (GDNF) (Ylikoski et al. 1998) can protect SG neurons and promote their survival after various types of insult, including ototoxic drugs.

Two studies are particularly relevant to our experiments. First, is a report by Walsh and Webster (1994) indicating 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) reported that exogenous administration of GM1 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 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 have been reported in the treatment of spinal cord injuries (Geisler et al., 1991), stroke and Alzheimers disease of early onset (Svennerholm, 1994).

Based upon the available literature, we hypothesized that GM1 treatment in our neonatally deafened animals would potentiate neurotrophins that sustain SG survival and thus ameliorate SG degeneration that occurs after deafening and prior to the time when electrical stimulation can be initiated. This Quarterly Progress Report presents the results of our study of GM1 in neonatally deafened cats.

METHODS

a. Neonatal Deafening and GM1 Treatment.

Table 1 presents data on the deafening and chronic stimulation histories for the animals in the GM1 treatment group. Also shown is a ***comparison*** group of 7 subjects, a subset of the group shown in Figure 1, that were selected to match the GM1 subjects as closely as possible for 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 first GM1 experimental group (K109 through K133), GM1 administration was initiated on the day that the profound hearing loss was documented. GM1 (monosialotetrahexosyl-ganglioside sodium salt, 99%) was supplied by FIDIA, Abano Terme, Italy. Kittens received daily subcutaneous injections (30 mg/kg, as per the protocol of Walsh and Webster, 1994) 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. *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. (Problems in importing GM1 at that time resulted in a shortage. GM1 is isolated from bovine brain, and the USDA had placed severe restrictions on importation of all such*

substances from Europe due to concerns about bovine spongioform encephalopathy. It was a lengthy process to obtain licensure from the USDA for continued importation of GM1 from FIDIA in Italy.)

In a second GM1 experimental group (K136 through K 145), GM1 administration was initiated simultaneously with ototoxic drug administration, beginning the day after birth and continued until the day of initial cochlear implant stimulation. Thus, these later subjects received up to 8 weeks of GM1 injections. In this group the GM1 dosage was reduced to 20 mg/kg, replicating the protocol of Parkins et al.(1999).

b. Chronic Stimulation.

Kittens in all 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). All groups received stimulation using electrical signals 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 groups 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. Subjects K125, 130, 133 and 136 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. (Specifically, for the first week, each channel delivered a simple, unmodulated 100 pps signal. In the second week, stimulation continued using a carrier rate of 300 pps, 100% sinusoidally amplitude modulated at 30Hz. In the third week, the signal was 500 pps/40 Hz AM. 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.) The remaining subjects wore an operational analogue cochlear implant speech processor, set as described in our previous publications (Leake et al., 2000b).

As mentioned previously, the comparison control group was a subset of subjects from the temporally challenging/high frequency stimulation groups which have been described in detail in previous publications (Leake et al., 1999, 2000a) and in a Quarterly Progress Report for our previous Contract #N01-DC-72105(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.

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 AD o **
			GM1 Ganglioside Group				
K109	60/19	7	**1,2:** 50-71 **3,4:** 12-158	25	300pps/30Hz 900pps/50Hz	32	AD
K117	60/18	8	**1,2:** max=45-71* **3,4:** max=36*	28	SP	36	AD
K119	60/17	7	**1,2:** 32-126 **3,4:** 100-141	34	300pps/30Hz 900pps/50Hz	41	AD
K125	60/21	7	**1,2:** 40-158 **3,4:** 63-71	30	100-800pps/50Hz	37	AD
K130	60/21	6	**1,2:** 50-112 **3,4:** 200-447	31	100-800pps/50Hz	38	AD
K133	60/21	8	**1,2:** 36-112 **3,4:** 224-355	36	100-800pps/50Hz	44	AD
K136	60/16	6	**1,2:** 40-141 **3,4:** 79-112	25	2 ch alt: Varied	31.5	GM1**
K137	60/16	6.5	**1,2:** 50-126 **3,4:** 50-100	26	SP: 2 ch sim.	33	GM1**
K138	60/16	7	**1,2:** 45-50 **3,4:** 100s-178	23	SP: 2ch sim	30	GM1**
K141	60/17	8.5	**1,2:** 50-89 **3,4:** 79-141	27.5	SP: 2ch sim	36	GM1**
K145	60/21	7.5	**1,2:** 126-251 **3,4:** 158-398	27	alt 2ch: 800/60	34.5	GM1**
MEANS (n=11):				**28.4 weeks**		**35.7 weeks**	
			Stimulation Only Control Group				
K84	60/19	10	**1,2:** 200-400	34	SP; Beh.	44	**No** GM1
K89	60/19	10	**1,2:** 80-100	27.5	300 pps/30 Hz	37	**No** GM1
K98	60/20	7.5	**1,2:** 50-100	32.5	SP; Beh	40	**No** GM1
K101	60/18	8	**1,2:** 79-200 **3,4:** 100-316	29	300 pps/30 Hz; Beh	37	**No** GM1
K105	60/20	9	**1,2:** 63-355	29	800 pps/20 Hz	38	**No** GM1
K106	60/20	9	**1,2:** 80-400	33.5	800 pps/20 Hz	43	**No** GM1
K107	60/18	9	**1,2:** 100-200 **3,4:** 71-100	22	800 pps/60 Hz	31	**No** GM1
MEANS (n= 7):				**29.6 weeks**		**38.7 weeks**	

* For subjects stimulated with the analogue implant signal processor (SP), the maximum amplitude is given.

Chronic stimulation periods in the 11 GM1 subjects ranged from 23 to 36 weeks, with a mean of **28.4 weeks**, and the comparison group had a similar range (22 to 34 weeks) and comparable mean stimulation period of **29.6 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, **35.7 weeks**) and the comparison group ranged from 31 to 44 weeks with a mean age at study of **38.7 weeks.**

RESULTS and DISCUSSION

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

We have previously published data showing that the protocol 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 total hair cell degeneration throughout the cochlea. Figure 2 illustrates SG density data from 3 control groups of animals studied either immediately after neonatal deafening or at 7-8 weeks of age at the time their littermates received a cochlear implant. At the left are data from 8 animals studied at 16-24 days postnatal, immediately after ototoxic drug treatment and confirmation of a profound hearing loss. Some degeneration of SG neurons has already occurred, and the morphometric data show that SG density is reduced to about **84%** of normal. Thus, significant neural degeneration occurs relatively rapidly in these animals.

Also shown in Figure 2 are comparison data for two groups of animals that were deafened by the identical ototoxic drug protocol and studied at 7-8 weeks of age, at the time their littermates received cochlear implants. Spiral ganglion density was further reduced to **66.2%** of normal in the control group. In contrast, the final group of 5 subjects received daily GM1 ganglioside injections (30 mg/kg, SQ) after profound hearing loss was confirmed and continuing until the time of study at 7 weeks of age. The survival in these older kittens is **78.4%** of normal, much better than the non-GM1 control group at this age, and fairly similar to the survival seen in the control group immediately after ototoxic drug treatment. These data suggest that most SG neurons that survived

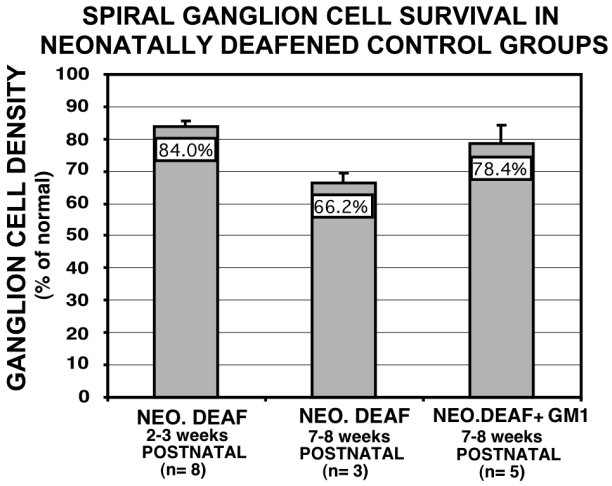

FIGURE 2. SG cell density (% of normal, averaged for the entire cochlea) in 3 control groups. Animals in the first group were studied at 2 to 3 weeks of age, immediately after documentation of profound hearing loss. Significant SG degeneration already is evident. The middle group of neonatally deafened animals were studied at 7-8 weeks of age, and SG density is further reduced. The final group received GM1 injections (30 mg/kg) and also were studied at 7-8 weeks of age. These data suggest that GM1 may maintain the SG neurons and ameliorate progressive degeneration after deafening.

after the ototoxic drug deafening procedure were maintained with GM1 treatment, until the time these animals would have undergone cochlear implantation. Thus, the starting point for neural survival is about 78% of normal, rather than 66%, at the time the deafened animals undergo cochlear implantation and begin chronic electrical stimulation protocols.

An interesting finding in the histological study of these GM1-treated subjects was the observation that initial SG cell loss seemed to occur predominantly in the upper portion of Rosenthal's canal, closer to the scala vestibuli. Figure 3 presents a histological section in which the lower part of ganglion (nearer the scala tympani) appears to have near-normal cell density, but there is substantial cell loss in the upper aspect of Rosenthal's canal. This result is of interest because our 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 upper, 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 suggested 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 result in a preferential effect of GM1 on this neural population.

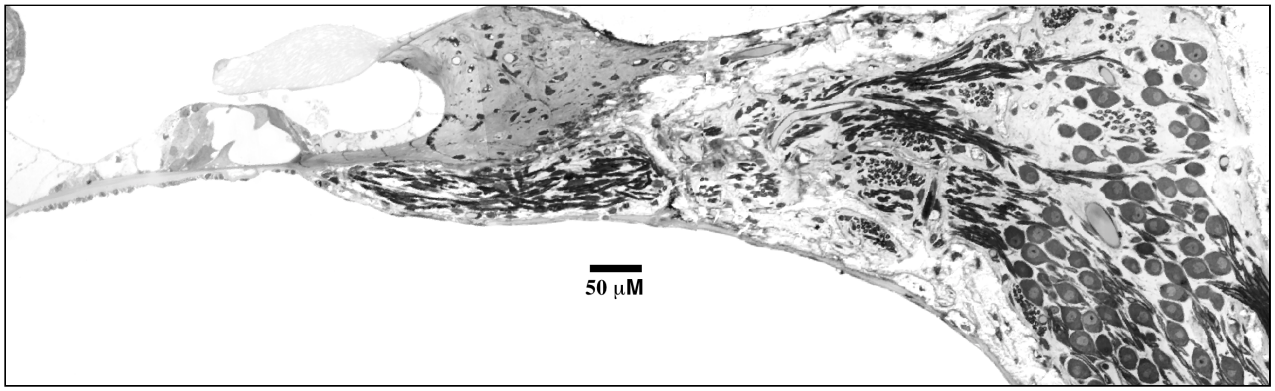

FIGURE 3. Histological section taken from the upper basal turn of the cochlea, about 11 mm from the base (40-50% sector), in a neonatally deafened, GM1-treated animal studied at 7 weeks of age. Note that substantial loss of SG cells is evident in the upper portion of the ganglion, but the lower aspect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

b. GM1 treatment and Chronic Electrical Stimulation: Group Comparisons

Figure 4a shows morphometric SG density data pooled for the group of 6 cats in the initial GM1 experimental group that received daily injections of 30 mg/kg GM1 over the period ***after*** deafening and ***prior*** to receiving a cochlear implant and undergoing 6 to 9 months of chronic stimulation. Spiral ganglion cell density is higher on the stimulated side than in the control deafened ears for all cochlear sectors examined. Particularly noteworthy is the robust neurotrophic effect of electrical stimulation seen in the basal cochlea (Figure 5), with SG density of >90% in the 0-10% sector and survival averaging 73% of normal for the regions 0=30% from the base. Averaged over all cochlear sectors, the mean SG survival in the stimulated ears was about 55% of normal, and survival on the control side was 34% of normal. This 21% increase in neural survival elicited by stimulation

was highly significant (P<0.001, Student's paired t-test). However, this overall value of 55% is still a relatively modest improvement over previous studies with electrical stimulation alone (Figure 1), and clearly a very significant decrement from the 78% of normal survival in GM1-treated subjects studied at 7 weeks of age, at the time their littermates received their cochlear implants.

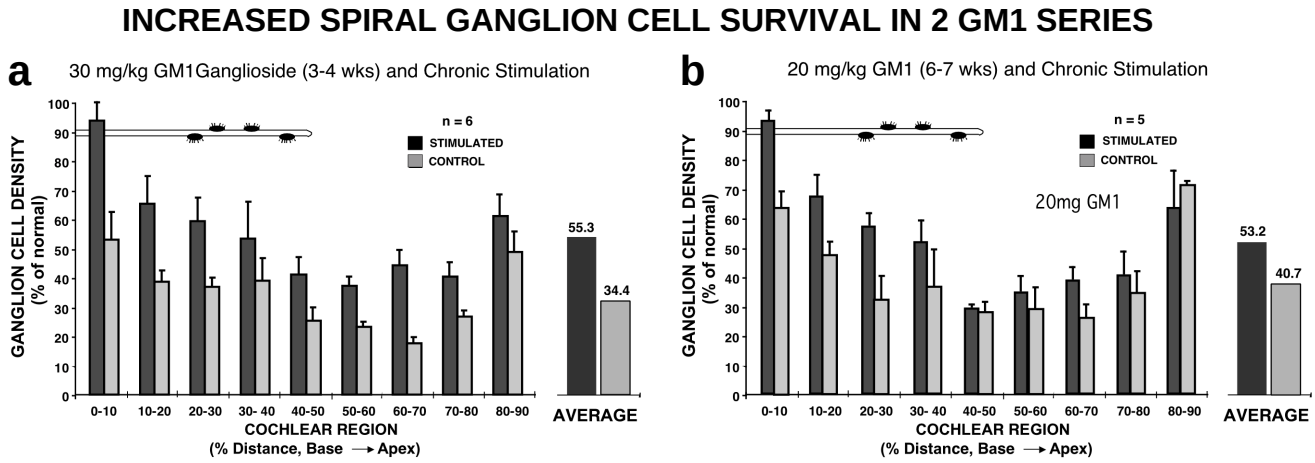

FIGURE 4. a. SG data pooled for the 6 subjects in the initial GM1 experimental group that received daily injections of 30 mg/kg GM1 *after* the profound hearing loss was confirmed. These animals subsequently completed chronic electrical stimulation periods of 6 to 9 months. Significantly higher SG cell density in the stimulated cochleae than in the control deafened ears. **b.** SG density data are shown for the 5 animals in the second GM1 experimental group which received daily injections of 20 mg/kg GM1 beginning on the second postnatal day and continuing until the time of implantation and initiation of chronic electrical stimulation. Results for the 2 experimental groups are very similar.

It should be noted that 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 the ototoxic drugs kanamycin and ethacrynic acid. In that study, GM1 was given at a dosage of 20 mg/kg (rather than 30 mg/kg as in our initial study and the Walsh and Webster [1994] report), and injections also were initiated concomitant with ototoxic drug treatment, whereas our study delayed GM1 injections 2-3 weeks until after profound hearing loss was confirmed. Therefore, we conducted a second experiment in which 20 mg/kg GM1 was administered to kittens beginning on the second postnatal day, continuing throughout the period of ototoxic drug treatment, and up until the time the animals underwent cochlear implant surgery and received their initial electrical stimulation. The quantitative SG density data for the 5 subjects in this second GM1 experimental group are illustrated in Figure 4b. The results observed with this modified GM1 protocol are very similar those of the initial study. Again, significantly higher SG density is observed in the cochleae that underwent implantation and subsequent chronic electrical stimulation, and overall survival in the stimulated ears is about 53% of normal, quite similar to the initial study group results.

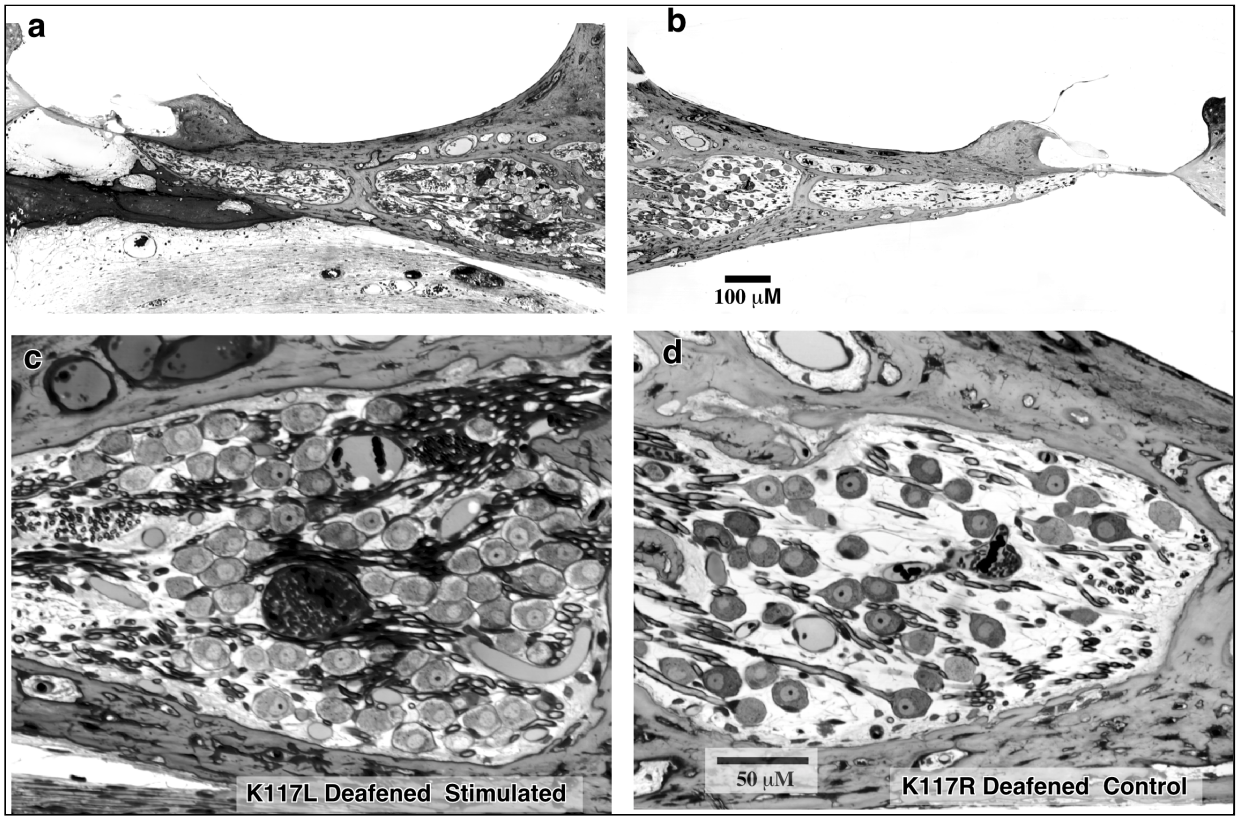

FIGURE 5. Histological sections taken from the cochleae of subject K117, illustrating the marked trophic effects of combined GM1 and chronic electrical stimulation on SG neural survival in the region 20-30% from the base. In the stimulated cochlea (left, panels **a, c**) mean SG density is about 84% 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.

Because the results with the two GM1 experimental protocols were so similar, we have pooled the data from the two groups to provide a larger *n* for statistical comparisons to the stimulation-only group. SG data from all 11 subjects in both GM1 experiments are illustrated in Figure 6a. The mean SG density in the stimulated cochleae is about 55% of normal, and survival on the control side is about 37% of normal. Figure 6b illustrates SG data from the comparison group of 7 neonatally deafened, chronically stimulated cats that <u>*did not*</u> receive GM1. These subjects are a subset of those shown in Figure 1 that were specifically selected to *match* the GM1 group for chronic stimulation parameters, duration of stimulation and age at study (see Materials and Methods; Table 1). The GM1 subjects show a slight improvement in SG survival over the stimulation-only subjects in both the stimulated ears (55 vs. 46% of normal) and the deafened-control side (37 vs. 30% of normal).

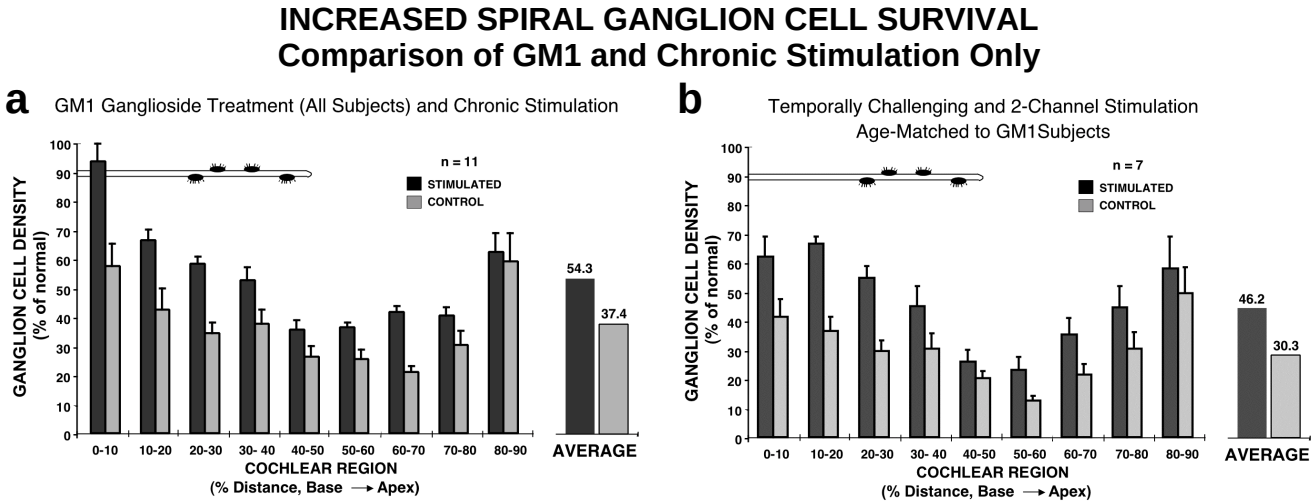

FIGURE 6. a. SG survival data pooled for all 11 subjects in two GM1 experimental groups. Again, significantly higher SG cell density is observed in the stimulated cochleae than in the control deafened ears. **b.** SG density data are shown for a *control* group of neonatally cats. These subjects were 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. Comparison of the two graphs suggests that the GM1 subjects showed modest improvement in SG survival over the stimulation-only subjects in both the stimulated ears and the deafened-control side.

Since survival values were improved on both sides in the GM1 group, we then averaged the data from the two sides in the two groups to provide an overall estimate of the GM1 effect on survival. Figure 7 illustrates this summary finding. The mean SG density in the GM1 group was 45.85% of normal, whereas the stimulation-matched non-GM1 group had a mean value of 38.23 % of normal. This difference of about 8% of normal neural survival between the two groups was statistically significant ($P<0.05$, Student's unpaired t-test).

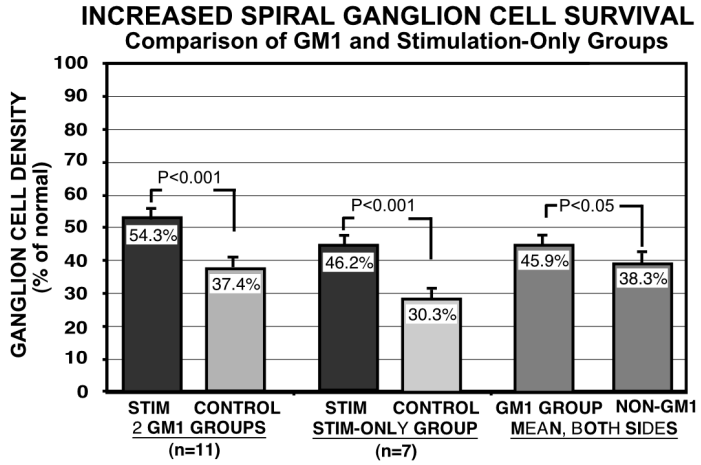

FIGURE 7. Summary SG survival data pooled for both GM1 experimental groups (n=11) are compared to the matched stimulation-only control group. Significantly higher SG cell density is shown in the stimulated ears as compared to the control deafened ears in both the GM1 and stimulation-only groups. When SG density data are averaged for both ears, the mean survival in the GM1 group is 8% higher than the stimulation-only group and this difference is statistically significant.

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 those results are expressed in *percent increase,* it is unclear what extent of SG neural survival (% of normal neural density) was actually seen in these animals. (For example, if SG survival in deafened untreated guinea pigs was 50% of normal, then a 77% increase in survival in the GM1 treated animals would be 85% of normal; but if neural survival in the deafened non-GM1 animals was only 20% of normal, then survival in the GM1-treated group would have been only about 35% of normal.) If our summary data are expressed in this fashion, the overall survival of 45.85% in the combined GM1 groups represents a 20% increase over the 38.23 % value seen in the control, stimulation-only group. (That is, stimulated less control/control). Thus, we conclude that either GM1 does not have as a marked effect in our kitten model, or alternatively, an initial more robust survival-promoting effect is *not maintained* over the subsequent 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 contrasting results. First, there are species and age differences. The Parkins study examined young adult guinea pigs that had normal sound experience during maturation, whereas our subjects are neonatally deafened 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 ameliorating effects of an acute ototoxic insult than one of more gradual onset. Finally, there is a major difference in the time course over which the neurotrophic effects were evaluated in the 2 studies: In the guinea pig study, animals were evaluated immediately following 8 weeks of GM1 treatment. In our kittens, neural survival was quite good initially, immediately after GM1 treatment (78.4%; see Figure 2) but this effect was fully not maintained over a subsequent prolonged period of chronic electrical stimulation with the cochlear implant. This suggests that GM1 and electrical stimulation may be promoting survival by different underlying mechanisms. These findings raise an interesting question: Would GM1 be more effective if treatment were continued throughout the subsequent chronic electrical stimulation period? 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 in conjunction with stimulation via 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.

c. Integrity of Supporting Cells of the Organ of Corti: Implications for Underlying Neurotrophic Mechanisms.

In the detailed examination of histological sections from cochlear specimens in the GM1 experimental series, it appeared that the extent of neurotrophic effects elicited in a given specimen might be related to the relative condition of supporting cells in the organ of Corti. In order to test

this suggestion, we examined the supporting cells and rated the extent of pathological alterations using a previously published rank order grading method (Leake et al., 1997), as illustrated in Figure 8. Our previous studies have shown that in animals deafened neonatally by neomycin sulfate administration, hair cell loss in the organ of Corti progresses from base to apex, and the supporting cell degeneration follows this same pattern. Initial pathological alterations are always most severe in the base. This is the classical pattern of aminoglycoside ototoxicity, which has been well described in the older literature in both animal studies and in human pathology.

EVALUATION OF INTEGRITY OF ORGAN OF CORTI SUPPPORTING CELLS AFTER NEONATAL DEAFENING

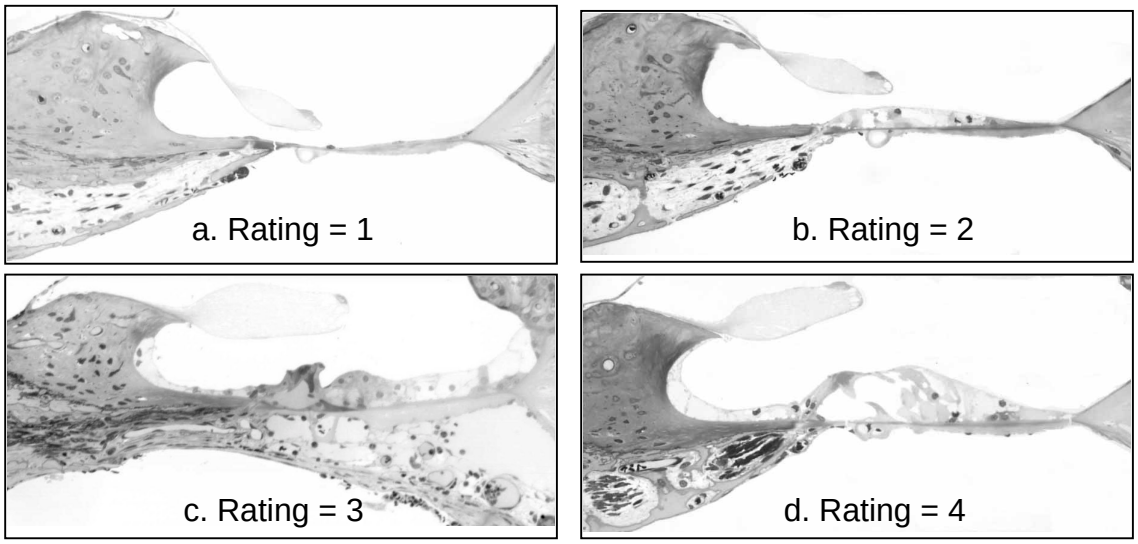

FIGURE 8. These micrographs illustrate the rank order rating scale used to evaluate the condition of supporting cells of the Organ of Corti in the cochleae of neonatally deafened, GM1 treated animals. Numbers are assigned to indicate the relative condition of the supporting cells as shown, from severe degeneration (**a**) to normal cytoarchitecture of the tunnel of Corti and all other supporting cells (**d**).

Figure 9 demonstrates that this same pattern of basal-to-apical degeneration occurs in the cochlear specimens of the initial GM1 experimental series. Supporting cell integrity is poorest in the basal cochlear sectors, and improves in progressively more apical regions. To test for a relationship between the supporting cell condition and neural survival, we averaged the regional supporting cell values for each ear and compared these values to the overall average spiral ganglion cell density. In the control ears of the GM1-treated subjects, a significant correlation (Spearman Rank Order correlation; R=0.89; P= 0.033) was observed between the overall integrity of supporting cells in a given ear, and the overall average survival of SG neurons in that ear. This finding suggests that GM1 may be promoting SG survival by helping to maintain better integrity of supporting cells. It's been reported that the supporting cells supply neurotrophins, which support the SG neurons, and these data suggest that relative extent of GM1 effect on neural survival is related to the supporting cell integrity.

In contrast, to the basal-to-apical progression of hair cell and supporting cell degeneration, the SG cell degeneration in neonatally deafened animals occurs first and is most severe in the middle cochlear sectors (Leake et al., 1997). Although the precise pattern varies somewhat among experimental groups, it is a consistent finding in all the neonatally deafened animals that regional

SG density is higher in the base and apex than in the middle cochlear sectors (see Figs. 1,4,6 above). Moreover, a particularly robust effect of stimulation in promoting SG survival is seen near the base of the cochlea, where the supporting cells are in the poorest condition. Thus, the electrical stimulation trophic effects clearly are NOT correlated with supporting cell condition, suggesting that the mechanisms underlying trophic support of neurons by electrical stimulation and GM1 are relatively independent. Thus, when we compared supporting cell condition and the overall SG density in the *contralateral, stimulated ears* of the same GM1 series for which the control ears are shown in Figure 10, there was no correlation (Spearman Rank Order, P> 0.05).

Thus, several lines of evidence now indicate that neurotrophins and electrical stimulation promote neural survival by ***different mechanisms***. This poses a significant challenge for clinical therapies designed to promote neural survival by modulating neurotrophins. For example, current research exploring the use of gene therapy to induce cells in the inner ear to express neurotrophins will probably have to induce ***long-term expression in conjunction with a cochlear implant*** to have a significant impact over the long term. But this definitely appears to be a promising area for clinical implants in the future. Once good methods are developed to promote neurotrophin expression, their effects will be additive with electrical stimulation effects.

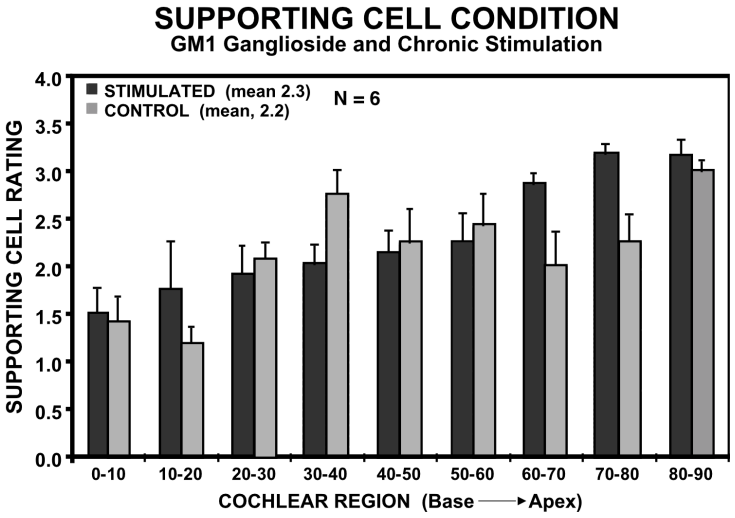

Figure 9. Summary graph illustrating data from evaluation of the condition of supporting cells in control (deafened, unstimulated) ears from the initial GM1 experimental group (n=6). After the cochlear hair cell degenerated, the supporting cells begin to degenerate as well. These data indicate that the supporting cell degeneration progresses from base to apex after neonatal deafening, reflecting the progression of hair cell degeneration. This is very different from the pattern of SG cell degeneration as illustrated in Figures 1,4, and 6 above.

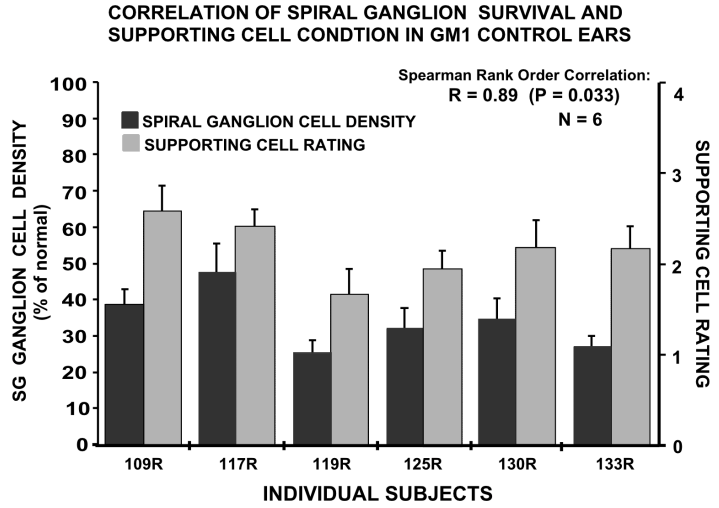

Figure 10. The values for regional supporting cell condition were averaged to provide a single value for each cochlea, as an estimate of overall integrity of supporting cells.

In the control, unstimulated cochleae of the GM1 subjects, the relative condition of supporting cells is correlated with the overall SG density for each cochlea in a Spearman rank order correlation (P<0.03). These data suggest that relative extent of GM1 effect on neural survival is related to the supporting cell integrity.

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Work Planned for the Next Quarter

1) During the current quarter 2 manuscripts comprising a two-part series by Dr. Peter Wardrop on temporal bone studies of perimodiolar electrodes were completed and submitted to the journal Otology and Neurotology for peer review. During the coming quarter, a third paper on the temporal bone studies will be drafted by Dr. David Whinney. This report will incorporat additional results obtained by Dr. Whinney and Steve Rebscher analyzing some of the factors underlying mispositioning and trauma with various electrode and summarizing results in all series to date.

2) Histolopathological studies of cochlear specimens from control and chronically stimulated experimental animals will be continued for our new series in which the anti-apoptoti drug desmethyldeprenyl (DES) has been administered over the period of several weeks in deafened neonates until the time cochlear implantation. Daily chronic electrical stimulation will continue for additional subjects in which the (DES) treatment is being continued throughout the chronic stimulation period.

3) Work will continue on further development of software for multichannel recording experiments examining spatial selectivity of electrical stimulation in guinea pigs and cats using the University of Michigan 16-electrode and 32-electrode multichannel recording probes.