Effects of Remaining Hair Cells on Cochlear Implant Function

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Neural Prosthesis Program

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I.   INTRODUCTION

Cochlear-implant candidates with residual hearing can maintain significant hair-cell integrity after cochlear implantation (von Ilberg et al., 1999), raising the possibility that the presence of hair cells can affect the response of auditory nerve fibers to electrical stimulation. Our previous contract research (N01-DC-9-2106) began investigations using animal models to explore how functional hair cells can interact with the electrical stimulation produced by a cochlear prosthesis. That contract focused exclusively on measures based on the electrically evoked compound action potential (ECAP), a potential that can be routinely recorded from research animal preparations as well as cochlear implant users equipped with neural response telemetry systems. Work in that contract demonstrated significant effects of viable hair cells on the response of auditory nerve fibers to electrical stimulation. We also demonstrated that the ECAP in response to single pulses or pulses trains could be modified both during and after the presentation of an acoustic noise stimulus.

Research to be conducted under this contract renewal will expand those findings to include more detailed ECAP measures and will also include a significant number of single-fiber measures. The general goal of this research is develop a better understanding of the effects of viable hair cells on the response to electrical stimulation of the cochlea in order to eventually develop more effective paradigms for stimulation with cochlear implant in individuals with residual hearing.

II.   SUMMARY OF ACTIVITIES IN THIS QUARTER

The first quarter of this contract extended from July 1 though September 30, 2002. During this time the following activities related to this contract were accomplished:

1. We performed several experiments with acute guinea pig preparations to investigate the effect of furosemide, a loop diuretic, on the electrically evoked compound action potential (ECAP). This agent reversibly impairs hair-cell function, providing both "pre" and "post" comparison conditions for assessing hair-cell effects. Of these experiments, four preparations maintained their acoustic sensitivity to within 10 dB through the course of the experiment. The results were pooled with those from previous experiments and form the primary topic of this quarterly report.

2. We began a new investigation on the additive effects of acoustic and electric stimuli presented simultaneously to the same ear. This investigation extends earlier work in which we investigated the extent and time course of adaptation-like effects. This initial work was and will be performed in acute guinea pig preparations using the ECAP.

3. We began a similar investigation of additive effects of acoustic and electric stimuli using acute cat preparations to explore responses at the single auditory nerve-fiber level.

III.   FOCUS TOPIC

The effects of furosemide on the electrically evoked compound action potential

A.   Introduction

Furosemide, an ototoxic diuretic drug, injected intravenously causes rapid and reversible changes in the inner ear that result in temporary hearing loss (Pike, DA., et al 1980, Sewell, 1984a,b). The mechanism underlying these changes is thought to relate to impairment of the function of the stria vascularis, resulting in a reduction in the endocochlear potential and hair cell function (Ruggero, et al., 1992, Sewell, 1984a,b). Such decreases in the endocochlear potential are correlated with decreases in the spontaneous activity recorded in individual auditory nerve fibers (Sewell, 1984a,b). We therefore hypothesized that temporary hearing loss due to furosemide injection would affect the stochastic response properties of nerve fibers, thereby altering neural responses to electrical stimulation.

Previous work in our laboratory using a kanamycin/ethacrynic acid deafening procedure demonstrated effects on the electrically evoked compound action potential (ECAP) in response to electric pulse trains that were consistent with a hypothesis of reduced stochastic properties due to loss of spontaneous activity (QPRs 1 and 3 Contract N01-DC-9-2106). We began a series of experiments using furosemide injections in order to assess responses with an alternative, temporary deafening procedure that would facilitate "ABA" comparisons within the same animal preparation. Preliminary results of those measurements using electric pulse trains showed that the unadapted ECAP amplitude (the response amplitude to the first pulse in the train) tended to increase with degree of acoustic threshold shift. In addition, the amplitude of the alternation component observed across responses to consecutive electric pulses in the train also tended to increase, as did the degree of adaptation measured over the duration of the 100 ms pulse train. In addition, these trends were shown to be reversible in three animals in which hearing recovered completely.

This report focuses on a detailed study of the time course of the observed changes in the ECAP measured before, during, and after administration of furosemide. We report data from seven animals that showed complete recovery of acoustically evoked compound action potential (ACAP) using acoustic clicks after furosemide treatment. Assessment of the efficacy of furosemide was assessed by repeated measures of ACAP.

B.   Methods

The animal preparation has been previously described (Miller, et al., 1998, Matsuoka, et al., 2000). The auditory nerve response was recorded with a bipolar electrode placed on or near the surgically exposed auditory nerve. Responses were amplified using a differential amplifier (gain = 10x). Acoustic click stimuli were produced with 100μs/phase biphasic rectangular pulses presented at rate of 33 clicks/s. The signal was passed through an attenuator to a Beyer DT-48 earphone, coupled to the ear canal through a speculum. Electric stimuli were delivered by a monopolar electrode. This electrode was simply an insulated platinum-iridium wire with the last 1 mm of its length stripped of insulation and inserted into the scala tympani through a small defect drilled in the bone of the cochlea near the round window. The return electrode for electrical stimulating was a needle electrode placed in the left forepaw.

Before administrating furosemide, we monitored hearing sensitivity for 2 hours to insure that both the acoustically evoked and the electrically evoked responses were stable. Furosemide was then injected intravenously through the right external jugular vein, with dosage of 80 or 100 mg/kg over a 1 minute interval. Both the acoustic and electric compound action potential (ACAP and ECAP) responses were measured

repeatedly both before and after furosemide treatment to assess the time course of changes. The ACAP was monitored over time for 10 -20 minutes before treatment with furosemide as well as during the recovery process after treatment. Threshold of ACAP was assessed using the 10 dB step. The ACAP amplitude to a click presented at either 86 or 96 dB SPL (peak equivalent) was assessed. ECAPs were measured in response to single pulses and constant-amplitude pulse trains. The single, biphasic, pulses were 40μs/phase in duration and were presented at a rate of 33 pps. The pulse trains consisted of the same biphasic pulses presented at 1000 pps for a total train duration of 100 ms. ECAP response amplitudes were measured from the N1and P2 peaks (Miller et al., 1998). Using growth functions to single pulses, stimulus levels were selected to assess pulse-train responses at several response amplitudes. In most cases, six levels were chosen that elicited responses that were 100%, 90%, 80%, 60%, 40% and 20% of the maximum ECAP amplitude.

Examples of ECAP amplitudes to successive pulses in the train are plotted as a function of time relative to stimulus onset in Figure 1. Data for three conditions are plotted: (1) pre-furosemide adminstration, (2) deafened (no response to acoustic stimulus), and (3) recovered (after the ACAP amplitude recovered from furosemide-induced reduction). Each plot displays a common feature of auditory nerve response to the electric pulse train: the response to the first pulse is largest and the response to the second and subsequent pulses display a decreasing amplitude tending to asymptote after approximately 100 ms. In some cases there is also an alternating pattern in response to successive pulses, typically over a limited time period as illustrated in these examples. A comparison of the three plots demonstrates the general trends that the first pulse response and alternating amplitude are larger in deafened condition than in the pre-furosemide or recovered conditions. To quantify changes in the ECAP responses to pulse trains, we defined four parameters that are listed below and are also illustrated in Figure 1.

   (1) ECAP response amplitude to the first pulse in the train;

   (2) Alternation amplitude, defined as the average of absolute difference in response amplitude to adjacent pulses for the 10th through 19th pulses in the train;

   (3) Adapted (or asymptotic) amplitude, defined as the averaged ECAP amplitude responses within the epoch from 80 to 90 ms relative to the first pulse response amplitude;

   (4) Refractory amplitude, defined as the ratio of the second pulse response amplitude to the first pulse response amplitude.

C.   Results

Data from seven animals are summarized in this report. The hearing thresholds of all subjects recovered to pre-injection levels within our 10 dB assessment step size. The extent and time-course of hearing loss and recovery, characterized by ACAP amplitudes and threshold, is illustrated for one subject (M50) in Figure 2. Four subjects (M28, M49, M52, and M55) received a second furosemide dose after the post-recovery period. In one subject (M50), time permitted administration of a third dose. The pattern of hearing loss and recovery in Figure 2 is illustrative of that observed in other subjects. Immediately after furosemide injection, response amplitudes of ACAP abruptly declined to zero and stayed at zero for several minutes. The recovery course showed ACAP amplitudes recovered fast at beginning, then recovered more slowly. Two hours after injection, the recovery reached a plateau. Threshold changes shown in the lower graph showed similar trends over time. The recovered hearing was defined with both the threshold of ACAP and the curve of ACAP amplitudes.

ECAP growth functions in response to single pulse stimuli are illustrated in Figure 3 for subject M50. They demonstrate reversible changes in response amplitude after treatment with furosemide. In the deafened condition, both the slope of the growth functions as well as saturation amplitude tended to be greater than what

was observed in the pre-furosemide and recovered conditions. The results obtained in each of the three separate furosemide treatments show similar trends.

The detailed time courses of our four response parameters are shown for the same subject in the graphs of Figure 4. In each case, the responses to five stimulus levels are shown. The legend expresses these five levels in terms of response amplitude relative to the pre-furosemide saturation amplitude. Also plotted is the ACAP amplitude produced by high level (96 dB SPL pe) clicks that was measured at various times through the experiment. Clear losses of acoustic sensitivity accompany each furosemide injection. The four electric-response parameters also show reversible trends. After hearing loss, the amplitudes in response to the first pulse consistently increase, alternation amplitudes tend to increase, adapted amplitudes and refractory amplitudes tended to decrease, particularly at low stimulus levels. During ACAP recovery, the response measures all tended to return to pre-furosemide levels. Similar trends occurred for the three injections of furosemide treatment as evident in the changes in each parameter compared to the changes in the ACAP. The changes observed with furosemide treatment are consistent with loss of hair-cell transduction and synaptic activity that have been reported with treatment with this loop diruetic.

In order to quantify furosemide effects across the subject pool, we characterized each of our four ECAP measures at three time intervals relative to each furosemide treatment. These three times corresponded to a time shortly before furosemide treatment ("pre-furosemide"), a time after administration when hearing loss was maximal ("deafened"), and a subsequent time when the ACAP reached plateau amplitude ("recovered"). Figures 5 through 8 summarize the furosemide effects on each of one of these four ECAP measures (first-pulse amplitude, alternation amplitude, adapted amplitude, and refractory amplitude, respectively). In each figure, six graphs are presented, corresponding to each of the six aforementioned ECAP response amplitudes. In each graph, both individual data for each subject and treatment as well as mean values, are plotted. Note that for each figure, each of the six graphs contains more than seven individual plots, as multiple furosemide treatments were administered to some subjects.

Figure 5 presents "pre", "deafened", and "recovered" data for the ECAP amplitude to the first stimulus pulse. Since some of the measures in data of subject M49 with the second injection and subject M55 with the first injection were far away from the group value, they did not show in Figure 5-8. A reversible trend can clearly be seen in that , at all stimulus levels, the first pulse response amplitudes increased as after deafening and decreased with recovery.

Alternation amplitudes from individuals along with the mean amplitudes are plotted in a similar manner in Figure 6. At higher stimulus levels (90%, 80% and 60%), there is a trend to an increase in alternation amplitudes with deafening and a corresponding decrease with recovery. The trends are not as clear saturation level or at lower stimulus levels.

The adapted amplitudes and refractory amplitudes are plotted in Figures 7 and 8 respectively. The changes of adapted amplitudes and the changes of refractory amplitudes at lower stimulus levels displayed similar reversible trends in that their amplitudes decreased with deafening and increased as hearing recovered.

D.   Discussion

This study characterized the effect of furosemide injections on several aspects of the electrically evoked compound action potential responses to pulse-train stimulation. Based on our previous work, we hypothesized that temporary loss of hair cell function would alter the ECAP responses in ways consistent with a loss of spontaneous neural activity. The hearing sensitivity in each animal was continuously monitored using ACAP over time, showing a consistent change affected by intravenous delivery of furosemide. Responses of the auditory nerve to electric stimuli illustrated changes related to the state of hair-cell function reflected by the loss or recovery of acoustic sensitivity. These changes were consistent with our hypothesis of a reduction of stochastic effects produced by synaptic activity. Specifically, the decreased stochastic activity is consistent with an increase in response amplitude and greater alternation in response amplitude. The results relative to adaptation also suggest that the temporal response properties may be affected by the presence of functional hair cells and/or synaptic activity.

The results in the animals treated with extra two or three injections of furosemide displayed consistent trends with each dose. In all cases the changes were reversible with hearing recovery. The reversible nature of the changes in electric response further support the causal relationship between hair cell function and responses to electrical stimulation.

The alternation of ECAP response amplitude has been reported in both deafened animal model studies and in cochlear implant patients stimulated with pulse trains at appropriate rates. This alternation can be considered a form of signal distortion and has been linked, in some cases, to altered perception (Wilson, 1997). Our observation of a reversible change in the degree of ECAP amplitude alternation suggests a positive attribute of functional hair cells, as they reduced this form of distortion in the experiments presented here. Our data suggest that functional hair cells indeed provide a source of "stochasticity" that could be of benefit to cochlear implant users.

E.   References

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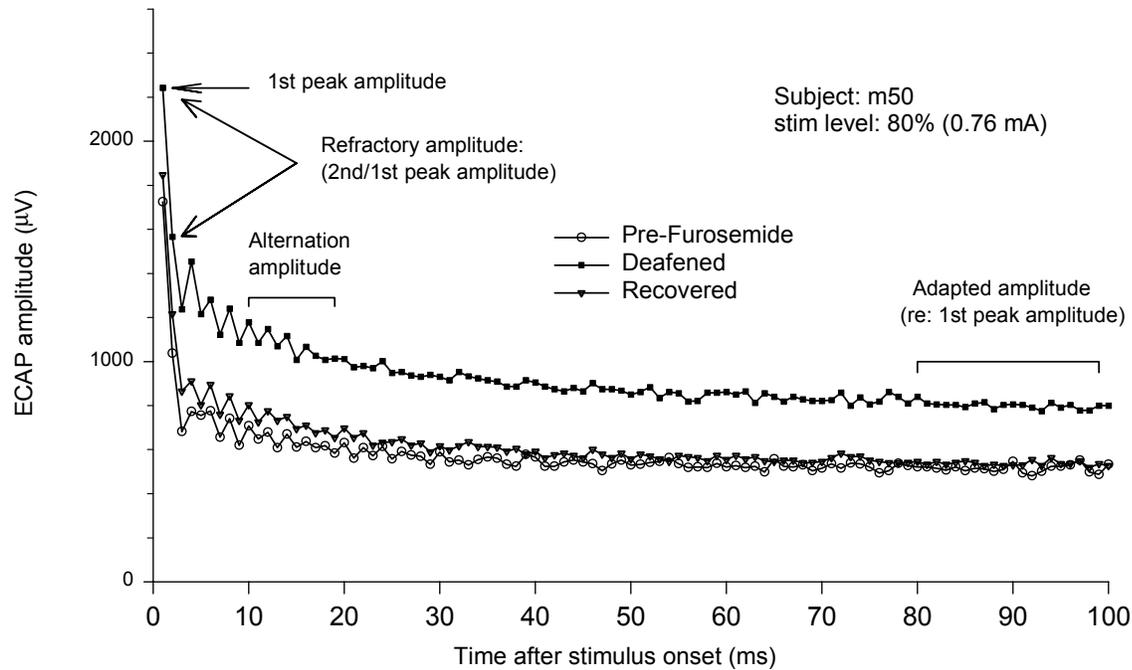
Figure 1. Amplitudes in response to each pulse in the train are plotted as a function of time after stimulus onset. Three plots correspond to measures before furosemide treatment, after deafening and after recovery of hearing as indicated by the legend. Data are plotted for a stimulus level of 0.76 mA, corresponding to a response at 80% of relative saturation amplitude. Four measurements were used to characterize the response to the pulse train (see text). Each is indicated on the graph.
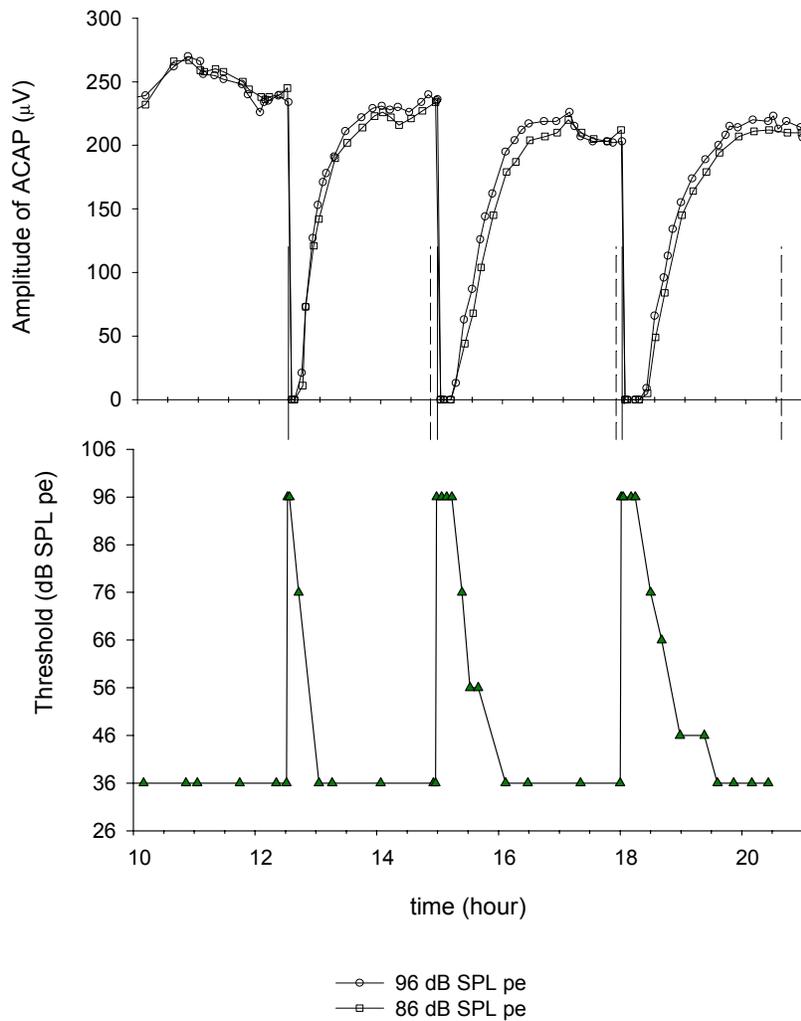
Figure 2. Response amplitudes and thresholds of ACAP are plotted as a function of time (subject M50). ACAP amplitudes in response to two different click levels are plotted in the upper graph. Thresholds of ACAP (triangle) are plotted in the lower graph. The vertical lines represent the times of furosemide injection. The dashed lines represent the time of data collection for recovered condition. Response amplitudes abruptly declined to zero after furosemide injection, and stayed at zero for several minutes. During the early recovery stage, ACAP amplitude recovered quickly, following by a time of slower recovery. Two hours after injection, the recovery reached a plateau. The changes of thresholds showed trends similar to amplitudes, but recovery was complete in a relatively short time.
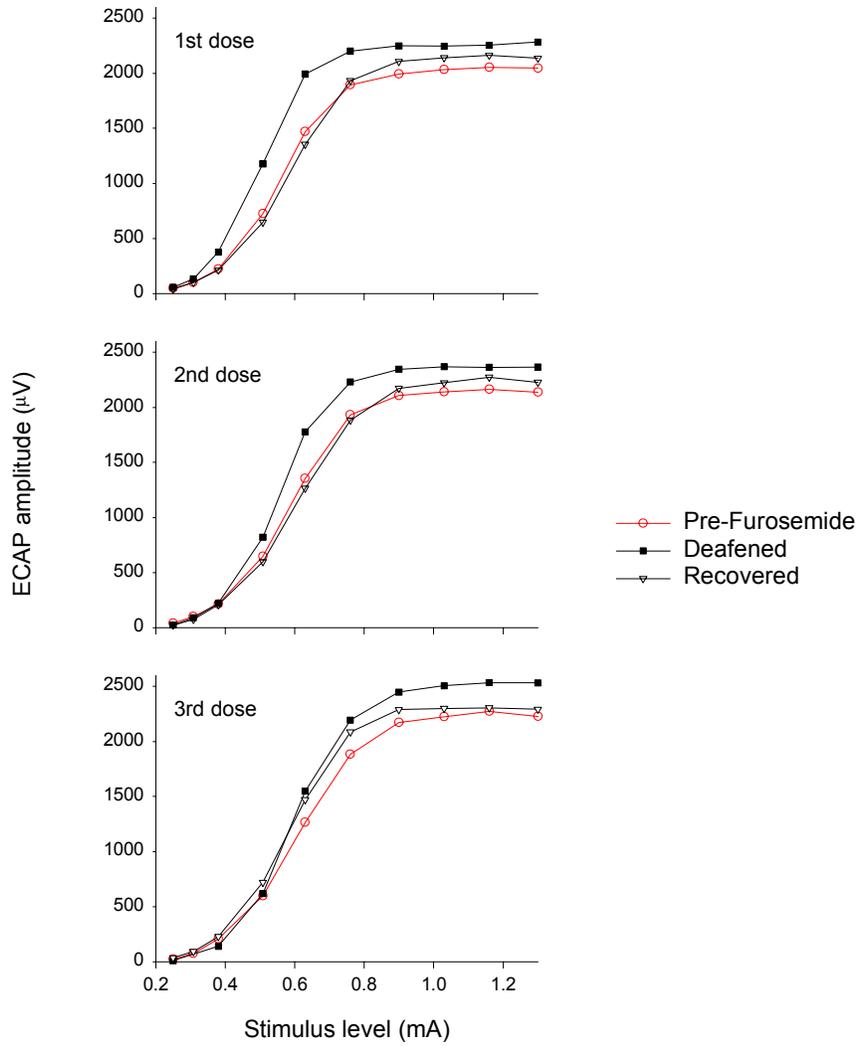
Figure 3. The ECAP growth functions to electric pulses obtained from subject M50 at different times relative to furosemide treatment. The three graphs correspond to three separate furosemide treatments given to this subject. Each graph shows functions obtained before ("pre-furosemide") and immediately after ("deafened") furosemide injection, as well as after hearing sensitivity was restored ("recovered").
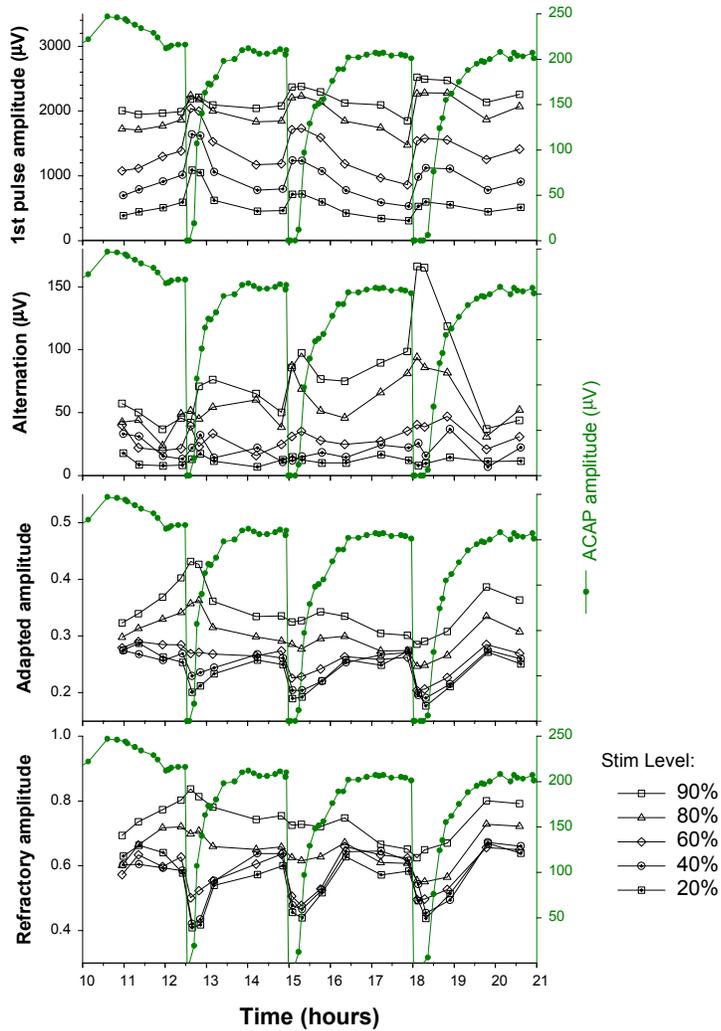
Figure 4. The time course of response to the first pulse, alternation amplitudes, adapted amplitudes (normalized to the first pulse response) and refractory amplitudes (the second pulse response relative to the first pulse response) are plotted as a function of time (subject M50). The stimulus levels for the pulse train were chosen from the ECAP growth function in response to the single pulse to produce amplitudes at 100%, 90%, 80%, 60%, 40% and 20% relative to the saturation amplitude. The response amplitudes of ACAP monitored with a click at 96dB are plotted with filled symbols over the same time course. The course spans three injections of furosemide and the corresponding periods of hearing loss and recovery.
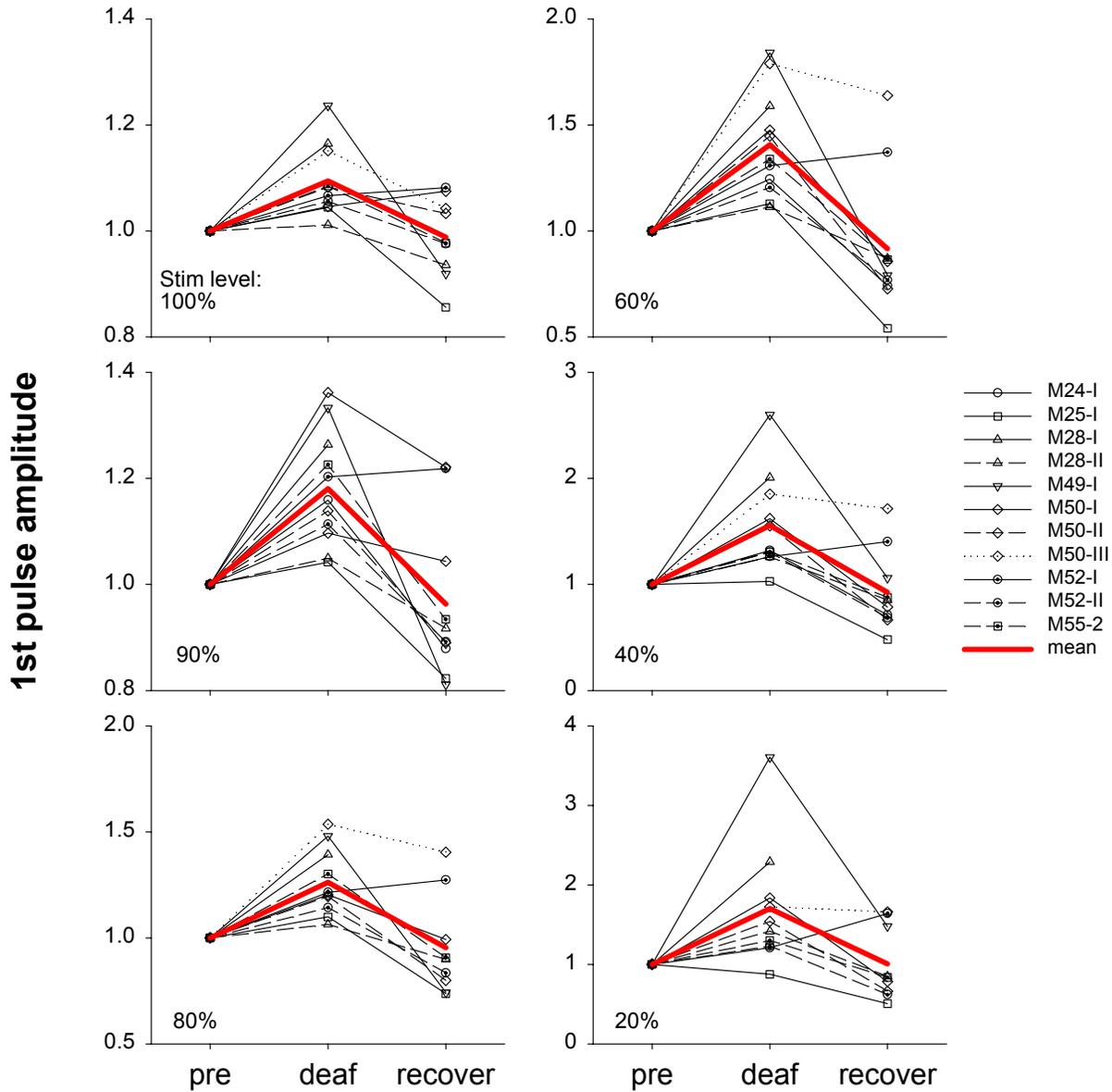
Figure 5. The amplitudes of response to the first pulse in the train for individual subjects, and in some cases for multiple injections of furosemide, are plotted for different hearing conditions: pre-furosemide, deafened and recovered. The mean value is plotted for each condition with the bold line. Each plot shows data for a single level which elicits a specified response relative to the ECAP saturation amplitude to single pulse stimulation.
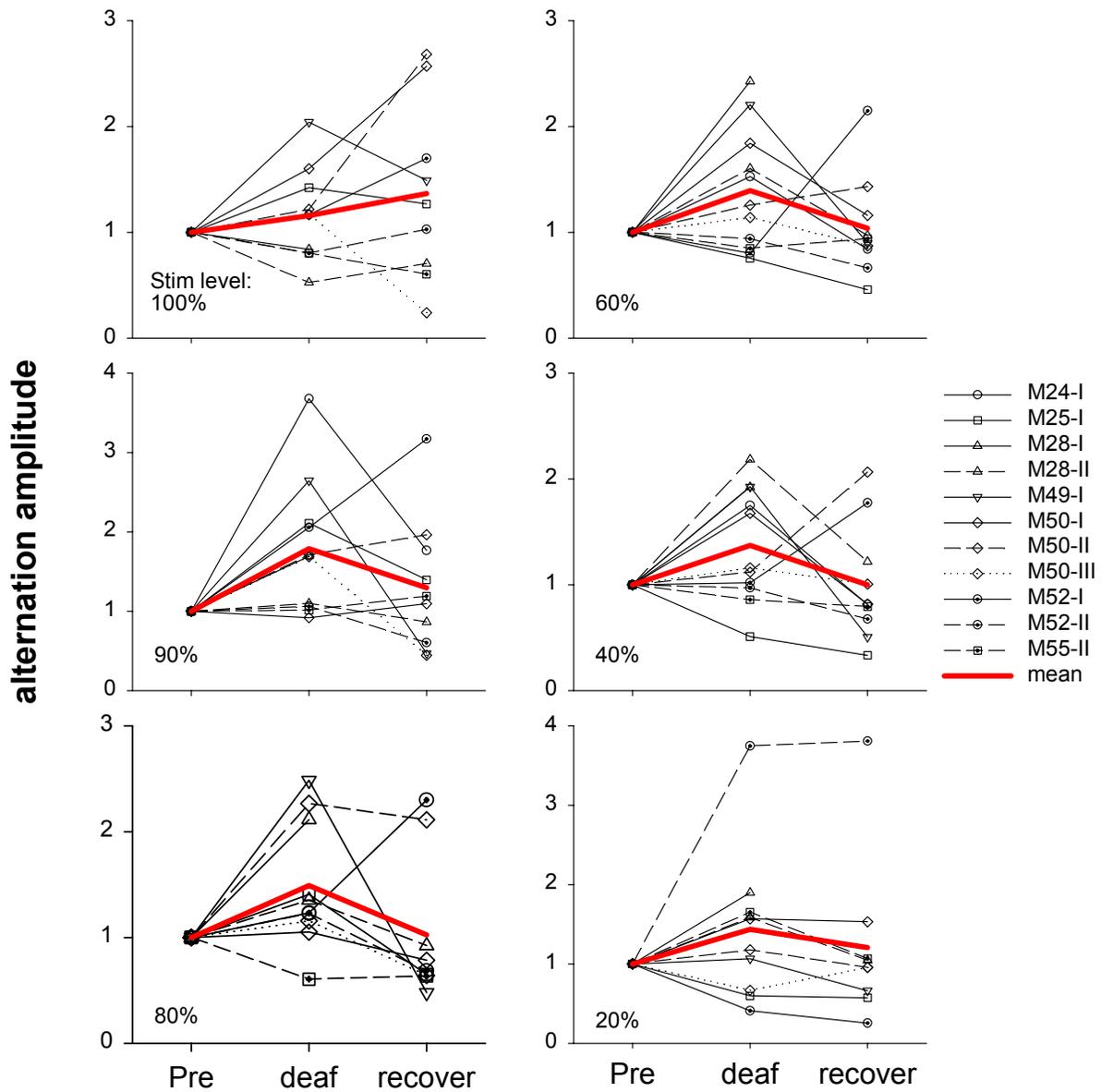
Figure 6. The amplitudes of alternation are plotted for different hearing conditions: pre-furosemide, deafened and recovered. Data are plotted in the same format as Figure 5.
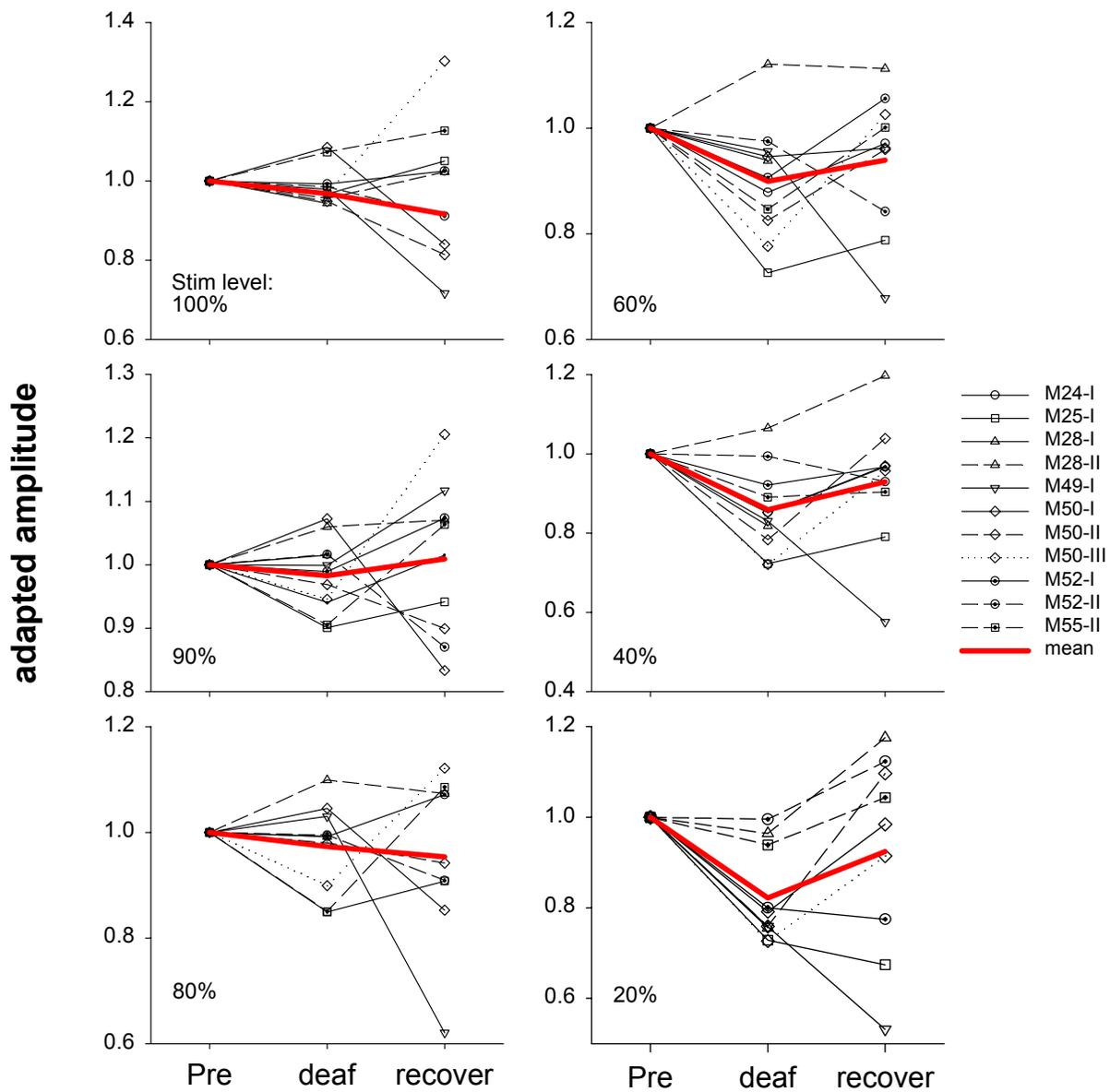
Figure 7. The adapted response amplitudes are plotted for different hearing conditions: pre-furosemide, deafened and recovered. Data are plotted in the same format as Figure 5.
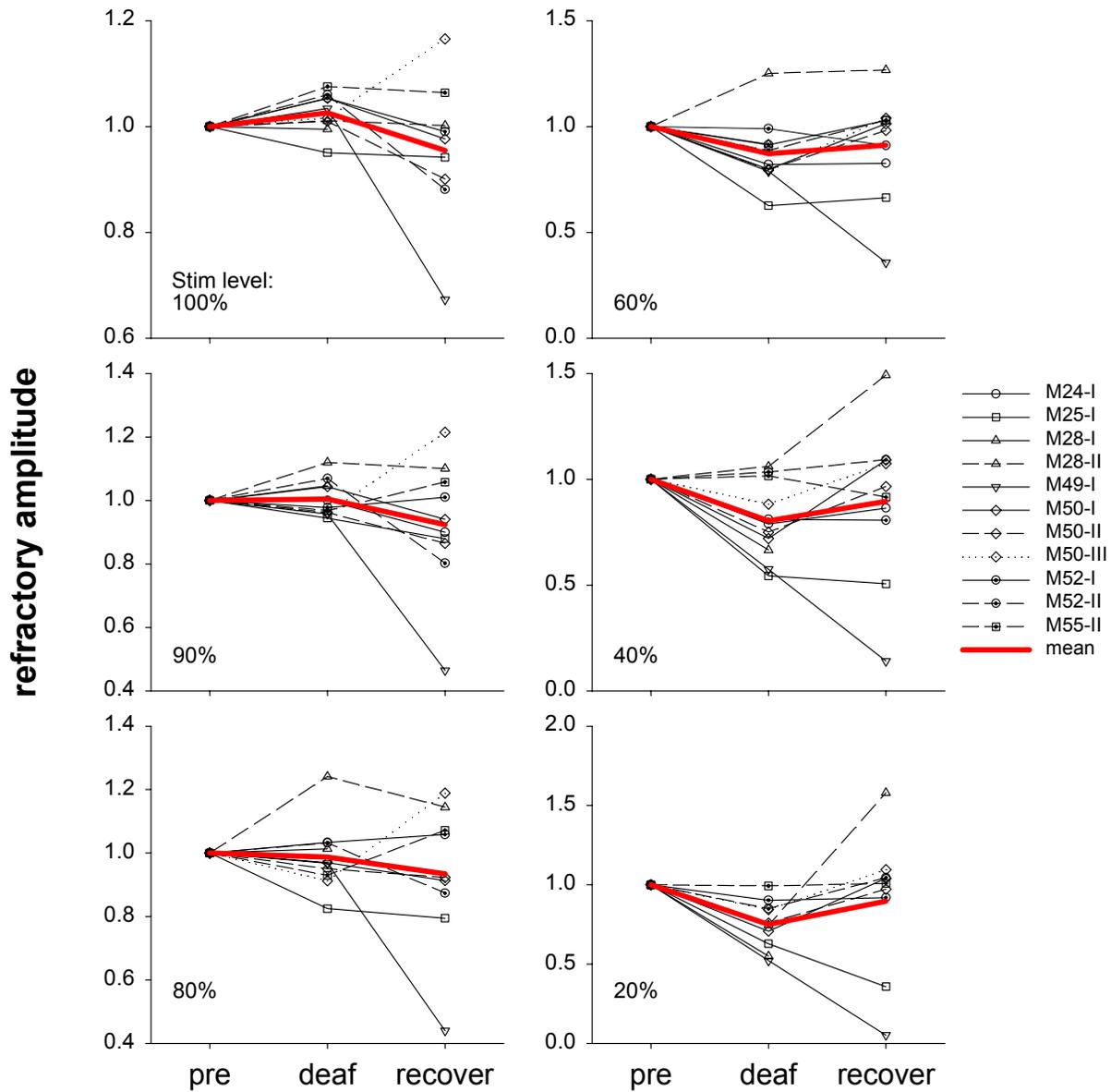
Figure 8. The refractory amplitudes (see text for explanation) are plotted for different hearing conditions: pre-furosemide, deafened and recovered. Data are plotted in the same format as Figure 5.

IV.   PLANS FOR THE NEXT QUARTER

In the next quarter, we plan to do the following:

1.   Perform additional analysis on the pre- and post-furosemide results that were described in this report. This will include characterization of the time-course of the deafening effect as well as a description of the recovery process.

2.   Begin preparing a manuscript for publication describing the focus topic of this quarterly report.

3.   Attend and present at the Neural Prosthesis Program Workshop at the NIH campus in Bethesda, Maryland.

4.   Conduct additional experiments using guinea pig preparations on the effects of simultaneous acoustic noise and electric pulse train stimulation, with focus on adaptation effects.

5.   Conduct additional experiments on feline single-fiber responses to simultaneous acoustic and electric stimulation. Focus will initially be on the influence of acoustically driven (and spontaneous) rate on the electric input-output functions to single-pulse stimuli.