Effects of Remaining Hair Cells on Cochlear Implant Function

13th Quarterly Progress Report

Neural Prosthesis Program
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1. Summary of activities in this quarter

During the thirteenth quarter of this contract (July 1 – September 30, 2005), we accomplished the following:

1. We attended the 2005 Conference on Implantable Auditory Prostheses (July 30 – August 4, 2005, Pacific Grove, CA) and made three presentations on the progress of our contract work (Miller *et al*., 2005; Nourski *et al.,* 2005b; Woo *et al*., 2005).

2. Dr. Miller visited Kresge Hearing Research Institute in September as an invited speaker as part of the KHRI Hearing and Chemical Senses Seminar series. While there, he worked with Dr. Bryan Pfingst to attempt to characterize the electrically evoked responses from chronically implanted guinea pigs with acoustic sensitivity. The goal was to evoke and record ECAPs using intracochlear electrodes in a fashion similar to that used in clinical approaches. The data obtained were preliminary and Dr. Miller advised Dr. Pfingst as to instrumentation that would enhance measures in any future attempts conducted by the Pfingst laboratory. However, positive results were obtained. One chronically implanted animal produced both direct (α-) as well as electrophonic (β-) responses, with the latter successfully masked by the presentation of wideband acoustic noise (as has been described in QPR #6). As the animals from Dr. Pfingst's laboratory are being used to assess psychophysical performance standards of implanted animals with residual hearing, the acquisition of the ECAP would be particularly valuable in identifying the mode of excitation responsible for the behavioral response and confirm the auditory origin of the responses.

3. We purchased two new acoustic drivers (Sennheiser model HD 590) and fabricated housings for them in order to improve the frequency response of our sound delivery system. Initial calibration runs indicated that these drivers have improved high-frequency response characteristics but somewhat lower maximum output levels overall. We will experiment with resonance damping materials (within the enclosure) prior to running full calibration checks on the new drivers.

4. We collected additional single-fiber recordings from two cats using electric masker stimuli to assess adaptation and recovery of electrically evoked responses. This work is being conducted in conjunction with the previously described ECAP measures in which electric maskers were used (QPR #10), with the goal being to better understand the non-monotonic recovery of the ECAP following acoustic masking (*cf.* QPR #3, 5). By comparing the effect of an electric masker to that produced by an acoustic masker, we can determine whether or not the unusual non-monotonic recovery patterns observed in the ECAP are unique to a mechanism related to functional hair cells or are intrinsic to auditory nerve masking by either acoustic or electric masking. A key rationale for obtaining single-fiber measures (in addition to the ongoing ECAP studies) is that they provide direct evidence of the influence of spontaneous activity and residual acoustic sensitivity on the electrically evoked responses.

5. We performed four acute guinea pig experiments that examined the effect of reversible deafening with furosemide on forward masking of the ECAP with high rate electric pulse trains. The present report summarizes results of that work.

2. Focus Topic: Forward masking of the electrically evoked compound action potential following furosemide treatment.

2.1. Introduction

Much of the work done under this contract has focused on a study of interactions between responses to combined electric and acoustic stimuli in cochleae with functional hair cells. One of the findings was a non-monotonic pattern of recovery of the electrically-evoked compound action potential (ECAP) following stimulation with acoustic noise (QPR #3). Specifically, a rapid recovery phase was followed by a transient depression of ECAP amplitude, and then a slower phase to complete recovery of ECAP amplitude. We further investigated this phenomenon by evaluating ECAP recovery following high-rate electric pulsatile stimulation (QPR #10). In those animals, cochlear function was impaired by administering an aminoglycoside to kill hair cells, so that we could better interpret the adaptation phenomenon as one arising primarily from neural membrane properties. It was demonstrated that electric maskers could produce similar non-monotonic ECAP recovery functions.

We hypothesized that recovery from masking in the auditory nerve could be associated with changes in driven firing rate and firing synchrony. An increase in driven rate over time following cessation of the masker would act to increase the amplitude of the ECAP. On the other hand, a decrease in firing synchrony among the auditory nerve fibers following the masker offset would produce a negative effect on the ECAP amplitude over time. Such a combination of changes in the firing properties (rate and synchrony) might account for the observed non-monotonicity of ECAP recovery.

An ongoing study in our laboratory involves the investigation of post noise-offset jitter in electrically stimulated single fibers. Data concerning these jitter measures will be presented in a later QPR; however, we can report that we have noted significant reductions in jitter in the post-noise interval in some fibers, providing direct evidence that enhanced synchrony to electric pulses are due, at least in some cases, to suppression of spontaneous activity. Such suppression of a desynchronizing influence – and hence reduced jitter – would be expected to increase ECAP amplitude (Miller *et al.*, 1999). Thus, modulation of spontaneous activity likely accounts for at least some of the ECAP non-monotonic effects reported by our group.

However, our current understanding of single-fiber responses from acoustically sensitive cats presents a more complicated picture. In our data set that is presently being collected, we have observed enhanced synchrony (as assessed by decreases in jitter) in fibers with low (<1 spike/s) spontaneous rates. This suggests that a second mechanism, in addition to spontaneous rate suppression, may contribute to the observed non-monotonic recovery functions of the ECAP in the post-noise-offset interval.

In the present report, we address this issue by using chemical treatments to impair hair cell functionality. By doing so, we create an animal model whose ECAP responses are dominated by response mechanisms located at the neural membranes rather than by a combination of hair-cell and membrane effects.

Two chemical treatments that we have previously used are the application of an aminoglycoside antibiotic (neomycin or kanamycin) and furosemide. Both types of treatments have distinct experimental advantages. Aminoglycoside poisoning causes irreversible damage to hair cells, causing them to be unresponsive to stimuli. Their use constitutes the primary means of producing a deaf animal model for cochlear-implant research. However, its irreversibility can be a significant limitation, particularly when coupled with the acute experimental animal models employed by our studies. For example, the intracochlear administration of neomycin requires direct replacement of the intrascalar fluid, which could

alter current paths and complicate before/after treatment comparisons. The use of furosemide offers two advantages. First, as it is applied systemically, there is no direct disruption of the cochlear fluids during application. Second, as it provides reversible effects on the endocochlear potential, A-B-A type comparisons can be made, which provides a means of assessing the overall stability of the preparation, as has been demonstrated by our group (Hu et al., 2003).

Furosemide affects the hair cell function in an indirect fashion by inhibiting the function of the stria vascularis (Pike & Bosher, 1980; Ruggero & Rich, 1991). This results in a reduction of the endocochlear potential, which, in turn, has been demonstrated to correlate with decreases in spontaneous activity in auditory nerve fibers (Sewell, 1984).

One disadvantage of using furosemide is a relatively brief (on the order of minutes) period of complete hearing loss following its administration (Hu *et al*., 2003). This required a modification of the stimulus presentation paradigm (see *Methods*) to make data collection during in the deafened condition more time-efficient.

Experiments that address responses of auditory-nerve single fibers to electric pulse trains are performed in our laboratory on neomycin-deafened cat subjects. Thus, comparisons of effects observed under neomycin treatment and furosemide treatment will provide us with information regarding how the method of deafening may affect the outcomes of our experiments. To that end, data obtained from one cat subject deafened with an intracochlear injection of neomycin are also presented in this report.

2.2. Materials and methods

Four adult guinea pigs with normal hearing and one deafened cat were used in acute experimental sessions. Animal preparation and surgical methods have been described elsewhere (Hu *et al*., 2003; Nourski et al., 2005a). Briefly, after inducing the surgical level of anesthesia, the cochlea and the auditory nerve trunk were exposed. In the guinea pig subjects, a Pt/Ir wire electrode was inserted into the scala tympani via a cochleostomy for intracochlear monopolar electric stimulation. In the data sets reported here, this insertion resulted in a decrease in acoustic sensitivity of 10 dB or less. The cat subject was stimulated in a monopolar mode with the most basal electrode of a stimulus eight-electrode array manufactured by Cochlear Corporation.

Stimuli were digitally generated by a 16-bit digital-to-analog converter (100,000 samples/s), controlled by custom-written software. Acoustic clicks, used to assess the subjects' hearing sensitivity, were produced by driving an earphone with 100 μs/phase biphasic electric pulses, presented using an interstimulus interval (ISI) of 30 ms. Sound pressure in the ear canal was monitored in the experiments using a probe-microphone system, described in QPR #4. Overall sound levels of the noise stimulus were determined by accounting for the system frequency response.

Cathodic-first biphasic (40 μs/phase) electric pulses were used as probe stimuli. Following the approach introduced in QPR #7, the pulses were presented with an interpulse interval (IPI) of 200 ms to avoid adaptation to the electric probe stimulus. To assess recovery of the ECAP from masking with electric stimulation, we used high-rate electric pulse trains (IPI 0.2 ms) as masker stimuli. To demonstrate post-stimulatory effects of acoustic stimulation on the ECAP, we used bursts of broadband acoustic noise as maskers. Masker duration was 400 ms in most experiments. The maskers were presented with intervals of 1200-1600 ms to minimize auditory fatigue (the ISI increased with masker stimulus intensity and duration).

The experimental paradigms used in the present study are summarized in Figure 1. The stimulus paradigm used to examine masking effects of acoustic noise on ECAP (Figure 1A) was introduced in QPR #7. A series of 15 *masker + probe* stimuli was presented with the electric probe onset being delayed relative to the masker. The delay, Δt, increased from 0 to 150 ms in approximately logarithmic steps (Δt = 0, 1, 2, 3, 4, 5, 7, 10, 15, 20, 35, 50, 70, 100, 150 ms) across the 15 stimuli. The 15 masker + probe conditions were presented under computer control to obtain 15 sets of time-averaged responses. In addition, a 16th *probe-alone* condition was presented to provide a comparison and monitor the stability of the animal preparation.

In the experiments that examined the effects of masking by high-rate electric stimulation on the ECAP, a similar approach was used (Figure 1B). When the effect of furosemide treatment on forward masking of the ECAP was studied, we modified the stimulus presentation paradigm to decrease data collection time. We used a series of 7 masker + probe stimuli (Δt = 0, 2, 4, 7, 15, 30, 100), followed by a probe-alone condition. This was done to minimize changes in the acoustic sensitivity of the subject during each data acquisition session. Refractory properties of the ECAP were addressed by presenting two electric pulses of equal amplitude with masker-probe intervals varying from 0.8 to 10 ms.

One percent furosemide solution was delivered intravenously at a dose of 80-100 mg/kg over a one minute interval. This treatment resulted in an abrupt hearing loss of at least 65-70 dB. In subjects J45 and J55, second doses were given after acoustic sensitivity had recovered from the previous dose, providing the opportunity for the collection of a second set of data comparison conditions. For data presented in this report that were obtained from these subjects, Roman numerals were added to the subject codes to distinguish between multiple doses of furosemide (e.g., J45-II). The cat subject D53, data from

which are presented in this report, was deafened with an intracochlear injection of 50 μL of 10% neomycin.

Auditory nerve evoked potentials were recorded using a ball electrode placed on the auditory nerve trunk. The responses were amplified (gain=100x), low-pass filtered at 30 kHz, and digitized by a 16-bit converter (sampling rate 50000 sample/s) for subsequent analysis. Acoustic sensitivity was assessed by measuring acoustically evoked compound action potentials (ACAP) in response to click stimuli and determining a threshold response level using visual criterion. ECAP amplitude growth functions were obtained by presenting alternating-polarity biphasic electric pulses at different levels. ACAP thresholds and ECAP growth functions were obtained repeatedly during each experiment to monitor the stability of the animal preparation.

ECAP amplitudes were measured using custom-designed software. In the experiments that addressed the refractory characteristics of the ECAP in a two-pulse forward masking paradigm, we performed waveform subtraction of the *masker-only* response waveform from the *masker + probe* waveform prior to measuring the probe-evoked ECAP. This was done to minimize contamination of the response to the probe by the response to the masker stimulus.

As we have previously done, the ECAP amplitudes of the *masker + probe* condition were subtracted from those of the *probe-only* condition to evaluate the absolute change in response amplitude and thus demonstrate the net effect of the masker. Decreases in ECAP amplitudes in response to the probe were plotted as functions of the masker-probe interval and described by fitting exponential functions to the ECAP amplitude decrease data using the Marquardt-Levenberg least squared error algorithm (see *Results* for the description of the models).

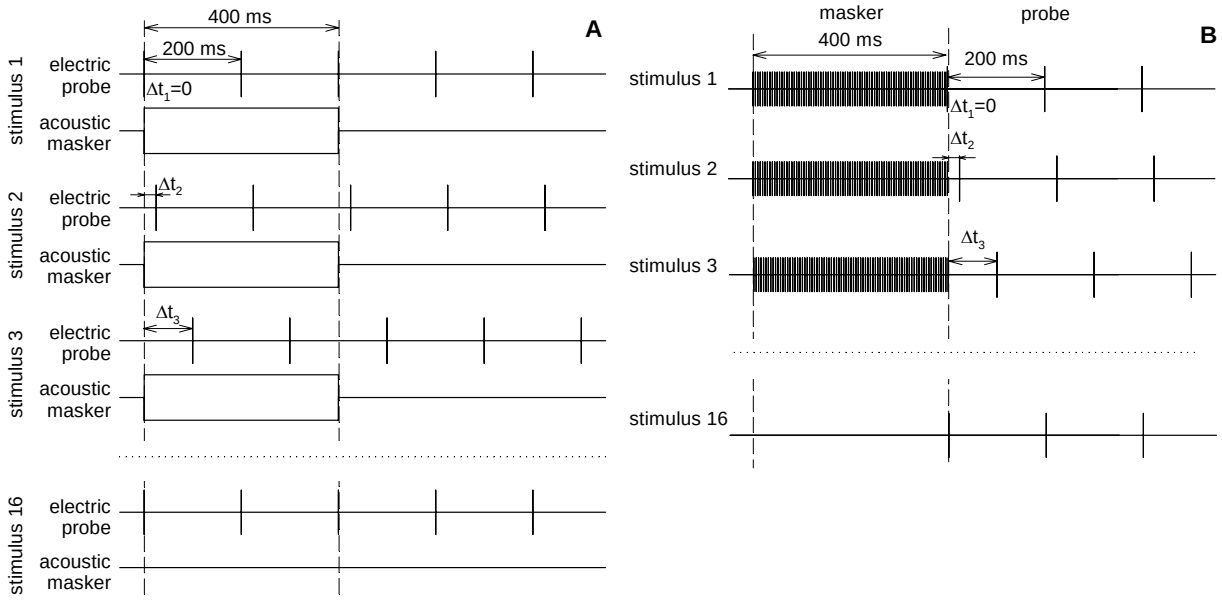

Figure 1. Schematic of stimulus presentation paradigms. A: Electric probe and acoustic masker. B: Electric probe and electric masker. Rectangles indicate bursts of noise; vertical bars indicate electric pulses, dashed lines indicate onset and offset of the masker stimulus. IPI, interpulse interval; Δt, probe onset delay time. See text for additional details.

2.3. Results

Figure 2 illustrates the time course of the loss and recovery of acoustic sensitivity as well as changes in the electrically evoked responses observed following furosemide treatment for subject J55. In Figure 2A, ACAP amplitudes in response to acoustic clicks presented at 90 dB SPL are plotted as a function of time after furosemide administration (80 mg/kg in this case). The dashed line represents the ACAP amplitude measured immediately prior to the drug delivery and corresponds to 0.478 mV.

Immediately after furosemide injection, ACAP amplitude declined to zero. The period of total hearing loss (as assessed by the ACAP) was relatively short; it was followed by a recovery of the ACAP amplitude. The time course of the recovery was fitted with an exponential function (solid line in Figure 2A), following the approach of Hu *et al.* (2003):

$$A(t) = A_0(1 - e^{-(t-t_0)/\tau}) \quad ,$$

where A(t) is the ACAP amplitude, t is the time after furosemide administration, A_0 is the asymptotic ACAP amplitude ($A_0>0$), t_0 is the delay of the recovery onset (i.e., the period of total deafness) ($t_0>0$), and τ is recovery time constant ($\tau>0$).

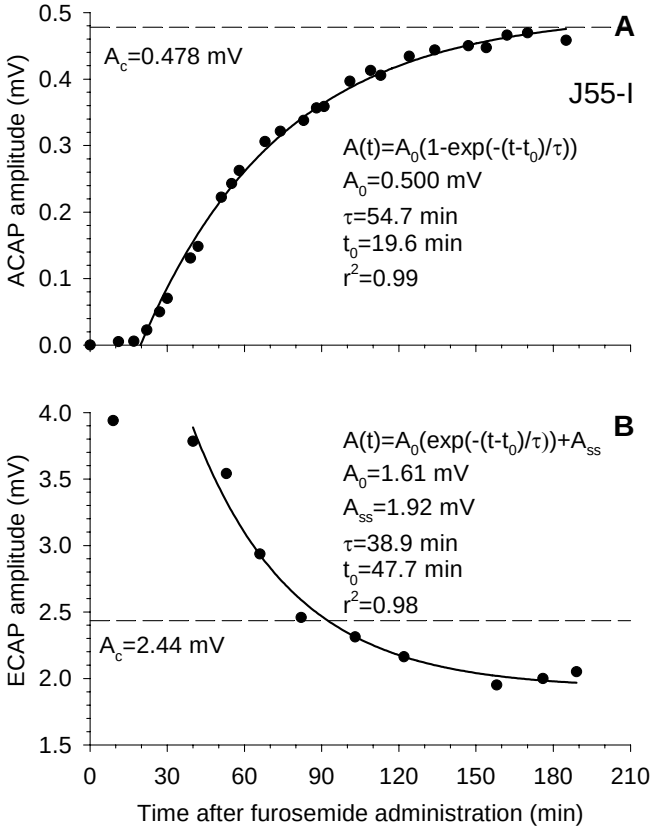

Figure 2. Evoked auditory nerve responses following furosemide treatment. Response amplitudes are plotted as functions of time after furosemide administration. **A**: ACAP in response to acoustic clicks (level 90 dB SPL). **B**: ECAP in response to electric pulses (I=0.56 mA). Solid lines indicate fitted curves obtained with regression analysis. Dashed lines indicate response amplitudes (A_c) obtained prior to furosemide administration.

The example shown in Figure 2A demonstrates that this model provides an adequate description of the time course of the ACAP amplitude recovery. The mean values of τ and t_0 obtained from five rounds of furosemide treatment obtained to date were 38.9 and 25.3 minutes, respectively.

Figure 2B shows changes in the ECAP observed following furosemide treatment. In this figure, ECAP amplitudes in response to electric pulses presented at 0.56 mA are plotted as a function of time after furosemide administration. The dashed line represents the ECAP amplitude measured immediately prior to the drug delivery and corresponds to 2.44 mV. Immediately after furosemide treatment, the ECAP amplitude exhibited a considerable increase, which was followed by a decrease of the ECAP to an asymptote, a process that could be described by a single decaying exponential function with a constant term (solid line in Figure 2B):

$$A(t) = A_0(e^{-(t-t_0)/\tau}) + A_{ss} \quad ,$$

where A(t) is the ECAP amplitude, t is the time after furosemide administration, A_0 is the magnitude coefficient ($A_0>0$), t_0 is the time offset ($t_0>0$), τ is the time constant ($\tau>0$) and A_{ss} is the asymptotic value of the ECAP amplitude ($A_{ss}>0$).

A comparison between the two functions presented in Figure 2 demonstrates that evoked responses to the two types of stimuli (ACAP and ECAP) undergo deafening-induced changes on a similar time scale.

Next, we addressed the hypothesis that the non-monotonicity of ECAP recovery could be affected by abolishing the hair-cell functionality. Figure 3 presents ECAP recovery functions following stimulation with acoustic noise (A) and electric pulse trains before, during and after furosemide treatment (B, C, and D, respectively). The intensities of the acoustic and electric masker were matched so that they produced a comparable amount of steady-state masking (approx. 0.8 mV). In this example, the non-monotonic pattern of recovery was observed both in hearing and in temporarily deafened conditions. However, the rising phase of the recovery function appeared to be slower in the deafened state (Figure 3C) than in either pre-furosemide or recovered conditions (Figures 3B and 3D, respectively). To quantify this observation, ECAP forward masking functions were obtained repeatedly over the course of recovery from deafening in this subject. Following an approach introduced in QPR #5, the forward masking functions were described by a three-component exponential function with two positive and one negative component:

$$A(t) = A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2} - A_3 e^{-t)/\tau_3} ,$$

where A is the decrease in ECAP amplitude, t is the masker-probe interval, A_1, A_2, A_3 are the magnitude coefficients ($A_1>0$, $A_2>0$, $A_3>0$), τ_1, τ_2 are the rapid and short-term recovery time constant, respectively (*i.e.*, $\tau_{p1}<\tau_{p2}$), and τ_{p3} is the time constant associated with the negative component of the function. An example of such a regression analysis, presented in Figure 4, demonstrates that the chosen model is adequate for the description of the observed recovery pattern.

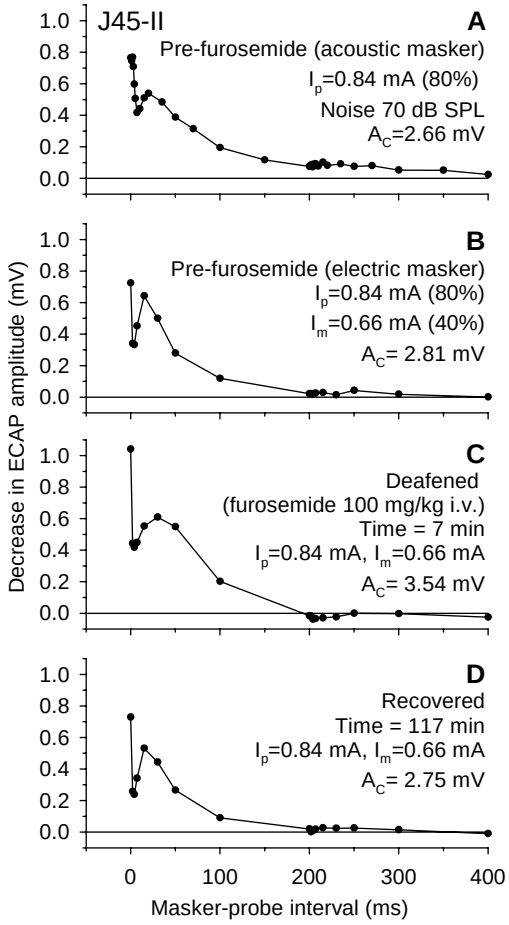

Figure 3. Forward masking of the ECAP with acoustic noise (**A**) and electric pulse trains (**B**-**D**) at different times relative to furosemide treatment. Decreases in ECAP amplitude in response to probe pulses are plotted as functions of masker-probe interval. Masker duration was 400 ms in all cases. I_p, probe stimulus current; I_m, masker stimulus current; A_c, ECAP in response to probe alone (unmasked control). Time in **C** and **D** indicates time after furosemide administration.

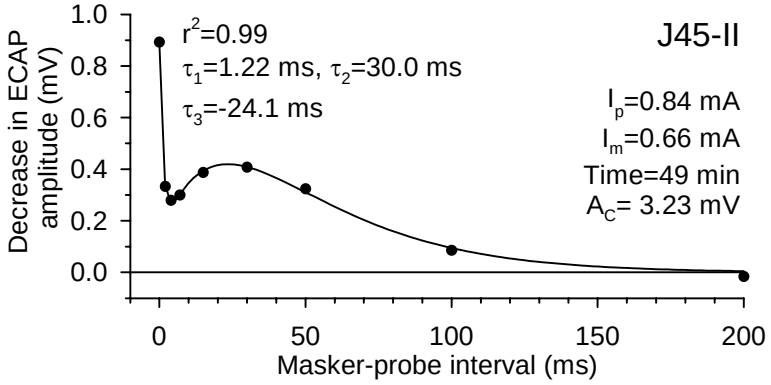

Figure 4. Exponential curve fitting of ECAP recovery from masking with a 400 ms electric pulse train. Decreases in ECAP amplitude in response to probe pulses are plotted as functions of masker-probe interval. I_p, probe stimulus current; I_m, masker stimulus current; A_c, ECAP in response to probe alone (unmasked control).

Changes in time constants that described ECAP recovery from forward masking relative to furosemide treatment are plotted as functions of time after furosemide administration in Figure 5. Following the first injection of furosemide (Figure 5A), the time constant associated with the negative component of the recovery function (τ_3) underwent an increase to values close to the short-term recovery time constant (τ_2), while the latter did not exhibit a systematic change relative to the furosemide treatment. This was followed by a gradual decrease of τ_3 to the pre-treatment range. The rapid recovery time constant (τ_1) decreased upon deafening. Similar changes were observed following the second administration of furosemide in that subject (Figure 5B).

To date, the non-monotonic pattern of recovery from masking has been observed in 5 out of 7 guinea pig subjects following deafening with either neomycin or furosemide. Figure 6 presents an example in which the non-monotonicity was observed in the hearing conditions (pre-furosemide and recovered), but not in the deafened condition.

Our previous observations indicate that the shape of ECAP recovery functions following acoustic as well as electric masker stimuli can change depending on the intensity of the masker and the probe (Nourski et al., 2005b; QPR #10). This is illustrated in Figure 7. Figure 7A shows recovery of the ECAP in response to a fixed-level probe following maskers presented at four different levels. In Figure 7B, ECAP recovery functions following a fixed-level masker are presented for four probe stimulus intensities. At probe levels considerably higher than the masker, the shape of the recovery function is dominated by the rapid recovery component (described by τ_1). When the intensity difference between the masker and the probe is decreased and they are presented at comparable levels, the shape of the recovery function is likely to be determined by the short-term recovery component (τ_2).

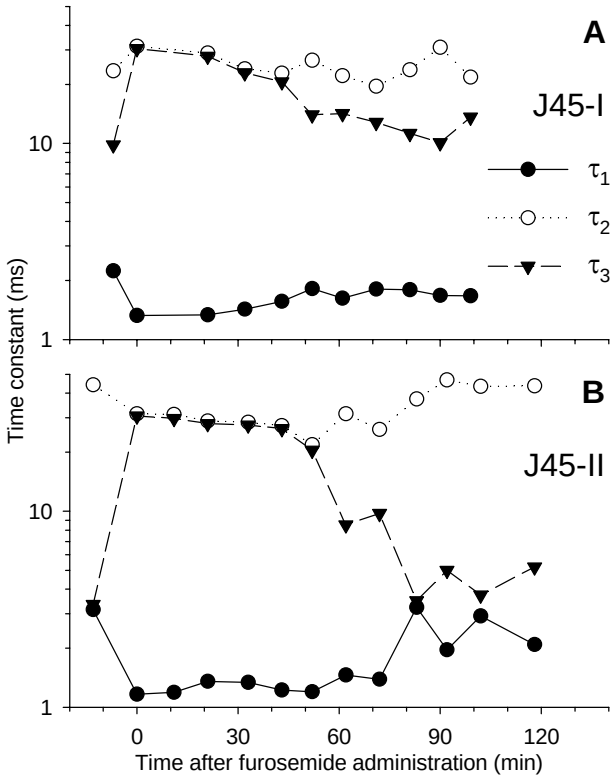

Figure 5. Time constants obtained with regression analysis of ECAP recovery from forward masking as functions of time after first (**A**) and second (**B**) furosemide administration.

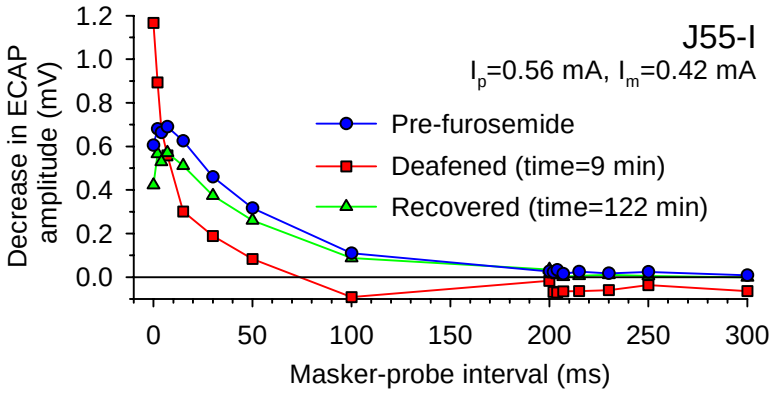

Figure 6. Forward masking of the ECAP with a 400 ms electric pulse train at different times relative to furosemide treatment. Decreases in ECAP amplitude in response to probe pulses are plotted as functions of masker-probe interval. I_p, probe stimulus current; I_m, masker stimulus current.

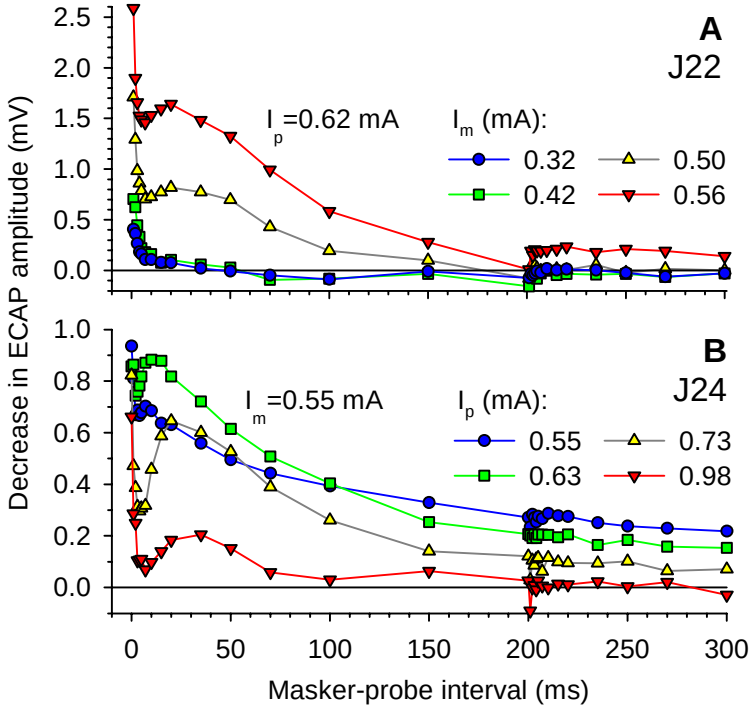

Figure 7. Effects of stimulus level on ECAP recovery from masking with 400 ms electric pulse trains. **A**: Effect of masker level (I_m). **B**: Effect of probe level (I_p). Decreases in ECAP amplitude in response to probe pulses are plotted as functions of masker-probe interval.

Figure 8 demonstrates the effect of masker duration on ECAP recovery from electric pulse-train masking. In the example presented here, the non-monotonic pattern of recovery was more pronounced at longer masker durations. In addition, recovery from masking with 400 and 800 ms pulse-train stimuli took noticeably longer compared to the two shorter masker durations.

It is evident that duration of the electric masker stimulus can affect the shape of the recovery functions. This finding motivated us to explore the extreme case of a short-duration masker: recovery of the ECAP from masking with a single electric pulse. Under such conditions, the effect of masker stimulus on response to the probe was expected to be limited to the refractory properties of the auditory neurons, providing us with the means of evaluating recovery free from the longer-term, adaptation-like, effects.

The results of the two-pulse forward masking experiment from two subjects (J55 and J57) are shown in Figure 9 (panels A and B, respectively). The data were obtained at four stimulus levels in each subject. The masker and the probe pulses were presented at equal levels in each condition. A notable feature of the recovery functions is a plateau/non-monotonicity that was observed at masker-probe intervals of about 1.5-2.5 ms. This feature was more pronounced at higher stimulus levels and was more evident in the data obtained from subject J57 (Figure 9A), in which recovery functions assumed a non-monotonic time course.

Such a timing is comparable with the latency of electrophonic (β-) hair-cell mediated response (Moxon, 1971; Lusted & Simmons, 1988). We therefore hypothesized that this feature of the recovery functions obtained in the two-pulse paradigm might be related to the electrophonic effect. Specifically, the "deviations" of the recovery functions from an otherwise approximately exponential time course might be reflecting refractoriness of the auditory neurons following β-responses evoked by the probe pulse. To address this possibility, we assessed the refractory properties of the ECAP in a two-pulse forward masking paradigm before, during and after treatment with furosemide. Figure 10 demonstrates that furosemide treatment abolished the plateau/non-monotonicity in the recovery functions.

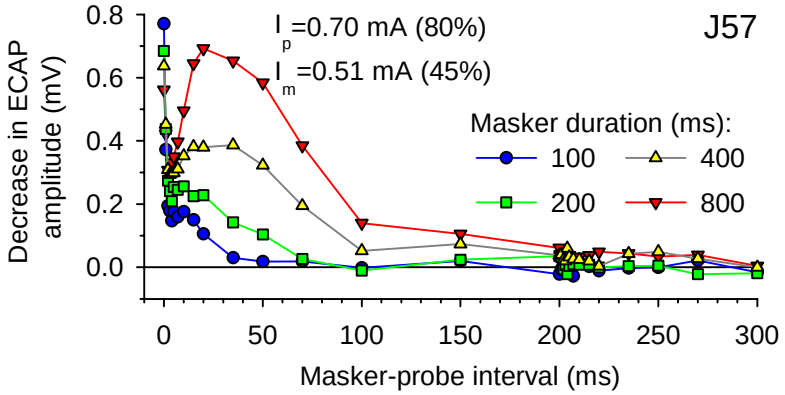

Figure 8. Effect of masker duration on ECAP recovery from masking. Decreases in ECAP amplitude in response to probe pulses are plotted as functions of masker-probe interval. I_p, probe stimulus current; I_m, masker stimulus current.

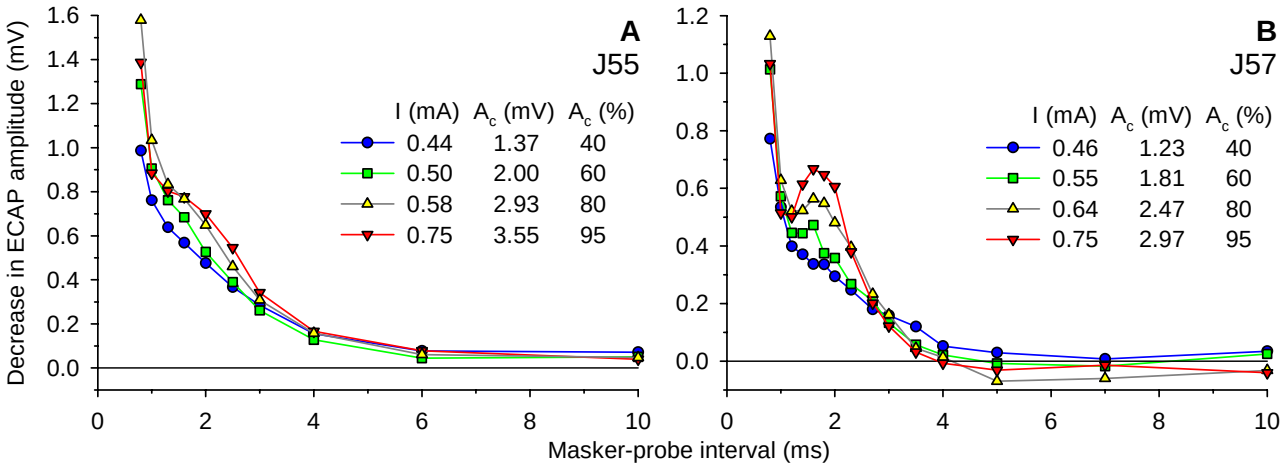

Figure 9. Recovery of the ECAP from masking with a single electric pulse. Data from two subjects (J55 and J57) are shown in panels A and B, respectively. Masker and probe pulses were presented at the same level in each case.

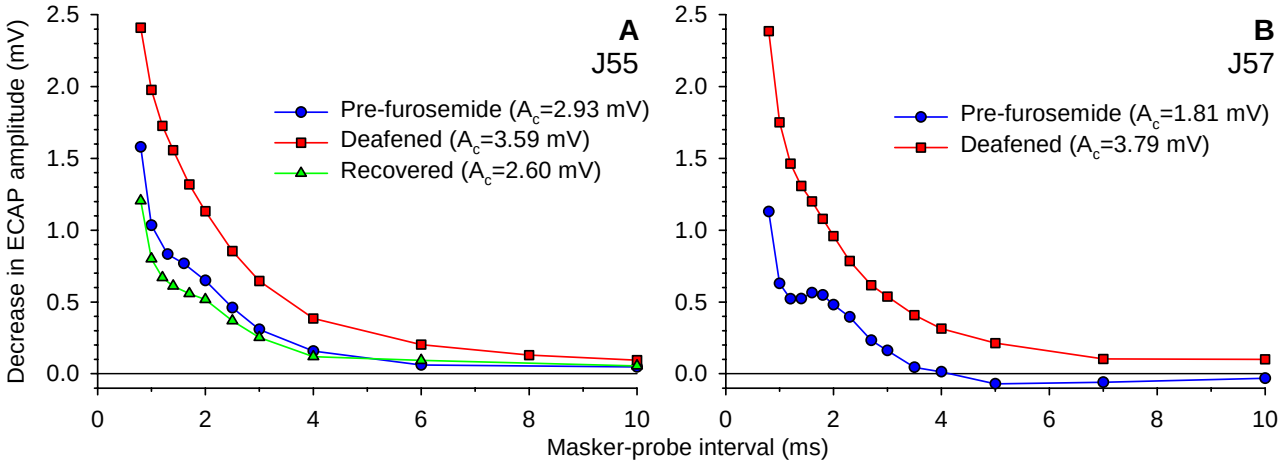

Figure 10. Effect of furosemide treatment on the refractory characteristics of the ECAP. Data from two subjects (J55 and J57) are shown in panels A and B, respectively. Masker and probe pulses were presented at the same level in each case.

We also evaluated recovery of the ECAP from masking in a cat preparation following acute deafening with neomycin. The results from that experiment are presented in Figure 11. The time course of ECAP recovery exhibited a dependence on masker level (Figure 11A) as well as duration (Figure 11B). It may be observed that as the masker level or duration is increased, the forward masking functions assume a relatively simple monotonic shape, feature a plateau and become non-monotonic. These results are consistent with our data obtained in guinea pig subjects deafened with furosemide (*cf.* Fig. 7, 8).

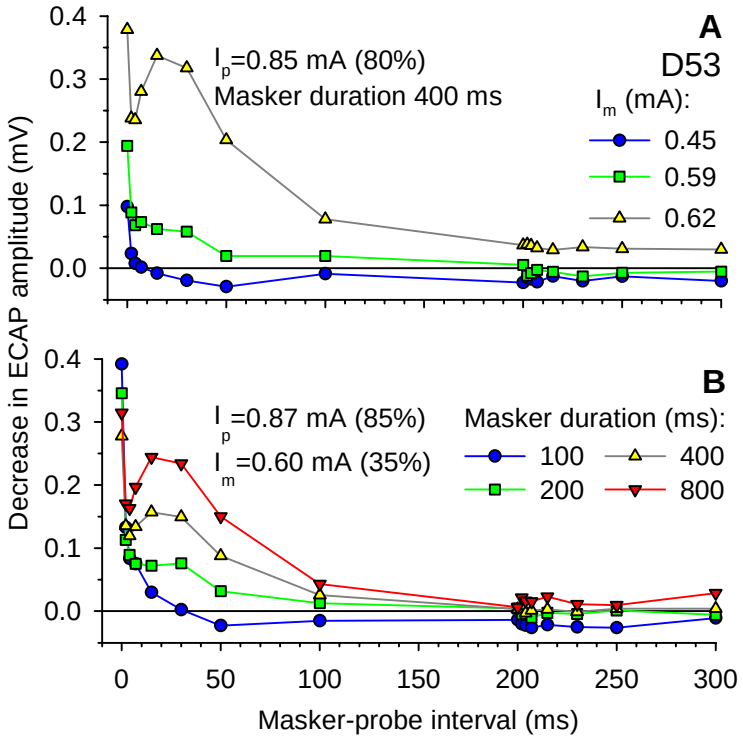

Figure 11. ECAP recovery functions obtained in a cat subject deafened with neomycin. **A**: Effect of masker level. **B**: Effect of masker duration. Decreases in ECAP amplitude in response to probe pulses are plotted as functions of masker-probe interval. I_p, probe stimulus current; I_m, masker stimulus current.

2.4. Discussion

The data presented in this report demonstrate that the ECAP can undergo non-monotonic recovery from masking in hearing as well as deafened conditions. This characteristic feature of recovery was observed in subjects deafened with furosemide as well as neomycin (see Figures 3, 11). Such a time course is comparable to the results of Killian et al. (1994), who reported a non-monotonic pattern of ECAP recovery from masking with a 16 kHz sinusoidal electric stimulus. While the origin of this non-monotonicity is unknown, our results suggest that it cannot be attributed solely to the recovery of spontaneous activity, as we assume that our chemical treatments abolish or greatly reduce that activity. However, changes in the time constants that described the recovery functions (see Figure 5) and disappearance of the non-monotonic pattern following deafening in some subjects (such as in Figure 6) indicate that recovery of the ECAP following electric masking can be affected by the hair-cell function.

Chatterjee (1999) reported non-monotonicities or plateaus sometimes observed in the psychophysical forward masking functions obtained from cochlear implant subjects. It was speculated that this phenomenon could have an origin in the central auditory system. Additionally, the probe stimuli used in the study were 20-ms pulse trains. This provided a possibility to suggest that the observed non-monotonic threshold recovery could be due to overlapping processes of *adaptation* to the probe stimulus and *recovery* from adaptation to the masker. We note that the timing of the phenomenon reported by Chatterjee (1999) is comparable with our observarions. As, in our experiments, the responses were recorded directly from the auditory nerve, we hypothesize that the non-monotonicity of the forward masking functions at least in part has a peripheral origin. Also, as a transient probe stimulus (single electric pulse) was used in our study, a combination of adaptation to the probe and recovery from the masker is not likely to account for the time course of forward masking in our experiments. In contrast to the results of Chatterjee (1999), Nelson and Donaldson (2002) did not observe non-monotonicities in psychophysical recovery from forward masking with pulse-trains.

The loss of non-monotonicity in the recovery functions following deafening in Subject J55 (see Figure 6) may be a result of changes in the ECAP growth functions associated with loss of spontaneous activity (steeper slope, greater threshold, greater saturation amplitude), described by Hu *et al.* (2003). Immediately after furosemide treatment, ECAPs underwent a noticeable increase in amplitude (see Figure 2B). As the shape of the forward masking functions depends on the levels of both the masker and the probe stimuli, it is possible that deafening changes the extent to which stimuli presented at the same levels as before treatment recruit the population of auditory neurons. The relatively brief period of total deafness following furosemide administration made it impossible to explore ECAP recovery further by adjusting the masker and the probe levels to account for the changes in single-pulse ECAP growth.

In the present report, we explored the effect of duration of the electric masker on post-stimulatory ECAP recovery (see Figures 8-11). We demonstrated that the complexity of ECAP forward masking functions increases with masker duration. This observation is consistent with our earlier findings obtained using acoustic masker stimuli (Nourski et al., 2005a ,2005b). We also studied an extreme case of short masker duration, in which the masker duration was limited to one electric pulse (see Figure 9). In this condition, changes in the probe stimulus-evoked ECAP amplitude were presumably limited to refractory effects. In this experiment, we encountered a different kind of non-monotonicity: the recovery functions demonstrated a deviation from an otherwise approximately exponential time course at masker-probe intervals of about 1.5-2.5 ms. This phenomenon was abolished by deafening (see Figure 10). The timing of this effect as well as its dependence on the hair cell function indicates that it is likely to be due to electrophonic response. We therefore note that hair-cell mediated responses can affect the refractory characteristics of the ECAP, as well as produce longer-term effects that have been previously reported.

3. Plans for the Next Quarter

In the next quarter, we will focus on the following major tasks:

1. Conduct additional experiments to study the effects of functional hair cells on recovery of the ECAP from masking with electric stimuli.

2. Conduct single-fiber experiments to study changes in the single-fiber response properties following electric masking. This will be done in cats using a stimulus paradigm comparable to that used in our guinea pigs preparations in which electric masking of the ECAP was studied.

3. Submit a manuscript on the feline single-fiber responses elicited by electric pulse trains from subjects with acoustic sensitivity. This manuscript will describe the performance of the cat model (i.e., the basic acoustic properties of fibers after implantation) as well as the response properties of single fibers to moderate-rate electric pulse trains.

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