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Protective and Plastic Effects of Patterned Electrical Stimulation on the Deafened Auditory System

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INTRODUCTION

Clinical studies have shown great intersubject variability in speech recognition performance of cochlear implant subjects. Congenitally deaf individuals implanted as adults generally demonstrate particularly poor speech discrimination, but they tend to improve gradually with increasing auditory experience. Studies of temporal processing in central auditory neurons may provide important clues for understanding the role of experience-induced plasticity for the discrimination of electrical stimuli.

Previous studies by our group have shown that in neonatally deafened cats, when a cochlear implant is introduced at 6-8 weeks of age and electrical stimulation is applied to the *developing* auditory system as the animals mature into adulthood, chronic electrical stimulation significantly alters the temporal resolution of inferior colliculus (IC) neurons to electrical pulses (Snyder et al., 1995; Vollmer et al., 1999). The goals of the present study were: 1) to examine the consequences of long-term congenital deafness, which results in very severe degeneration of the cochlear spiral ganglion and cochlear nuclei; and 2) to study the effects of chronic electrical stimulation initiated in neonatally deafened animals as adults after prolonged periods of deafness (>2.5 years). Two experimental groups of neonatally deafened animals are included in this report: 'Unstimulated' cats were implanted and studied acutely; a second 'stimulated' group received a unilateral chronic implant and several weeks to months of electrical stimulation. Both groups demonstrated severe degeneration of spiral ganglion cells (density=1-18% of normal). A group of normal adult cats, acutely deafened at the time of study, served as controls. To assess temporal processing in these groups we compared maximum following frequencies (Fmax) and first spike latencies of single neurons and multiunit clusters within the IC or in primary auditory cortex (AI).

METHODS

Deafening, Implantation, Chronic Electrical Stimulation.

Cats (n=11) were deafened neonatally by administration of neomycin sulfate (60 mg/kg, SQ) beginning the day after birth and continuing for 16-21 days. As soon as deafness was confirmed by the absence of click-evoked auditory brainstem responses at 105 dB SPL, neomycin injections were discontinued. Since kittens are deaf at birth because of the immaturity of the auditory system, we consider these animals to model congenital deafness. The animals were studied as adults after prolonged periods of 2.5 to >7 years of profound deafness. An intracochlear electrode containing two offset-radial bipolar pairs of stimulating contacts was surgically implanted into the left scala tympani under isoflurane anesthesia and using aseptic surgical procedures. Six of these cats were studied acutely, comprising the long-deafened unstimulated (LDU) group. Five subjects received chronic stimulation for 4 hr/day, 5 day/week, with the intensity of the electrical stimulus set at 2dB above the electrically evoked auditory brainstem response (EABR) threshold, and were studied thereafter. Fourteen normal adult cats, acutely deafened at the time of study, served as controls.

Animal ID	Age @ Initital Stimulation (mo)	Duration of Stimulation (wks)	Age at Study (mo)	Spiral Ganglion Survival (% normal)	Stimulation Characteristics
Long Deafened Unstimulated (LDU)					
K16	NA	NA	44	4.5	NA
K24	NA	NA	30	3.1	NA
K33	NA	NA	51	5.1	NA
K51*	NA	NA	78	4.9	NA
K73*	NA	NA	38	18.3	NA
K111	NA	NA	38	11.9	NA
Mean			46.5	7.97	
Long Deafened Stimulated (LDS)					
CH611	42	28	50	3.1	300/30 SAM, 80 pps, SP; Beh
CH618*	52	34	60	1.3	300/30 SAM, SP; Beh
CH539*	65	13	69	2.7	SP, 300/30 SAM; Beh
K56*	84	7	86	5.1	300/30 SAM
CD393*	73	24	79	3.5	300/30 SAM
Mean	63.2	21.4	68.8	3.1	

TABLE 1: Duration of deafness and stimulation histories are shown for all long-term neonatally deafened animals. The control animals (n=14) are not included in this table. Asterisks denote animals for which cortical data were available. 300/30 SAM denotes stimulation with biphasic pulses (200 µsec/phase) delivered at a carrier rate of 300 pps and 100% sinusoidally amplitude modulated at 30 Hz. 80 pps denotes stimulation with a continuous train of unmodulated electrical pulses. Beh. denotes behavioral training. SP, stimulation with speech processor. Spiral ganglion survival is indicated as the overall average cell density for all sectors of the cochlea, expressed in percentage of normal. (NA=not applicable)

Electrophysiological Recording.

Responses from single neurons isolated within the IC clusters were elicited by trains of biphasic pulses (200 µsec/phase) delivered to an intracochlear bipolar pair of electrodes. Responses were recorded using tungsten microelectrodes, as previously described, using a differential recording technique (i.e., two impedance-matched microelectrodes, with the return electrode positioned at the surface of the brain while the recording electrode is advanced through the IC). For IC data collection, several penetrations through the IC were made in a trajectory parallel to the frequency gradient in the central nucleus. Response thresholds were recorded at 100 µm intervals, and single units were isolated and recorded whenever possible (Snyder et al, 1995; Vollmer et al., 1999). Responses were averaged for 20 repetitions of each electrical stimulus, beginning with a pulse train at 10 pps and increasing frequency in steps of 10 pps until the unit failed to respond or responded with only to the onset of the stimulus. Poststimulus time histograms (PSTH) were constructed, and two response variables were measured: The maximum frequency (Fmax) to which the neuron followed in a synchronized manner (Raleigh test, $p<0.01$) and the first spike response latency at 20 pps (IC) or 4 pps (AI). Examples of PSTH and the Fmax determined for 2 single IC neurons are shown in Figure 1.

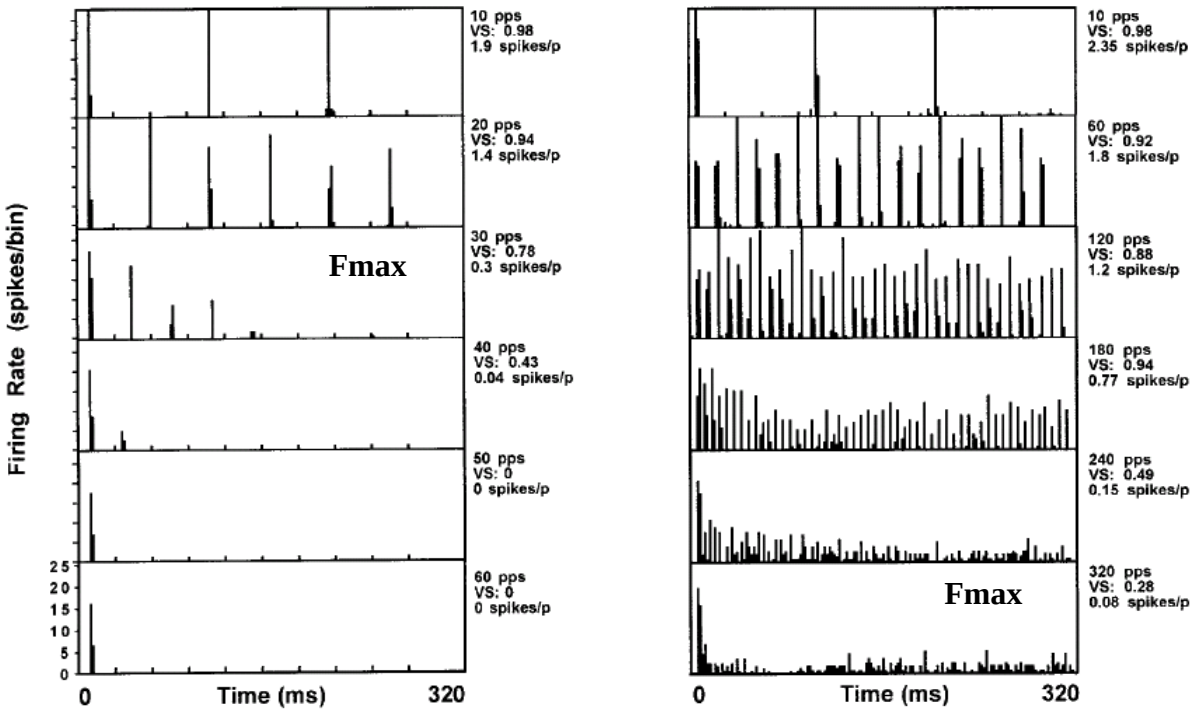

Figure 1: Poststimulus time histograms for 2 single units in the IC. A relatively low frequency neuron is shown in the panel on the left. The maximum frequency at which the unit exhibited valid phase-locking to the electrical stimulus was 30 pps. A unit which showed following to much higher frequencies is shown at the right. The Fmax for this IC neuron was 320 pps. (VS=vector strength)

Following completion of the IC experiment, in 6 of the long-deafened subjects we were able to conduct a second experiment, recording from AI auditory cortex, as previously described (Schreiner and Raggio, 1996). A craniotomy was made in the parietal bone to expose the anterior and posterior ectosylvian sulci, and the overlying dura was excised and reflected. A video image of the cortex was obtained and used to mark the locations of multiple electrode penetration sites that were made at closely spaced intervals across the auditory cortex. Responses of single neurons and multineuronal clusters in AI were recorded using a differential recording technique with two impedance-matched microelectrodes analogous to the one described above for the IC. The return electrode was placed at the surface of the cortex, and the recording electrode was advanced into layers IIIb or IV of the cortex, a depth of approximately 750 to 1050 µm. At each location, the threshold and temporal response profiles were recorded for selected stimulating channels. As in the IC, responses to trains of biphasic pulses (200 µsec/phase) delivered to an intracochlear bipolar pair of electrodes at varying rates were saved as PSTH. From these PSTH, the Fmax of AI units were determined for as many penetration sites as possible.

Cochlear Histology.

After completion of the terminal electrophysiological experiment in each long-deafened cat, the animal was euthanized humanely by an overdose of barbiturate. Cochlear and transcardiac perfusions were performed with histological fixative (2.5 % paraformaldehyde and 1.5% glutaraldehyde in 0.1M phosphate buffer), and the temporal bones and brain were removed for histological examination using previously described methods (Leake et al., 1999). Briefly, specimens were dissected, embedded in epoxy resin, and reconstructed in surface preparations to measure the basilar membrane. Radial semi-thin sections (2 µm) were cut at 50 µm intervals, and spiral ganglion (SG) cell density was determined using a point-counting method and expressed as percentage of normal for every 10%-sector of the cochlea in each animal.

RESULTS

Cochlear Histopathology.

Figures 2 and 3 illustrate the severe degeneration of SG cells observed in the long-term congenitally deafened subjects included in this study. Neural survival is shown in Figure 3 as percent of normal for 10% sectors of the cochlea from base to apex. The mean SG cell density for all sectors throughout the cochlea in both groups was less than 15% of normal. Table 1 lists the mean values obtained for overall neural survival (i.e., averaged for all cochlear sectors) in the 11 individual long-deafened subjects. These data indicate that degeneration of the spiral ganglion was very severe in all these animals. Overall SG survival in the LDU group was about 8%, and in the LDS group neural survival averaged about 3% of normal. For comparison, we have conducted many previous studies examining the effects of stimulation delivered during development, beginning at 6-8 weeks of age in neonatally deafened cats (Leake et al., 2000a,b; Snyder et al., 1995; Vollmer et al., 1999), and the SG survival in the stimulated ears of these animals averaged about 50% of normal (Leake et al., 1999, 2000a).

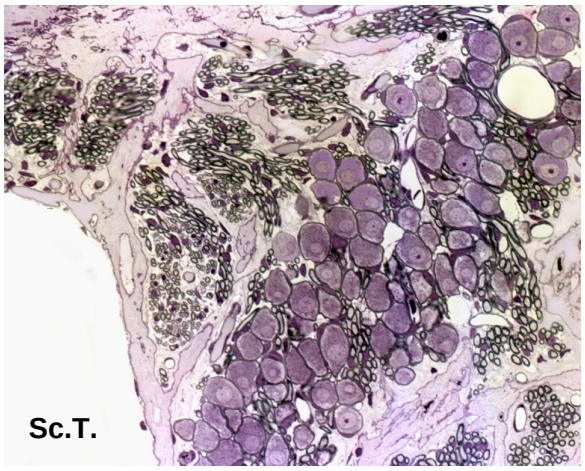

Figure 2a. Micrograph of Rosenthal's canal in a normal hearing control animal, illustrating the densely packed spiral ganglion cells, the myelinated radial nerve fibers coming from the organ of Corti (at left), and the central axons projecting into the modiolus to form the auditory nerve at the right of the image. (Sc.T.=Scala tympani)

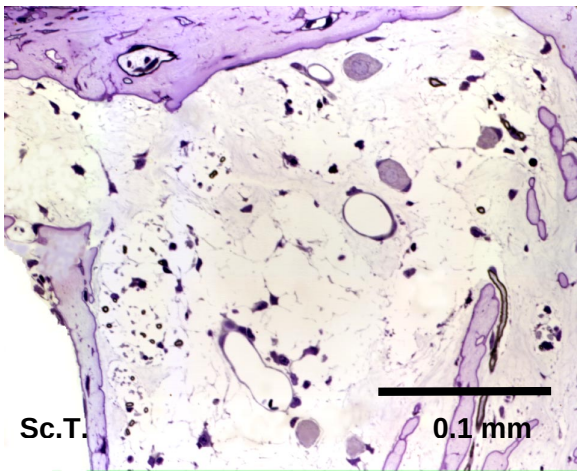

Figure 2b. Rosenthal's canal in a long-deafened cat (K56) illustrating severe loss of spiral ganglion cells (density <5% of normal) and degeneration of the radial nerve fibers and central axons of the auditory nerve. Note also that the few residual ganglion cells are pathologically altered, in that they appear severely shrunken and have lost the myelin which normally surrounds most of the cell somata.

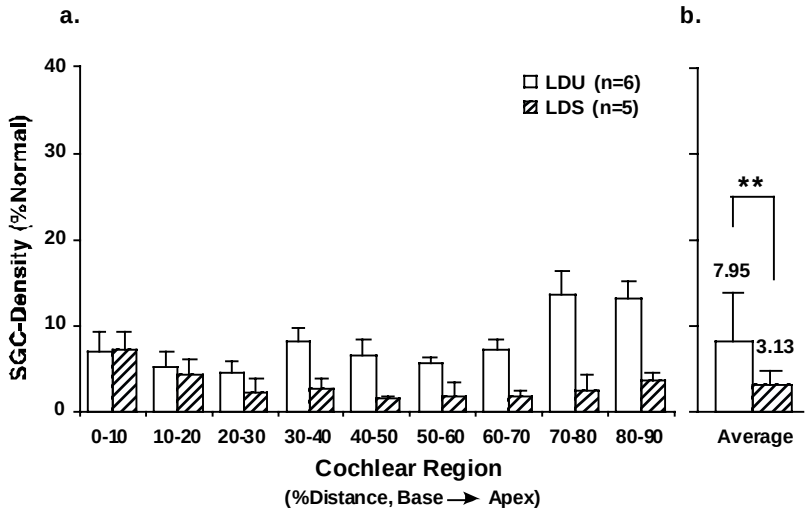

Figure 3. Quantitative data illustrating SG cell density in the left cochleae of 2 groups of congenitally deafened cats studied as adults after prolonged periods of deafness ranging from 2.5 to 7 years. SG survival is shown for 10% sectors of all cochleae from base to apex (a) and expressed as percentage of the normal SG density, with the overall averages for the two groups shown in (b). Asterisks indicate statistical significance (Student's t-test; $p<0.01$:**).

Fmax and First Spike Latencies

Figure 4a presents a scatter plot of first spike response latencies as a function of maximum following frequencies (Fmax) for single neurons in the central nucleus of IC. The data suggest that neurons from all three experimental groups exhibited relatively similar ranges of Fmax and latencies. Fmax for IC neurons ranged from 10 to 330 pps in control subjects; the highest Fmax value was recorded in a long-term deafened unstimulated animal (620 pps). The logarithmic regression function is shown and suggests a modest tendency for shorter first spike latencies to be correlated with higher Fmax.

First spike response latencies for single neurons and multineuron clusters in the AI primary auditory cortex are plotted in Figure 4b for all three experimental groups. In the AI, the data for neurons from long-deafened stimulated animals covered the broadest range of represented Fmax and latencies. For the LDS group, latencies ranged from roughly 5 to 15 ms, and Fmax ranged from 4 to 76 pps. The distributions for neurons from long-deafened unstimulated animals (relatively low Fmax and long latencies) and control animals (moderate to high Fmax and short latencies) barely overlap. However, it should be noted that the data for control subjects are very limited at present. As was the case for the IC neurons, there is a modest tendency in cortical neurons for shorter first spike latencies to be correlated with higher Fmax, as indicated by the logarithmic regression function.

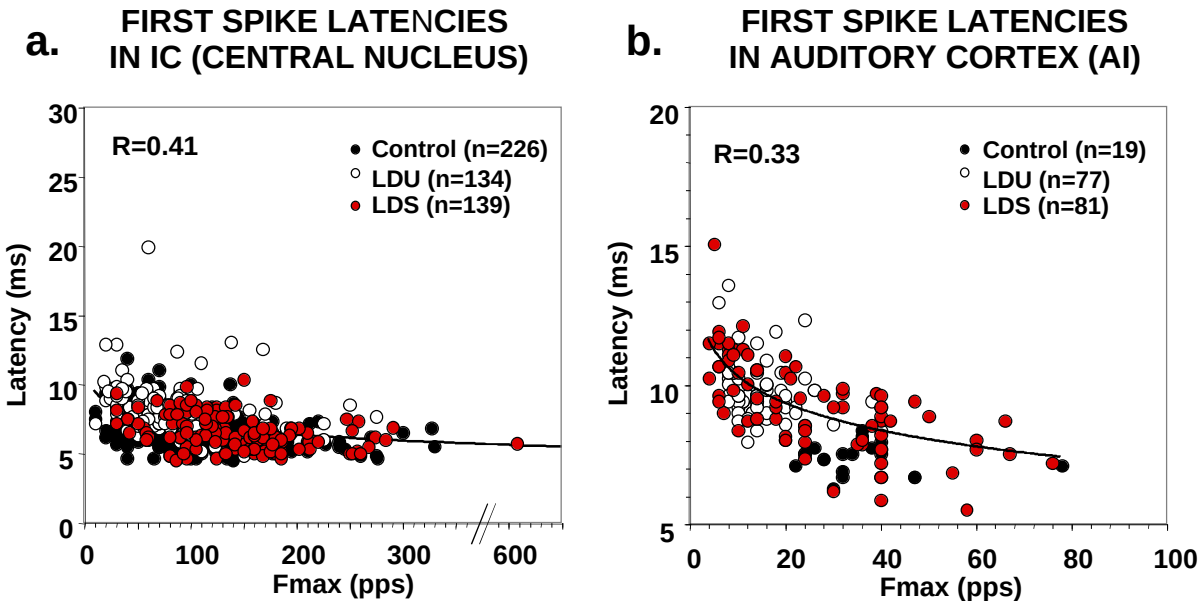

Figure 4. Scatter plots illustrating latency vs. Fmax distributions for single units in the IC (a) and for single neurons and multi-unit clusters in AI auditory cortex (b). Data include control subjects deafened as adults at the time of study and the 2 groups of congenitally deafened cats studied as adults after prolonged periods of deafness ranging from 2.5 to 7 years. (LDU, long-deafened unstimulated cats with no prior experience of electrical stimulation prior to the acute experiment; LDS, long-deafened cats that received several weeks to months of electrical stimulation from their implant prior to study. (n, number of neurons; R, correlation coefficient)

The data for Fmax are presented in Figure 5. The box plots compare median and percentile data (Mann-Whitney U test) for the control group and the two groups of long-term congenitally deafened animals. In the central nucleus of the IC in the control group, the median Fmax value was 95 pps. In the LDU group that had no prior experience with electrical stimulation until the time of the study, Fmax was reduced to a median value of 70 pps, and this apparent degradation in temporal resolution was statistically significant. In contrast, LDS long-deafened animals that received chronic electrical stimulation from a cochlear implant exhibited an increase in Fmax to a value of 135 pps that was not only significantly higher than the Fmax of the LDU group but also higher than that of normal control subjects.

Similar but not identical results were observed in AI. In normal control subjects the median Fmax value for AI was 32 pps. Temporal resolution was significantly lower in the LDU animals to a median value of 13 pps. The LDS group exhibited an intermediate median value for Fmax of 22 pps – a value that was significantly *higher* than that for the LDU, but significantly *lower* than that observed in normal controls. In addition, the AI Fmax data of LDS animals showed a much broader range than the data from either of the other groups, indicating much greater variability in the capacity of AI neurons to code repetition rates of the electrical stimuli. It should be noted that a single subject in the LDS group with extremely low temporal resolution and weak AI responses contributed greatly to the large variability observed in this group. It is interesting that this subject, K56, had the longest duration of deafness (>7years) and also the *shortest* duration of stimulation of all the long-term deafened animals (see Table 1). It seems likely that these factors account for the reduced effect of stimulation in the AI of this individual.

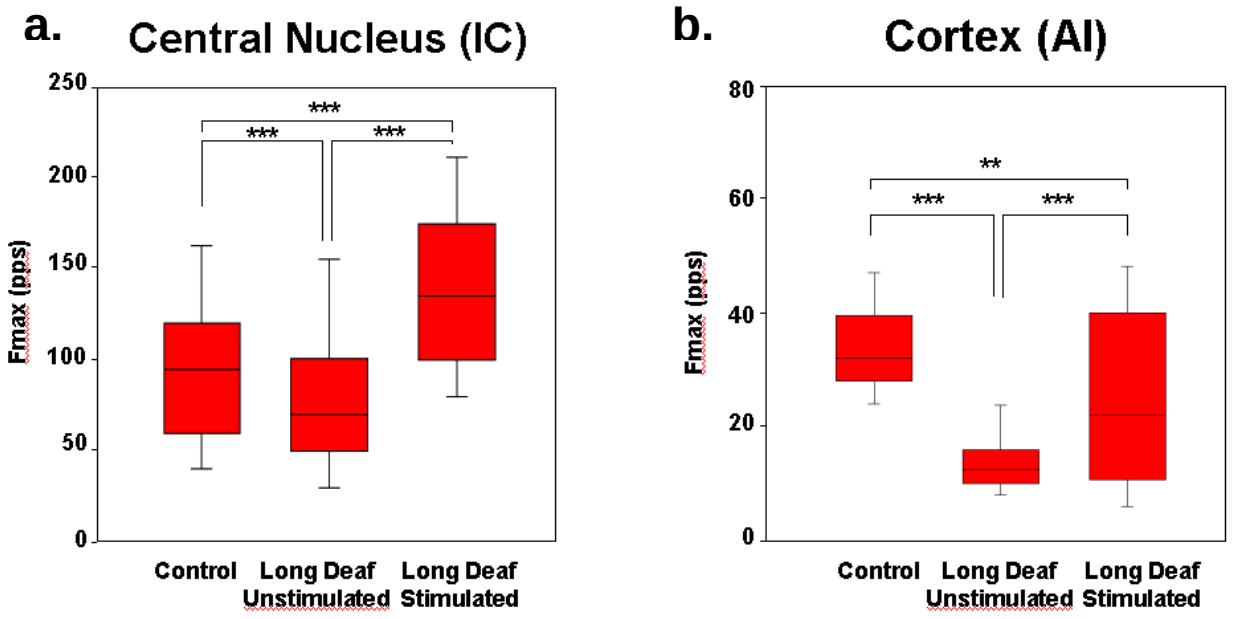

Figure 5. Fmax data for IC (a) and AI (b) are shown as box plots for median and 10, 25, 75 and 90 percentiles of all values for control subjects and also for the 2 groups of long-term neonatally deafened animals. (Mann-Whitney U test; p<.001:***; p<.01:**, p<.05:*).

The first spike latencies for these same data sets are presented in Figure 6. Again, box plots compare median and percentile data for the group of prior-normal control subjects and the two groups of long-term congenitally deafened animals. As expected, the latency data for both IC and AI showed results that closely paralleled the Fmax data shown in the previous figure. That is, shorter first spike latencies were observed in the control and LDS groups with higher Fmax values, and longer first spike latencies corresponded to lower Fmax and poorer temporal resolution observed in the LDU group. In AI, the control and LDU groups had latency ranges that were completely non-overlapping. The LDS group showed a much broader range of latencies than the data from the other two groups. Again, this large variability in the data is mainly due to the results from a single animal in this group, K56, that had extremely long latencies in the AI.

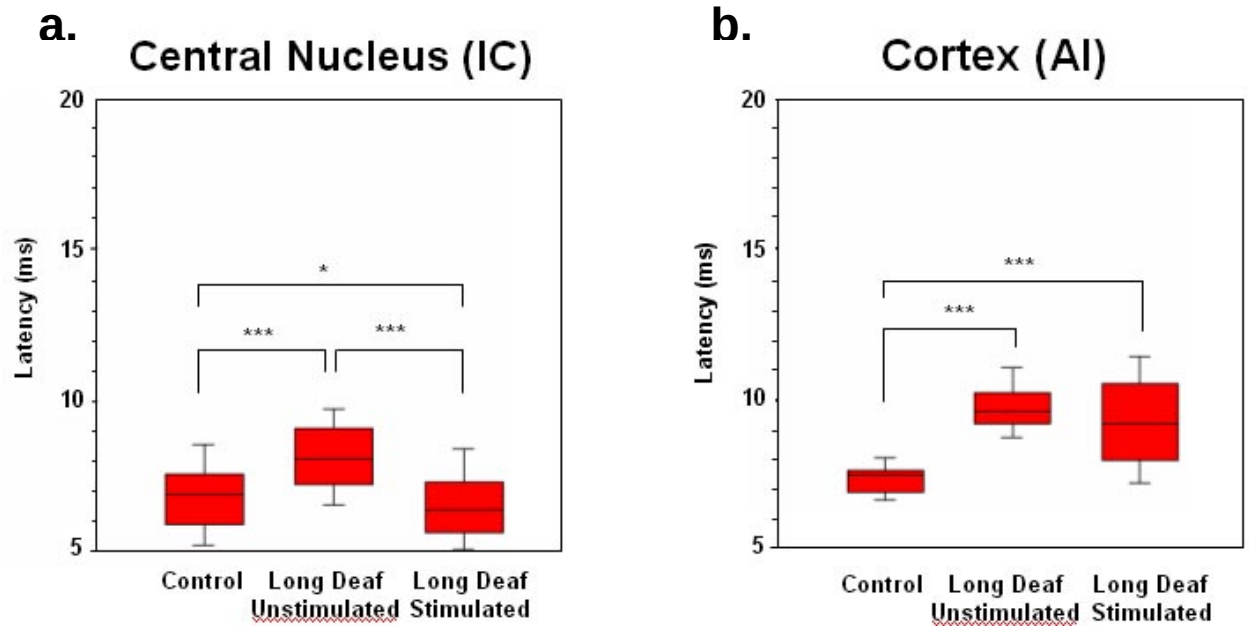

Figure 6. First spike latencies for single neurons in the IC (**a**) and single units and multineuron clusters in the AI (**b**) are shown as box plots for median and percentiles for the same control subjects and 2 groups of long-term neonatally deafened animals as in Figure 5. (Mann-Whitney U test; p<.001:***; p<.01:**, p<.05:*).

SUMMARY AND CONCLUSIONS

These data indicate that neurons in the central nucleus of the IC and AI cortical neurons in early deafened animals, studied as adults after prolonged durations of deafness, have significantly lower median Fmax and longer median latencies than controls. We assume that these findings are the direct result of the severe cochlear pathology observed in these animals, which included reduction in SG cell density to averages of 8% of normal in the LDU and 3% in the LDS group, demyelination and shrinkage of the remaining cell somata, and degeneration of virtually all radial nerve

fibers. Moreover, a previous study of the cochlear nuclei in these long-term deafened animals showed severe reductions in both cochlear nucleus volume and spherical cell size (Lustig et al., 1994). Given the severity of the pathology recorded in these animals, we hypothesized that the degradation in temporal resolution of IC neurons and decreased repetition rate coding in AI would be largely irreversible when chronic electrical stimulation was introduced after such long durations of deafness. In contrast to our expectations, however, chronic electrical stimulation resulted in a significant increase in temporal resolution in the IC neurons in these long-deafened animals. In fact, the LDS group exhibited significantly higher Fmax and shorter latencies than both long-deafened unstimulated animals *and* the prior normal control group.

The data for AI neurons also suggest that chronic stimulation led to a significant increase in repetition rate coding in the primary auditory cortex. However, Fmax of AI neurons in the LDS group still remained lower than those recorded in the prior normal control subjects. Further, median latencies in the *unstimulated* and *stimulated* long-deafened animals were virtually identical, and latencies in both deafened groups were significantly longer than those of control animals.

Together, these findings suggest that, despite severe peripheral and central pathology, chronic electrical stimulation can partially reverse the deleterious effects of long-term congenital deafness on temporal processing in the central auditory system, but the changes elicited are more pronounced in the IC than in the AI.

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Work planned for the next quarter

1) Daily chronic stimulation and drug treatment will continue in 2 subjects in our new experimental series in which the anti-apoptotic drug desmethyldeprenyl (DES) has been administered in deafened neonates both prior to implantation and continuing throughout the chronic stimulation period.

2) Two additional subjects will be implanted and daily chronic electrical stimulation initiated using the Advanced Bionics BDCS processor.

3) Two new litters of kittens born during the current quarter will provide additional controls for both the neomycin-only group and the DES treatment group, studied at 6-8 weeks of age, at the time their littermates undergo cochlear implantation.