
5th Quarterly Progress Report

October 1 through December 31, 2004

Neural Prosthesis Program Contract #N01-DC-3-1006

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

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INTRODUCTION

One objective of this Contract research is to examine functional consequences of electrical stimulation delivered by a cochlear implant in the central auditory system. This Quarterly Progress Report presents data from electrophysiological experiments conducted in the primary auditory cortex (AI) of cats, characterizing temporal responses to electrical stimulation. In most subjects, AI recording was carried out following completion of a recording experiment in the inferior colliculus, which will ultimately allow us to compare temporal processing in the auditory midbrain and cortex.

The AI is an essential processing station for species-specific vocalizations (Benson and Geschwind 1969; Heffner and Heffner 1986; Wang et al., 1995). Considering the spectral and temporal diversity of natural sounds and the necessity to selectively process sound that is behaviorally relevant, it is not surprising that this auditory cortical field can be reorganized and functionally modified by experience and learning (Buonomano and Merzenich 1998; Scheich 1991; Weinberger 1993). Studies in humans using stimuli with degraded spectral cues and preserved temporal envelope cues have shown that temporal cues are necessary and sufficient for speech recognition (Drullman 1995; Shannon et al. 1995). Temporal cues are required also for normal responses in AI neurons to spectrally degraded vocalizations in monkeys (Nagarajan et al. 2002).

Recently, experiments in animals with normal hearing have suggested that auditory discrimination learning based on temporal cues can significantly affect temporal processing in AI (Beitel, et al 2003; Bao et al. 2004). Clinical and psychophysical evidence indicates that deaf cochlear implant users typically show improvement in speech recognition over time, indicating that experience is an important factor for enhanced performance in electrical hearing as well (Busby et al. 1991; Syka 2002). Improved performance suggests that reorganization of auditory processing capacities has occurred, presumably reflecting the effects of plastic mechanisms in the central auditory system. Hypothetically, experience-induced plasticity can lead to improved functional representation of the electrical signals in the brain and to better speech recognition.

At UCSF, we have developed several deaf animal models to evaluate the effects of auditory experience and the duration of deafness on temporal processing in the central auditory system. These studies have shown that: 1) in the absence of intracochlear electrical stimulation, temporal resolution deteriorates during long-term deafness (Vollmer et al. 2005); 2) chronic electrical stimulation in deafened cats maintains or increases temporal resolution of neurons in the inferior colliculus (Moore et al. 2002; Snyder et al. 1995; Vollmer et al. 1999; Vollmer et al. 2005); 3) deaf cats can be trained to detect and discriminate temporally modulated signals (Beitel et al. 2000; Vollmer et al. 2001); 4) in the neonatally deafened cat, training may improve neuronal temporal processing in the inferior colliculus (Beitel et al. 1997) and 5) temporal resolution in cortical neurons to electric or acoustic stimulation appears to be determined primarily by central processing mechanisms rather than by differences in the peripheral excitation patterns of the two modes of stimulation (Schreiner and Raggio 1996; Schreiner et al. 1997).

In the present study, temporal processing in AI neurons was evaluated and compared among several groups of cats with different histories of deafness, chronic stimulation and behavioral training. The results show that long-term deafness degraded temporal processing in AI, whereas both chronic passive and behaviorally relevant electrical stimulation of the cochlea significantly enhanced temporal processing in AI of the deaf cat.

METHODS

Table 1 summarizes the deafness, chronic stimulation and behavioral training histories of the six groups of cats used in the present study. Prior to all surgical procedures an animal was sedated (ketamine: 22-33 mg/kg; acepromazine maleate: 0.1 mg/kg), and anesthesia was induced by pentobarbital sodium (7-10 mg/kg) delivered via an intravenous catheter. An areflexic level of anesthesia was maintained during surgery by intravenous infusion of pentobarbital sodium in Ringer's solution. In the present study, all procedures followed NIH and UCSF guidelines for the care and use of laboratory animals.

		Deafness		**Chronic Stimulation History**				
Group	**Cat**	**Age**	**Duration (wk)**	**Type**	**Intensity (μA)**	**Period (wk)**	**Electrode**	**Behavior**
A	**C958**	**Adult**	**2**	**-**	**-**	**-**	**-**	**-**
	C617	**Adult**	**2**	**-**	**-**	**-**	**-**	**-**
B	**C158**	**Adult**	**24**	**300/30**	**100**	**22**	**1,2**	**-**
	C087	**Adult**	**33**	**300/30**	**100-251**	**32**	**1,2**	**-**
C	**K51**	**Neonate**	**69 (mo)**	**-**	**-**	**-**	**-**	**-**
	K73	**Neonate**	**33 (mo)**	**-**	**-**	**-**	**-**	**-**
D	**K92**	**Neonate**	**32**	**300/30**	**33-150**	**24**	**1,2**	**-**
	K89	**Neonate**	**37**	**300/30**	**80-100**	**26**	**1,2**	**-**
E	**K99**	**Neonate**	**45**	**300/30**	**32-100**	**37**	**1,2**	**+**
	K91	**Neonate**	**38**	**300/30**	**30-400**	**32**	**1,2**	**+**
	K102	**Neonate**	**46**	**300/30**	**79-158**	**38**	**1,2**	**+**
F	**K80**	**Neonate**	**35**	**30 pps**	**250-315**	**27**	**RW**	**+**
	K82	**Neonate**	**36**	**30 pps**	**315-400**	**28**	**RW**	**+**

TABLE 1: Summary of onset and duration of deafness, chronic stimulation history and behavioral training. Electrodes: 1,2 (apical bipolar intracochlear) or RW (monopolar electrode just inside the round window).

Deafening. Adult cats (n=4, Table 1, Groups A, B) were deafened by co-administration (SQ) of kanamycin and ethacrynic or aminooxyacetic acids; kittens (n=9, Groups C-F) were deafened by injections (IM) of neomycin sulfate for 16-21 days beginning at birth (Leake et al. 1987; 1991). Neomycin injections were terminated when profound hearing loss (>108 dB) was confirmed by the absence of auditory brainstem responses to clicks (0.2 ms/ph, 20 pps). None of the animals demonstrated any residual hearing.

Implantation. Cochlear electrodes were fabricated from Teflon coated platinum-iridium wires embedded in a silicone rubber carrier and were implanted into the left scala tympani in anesthetized animals using sterile surgical procedures. Intracochlear electrode arrays implanted in most of the animals (Groups A, B, C, D, E) had four bipolar contacts (250 µm diameter) arranged as two offset-radial pairs. In two of the cats (Group F), the implant was a single intracochlear monopolar electrode positioned just inside the round window (RW).

Chronic stimulation. With the exception of cats in Groups A and C, in each cat a regimen of chronic electrical cochlear stimulation (4 hr/d, 5 d/wk, 2 dB above EABR threshold) was initiated, usually within one week after surgery. Chronic electrical stimuli consisted of charge-balanced, biphasic rectangular current pulses (0.2 ms/phase). For Groups B, D and E, pulses were delivered to the apical electrode pair (1,2) located 10 to 12 mm from the round window at 300 pulses/s, amplitude-modulated at 30 Hz (300/30). For Group F, 30 pulses/s were delivered to the RW electrode. Electrically evoked auditory brainstem response thresholds and electrode impedances were measured at regular intervals to assess the stability and reliability of the cochlear implants.

Psychophysical procedures. Training was based on a conditioned avoidance procedure (Beitel et al., 2000). A cat was placed in a wire cage (56 x 33 x 33 cm) located inside an acoustical chamber (Industrial Acoustics Company) and was trained to lick a metal spoon located at one end of the wire cage to obtain a preferred food reward (meat puree). A computer monitored contact with the spoon (sampling rate=50Hz), and the puree was delivered at a constant rate on Safe trials (80% of trials) from a motor driven syringe-pump during periods when the cat was licking the spoon. On Warning trials (20% of trials), a warning signal or conditioned stimulus (CS) was presented, and the cat was required to interrupt licking to avoid a mild electrocutaneous shock or unconditioned stimulus (UCS). On Safe trials, electrical signals were never delivered to the cochlea. The UCS was a 140 to 210 ms duration sinusoidal pulse train (60 Hz), adjusted for each cat to the minimum current intensity (0.5-2.0 mA) required to maintain avoidance behavior.

Amplitude modulated (300 pps modulated at 30 Hz; Group E) or unmodulated (30 pps; Group F) charge-balanced biphasic rectangular pulses were used as the CS in this study. These stimuli were computer generated (National Instruments LabView) and were delivered through an audio attenuator (HP 350B) to an optically isolated constant current stimulator. The output from this stimulator was delivered to an implanted cochlear electrode through a percutaneous cable. Prior to each behavioral session, this system was calibrated to a common reference level.

Warning trials occurred randomly during a session, with the restriction that they could not occur successively. After several suprathreshold Warning trials, a cat typically avoided the noxious UCS by breaking contact with the spoon during the CS. The intensities of the CS were then gradually reduced over several training sessions. Once performance stabilized from session to session, threshold testing was begun.

To estimate threshold performance, the method of constant stimuli (MCS) was used. The CS was presented randomly during a session within a range (5-10 dB, 1-dB steps) that bracketed the estimated threshold. The minimum difference between successive CS presentations was a 1-dB step. During a testing session, only the amplitude of the CS was varied. Testing continued until threshold performance from session to session was within 2 dB for a particular stimulus condition.

At the conclusion of testing, the following response measures were obtained by pooling data from 3 to 5 testing sessions: a) False Alarm rate: p(FA). False Alarms occurred whenever a cat was not in contact with the spoon during the final 200 ms of a Safe trial. The p(FA) was calculated: p(FA)=total FA/total Safe trials; b) Hit rate at each level of the CS: p(H). Hits occurred whenever a cat was not in contact with the spoon during the final 200 ms of a Warning trial, i.e., whenever a cat successfully avoided the UCS. The p(H) was calculated separately for each intensity of the CS: p(H)=total

Avoidance Responses at each level of the CS/total Warning trials at each level of the CS. A correction based on p(FA) was calculated to obtain the probability for detection at each level of the CS: p(DETECTION)=p(H)-p(FA)/1-p(FA).

Figure 1 illustrates typical psychometric functions in a deaf cat. The abscissa shows the levels of the CS in dB. A 50% detection or avoidance threshold was estimated by linear interpolation from the intersection of the dashed line with a psychometric function. The figure shows three psychometric functions for stimuli with different pulse rates and/or modulation frequencies. The arrows indicate the range of thresholds.

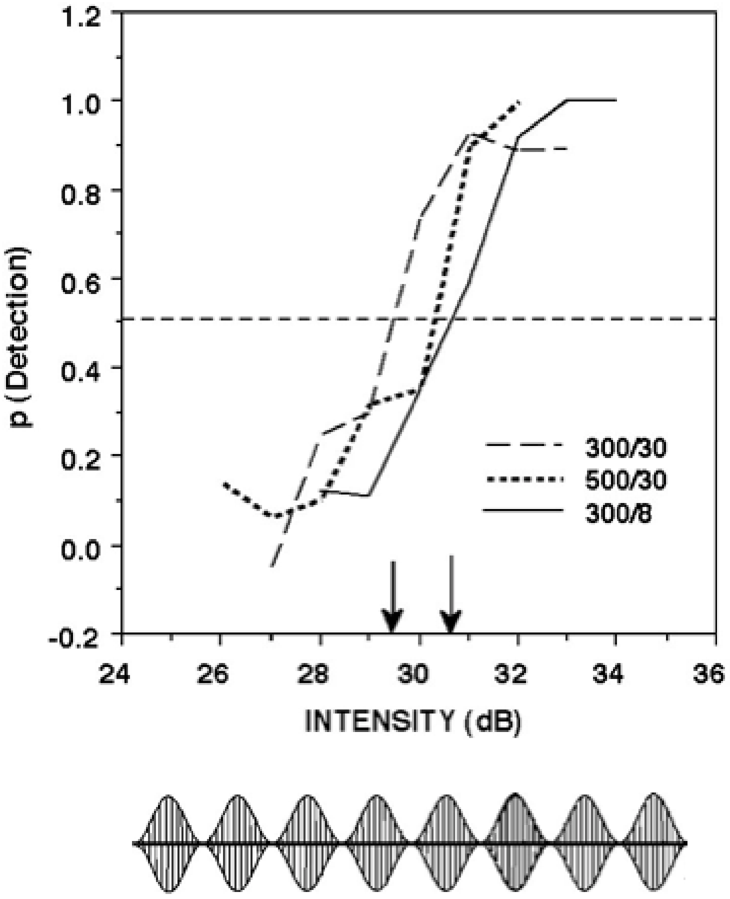

FIGURE 1: In behavioral sessions, animals were trained to detect electrical signals delivered to the cochlea at several intensities (Method of Constant Stimuli). Psychometric functions are shown for pulse rates of 300 and 500 pps modulated at 8 or 30 Hz. Arrows indicate the range of thresholds estimated in a deaf cat. Drawing below graph illustrates 8 cycles of a sinusoidal amplitude modulated pulsatile carrier. Modulation depth is 100%.

<u>Electrophysiological procedures</u>. 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 A1. Responses of multi-neuronal clusters in the AI of each cat were recorded using a differential recording technique with two impedance-matched tungsten microelectrodes. 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 at intracortical depths of approximately 600 to 1200 µm. Response thresholds to biphasic pulses delivered at intensities just sufficient to activate the unit were determined using audio-visual criteria. Responses of neuronal clusters evoked by trains of biphasic current pulses (0.2 ms/phase; 2 to ~40 pps; 2-10 dB above threshold) were recorded from locations distributed across AI. Spikes were discriminated and stored in a computer. Data were displayed in post stimulus time histograms (PSTHs) and were analyzed quantitatively to obtain the number of phase-locked spikes (Total Spikes*Vector Strength) evoked by each pulse rate at each recording site in each cat. For each recording site (N=455), three phase-locked response variables were measured: the pulse frequency that evoked the largest number of phase-locked spikes (best modulation frequency, BMF); the stimulus frequency at which the number of phase-locked spikes was just less than 50% of the number at BMF (Cutoff Frequency); and the Peak Latency of the period histogram at the lowest stimulus frequency (2 pulses/s). An example of the BMF and the Cutoff Frequency for one recording location is shown in Figure 2.

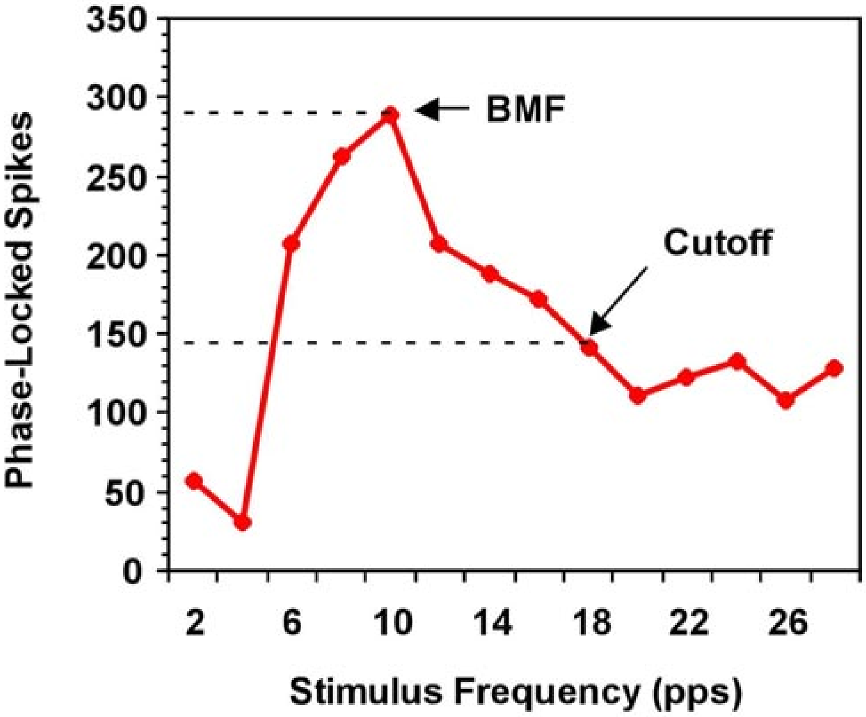

FIGURE 2: Total number of phase-locked spikes versus pulse frequency (pps). Pulses (0.2 ms/phase) were presented at rates from 2 up to 40 pps, 2-10 dB above threshold in ascending order (20 repetitions/frequency). Data shown were recorded at one penetration site in AI of a deaf cat. The best modulation frequency (BMF, 10 pps) and the stimulus frequency at which the number of phase-locked spikes was just less than 50% of the number at BMF (Cutoff Frequency, 18 pps) are identified in the figure.

RESULTS

Approximately 90% of the neuronal clusters responded at lower stimulus frequencies (2 to 6 pulses/s), and about one-third of the clusters responded to pulse trains with a rebound, oscillation or afterdischarge. The PSTHs in Figure 3A are characterized by oscillatory responses to low frequency pulse trains. However, the

PSTHs in Figure 3B are characterized by a simple response at low frequencies. In both panels, only an onset response to the first pulse and the stimulus artifact are seen at frequencies above 12 or 14 pps.

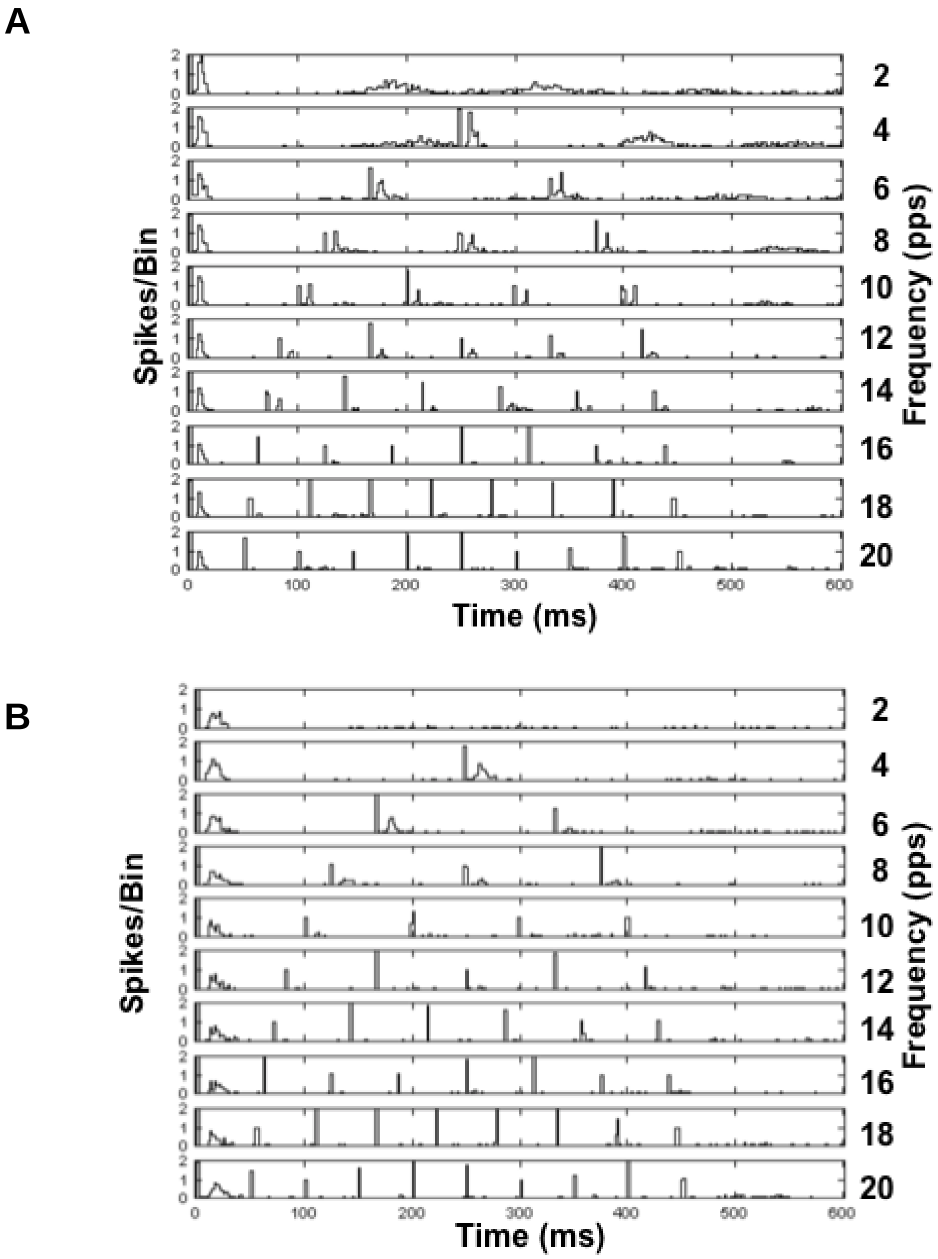

FIGURE 3: Post stimulus time histograms (PSTHs). Left ordinates: Spikes/bin; right ordinates: Pulse frequency (pps). A) Low frequency pulse trains evoke oscillatory responses at this recording site. B) At this site, low frequency pulse trains evoke a simple response to each pulse. Bin width: 2 ms.

In Figure 4, the main results are shown in rows that correspond to the response variables (BMF, Cutoff Frequency, Peak Latency) and columns that compare median and percentile data (***, p<.001; **, p<.01; Mann-Whitney Rank Sum Test) for groups of cats represented by box plots. Letters on the abscissas and in Table 1 identify the groups. The major findings indicating effects of deafening, chronic stimulation and training on temporal processing in the AI are summarized within each column.

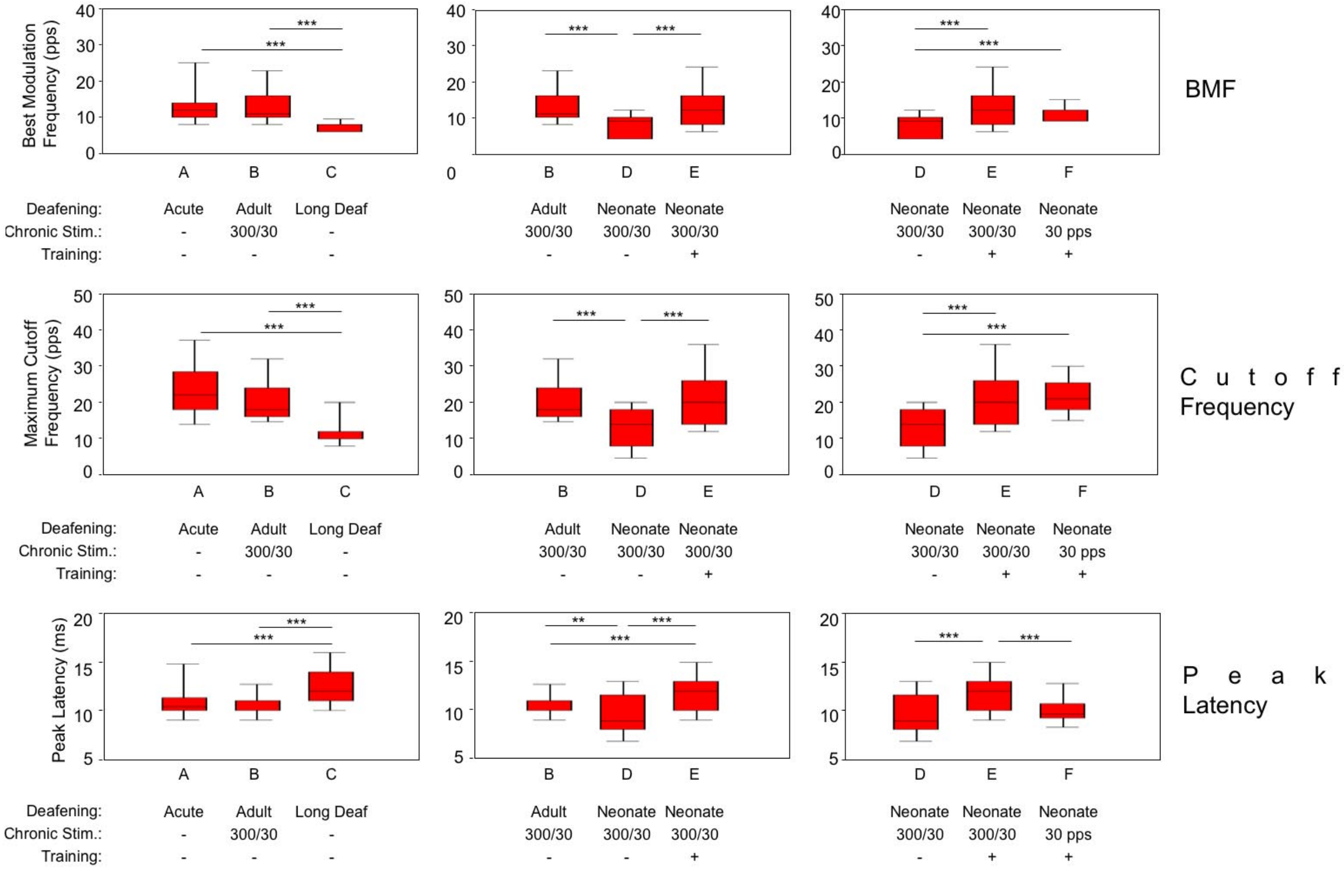

FIGURE 4: Box plots summarizing results for response variables BMF, Cutoff Frequency and Peak Latency (Mann-Whitney Rank Sum Test).

Left column: Cats with extensive hearing histories (Groups A and B) and extensive chronic stimulation (Group B) have higher BMFs, higher Cutoff Frequencies and shorter Peak Latencies than long deaf cats without a history of hearing or chronic stimulation (Group C). This column shows the deleterious effects of long-term deafness on temporal processing in AI.

Middle column: Neonatally deafened cats given chronic passive stimulation (Group D, 300/30) have lower BMFs and lower Cutoff Frequencies than cats with a history of hearing and chronic stimulation (Group B, 300/30). However, neonatally deafened cats given comparable chronic stimulation (300/30) *and* behavioral training (Group E) have BMFs and Cutoff Frequencies that are significantly higher than those for chronically stimulated, untrained Group D and similar to those for cats with a history of hearing and chronic stimulation (Group B).

Right column. Neonatally deafened cats (Groups E and F) that were trained *and* chronically stimulated with either 300/30 or 30 pulses/s have similar BMFs and Cutoff Frequencies, and these response variables are significantly higher that those for neonatally deafened, chronically stimulated, untrained cats (Group D, 300/30).

SUMMARY AND CONCLUSIONS

The results demonstrate the significant impact of long-term deafness, the limitation of chronic stimulation alone and the importance of the behavioral context for modification of temporal processing in the primary auditory cortex. Long-deafened cats showed significant degradation in temporal following and longer latencies than all other studied groups. Chronic (passive) electrical stimulation of the cochlea applied for hundreds of hours over a period of several months was not sufficient to maintain temporal processing at levels observed in deaf cats with an extensive history of hearing. Adding a few minutes of behaviorally relevant stimulation per day, however, resulted in a significant increase in neuronal BMFs and Cutoff Frequencies in the groups of behaviorally trained deaf cats.

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WORK PLANNED FOR NEXT QUARTER

1) Ongoing morphological data analyses of spiral ganglion survival and cochlear nucleus projections will continue in subjects that were studied in terminal experiments during the last 2 quarters. This includes 2 subjects in which the anti-apoptotic drug desmethyldeprenyl (DES) was administered in deafened neonates both prior to implantation and continuing throughout the chronic stimulation period, and one chronically stimulated subject that was deafened at 30 days of age, rather than neonatally.

2) Two additional subjects will be deafened at 30 days of age, rather than neonatally as in most of our earlier studies. One subject will be euthanized as a control at 8 weeks of age, and the second will be implanted unilaterally at the same age and will undergo chronic daily 2-channel intracochlear electrical stimulation. This new experimental series is designed to evaluate the potential critical period effects of a short period of normal hearing early in life.

3) One additional normal-hearing control subject will be deafened acutely, implanted for a short period to allow stabilization of thresholds, and studied in an acute electrophysiological experiment. We have recently finalized the design of a new feline intracochlear electrode with stimulating contacts positioned along the inner radius of the electrode, reflecting the design of contemporary human electrodes. This cat will be studied as a control to evaluate the new electrode.

4) During the next quarter two additional control animals will be deafened neonatally and euthanized at 8 weeks of age as controls for our current histological studies in the DES series.

5) Several of the investigators will attend the annual midwinter meeting of the Association for Research in Otolaryngology to present findings from this Contract research. Abstracts will be included in the next Quarterly Progress Report.