11th Quarterly Progress Report
April 1, 2003 to June 30, 2003

Fundamental Neurosciences Contract N01-DC-0-2108

Protective Effects of Patterned Electrical Stimulation on the Deafened Auditory System

Submitted by:

Patricia A. Leake, Ph.D.
Gary T. Hradek
Stephen J. Rebscher, M.A.
Russell L. Snyder, Ph.D.

Epstein Hearing Research Laboratories
Department of Otolaryngology, Room U490
University of California, San Francisco
San Francisco, Ca 94143-0526

Neurotrophic Factors: GM1 Ganglioside and (-)-Desmethyldeprenyl

Several aspects of the data on survival of spiral ganglion (SG) neurons from our neonatally deafened and chronically stimulated animals suggest that mechanism(s) other than direct depolarization may mediate at least part of the conservation of SG neurons *in vivo*. First, the increases in neuronal density appear to be more broadly distributed throughout the cochlea than would be expected with discrete activation produced by closely spaced pairs of bipolar scala tympani electrodes as employed in these experiments. Yet, increased survival of neurons is *not* seen when electrodes are implanted but not activated, indicating that vascular changes and inflammatory processes which accompany implantation alone are not sufficient to promote survival (Leake et al., '91). Moreover, the extent and spatial distribution of neurotrophic effects were not diminished when the intensity of electrical stimulation was reduced from 6 dB in earlier experiments to only 2 dB above EABR threshold (Leake et al., '92). These results suggest that there may be subthreshold effects of the electrical fields generated by the implanted electrodes or other factors that ameliorate the slow, retrograde degeneration of spiral ganglion neurons which otherwise is progressive for years after deafening (Leake and Hradek, '88). Moreover, although we have demonstrated highly significant effects of electrical stimulation in promoting neural survival, SG survival is still far from normal in our chronically stimulated animals. We are thus very interested in exploring other potential neurotrophic factors, which may further augment neural survival when used in conjunction with electrical stimulation.

In our view, the key intracellular signaling mechanisms and pathways underlying the survival-promoting effects of electrical stimulation and other neurotrophic agents can be investigated most efficiently in cell culture preparations. Research on cultured SG neurons by Green and co-workers (Hansen et al., '01; Hegarty et al., '97) has demonstrated that neuronal survival is supported *both* by membrane depolarization and by neurotrophins: Their research has shown that the survival-promoting effect of depolarization is mediated by L-type voltage gated Ca^{2+} channels and involves multiple distinct signaling pathways, including 1) an autocrine *neurotrophin* mechanism; 2) cAMP production; and 3) CAM kinase-mediated phosphorylation of CREB. The neurotrophins BDNF and NT-3 are expressed by SG neurons and promote survival by an autocrine mechanism that is *additive* with the survival-promoting effect of depolarization.

Over the past several years, a number of *in vivo* studies have shown that several neurotrophic factors (usually administered via perilymphatic infusion) can reduce SG loss following deafness. The best-characterized neurotrophic factors are members of the nerve growth factor (NGF) family of proteins, and are called neurotrophins. Neurotrophins include NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3, and neurotrophin-4/5, each of which binds to specific high-affinity receptors, the Trk family of receptors. Neurotrophins are particularly relevant to our studies of spiral ganglion cell survival in neonatally-deafened animals because they regulate neuronal differentiation and survival during development (Korsching, '93; Gao et al.,'95) and also are known to protect neurons from injury and toxins in adults (Apfel, et al., 1991; Kanzake et al., '02; Miller et al., '97; Yan et al., '92; Zheng et al, '95; Zheng and Gao, '96).

Recent studies suggest that release of neurotrophins and activation of the Trk receptors underlie the protective effects of GM1 ganglioside (Rabin et al., '02; Duchemin et al., '02), a glycosphingolipid, which has been shown to ameliorate degeneration of spiral ganglion neurons and ventral cochlear nucleus neurons after unilateral conductive

hearing loss (Walsh and Webster, '94) or aminoglycoside deafening (Parkins et al., '99). Because GM1 can be administered exogenously, rather than via cochlear infusion, and because it has already been applied in a number of clinical trials, we considered it an attractive candidate for initial studies in our chronic animals. As reported in a previous Quarterly Progress Report (QPR#9, Oct. 1 – Dec. 30, 2002), results from our recently completed study suggest that GM1 treatment in neonatally deafened animals *prior to implantation* and chronic stimulation, provides a modest increase in neural survival in control deafened ears, and is *additive* to the effects of electrical stimulation in promoting SG survival. The increase was about 8% over the effects of stimulation alone (Fig. 1). SG survival in these ears, however, is still far from normal (about 54% of normal). Thus, we are very interested in continuing to explore neurotrophic agents in protocols that model potential clinical interventions for use in conjunction with a cochlear implant.

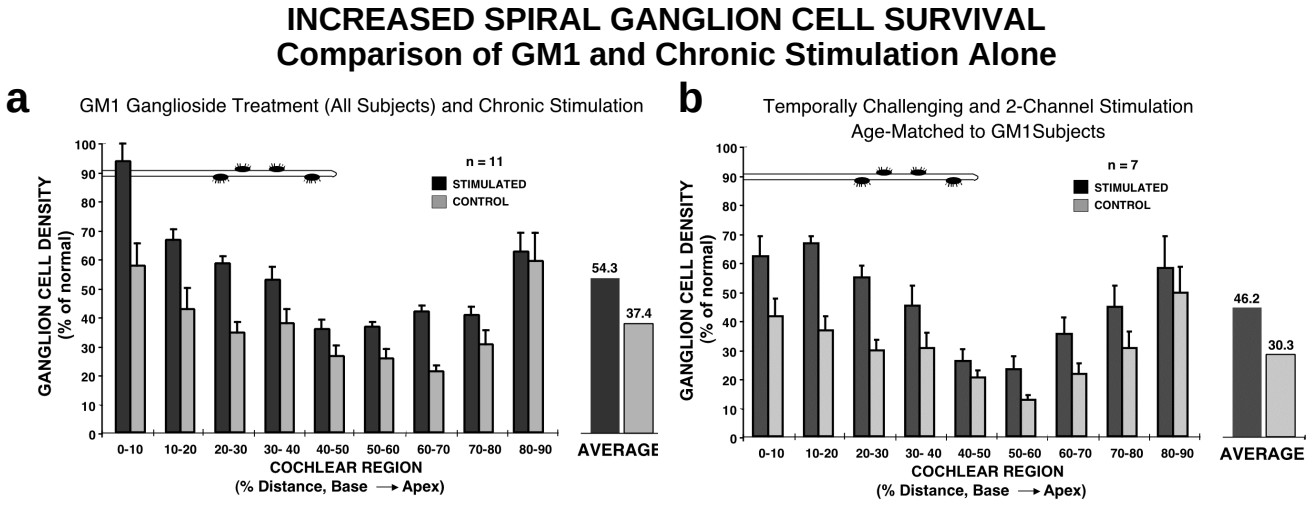

FIGURE 1. **a.** SG survival densities are shown as percentage of normal for cochlear sectors from base to apex. Data are pooled for 11 subjects in the GM1 experimental group . Significantly higher SG cell density is observed in the stimulated cochleae than in the control deafened ears. **b.** SG density data are shown for a *control* group of neonatally deafened 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.

One interesting aspect of the GM1 experiments is the finding that in animals studied *immediately after* GM1 treatment at about 8 weeks postnatal (at the time their littermates were implanted), SG survival averaged 78.4% of normal, a value that is more than 10% higher than survival in the non-GM1 group (66.2%) at this time (Figure 2). However, this excellent initial survival clearly was *not* fully maintained over a subsequent prolonged period of chronic electrical stimulation by the cochlear implant. This suggests that the GM1 treatment might be more effective if treatment were continued *throughout the subsequent chronic electrical stimulation period.* While the data suggest that GM1 ganglioside can ameliorate the initial SG degeneration resulting from ototoxic drug insult, it is important to determine if the survival-promoting effects can be maintained over the long-term in conjunction with stimulation via a cochlear implant. Otherwise, GM1 and other agents that modulate neurotrophic factors may be of little practical value clinically if "rescued" neurons are not viable over the long term.

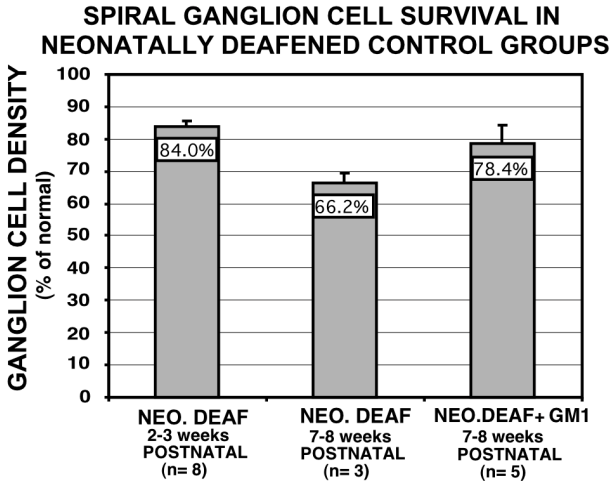

FIGURE 2. SG cell density (% of normal, averaged for the entire cochlea) in 3 control groups. Animals in the first group were studied at 2 to 3 weeks of age, immediately after documentation of profound hearing loss.

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/day) and also were studied at 7-8 weeks of age. These data suggest that GM1 may promote survival of the SG neurons and ameliorate progressive degeneration after deafening.

Therefore, we are strongly interested in studying the *long-term* effects of gangliosides in our animal model, specifically, using a new semi-synthetic derivative of GM1 ganglioside, LIGA20. LIGA20 has several advantages over GM1: First, recent studies have shown that GM1 induces secretion of NT-3 and activates TrkC, whereas LIGA20 differentially induces BDNF secretion and activates the TrkB receptor (Rabin et al., '02; Duchemin et al., '02). This differential effect is important because studies of SG neurons, both in cultured preparations (Hansen et al., '01; Hegarty et al., '97; Zha et al., '01; Mou et al., '97; Lalwani et al., '02) and *in vivo* (Miller et al., '97; Staecker et al., '96; Zheng and Gao, '96) suggest that BDNF is ***more potent*** than NT-3 in promoting survival of SG neurons. In addition, because it is semi-synthetic, LIGA20 may be more stable and less variable in specific activity than GM1. Finally, LIGA20 can be administered ***orally*** rather than by injection, which is a distinct advantage for the extended chronic administration required for our experiments. Unfortunately, however, LIGA20 is not currently available in the United States, due to problems that the manufacturer FIDIA has faced in exporting it. LIGA20 is produced by isolation from bovine brain, and the USDA has placed severe restrictions on importation of all such substances from Europe due to concerns about bovine spongioform encephalopathy.

Thus, until LIGA 20 becomes available again, we have recently initiated a study of a new selegeline drug in collaboration with Dr. William Tatton at Mt. Sinai School of Medicine. The selegeline (-)-deprenyl has been used clinically for many years to treat Parkinson's disease and Alzheimer's disease (Tatton, '99). Deprenyl was originally thought to act as an MAO-B inhibitor, but Tatton recently has suggested that it is actually the drug's primary metabolite, (-)-desmethyldeprenyl (DES), which reduces neuronal apoptosis by a mechanism that requires gene transcription and involves maintenance of mitochondrial membrane potential (130). Mediated by GAPDH binding, DES is reported to increase mitochondrial BCL-2 and BCL-Xß levels, to decrease BAX levels, and thereby preventing the permeability transition pore from opening and preventing apoptosis (Carlile et al., '00; Tatton, '99; Tatton and Chalmers-Redman, '96; Tatton et al., '99). In evaluating this information, we consulted Dr. Steven H. Green, who is an expert on apoptosis and has conducted several definitive studies in *cultured SG neurons* that have identified key intracellular signaling mechanisms underlying the survival-promoting effects of depolarization on neural survival (Hansen et al., '01; Hegarty et al., '97; Zha et al., '01). Dr. Green was interested in the selegelines and thought it likely that the mechanisms by which DES prevents apoptosis might also be involved in spiral ganglion

cell death following trophic factors. Preliminary *in vitro* studies were then conducted in Dr. Green's lab. Several experiments evaluating SG neural survival at 48 hours, 72 hours and 96 hours consistently suggest that both DES and deprenyl promote significant improvement in survival that is ***additive*** to the effect of depolarization, eliciting an increase to about 120% of the level of survival seen after depolarization alone.

Based upon these findings, we initiated an ***in vivo*** study of DES, to determine if it is effective in reducing apoptosis of the cochlear neurons after neonatal deafness in our animals. DES was administered by daily injections (100 µg / kg / SQ) to littermate pairs of kittens, beginning on the day after birth. The DES was given concurrently with the neomycin sulfate injections until a profound hearing loss was confirmed by ABR testing at 16-21 days of age. DES injections continued until animals were 7-8 weeks of age. At this time control subjects were euthanized for study, and at the same time their littermates underwent cochlear implant surgery. Table 1 details the individual histories of the initial DES subjects included in this QPR. SG data have been evaluated to date for 4 control subjects studied at 6-8 weeks of age and 4 initial subjects studied after several months of chronic electrical stimulation delivered by a unilateral cochlear implant.

Table 1. DES ADMINISTRATION AND CHRONIC STIMULATION HISTORIES

Cat #	Neomycin mg-kg/days	Age at First Stimulation (weeks)	Stimulation Intensity electrode pair/µA	Stim. Period (weeks)	Stim. Frequency	Age at Study (weeks)	DES: mg-kg/ days
DES Control Group							
K159	60/16	--	Littermate of K158	--	--	8	0.1/47
K161	60/16	--	Littermate of K160	--	--	6	0.1/47
K164*	60/16		Littermate of K165	--	--	6	0.1/43
K165	60/21	--	Littermate of K164	--	--	6	0.1/45
*severe congenital cardiac pathology							
DES and Two-Channel Stimulation Pilot Study							
K158	60/19	7	1,2: 22-251 3,4: 178-355	34	325/60 sim.	41	0.1/47
K160**	60/19	6	1,2: 89-348 3,4: 112-316	28	325/60 sim.	34	0.1/56
K163	60/19	6	1,2: 112-178 3,4: 100-141	14	325/60 sim.	20	0.1/73
K166	60/19	7	1,2: 63-282 3,4: 89-562	16	SP alt.	23	0.1/161
** severe, chronic abscess, labyrinthitis							

We have previously published data demonstrating that the protocol of ototoxic drug (neomycin) administration applied in our neonatally deafened cats results in 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 3 illustrates SG density data from 3 control groups of these neonatally deafened animals. 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.

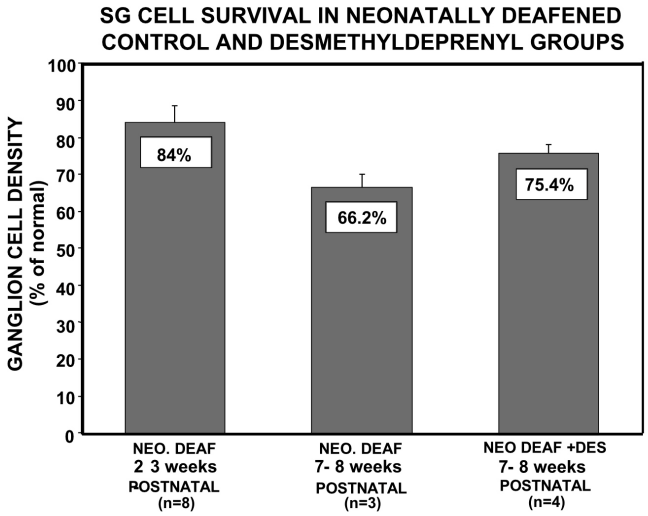

Figure 3. Cats studied immediately after neonatal deafening by ototoxic drugs already show significant SG loss (left data bar). Degeneration is progressive and further loss occurs by 7-8 weeks of age (center) when deafened animals usually receive a cochlear implant. At right are preliminary data showing modest improvement in survival of SG neurons at 7-8 weeks in neonatally deafened subjects who received daily DES injections (0.1 mg/kg SQ).

Also shown in Figure 3 are comparison data for 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 this second control group. In contrast, the final group of 4 subjects received daily DES injections (100 µg / kg, SQ) continuing again until the time at 6-8 weeks of age when their littermates received a cochlear implant. The survival in this group is 75.4% of normal, about 9% better than the non-DES control group at this age. These data suggest that more of the SG neurons that survived after the ototoxic drug deafening procedure were maintained with DES treatment, until the time these animals would have undergone cochlear implantation. Thus, the starting point for neural survival is about 75% of normal, rather than 66%, at the time these deafened animals would begin chronic electrical stimulation protocols.

Figure 4 shows preliminary SG data for 4 individual neonatally deafened, DES-treated subjects that also received a cochlear implant and underwent several months of chronic intracochlear electrical stimulation:

Subject #K158: The first subject in the DES / stimulation group completed more than 8 months of chronic electrical stimulation and was studied in the final acute electrophysiological experiment at 41 weeks of age. The pattern of SG survival in the control ear is typical of animals deafened neonatally by systemic administration of neomycin, with more severe degeneration in the middle sectors of the cochlea and better neural survival in the most apical and basal regions (Fig. 4a).

SPIRAL GANGLION SURVIVAL IN NEONATALLY DEAFENED CATS
FOLLOWING DES ADMINISTRATION AND CHRONIC ELECTRICAL STIMULATION

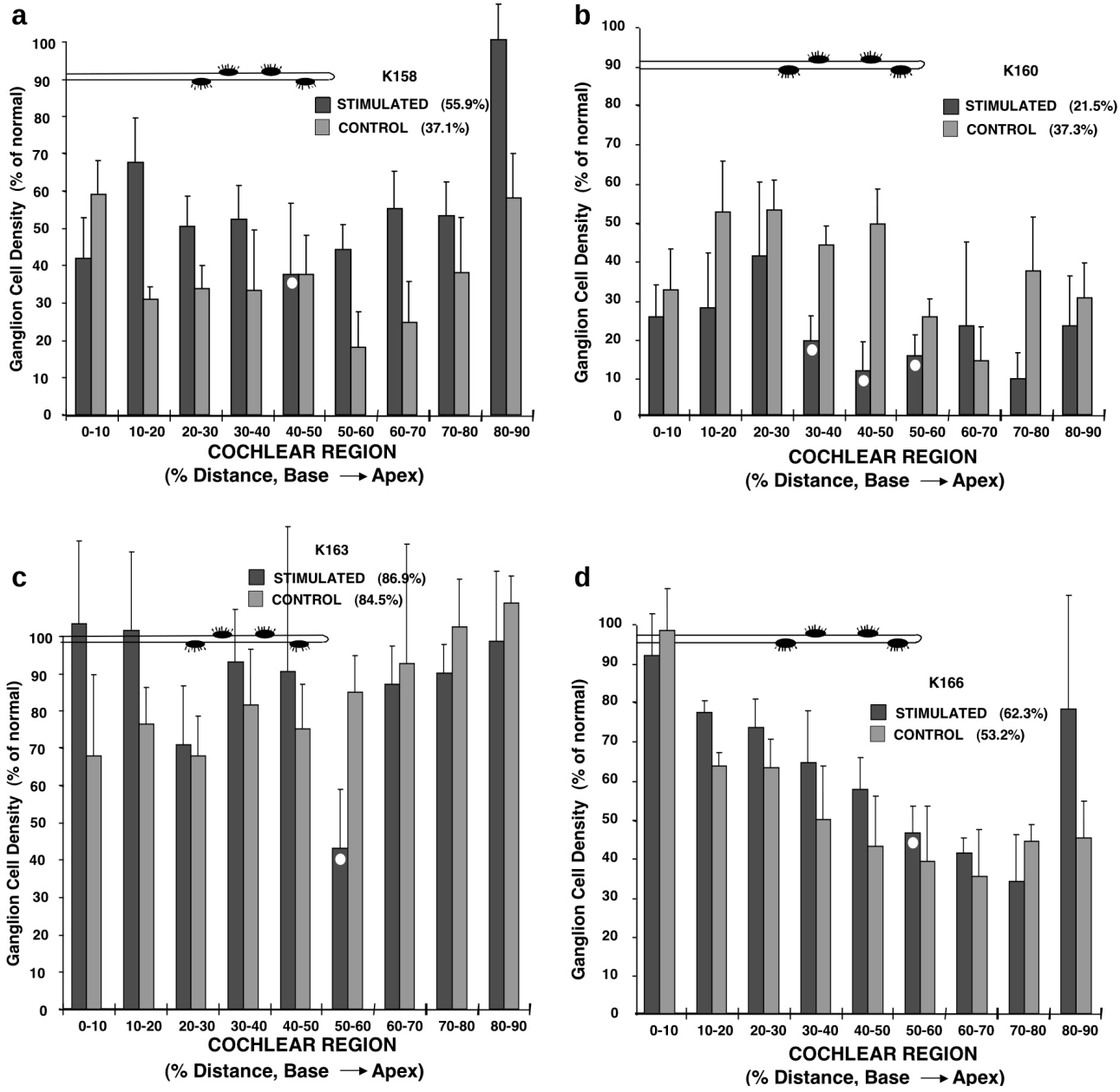

FIGURE 4. SG survival data are shown for 4 individual subjects following administration of desmethyldeprenyl and several months of chronic electrical stimulation. Individual histories are provided in Table 1 and findings are presented in the text. Significantly higher SG cell density is observed in the stimulated cochleae than in the control deafened ears in 2 of the 4 cases, subjects K158 and subject K166, as shown in **a** and **d** respectively. In subject K160 a severe chronic labyrinthitis resulted in fibro-osseous sclerosis that obviously compromised SG survival in the implanted cochlea as seen in panel **b.** The final subject K 163 shown in **c** demonstrated unusually high SG densities in both cochleae after 14 weeks of chronic stimulation and extended DES administration. White symbols on some of the data bars indicate cochlear sectors where mechanical trauma from surgical insertion of the electrode array was observed.

Chronic electrical stimulation in subject K158 apparently elicited increased survival of the SG neurons in the implanted cochlea, with an overall mean of 56% of normal as compared to 37% of normal in the contralateral deafened ear. SG neural survival was higher on the stimulated side in all but 2 of the 9 cochlear sectors examined: At the extreme cochlear base SG survival was markedly reduced in association with extensive ectopic new bone formation within the scala tympani near the round window; and in the 40-50% sector where insertion trauma to the osseous spiral lamina was observed, the chronic stimulation also failed to promote increased SG survival in the stimulated ear as compared to the same region on the contralateral side.

Subject #K160: The second chronically stimulated subject in the series suffered a severe abscess at the percutaneous cable exit site of the implanted device (at the nape of the neck) about a month after the implant surgery. The infection appeared to respond satisfactorily to antibiotic treatment, but consistently recurred several times soon after antibiotics were discontinued. Throughout the 7 months of the chronic stimulation period this subject exhibited progressive elevation in EABR thresholds to stimulation delivered by both the apical (electrodes 1,2) and basal (electrodes 3,4) bipolar channels. After the final acute electrophysiology experiment, when the implanted device was removed in order to perfuse the cochlea, it was clear that the infection had tracked down the cable into the middle ear, and the round window was occluded by dense, reactive connective tissue, which also encapsulated the electrode within the scala tympani. Histological sections from the implanted cochlea in this subject indicated chronic labyrinthitis with partial fibro-osseous sclerosis extending throughout the entire cochlea. Given this severe pathology, it is not surprising that the SG neural degeneration was severe in this cochlea (Fig. 4b). Averaged over all sectors of the cochlea, SG survival was reduced to 21.5% of normal. In fact, neural survival in the implanted cochlea was actually lower than that observed in the control, deafened ear of this subject, in which SG density was 37.3 % of normal. It should be noted that this value for the control ear was virtually identical to that measured in the control ear of the first subject K158. Because of the obvious deleterious effect of the severe labyrinthitis upon the SG survival in the implanted ear of this subject, it will be deleted from pooled group data for evaluating the effects of DES and chronic stimulation.

Subject #K163: The third subject in the DES/chronic stimulation pilot series presents SG data that sharply contrast with the previous subject. As illustrated in Figure 4c, both cochleae of this animal had excellent SG survival, with a mean value of about 87% of normal in the stimulated ear and about 84% in the control ear. There is no significant difference between the sides, but this appears to be mainly because so little degeneration has occurred in the control ear. The data from the basal half of the cochlea suggest that some effect of stimulation may be emerging, but the higher densities seen in several basal sectors in the stimulated cochlea is offset by the insertion trauma in the 50-60% region of the implanted ear which resulted in markedly lower SG survival in that region and lowered the overall average for this ear. (In fact, if the 50-60% sector is omitted from the averages, the SG survival in the stimulated cochlea is about 98% of normal as compared to 84% on the control side.)

This cat had an abbreviated chronic stimulation period of only 14 weeks (Table 1), at which time the animal badly damaged its implant cable and was studied at 20 weeks of age. The wire breakage was internal and although the device could be repaired, the repaired cable would not be as sturdy as the original, and continued chronic stimulation would have run the risk that a second break could not be repaired, thus preventing conduct of any electrophysiological experiment in this subject.

Therefore, we decided to repair the electrode and study this subject immediately in a premature final electrophysiological experiment. However, in addition to the relatively brief chronic stimulation period in this subject, the daily DES injections also were continued for a longer period (total of 73 days) in this subject, continuing for 4 weeks after cochlear implantation and concurrent with the initial month of chronic electrical stimulation (Table 1). Thus, it is unknown what (if any) contribution to the excellent SG survival in this subject was made by the extended DES treatment, the shorter survival time post-deafening, or simple individual variability in the neural degeneration elicited by the ototoxic drug regimen.

Subject #K166: The fourth and final subject in this initial pilot series also showed fairly good neural survival overall with a mean of 62% of normal SG density in the implanted, stimulated cochlea and 53% in the control ear (Fig. 4d). The DES treatment was continued in this subject throughout the entire chronic stimulation period until the time of study (Table 1) and may have contributed to the level of neural survival maintained in this subject

However, it should be noted that this cat also was studied early, at about 6 months of age and after 16 weeks of chronic stimulation. Our goal in these experiments has been 6-8 months of chronic stimulation, in order to match stimulation periods in previous experimental groups that received only chronic stimulation for comparison to the present DES/stimulation series. However, we were in the process of developing a new subcutaneous attachment cuff for the percutaneous connector, in the hope of reducing the incidence of infection at the cable exit site. It should be noted that the new cuff is now working very well, but the iteration used in K166 was too thick and the cuff avulsed through the skin 15 weeks after implantation. We then removed the cuff, but without the strain relief and fixation provided by the subcutaneous cuff, the animal was found to have severed the cable about a week later. Again, we elected to repair the device and immediately conduct an early final electrophysiological experiment. The shortened stimulation period would have allowed less time for SG degeneration in this subject. We believe that is the likely explanation for the finding in this subject of only a relatively modest difference in neural survival elicited by chronic stimulation, with about 9% higher SG density in the stimulated ear (e.g., as compared to a difference of 19% in K158 and 16-17% in the data shown in Fig. 1).

Figure 5 shows the SG ganglion data pooled for the 3 subjects (omitting K160, the subject with the severe infection and labyrinthitis) in this group in which we evaluated some possible protocols for combining DES administration and chronic electrical stimulation. Averaged for this rather disparate group, the mean SG survival was about 68% of normal in the stimulated ears and about 10% less at 58% of normal for the control deafened side.

These initial results are quite encouraging, especially in consideration of the data presented in Figure 2 above, indicating that SG survival in neonatally deafened control subjects (without DES administration) averaged 66% of normal at 7-8 weeks of age at the time the DES animals received an implant and stimulation was initiated. Thus, our pilot group actually maintained equivalent SG survival in the stimulated cochleae after surgical implantation of a cochlear implant and electrical stimulation delivered over several months.

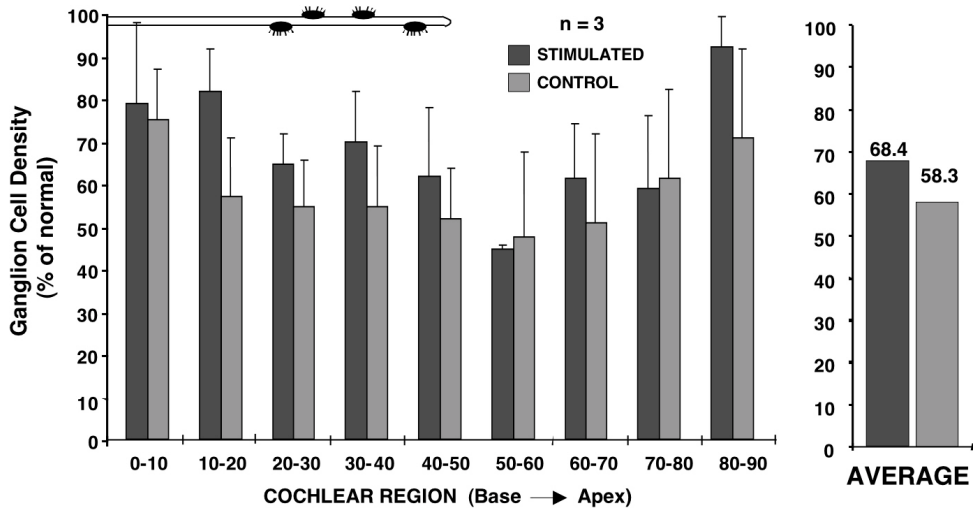

FIGURE 5. SG data are shown for 3 neonatally deafened cats that received DES combined with several months of electrical stimulation from an implant. (K160 has been excluded from the group due to a severe labyrinthitis that compromised SG survival in the implanted ear.) SG cell density is about 10% higher in the stimulated cochleae than in the deafened control ears, and overall neural survival on both sides is excellent. However, the relatively short survival times in 2 of the subjects confound the interpretation of these otherwise promising results of combined DES and electrical stimulation.

Obviously, given the small *n* of the pilot group examined so far and the great individual variability observed in the SG data from these subjects, no conclusions can be drawn regarding the importance of several different factors that may have contributed to promoting the excellent neural survival seen in this subjects. However, the results presented here suggest that the extended DES treatment, the duration of survival times post-deafening, and intersubject variability in SG degeneration after ototoxic drug deafening are all potentially important contributing factors that should be further examined in future studies of the *in vivo* effects of chronic electrical stimulation in promoting SG neural survival after deafness.

Literature Cited

Apfel, S.C., Lipton, R.B. Arezzo, J.C. Kessler, J.A. 1991. Nerve growth factor prevents toxic neuropathy in mice. Ann. Neurol. 29:87-89.

Carlile, G.W., Chalmers-Redman, R.M.E., Tatton, N.A., Pong, A., Borden, K.E., Tatton, W.G. 2000. Reduced apoptosis after nerve growth factor and serum withdrawal: Conversion of tetrameric glyceraldehyde-3-phosphate dehydrogenase to a dimer. Mol. Pharm. 57:2-12.

Duchemin, A. M., Ren, Q., Mo, L.. Neff, N. H., Hadjiconstantinou, M. 2002. GM1 ganglioside induces phosphorylation and activation of Trk and Erk in brain. J. Neurochem 8: 696-707.

Gao, W-Q, Zheng, J.L., Karihaloo, M. 1995. Neurotrophin-4/5 (NT-4/5) and brain-derived neurotrophic factor act at later stages of cerebellar granule cell differentiatiation. J. Neurosci. 15: 2656-2667.

Hansen, M.R., X.-M. Zha, J. Bok and S.H. Green 2001. Multiple distinct signal pathways, including an autocrine neurotrophic mechanism contribute to the survival-promoting effect of depolarization on spiral ganglion neurons *in vitro.* J. Neuroscience: 21:2256-2267.

Hegarty, J.L., A.R. Kay and S.H. Green. 1997. Trophic support of cultured spiral ganglion neurons by depolarization exceeds and is additive with that by neurotrophins or cAMP and requires elevation of $[CA^{2+}]i$ within a set range. J. Neurosci. 17: 1959-1970.

Kanzake, S., Stover, T., Kawamoto, K., Priskorn, D.M., Altschuler, R. A., Miller, J.M., and Raphael, Y. 2002. GDNF and chronic electrical stimulation prevent VIII cranial nerve degeneration following denervation. J.Comp Neurol 454: 450-360.

Korsching, S. 1993. The neurotrophic factor concept: A reexamination. J.Neurosci. 13: 2739-2748.

Lalwani, A. K., Han, J. J., Castelein, C. M., Carvalho, G. J., Mhatre, A. N. 2002. In vitro and in vivo assessment of the ability of adeno-associated virus-brain-derived neurotrophic factor to enhance spiral ganglion cell survival following ototoxic insult. Laryngoscope: 112: 1325-1334.

Leake, P.A. and Hradek, G.T. 1988. Cochlear pathology of long term neomycin induced deafness in cats. Hearing Res., 33:11-34.

Leake, P.A. and Hradek, G.T., Snyder, R.L., Rebscher, S.J. 1991. Chronic intracochlear electrical stimulation induces selective survival of spiral ganglion cells in neonatally deafened cats. Hearing Res. 54:251-271.

Leake, P.A.,Snyder, R.L., Hradek, G.T., and Rebscher, S.J. 1992. Chronic intracochlear electrical stimulation in neonatally deafened cats: Effects of intensity and stimulating electrode location. Hearing Res. 64:99-117.

Miller, J.M., Chi, D.H. O'Keefe, L.J., Kruszka, P., Raphael, Y. and Altschuler, R.A. 1997. Neurotrophins can enhance spiral ganglion cell survival after inner hair cell loss. Int. J. Devl. Neurosci. 15: 631-643.

Mou, K., Hunsberger, C. L., Cleary, J. M., Davis, R. L. 1997. Synergistic effects of BDNF and NT-3 on postnatal spiral ganglion neurons. J. Comp Neurol. 386: 529-539**.**

Parkins, C.W., Li, L., Webster, D.W. 1999. Comparison of GM1 ganglioside treatment and electrical stimulation for preventing spiral ganglion cell loss in pharmacologically deafened guinea pigs. Abst. of ARO 22nd Midwinter Research Meeting, 660:167.

Rabin, S. J., Bachis, A., Mocchetti, I. 2002. Gangliosides activate Trk receptors by inducing the release of neurotrophins. J Biol Chem 277 (51):49466 –49472.

Staecker, H. Kopke, R., Malgrange B., Lefebvre P., Van de Water, TR. 1996. NT-3 and/or BDNF therapy prevents loss of auditory neurons following loss of hair cells. Neuroreport 7:889-894.

Tatton, W.G. 1999. Apoptotic mechanisms in neurodegeneration: Possible relevance to glaucoma. Eur. J. Opthalm. 9 (Suppl.1) S22-S29.

Tatton, W.G., and Chalmers-Redman, R.M.E. 1996. Modulation of gene expression rather than monoamine oxidase inhibition: (-)-Deprenyl-related compounds in controlling neurondegeneration. Neurol.47(3): S171-183.

Tatton, W.G., Chalmers-Redman, R.M.E., Pong, A., Rideout, H.J., Tatton, N.A. 1999. Neuronal death and deprenyl in Parkinson's disease: A conceptual journey from MAO-B to glyceraldehyde-3-phosphate dehydrogenase. GreyCell Pharm.Res. 914:1-13.

Walsh, M.E. and D.B. Webster. 1994 Exogenous GM1 ganglioside effects on conductive and sensorineural hearing losses. Hearing Res. 75: 54-60.

Yan, Q. Elliott, J.L., Snider, W.D. 1992. Brain-derived neurotrophic factor rescues motoneurons from axotomy induced cell death. Nature 360: 753-755.

Zha, X. M., Bishop, J. F., Hansen, M. R., Victoria, L., Abbas, P. J., Mouradian, M. M., Green, S. H. 2001 BDNF synthesis in spiral ganglion neurons is constitutive and CREB-dependent. Hear Res: 156: 53-68.

Zheng, J.L., Stewart, R.R. and Gao, W-Q. 1995. Neurotrophin-4/5 enhances survival of cultured spiral ganglion neurons and protects them from cisplain ototoxicity. J. Neurosci. 7:5079-5087.

Zheng, JL., Gao, W-Q. 1996. Differential damage to auditory neurons and hair cells by ototoxins and neuroprotection by specific neurotrophins in rat cochlear organotypic cultures. Eur. J. Neurosci. 8: 1897-1905.

WORK PLANNED FOR THE NEXT QUARTER

1) During the next quarter a manuscript reporting data from the GM1 ganglioside experiments will be prepared for submission to JARO.

2) Histopathological studies of cochlear specimens from chronically stimulated and control experimental animals will be continued for our new series in which desmethyldeprenyl (DES) has been administered over the period of several weeks in deafened neonates until the time their littermates undergo cochlear implantation.

3) Two new littermate pairs of kittens will be selected for neonatal deafening and DES treatment during the next quarter, with one subject of each pair undergoing subsequent chronic stimulation. In these subjects DES treatment will be continued throughout chronic stimulation periods, rather than just in the interim period after deafening and prior to implantation.