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Protective Effects of Patterned Electrical Stimulation on the Deafened Auditory System

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ABSTRACT

During this first quarter of the new Contract, we have focused upon completing further analyses and synthesis of data from previous experiments, in order to help us set priorities and select specific protocols and parameters for future experimental series. This Quarterly Progress Report presents new results from studies comparing -- in the same individual experimental animals -- psychophysical and neurophysiological thresholds to various stimuli. Previously reported data have now been supplemented by the study of several additional subjects, and also by extending the repertoire of electrical stimuli tested to include studies of effects of waveform (pulses vs. sinusoids), stimulus rate, stimulus duration and phase duration.

Results show that for pulsatile stimuli over a wide range of pulse rates, EABR and psychophysical thresholds are strongly correlated. Moreover, when measurements are all made in the same animal, psychophysical thresholds are, in turn, identical (or very close) to minimum thresholds for single unit neural responses (for the most sensitive units) in the central nucleus of the inferior colliculus (ICC) and in the primary auditory cortex (A1). Results of studies using sinusoidal stimuli indicate that psychophysical thresholds are always lower than neurophysiological thresholds for short duration (0.03 sec) sinusoidal stimuli, although this difference is not statistically significant. In contrast, psychophysical thresholds for longer duration (1.0 sec) sinusoidal stimuli are significantly lower than either psychophysical or neurophysiological thresholds for short duration (0.03 sec) sinusoidal stimuli.

Also during this quarter, two adult-deafened cats have been implanted for short periods with new model scala tympani arrays designed to provide: i) more optimal placement of stimulating contacts with respect to the spiral ganglion so that contacts in the basal region near the round window will have thresholds similar to those of more apical contacts; ii) longer safe insertion to allow selective stimulation over more of the frequency range of the cat cochlea; and iii) increased number of stimulating electrode contacts to permit 3 to 4 bipolar channels of electrical stimulation. These cats have been studied in acute electrophysiological experiments in order to characterize the new electrode arrays. In addition, histological preparations of cochlear nucleus specimens from our 4 most recent chronic intracochlear stimulation cases have been completed and analysis will be undertaken during the next quarter.

Comparison of Psychophysical and Physiological Thresholds for Electrical Stimulation.

Previously published reports from other investigators comparing psychophysical thresholds (as measured in macaque monkeys) with auditory nerve fiber thresholds (measured in both cats and squirrel monkeys) have suggested that psychophysical thresholds are consistently lower than neural thresholds for electrical stimulation of the cochlea (Pfingst, 1988; Pfingst, et al., 1991). However, these comparisons suffer not only from possible species difference, but also differences in intracochlear electrodes and various other experimental parameters such a duration of implantation, manner of inducing deafness, etc. Therefore, one focus of our Contract research has been to compare psychophysical and neurophysiological thresholds for electrical stimulation of the cochlea in the same experimental animals. In this report we summarize results obtained to date, including data on some effects of waveform (pulsatile vs. sinusoidal), stimulus rate (pps, Hz), stimulus duration, and phase duration on psychophysical thresholds, EABR thresholds, and minimum threshold responses of single units and multiunit neuronal clusters in the central nucleus of the inferior colliculus (ICC) and the auditory cortex (A1).

1. Methods

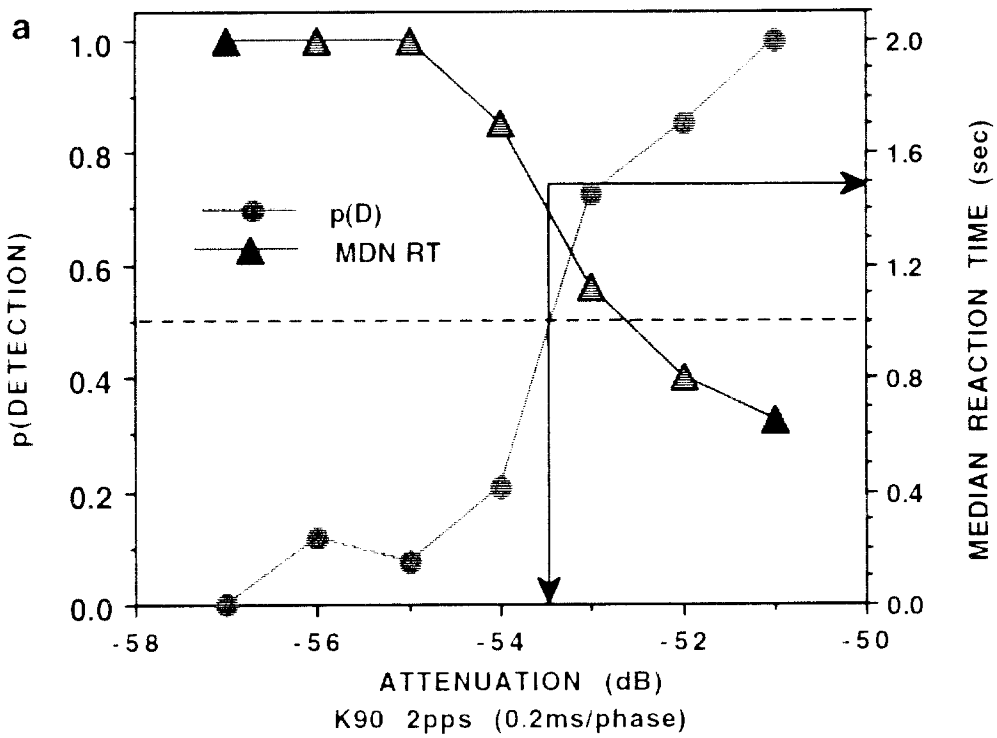
Experimental animals studied to date include cats that were deafened neonatally (n=10) and one cat deafened during adulthood by injection of ototoxic drugs. Cats were chronically implanted either with feline intracochlear electrodes (n=7) consisting of four electrode contacts driven as bipolar pairs, or a round window electrode (n=4). These profoundly deaf cats were trained in a conditioned avoidance paradigm to lick a metal spoon on "safe" trials to obtain a preferred food reward (meat puree) and to interrupt licking on "warning" trials to avoid a mild electrocutaneous shock. During training, biphasic square-wave pulses (charge-balanced; 0.2 msec/phase; 1-2 sec duration train) were delivered to the cochlea as warning signals. Once performance was stable, threshold estimates (50% avoidance, corrected for false alarms) and reaction times were obtained for a variety of pulsatile and sinusoidal stimuli using the psychophysical method of constant stimuli.

Evoked auditory brain stem response (EABR) thresholds to electrical biphasic pulses (0.2 msec/phase; 2 pps; responses averaged over 500 trials) were determined at regular intervals. At the conclusion of behavioral testing, acute electrophysiological mapping experiments were conducted to determine threshold response distributions for single units and multiunit neuronal clusters in the ICC and the A1 in each cat. For comparisons with psychophysical data, neurophysiological thresholds were defined *as the minimum amplitude stimulus that evoked a response* (audio-visual criteria) in the ICC and the A1, as determined by analysis of data from all penetrations in the respective maps of these central auditory structures. In each cat, the implanted cochlear electrode used in psychophysical testing was left in place at least during initial part of the acute physiological mapping experiments, and minimum threshold values were derived from the maps for this electrode or pair of electrodes. For all psychophysical and neurophysiological thresholds reported here, the reference level is -60 dB=50 μA_{peak} for pulsatile stimuli and -60 dB=50 μA_{rms} for sinusoidal stimuli.

2. Basic Psychophysics

Figure 1a illustrates typical psychometric functions used to derive psychophysical thresholds in experimental cats. The threshold and median reaction time are estimated by interpolation (arrows) from the dashed line which represents 0.5 probability for detection (corrected for false alarms). In this example (K90), the threshold for detection of 0.2 ms/phase biphasic pulses delivered via stimulation with a chronically implanted extracochlear monopolar electrode was 53.5 dB (attenuation) and the median reaction time was 1.6 sec.

1a



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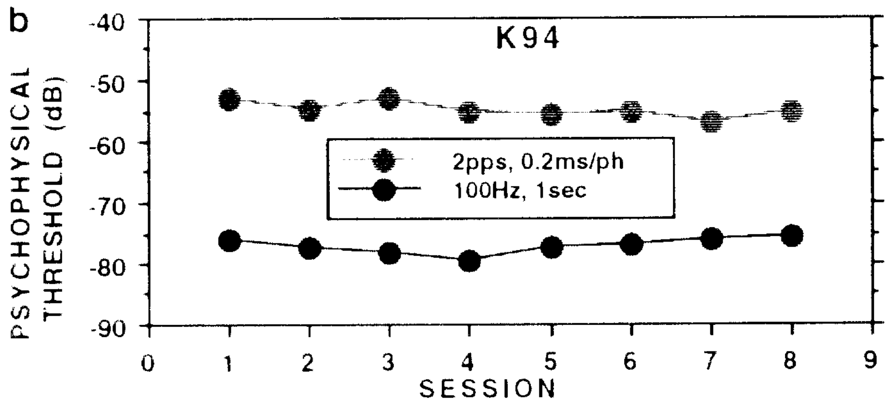


1c

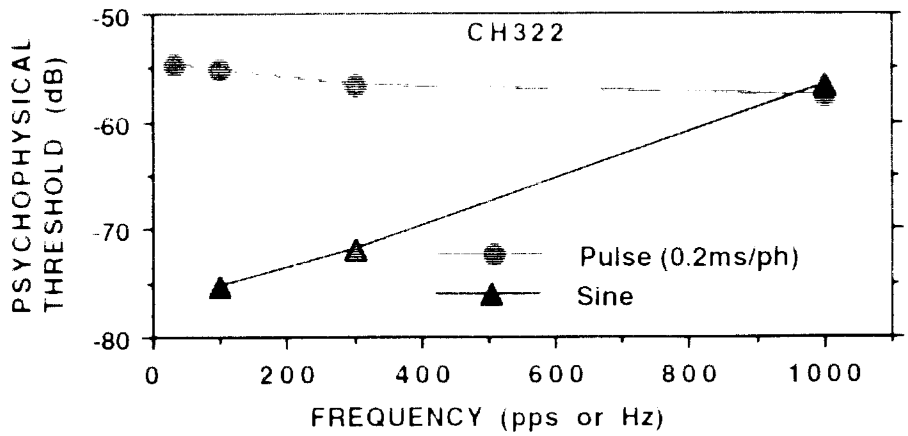


Figure 1 a. Typical psychometric functions for one implanted cat (K90), illustrating method of estimating threshold and median reaction time. b. Psychophysical thresholds for pulses and sinusoids measured in one cat during several training sessions showing stable performance over time. c. Thresholds for pulses (dots) and sinusoidal stimuli (triangles) as a function of increasing frequency.

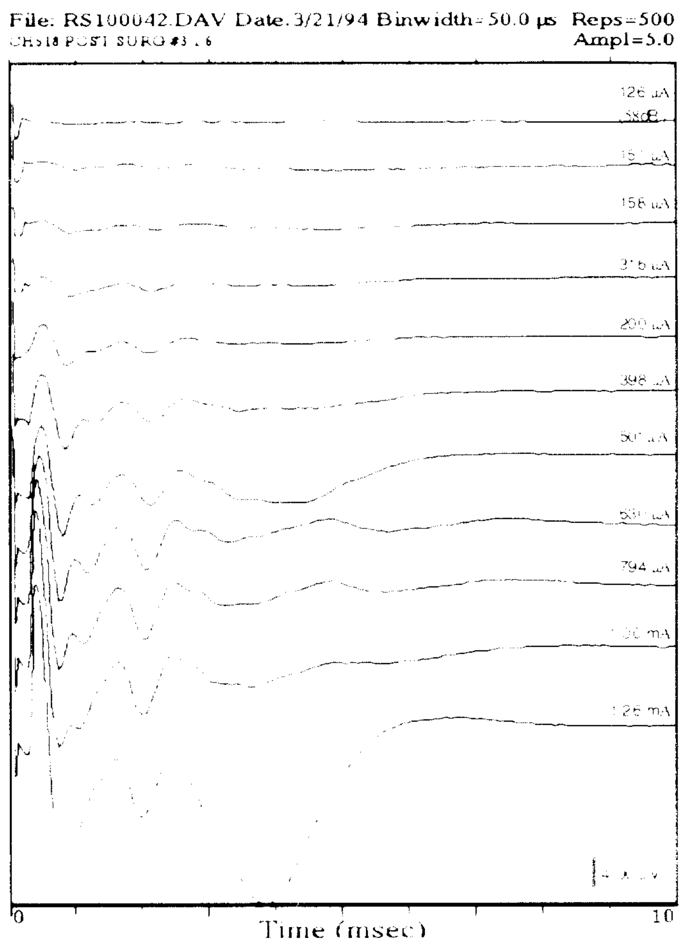
In most cats, psychophysical thresholds were stable over many weeks to months of implantation and behavioral testing. In a few animals, elevation of both EABR and pyschophysical thresholds was observed a few weeks after implantation. In these animals, thresholds then stabilized again, at new levels and remained quite stable over the ensuing months of testing. These animals were subsequently determined to have experienced profound stimulation induced damage to the cochlea and spiral ganglion neurons, due to a few episodes of EABR testing with a faulty new stimulator. (Histopathological results from this experimental group will be presented in the next Quarterly Progress Report.) Figure 1b shows thresholds in one of these cats, subsequent to the induced change in threshold and at a time when thresholds had once again stabilized at the higher level. Results show stable performance across 8 test sessions, representing a period of approximately 2 weeks. The mean threshold for 0.2 msec/phase pulses (stimulus duration = 1sec) was -54.9 dB (±1.2 dB). The mean threshold for sinusoidal stimuli (100 Hz; stimulus duration = 1 sec) was -77.2 dB (±1.3 dB).

In Figure 1c the psychophysical thresholds for one animal are shown as a function of stimulus rate (pps) or frequency (Hz). The thresholds for pulsatile stimuli were relatively invariant over rates from 30 pps to 1000 pps. The thresholds at 30 pps and 1000 pps were -54.7 dB and -57.6 dB respectively. In contrast, the thresholds for sinusoidal stimuli increased with increasing stimulus frequency, reflecting the progressive reduction in phase duration at higher frequencies. The thresholds at 100 Hz and 1000 Hz are -75.4 dB and -56.7 dB respectively. The duration for both pulsatile and sinusoidal stimuli was 2 sec.

3. Comparison of EABR, Neurophysiological and Psychophysical Thresholds for Pulsatile Stimuli

Figure 2a illustrates typical examples of the EABR waveforms evoked by biphasic pulses (0.2 msec/phase, 2 pps) in one implanted cat. Stimulation with intracochlear bipolar electrodes was delivered at increasing intensities in 2 dB steps from -58 dB (126µA) to -38 dB (1.26 mA). Each analog waveform is the average response to 500 repetitions of the stimulus. The threshold in this cat was -58 dB. In Figure 2b, the psychophysical thresholds for pulsatile stimuli (0.2 msec/phase) delivered at rates varying from 2 pps to 1000 pps are plotted against EABR threshold for pulsatile stimuli (0.2 msec/phase) delivered at a rate of 2 pps. These two threshold measures are significantly correlated (r=.90; p<.001). Moreover, psychophysical thresholds at each pulse rate tested were always lower than EABR threshold. For within cat comparisons, the mean threshold difference was -4.8 dB (±2.8 dB). The relatively small value of this difference is noteworthy, since within the sample of cats, differences in type of implanted electrodes, post-implant survival times, and extent of nerve survival were potentially confounding variables. These results indicate that EABR threshold is a robust predictor of psychophysical threshold for a wide range of pulse rates.

Figure 2a



2b

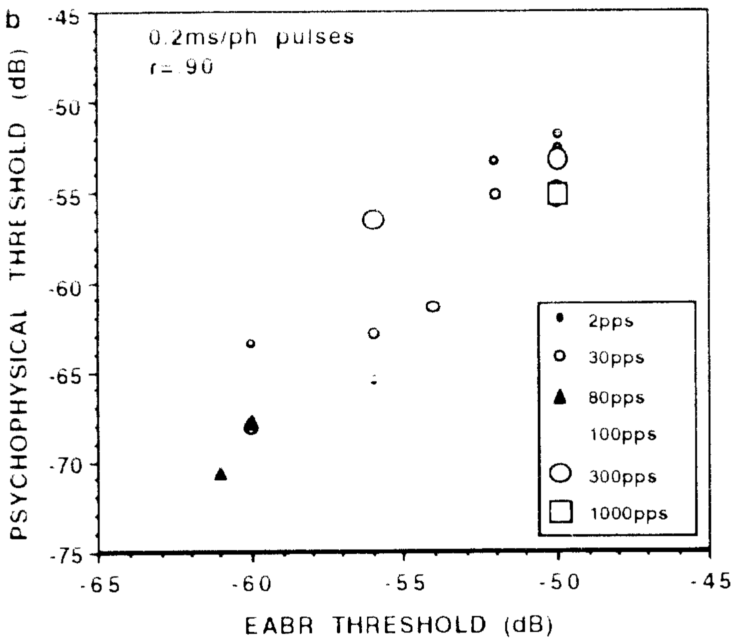


Figure 2 a. Example of EABR responses to biphasic pulses of increasing intensities (2 dB steps). Threshold was estimated at -58 dB. b. Correlation of psychophysical threshold for pulsatile stimuli (varying rates from 2 to 1000 pps) and EABR threshold.

Figure 3 shows individual data for four chronically implanted, behaviorally trained cats, comparing the psychophysical thresholds and the minimum ICC and A1 single unit thresholds for pulsatile stimuli (0.2 msec/phase; 2 pps). The results shown in panels a, b, and d are from three cats implanted with intracochlear electrodes and trained with bipolar stimulation delivered by the apical most pair of electrodes; the results shown in panel c are from one cat (K90) implanted with an electrode that was located near the round window and stimulated against a distant ground (monopolar stimulation). The dashed line in each panel represents the EABR threshold to 0.2 msec/phase pulses. It should be noted that the A1 in cat K94 (see panel a) was damaged during surgery, prior to the cortical mapping experiment, probably accounting for the reduced sensitivity and higher minimum threshold seen in the cortex of this particular animal. The mean psychophysical threshold for this group of experimental animals was -56.0 dB (±5.0 dB), while the mean minimum neural threshold for single units (combined data for ICC and A1) was -55.4 dB (±8.3 dB). This difference between psychophysical and neurophysiological thresholds was not significant ($p > .05$).

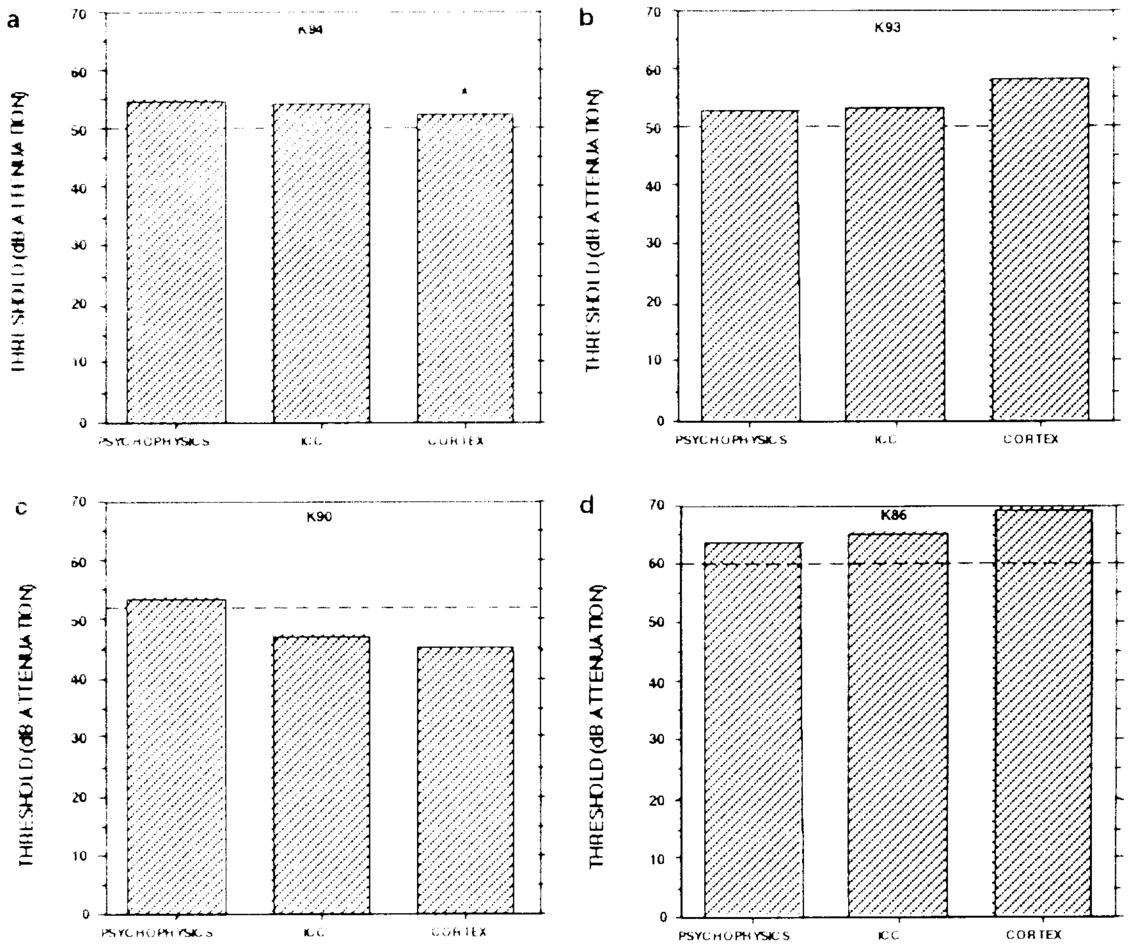


Figure 3. Comparison of psychophysical and neurophysiological thresholds in the ICC and AI for pulsatile stimuli as measured in 4 individual cats. Dashed lin in each panel represemts the EABR threshold (0.2msec phase)

The effect of phase duration on psychophysical and neurophysiological threshold for pulsatile stimuli was also examined, and these results are illustrated in Figure 4. In panel 4a the psychophysical and ICC neurophysiological threshold functions for one experimental animal (K93) are shown. Stimuli were biphasic pulses, and thresholds are plotted as a function of increasing phase duration. The pulse rate was 2 pps. The psychophysical threshold curve (large filled symbols) and the threshold curves (n=41) for most units recorded from the ICC decreased monotonically as phase duration was increased. At each phase duration, unit thresholds were usually higher than the psychophysical threshold and the range of unit thresholds increases with increasing phase duration. However the most sensitive single unit thresholds and the psychophysical thresholds were fairly close. Figure 4b compares the psychophysical threshold curve (large filled symbols) and the threshold curves (n=23) for units recorded from the A1 in this same cat. Results here are similar although the AI minimum single unit threshold do not match the psychophysical thresholds quite as well as the ICC thresholds did.

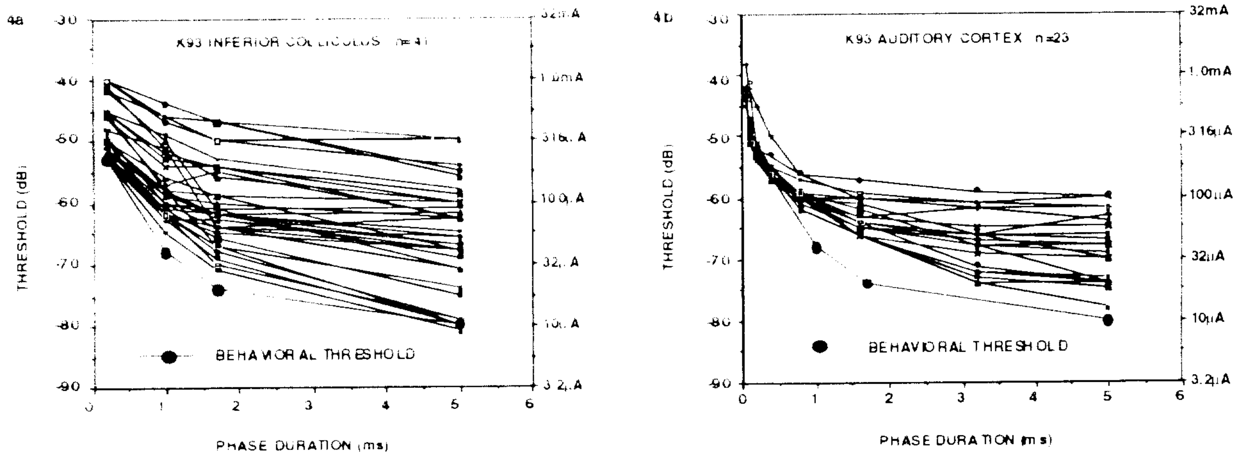
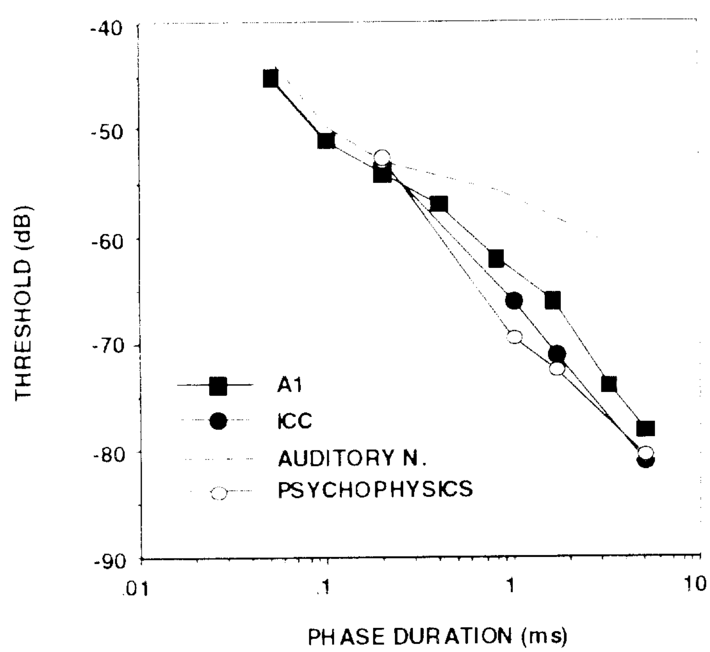


Figure 4. Comparison of psychophysical thresholds and neurophysiological single unit thresholds in both ICC (panel a) and AI (b)

These results comparing minimum threshold evoked neural responses for units in the ICC and the A1 with psychophysical thresholds in cat K93 are replotted together in Figure 5 as functions of phase duration for biphasic pulses (delivered at a rate of 2 pps). Virtually identical results were obtained in another cat K94 (not shown). The dashed curve depicts auditory nerve fiber minimum thresholds evoked by biphasic pulses in the squirrel monkey (Parkins and Colombo, 1987). At phase durations longer than 0.2 msec/phase, the slopes of the threshold curves for K93 are approximately parallel and are steeper than the threshold curve for auditory nerve fibers.

Figure 5. Comparison of minimum threshold for units in the ICC and AI with psychophysical thresholds in one cat as a function of phase duration for biphasic pulses. Dashed line represents auditory nerve data from Parkins and Colombo



Parkins and Colombo (1987) hypothesized that the differences between psychophysical and auditory nerve fiber thresholds at longer phase durations may be attributed to central processing. Our data suggest that unit thresholds in the ICC and AI are identical (or nearly so) to psychophysical detection thresholds and thus, that neural activity within the classical or mainline central auditory system underlies the psychophysical detection of these pulsatile stimuli.

4. Comparison of EABR, Neurophysiological and Psychophysical Thresholds for Sinusoidal Stimuli

Figure 6 compares the psychophysical, ICC, and A1 thresholds for sinusoidal stimuli (100 Hz; 0.03 sec and 1.0 sec duration). The results shown in panels a, b, c, and d are for the same individual experimental cats whose pulsatile thresholds were illustrated in Fig. 3.

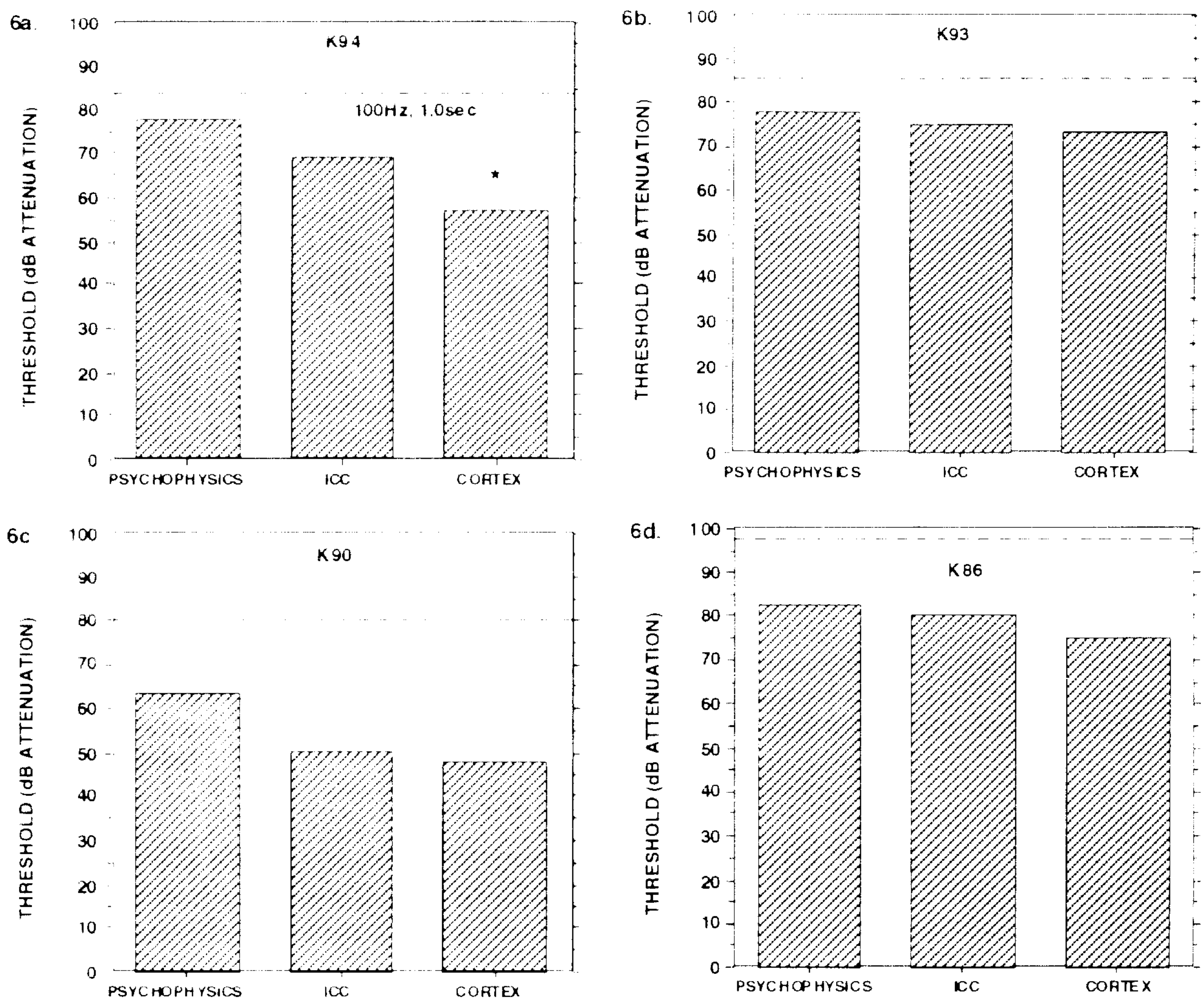


Figure 6. Data bars indicate psychophysical, ICC, and AI thresholds for 100 Hz sinusoidal stimuli (0.03 sec duration) for the same 4 individual cats as were shown in Figure 3. Dashed lines represent psychophysical threshold for each cat to longer duration (1.0 sec) 100 Hz sinusoidal stimuli.

are shown only for Cat K85, this result was confirmed by data obtained in the other cat (K90). Similar effects have been reported for acoustic stimulus duration on reaction times in normal hearing cats (Costalupes, 1983; Gerkin and Sandlin, 1977). These results suggest that behaviorally significant temporal integration occurs within 100 msec of stimulus onset for sinusoidal stimuli in these deaf cats.

Conclusions

We have developed a robust and reliable deaf animal model to estimate psychophysical and neurophysiological thresholds for electrical stimulation of the cochlea. Based on this model, the results presented above indicate that:

1. Over the wide range of pulse rates used in the behavioral experiments, EABR and psychophysical thresholds are strongly correlated;
2. For pulsatile stimuli, psychophysical thresholds and the minimum threshold single unit neural responses in the ICC and the A1 are similar at each pulse duration tested;
3. Psychophysical thresholds are always lower than neurophysiological thresholds for short duration (0.03 sec) sinusoidal stimuli, although the disparity is not statistically significant;
4. Psychophysical thresholds are significantly lower for long duration (1.0 sec) sinusoidal stimuli, compared to either psychophysical thresholds or neurophysiological thresholds for short duration (0.03 sec) sinusoidal stimuli.

The results presented suggest that behaviorally significant temporal integration occurs for sinusoidal stimuli over a range of stimulus durations from 0.1 sec to 1.0 sec. Our working hypothesis is that spatiotemporal integration of neural activity in the deafened auditory system is necessary for threshold psychophysical detection of sinusoidal stimuli. Further experiments are planned to test this hypothesis.

References

Costalupes, J.A. Hearing Res. 9:43-54, 1983.

Gerken, G.M. and Sandlin, D. JASA 61:602-607, 1977.

Parkins, C.W. and Colombo, J. Hearing Res. 31:267-286, 1987.

Pfingst, B.E. Hearing Res. 34:243-252

Pfingst, B.E., DeHaan, D.R., and Holloway, L.A. JASA 90: 1857-1866, 1991.

Work Planned for the Next Quarter

1) Two adult-deafened cats will be implanted with a new model UCSF cat electrode for periods of 2-3 weeks, until EABR thresholds stabilize at acceptable levels. As for the 3 previous cats in "normal adult" series, these animals will be studied in acute electrophysiological experiments to evaluate spatial selectivity of different electrode configurations with this new device. It is clear that the new electrode design permits electrodes to be inserted farther and will access to lower frequency (more apical) sectors of the cochlear nerve. In addition, specific design features improve positioning of the most basal electrodes, thereby decreasing thresholds on these electrodes and providing several channels with equivalent selectivity, distributed across a broader frequency range. Several issues relating electrode position and configuration to thresholds and selectivity of electrical stimulation are being evaluated in this series.

2) Histological processing and morphometric evaluation of spiral ganglion survival will be completed for our latest series of cats stimulated with temporally challenging electrical stimuli (300 pps with sinusoidal AM @ 30 or "speech" processor). These data will be presented in the next QPRs.

3) Further analyses of results from electrophysiological threshold mapping experiments in primary auditory cortex are underway and will be included in upcoming progress reports.

4) Histological processing of the cochlear nucleus from several chronically stimulated cats has been completed during the current quarter. During the next quarter work will focus on morphometric analyses of these specimens.