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The Effect of Intense Acoustic Stimulation on Basilar-Membrane Vibrations

M. A. RUGGERO†*, N. C. RICH and A. RECIO†

*†The Hugh Knowles Center and *Institute for Neuroscience, Northwestern University, Evanston, IL*

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Exposure of the mammalian ear to intense sounds causes elevations of hearing thresholds and reduces the responses of hair cells and auditory-nerve fibers in a frequency-specific manner. We tested the hypothesis that the acute effects of acoustic overstimulation on auditory-nerve and hair-cell responses reflect, at least partly, diminished sensitivity of basilar-membrane vibrations. Using laser velocimetry, basilar-membrane responses to sound were studied at a site of the chinchilla cochlea with characteristic frequency (CF, the most effective stimulus frequency) of 9–10 kHz. Test stimuli, tones and clicks, were presented preceding and following exposures to intense tones whose characteristics (4-minute duration, 100 dB SPL, 7-kHz) were chosen to induce substantial and long-lasting, but reversible, threshold elevations at CF. Following the exposures, basilar-membrane responses were reduced at near-CF frequencies but were unaffected at other stimulus frequencies. Response-magnitude reductions were largest at low stimulus levels and insignificant at intense stimulus levels. Thus, acoustic overexposure tended to abolish the CF-specific compressive nonlinearity that characterizes normal basilar-membrane responses, broadening the frequency tuning and shifting the apparent CF toward lower frequencies. The response-magnitude changes were accompanied by phase lags which were largest (45–180 degrees) at frequencies near, or somewhat higher than, CF. The alterations of basilar-membrane function by intense sounds closely resemble those induced by death, surgically-induced cochlear trauma or ototoxic drugs. These alterations are consistent with previous findings that acoustic overstimulation reduces the receptor potentials of outer hair cells: such reduction should decrease the mechanical feedback which outer hair cells presumably exert on basilar-membrane vibration.

Key words: basilar membrane, cochlea, temporary threshold shift, TTS, outer hair cells, hearing loss, laser velocimetry, chinchilla, compound action potentials

EXPOSURE OF THE EAR to intense sounds can cause transient and permanent elevations of hearing thresholds (Schmiedt, 1984; Clark, 1991; Saunders *et al.*, 1991; and Patuzzi, 1992). It is clear that the threshold elevations originate in the cochlea, since acoustic overexposure causes threshold shifts in the responses to sound of auditory-nerve fibers (e.g., Cody and Johnstone, 1980; Liberman and Mulroy, 1982; Salvi *et al.*, 1983) and hair cells (Cody and Russell, 1988) which are sufficiently large to account for most of the psychophysical deficits.

The manner in which intense sounds exert their cochlear effect is the subject of considerable debate. There is some evidence that the stereocilia of inner hair cells (Hunter-Duvar, 1977) and the synapses of inner hair cells with auditory-nerve dendrites (Spoendlin, 1971; Lenoir and Pujol, 1980; Liberman and Mulroy, 1982; Robertson, 1983; Henry and Mulroy, 1995) are implicated. However, a dominant involvement of the inner hair cells or their synapses seems unlikely for all but the most extreme exposures since, in both auditory-nerve fibers and inner hair cells, acoustic overexposure usually causes response reductions that are largely confined to the neighborhood of the characteristic frequency (CF, the most effective stimulus frequency). Since frequency tuning in the mammalian cochlea is established in the vibration of the basilar membrane (see reviews by Patuzzi and Robertson, 1988, and Ruggero, 1992), many believe that the neural effects of acoustic overstimulation largely reflect a loss of sensitivity of basilar-membrane vibration (see reviews by Schmiedt, 1984; Saunders *et al.*, 1991; and Patuzzi, 1992). Direct evidence for this hypothesis has so far been meager and

Corresponding author: Mario A. Ruggero, Northwestern University, 2299 North Campus Drive, Evanston, IL 60208-3550.

not fully consistent. *In-vivo* measurements from two guinea pigs (Sellick *et al.*, 1982; Patuzzi *et al.*, 1984) and one cat (Cooper and Rhode, 1992) show that acoustic overstimulation tends to reduce and linearize basilar-membrane responses to CF tones. In stark contrast, overstimulation of *in-vitro* preparations of the guinea-pig cochlea enhances the vibrations of the organ of Corti (Ulfendahl *et al.*, 1993). A discrepancy also exists between the two sole measurements of basilar-membrane response phases in *in-vivo* cochleae following overstimulation: one showed phase leads (Patuzzi *et al.*, 1984) and the other phase lags (Cooper and Rhode, 1992).

We present here findings from an investigation in chinchilla cochleae in which the responses of the basilar membrane to tones and clicks were measured before and after exposures to intense tones which induce substantial and long-lasting (but reversible) threshold shifts in normal cochleae. Our results strongly support the mechanical hypothesis, showing that such exposures lead to frequency-specific alterations of basilar-membrane responses that resemble those caused by death, surgical trauma or the administration of furosemide (e.g., Rhode, 1973; Sellick *et al.*, 1982; Robles *et al.*, 1986; Ruggero and Rich, 1991b). Preliminary results of this investigation have been published in abstract form (Ruggero *et al.*, 1993a) and in the proceedings of a symposium (Ruggero *et al.*, 1993b).

METHODS

Animal Preparation

The effects of acoustic overstimulation on basilar membrane responses to sound were studied, using laser velocimetry, at a site of the chinchilla cochlea located 3.5 mm from the oval window. Chinchillas, anesthetized with sodium pentobarbital (initial dose: 65 mg/kg; injected intraperitoneally), were tracheotomized and intubated, but forced respiration was usually not used. Normal body temperature was maintained by means of a heating pad servocontrolled by a rectal probe. The left pinna was resected, the bulla was widely opened, the tensor tympani muscle was cut and the stapedius muscle was detached from its anchoring. A silver-wire electrode was placed on the round window to record compound action potentials evoked by tone bursts. Compound action potential thresholds (sound pressure levels—SPLs—required to elicit 10 μV N1 responses) served to monitor the physiological state of the cochlea. A small hole made in the basal turn of the otic capsule allowed direct visualization of the basilar membrane and placement on it of a few glass microbeads (diameter: 10–30 μm), which served as reflecting targets for the light beam of the laser velocimeter.

Acoustic Stimulation

Acoustic stimuli were produced under computer control by a custom-built arbitrary waveform generator (Ruggero and Rich, 1983) and were delivered through a Beyer DT-48 earphone. This was mounted on the back of a plastic speculum sealed to the bony ear canal by means of ear-impression compound. A Knowles (1842 or 1785) miniature microphone equipped with a probe tube was used to measure the sound pressure within 2 mm of the tympanic membrane. Single-tone stimuli were gated tones modulated at onset and offset by 1/2 period of a cosine waveform (1.16 ms rise/fall time). To ensure that the tone stimuli did not cause threshold shifts, tone pips were of short duration (10 or 25 ms) and had relatively long repetition periods (50 or 100 ms, respectively). Acoustic clicks were generated by exciting the earphone with electrical 50-microsecond pulses (25-ms repetition period).

Laser Velocimetry

Laser velocimetry measures the velocity of a vibrating object by detecting the Doppler frequency shift of light reflected from it. In our application of this method, the laser beam is reflected from glass microbeads placed on the basilar membrane. The velocimeter used in these experiments consisted of a 20-mW He-Ne laser (Spectra Physics 106-1), a Dantec 41 × 60 fiber vibrometer and a Dantec 55N20 Doppler frequency tracker. The velocimeter head was coupled to a compound microscope (Olympus BHMJ) equipped with 5X and 20X ultralong working-distance objectives (Mitutoyo Plan Apo 5X, N.A. 0.14, and 20X, N.A. 0.42). The electrical output of the Doppler frequency tracker, a voltage (1–10 V) proportional to velocity, was frequency filtered with a pass band of 1–15000 Hz before analog-to-digital conversion under computer control (maximum sampling rate of 40 kHz). Responses were averaged over 512 or 1024 stimulus repetitions and velocity spectra were computed off-line by Fourier transformation. For more details on the application of laser interferometry to the measurement of basilar-membrane vibration see Ruggero and Rich (1991a).

RESULTS

Basilar-membrane responses to tones and clicks were measured in 4 chinchillas at a cochlear site with CFs of 9–10 kHz located approximately 3.5 mm from the oval window. Recordings were made preceding and following 4-minute exposures to 7-kHz tones presented at 100-dB SPL. The exposure frequency was selected on the basis that intense tones produce threshold shifts that are maximal at frequencies about $^1/_2$ octave above the exposure frequency (e.g., Lonsbury-Martin and Meikle, 1978; Cody and Johnstone, 1981). The expo-

sure intensity was selected to produce a large but reversible threshold elevation (see below).

Effects of Overstimulation on Basilar-Membrane Responses to CF Tones

Figure 1 shows the magnitudes of basilar-membrane responses to CF tones (10-kHz in this case), as a function of stimulus intensity, recorded from a relatively-sensitive chinchilla cochlea. As estimated by CAP thresholds, at the time of overexposure the basilar-membrane recording site had suffered a sensitivity loss of only 8 dB due to the preparatory surgical procedures. The pre-exposure input-output functions for the CF tone were highly nonlinear, growing between 40 and 70 dB at rates of about 0.23 dB for every dB of stimulus increase. Following a 4-minute exposure to a 7-kHz, 100-dB SPL tone, responses to CF tones were reduced in magnitude at low stimulus levels but minimally affected above 70 dB. As a result, the average slope of the velocity-intensity function was increased to 0.67 dB/dB. The exposure caused a decrease in sensitivity of about 25 dB, measured "horizontally" as the intensity increment necessary to maintain a response criterion of 50–100 μm/sec.

The intensity-specific effect of overexposure, whereby only lower-level responses were significantly reduced, increased the rate of growth of CF input-output functions and therefore tended to linearize them. Figure 2 illustrates the effect of overstimulation in two chinchilla cochleae that were somewhat less sensitive than that of Figure 1. Except for a 10-dB sensitivity difference, both the pre-exposure input-output functions and the effect of overexposure were very similar in both cochleae. Before the exposure, responses grew at rates of 0.40–0.47 dB/dB in the 50–80 dB range. Overexposure reduced responses to low-level stimuli by as much as 25 dB but responses at intensities of 80 dB or greater were only minimally affected. Thus, the slopes of the velocity-intensity functions were sharply increased (to 0.97 and 0.79 dB/dB in cochleae L83 and L84, respectively).

The effect of overstimulation was relatively small in L110, a cochlea which had suffered substantial threshold elevation as a result of surgical damage (Fig. 3). This cochlea was (exceptionally) exposed to two 4-minute presentations of the 100-dB, 7 kHz tone within an interval of 40 minutes. In advance of the first exposure, two intensity-velocity functions were measured (solid symbols and solid or dashed lines), separated by a time interval of 3 hours. During this time there was some deterioration of the cochlea, amounting to 7 dB (measured "horizontally") for responses to low-level CF tones. Since this cochlea had already suffered a 24-dB sensitivity loss due to surgical procedures, the overall sensitivity loss at the time just preceding overexposure can be estimated at 31 dB. Following the first exposure, low-level CF responses were reduced by slightly more than 9 dB. Following a second exposure, the CF responses were further reduced, yielding a cumulative total of 19 dB. As was the case for the other cochleae (Figs. 1–2), the response reductions were

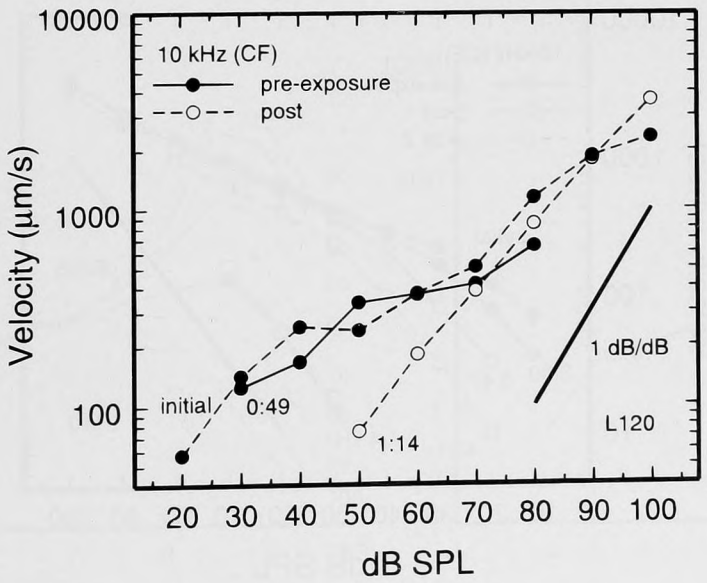

FIGURE 1 **The effect of acoustic overexposure on basilar-membrane responses to CF tones.** The input-output functions display peak velocities of responses to 10-kHz tone pips preceding and following a 4-minute exposure to a 7-kHz, 100 dB tone. Two pre-exposure velocity-intensity functions are shown, one recorded early in the experiment (dashed line, filled symbols) and the other (continuous line, filled symbols) just preceding the exposure. The times (hours and minutes) when the velocity-intensity functions were completed, relative to the time of completion of the initial pre-exposure curve, are shown adjacent to the curves. The thick continuous line exemplifies a linear input-output function.

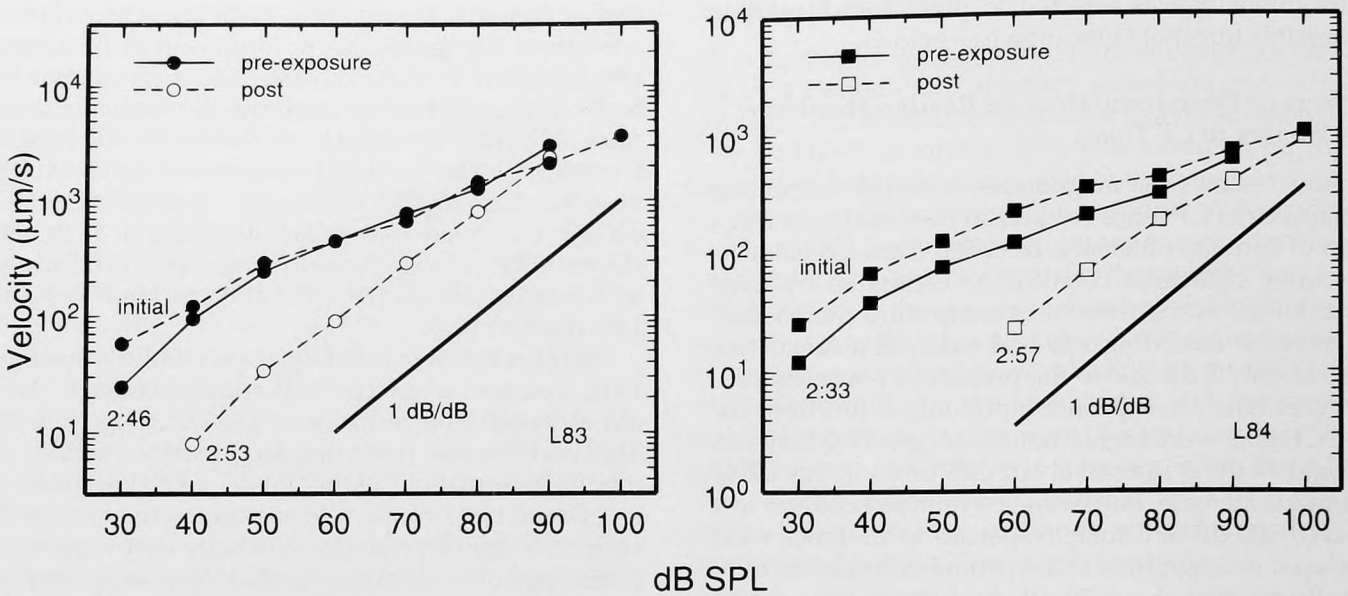

FIGURE 2 **The effect of acoustic overexposure on basilar-membrane responses to CF tones in two cochleae.** The input-output functions display peak velocities of responses to 9-kHz tone pips preceding (filled symbols) and following (open symbols) a 4-minute exposure to a 7-kHz, 100 dB tone. For each cochlea two pre-exposure velocity-intensity functions are shown, one recorded early in the experiment (dashed line, filled symbols) and the other (continuous line, filled symbols) just preceding the exposure. The times (hours and minutes) when the velocity-intensity functions were completed, relative to the time of completion of the initial pre-exposure curve, are shown adjacent to the curves. The thick continuous line exemplifies a linear input-output function.

largest at the lowest stimulus level and became progressively smaller as stimulation levels increased. In contrast with the other cochleae, L110 retained substantial nonlinearity in the range of 50–80 dB after overstimulation.

An issue of concern in the interpretation of the effects of overstimulation on basilar-membrane responses is the extent to which the measurement of such effects may be contaminated by the cochlear deterioration that usually occurs with the passage of

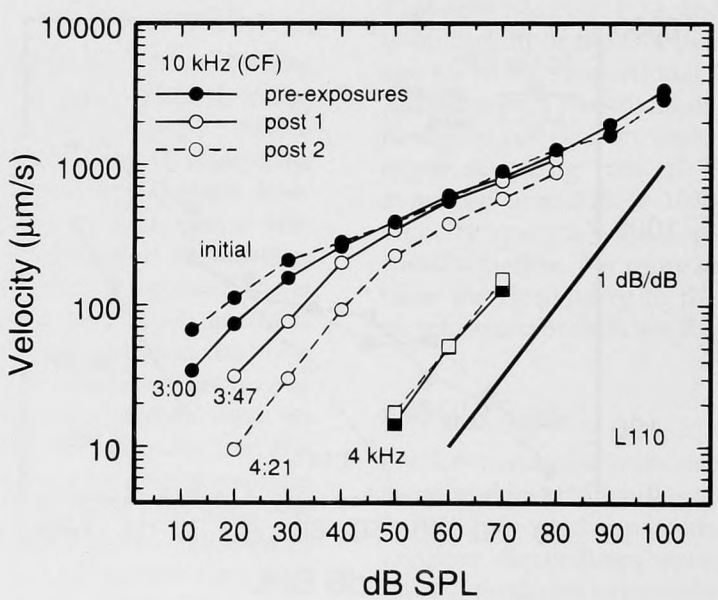

FIGURE 3 **The effect of acoustic overexposure on basilar-membrane responses to tones.** The input-output functions display peak velocities of responses to CF (10-kHz; circles) and 4-kHz (squares) tone pips preceding and following two 4-minute exposures to a 7-kHz, 100 dB tone. For the CF tones, two pre-exposure velocity-intensity functions are shown, one recorded early in the experiment (dashed line, filled symbols) and the other (continuous line, filled symbols) just preceding the exposure. The times (hours and minutes) when the velocity-intensity functions were completed, relative to the time of completion of the initial pre-exposure curve, are shown adjacent to the curves. The thick continuous line exemplifies a linear input-output function.

time. An estimate of the deterioration can be made by comparing the rate of deterioration preceding the exposure with the time intervening between the pre-exposure and post-exposure recordings. Figures 1–3 provide data for such estimation. For each of 4 cochleae, two pre-exposure velocity-intensity functions are shown, one recorded early in the experiment ("initial") and the other just preceding overstimulation. The times, relative to that for the initial curve, are indicated (in hours and minutes) for all other curves. In general, the pre-exposure deterioration was much smaller than the apparent effect of the exposure, even though the corresponding elapsed times were much greater for the recordings preceding the exposure. For example: in cochlea L84 (Fig. 2) there was a pre-exposure deterioration amounting to 6 dB over 153 minutes, whereas the apparent effect of overexposure was 25 dB measured over 24 minutes. Linearly extrapolating from the initial rate of deterioration (0.04 dB/min), one can estimate that less than 1 dB (out of 25 dB) of the apparent effect of overexposure in cochlea L84 resulted from deterioration. Computations for cochleae L120 and L83 yield a similar conclusion: deterioration could have contributed only trivially (< 1dB) to the apparent effects of overstimulation (25 and 17 dB). In the case of cochlea L110 (Fig. 3) the estimated contamination by deterioration was somewhat larger, but still small, amounting to 1.8 dB out of an overall apparent loss of 9 dB for the first exposure, or 3.2 dB out of 19 dB for the two exposures combined.

Frequency Specificity of the Effects of Overstimulation on Basilar-Membrane Responses to Tones

In addition to velocity-intensity curves for CF tones, Figure 3 shows input-output functions for responses to 4-kHz tones. In contrast with the responses to 10-kHz tones, the pre-exposure responses to 4-kHz tones grew linearly with stimulus intensity (1 dB per dB) and they were unaffected by overstimulation. The frequency selectivity of the effects of acoustic overexposure on responses to tones can be viewed comprehensibly by comparing families of isointensity functions (Fig. 4). The data for the left panel of Figure 4—responses to tones as a function of stimulus frequency and intensity—were obtained in the same cochlea represented in Figure 3 during the time interval between the acquisition of the two pre-exposure input-output functions for CF tones (filled circles in Fig. 3). In spite of a substantial pre-existing sensitivity loss, the responses of this cochlea remained highly nonlinear at the time of overexposure. To emphasize the effect of nonlinear growth, the velocity data have been normalized to stimulus intensity, thus yielding gains (velocity per stimulus pressure). Had responses grown linearly with stimulus intensity, the isointensity gain curves would completely superimpose. In fact, the gain curves measured pre-exposure (left

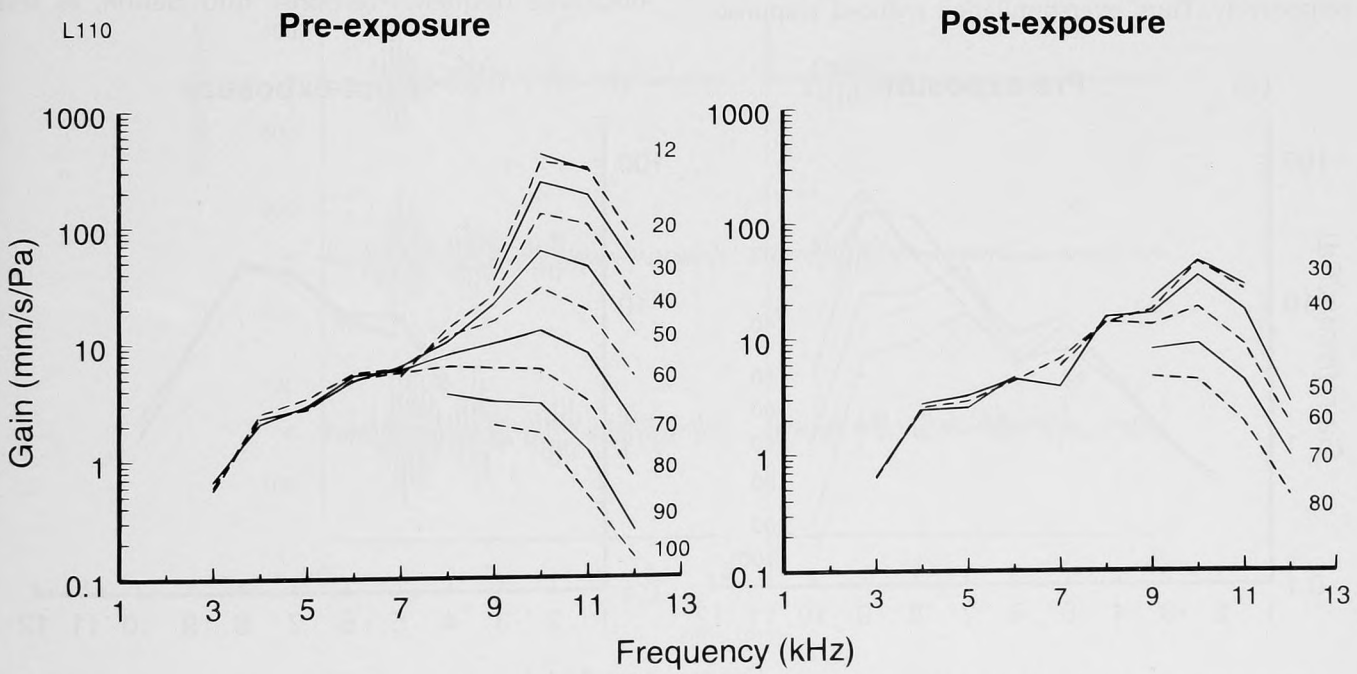

FIGURE 4 **Effects of acoustic overexposure on the frequency response of basilar-membrane vibration.** Each panel consists of a family of isointensity curves representing the velocity gains (velocity divided by acoustic pressure) of basilar-membrane responses to tone pips as a function of frequency (abscissa) and intensity (parameter, in dB SPL). Left panel: responses to tones preceding two 4-minute exposures to a 7-kHz, 100-dB tone. Right panel: responses following the second exposure. Data from cochlea L110, also represented in Figure 3.

panel) superimpose only at frequencies of 7 kHz and below. Pre-exposure gain at CF and at frequencies just above CF (at least up to 12 kHz) grew systematically *larger* as a function of *decreasing* stimulus level. Between 12 and 100 dB SPL, gains at CF changed by a factor of nearly 150 or, equivalently, 43 dB. Because of the frequency specific and intensity-dependent changes in gain, frequency tuning also changed systematically with stimulus intensity, being sharpest at low stimulus levels and broad for intense stimuli. For 80-dB tones, response magnitudes had no clear peak and were essentially uniform between 6 and 10 kHz. For 90-dB tones, responses to 6-kHz tones were slightly larger than at any other measured stimulus frequency, including CF (10 kHz).

After acoustic overexposure (right panel), responses to tones which originally grew linearly—i.e., those with frequency 7 kHz or lower—were hardly affected but responses to near-CF tones, including those with frequency as high as 12 kHz, had reduced peak gains. The reduction in gain was maximal for the lowest stimulus intensities and diminished systematically with stimulus intensity. The post-exposure gains for responses to CF tones presented at 30 and 40 dB were identical (47 mm/s/Pa), indicating linear response growth at intensities lower than 40 dB and implying that the exposure reduced the maximal attainable gain to 47 mm/s/Pa. In contrast, in advance of the exposure responses to CF tones grew at a substantially compressive rate at intensities as low as 12–20 dB. At these intensities gains were 429 and 367 mm/s/Pa, respectively. Thus, overstimulation reduced response sensitivity at CF by a factor of at least 9.1 times or, equivalently, 19.2 dB.

Figure 5 presents families of isointensity gain curves for a cochlea (L83; see Fig. 2) in which the effects of overexposure were somewhat more severe than those shown in Figure 4. Pre-exposure (left panel), the response gains at frequencies near CF (9 kHz) were largest at low stimulus intensity and decreased systematically with increasing intensity. The compressive nonlinearity was largest at 10 kHz and diminished systematically with decreasing stimulus frequency. As a result, the peak responses shifted downward as intensity was raised: 9 kHz at 30–50 dB, 8 kHz at 60 dB and 7 kHz at 70 dB and higher intensities. As in the case of cochlea L110, responses that grew linearly in advance of the exposure (those for stimulus frequencies below 6 or 7 kHz) were not affected by the exposure (right panel). However, in contrast with cochlea L110 (Figs. 3 and 4), nonlinear growth in cochlea L83 was nearly abolished at all frequencies, so that the isointensity gain curves largely overlap. Acoustic overstimulation reduced substantially the responses to low-level CF (9-kHz) tones and to a lesser extent also responses to 8- and 10-kHz tones and thus shifted the apparent CF to 7 or 8 kHz (right panel).

Effects of Overstimulation on Basilar-Membrane Responses to Clicks

The effects of exposure to intense tones were also studied using clicks, which are useful for rapidly obtaining frequency-response information, as test

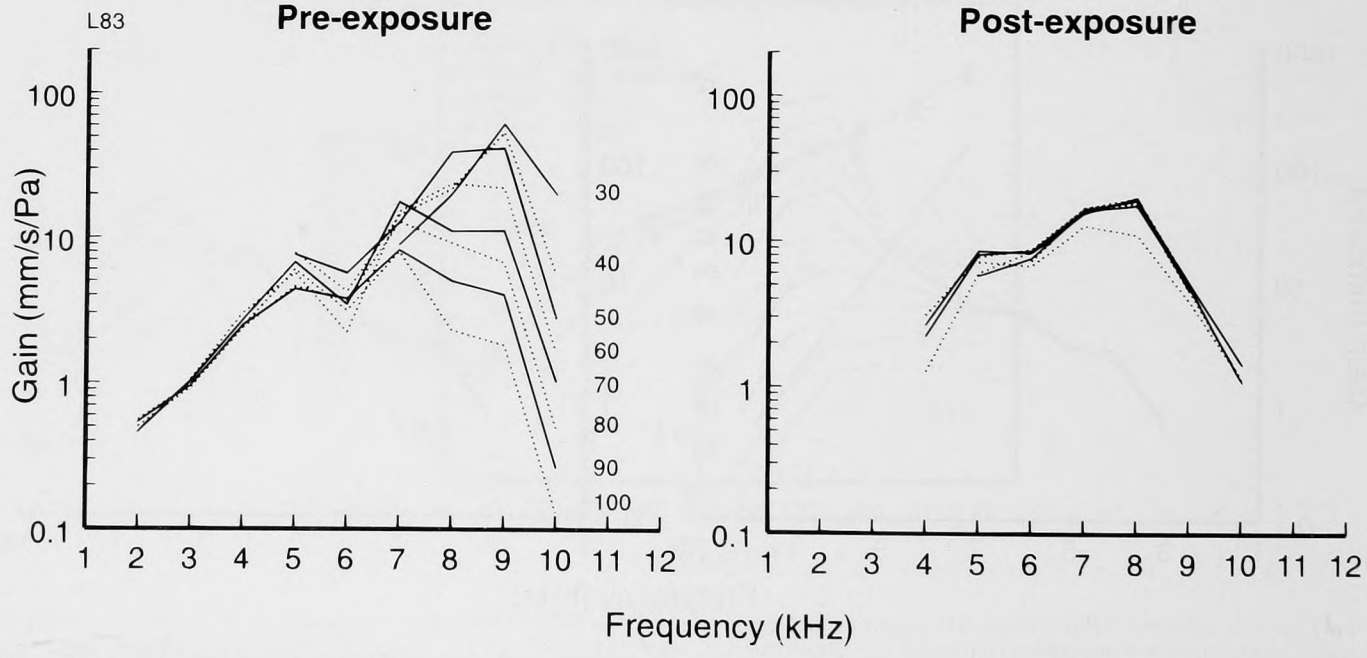

FIGURE 5 **Effects of acoustic overexposure on the frequency response of basilar-membrane vibration.** Left panel: responses to tones preceding a 4-min exposure to a 7-kHz, 100-dB tone. Right panel: responses following the exposure. The families of isointensity gains represent responses of cochlea L83, also illustrated in Figure 2. Other details as for Figure 4.

stimuli (Fig. 6). The traces in the left column of Figure 6 show responses to clicks with peak intensities of 52–92 dB SPL, recorded just before overstimulation. As is the case in healthy cochleae, the basilar-membrane responses to low-level clicks consisted of relatively undamped oscillations with fundamental frequency close to the CF (Ruggero and Rich, 1991a; Ruggero *et al.*, 1992a). The short-lasting initial component of the oscillation has a lower fundamental frequency than later cycles, grows linearly, and hence becomes prominent at high stimulus levels. A later, longer-lasting component grows with stimulus intensity at rates lower than 1 dB/dB and is dominant at low stimulus levels. This intensity-dependent nonlinearity manifests itself in systematic changes in the envelope of the responses. To highlight the changes in envelope shape, the traces of Figure 6 have been scaled so that the absolute sizes of the responses are de-emphasized: the scales are compressed by a factor of 2 (6 dB) for every 10 dB increment in click intensity. As exemplified in Figure 6, the envelopes of responses to clicks in healthy cochleae are often characterized by conspicuous lobes (three or four in this case) delimited by envelope nodes occurring at 1.5, 2.2 and 3.1 ms. The lobes are most prominent at low stimulus levels.

Following two exposures to 100-dB SPL, 7-kHz tones, responses changed drastically for low-level clicks but very little for intense clicks (right column in Fig. 6). For 52-dB clicks, the entire response was buried in the noise. For higher click levels (62–82 dB), the ear-

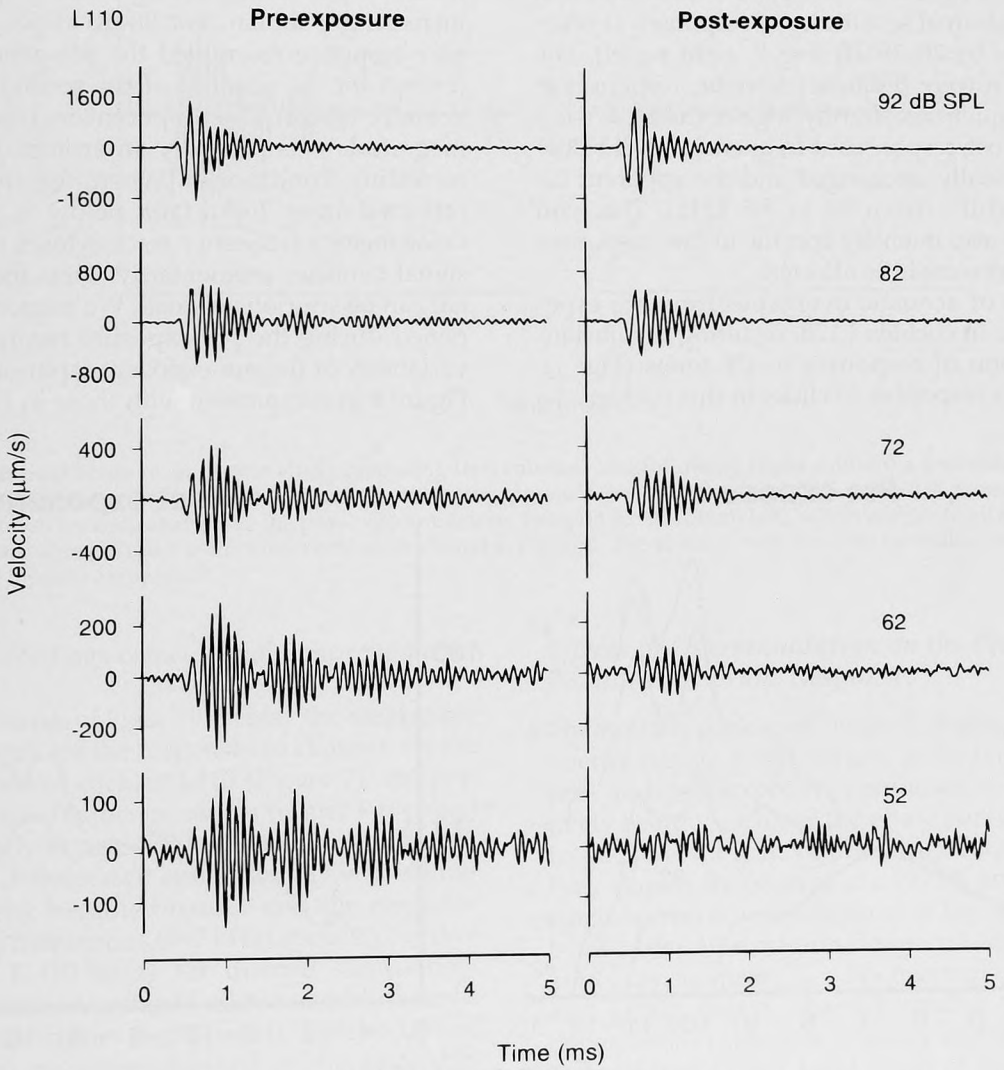

FIGURE 6 **Basilar membrane responses to clicks preceding (left column) and following (right column) two 4-minute exposures to a 7-kHz, 100-dB SPL tone.** Peak intensities of the click stimuli are indicated above the post-exposure traces. The abscissa indicates time (in milliseconds) elapsed from onset of electrical input to earphone. The ordinates have scales that are progressively compressed: they are shrunken by a factor of 2 (i.e., 6 dB) for every 10-dB increase in stimulus intensity. The data were obtained in the same cochlea illustrated in Figures 3 and 4.

liest 1–2 cycles of oscillation were little affected but successively later cycles were increasingly reduced. For the highest click level (92 dB), the effects were small. Overall, the most conspicuous effect of overstimulation was the elimination of all but the earliest envelope lobe.

The frequency- and level-specific effects of acoustic overstimulation are best seen in the frequency domain. Figure 7 displays the gain spectra computed by Fourier transformation of the waveforms of Figure 6. The left panel makes evident the marked nonlinear growth of responses at spectral frequencies near CF: the gain peaks are largest for the weakest stimuli and become progressively smaller with increasing click intensity. As a result of the frequency-dependent nonlinear growth the response peaks shift by nearly 1/2 octave, from 10–11 kHz for low-level clicks, to about 6.5 kHz for 102-dB clicks. Acoustic overstimulation reduced the maximal sensitivity of responses at near-CF frequencies by 20–25 dB (Fig. 7, right panel). The changes in gain were frequency specific: responses at CF and at frequencies slightly higher than CF were reduced the most, responses at frequencies well below CF were essentially unchanged and the apparent CF decreased slightly (from 10 to 9.5 kHz). The gain reduction was also intensity specific in that responses to intense clicks were little affected.

The effects of acoustic overexposure were especially dramatic in cochlea L120, resulting in substantial linearization of responses to CF tones (Fig. 1). Figure 8 shows responses to clicks in this cochlea. To permit a more direct check on the extent of linearization, the traces are depicted here after normalization to stimulus intensity. In other words, the traces are shown with a constant scale of gain (lower left), with units of velocity per unit pressure. For responses that grow linearly, the normalized traces should be identical. In fact, the waveshapes (envelopes) of the pre-exposure responses changed substantially with stimulus intensity and the gains decreased in magnitude. For example, the oscillation cycle peaking at 0.98 milliseconds (vertical line) was many times smaller at 98 dB than at 58 dB. In contrast with the pre-exposure responses, the post-exposure responses (right column) were fairly similar in waveshape and the gains changed little with intensity. For 58-dB clicks, the exposure reduced drastically all but the two earliest oscillation cycles. At higher click intensities, the waveforms of pre- and post-exposure responses became increasingly similar. For 98-dB clicks, the post-exposure response resembled the pre-exposure response (except for the absence of the second lobe) but was actually larger. This apparent increase in response magnitude was probably an artifact due to unstable recording conditions. [When the intensity of the reflected laser light falls below a threshold, the velocimeter's frequency tracker loses its lock and the signal vanishes momentarily. Thus the averaged signal can be spuriously small. We suspect that this happened during the pre-exposure recordings (note the variability of the pre-exposure input-output curves in Figure 1 in comparison with those in Figures 2 and 3)

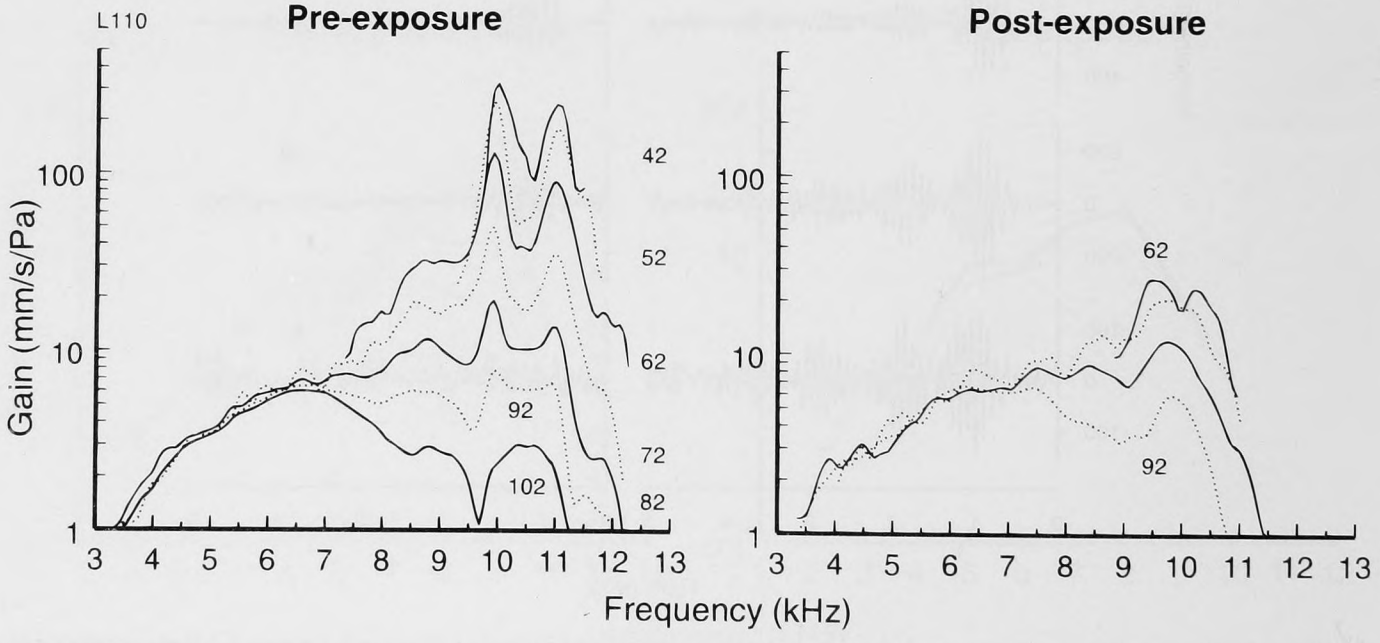

FIGURE 7 **Gain spectra of responses to clicks computed by Fourier transformation of the time traces of Figure 6.** Each panel consists of a family of curves representing the gain (velocity divided by sound pressure) of basilar-membrane responses to clicks as a function of spectral frequency (abscissa) and intensity (parameter, in dB SPL). Left panel: gain spectra for responses preceding two 4-minute exposures to a 7-kHz, 100 dB tone. Right panel: gain spectra for responses following overexposure.

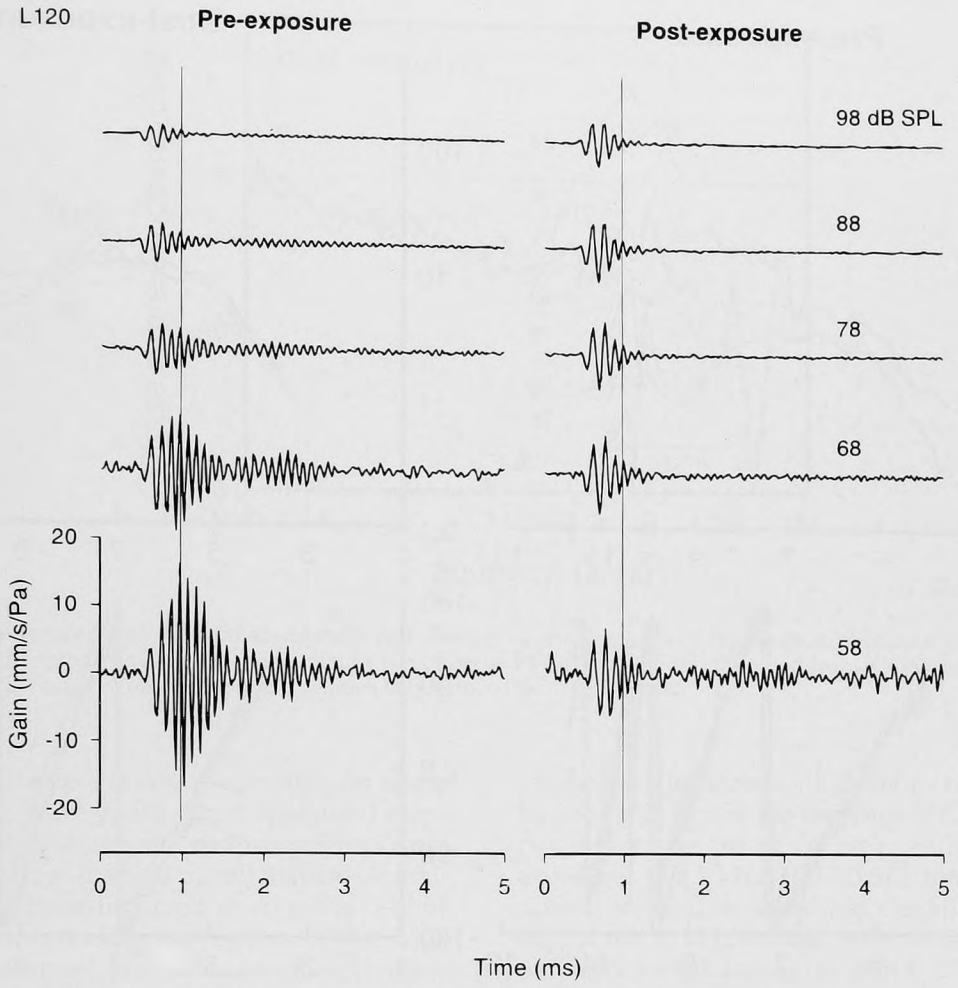

FIGURE 8 **Basilar membrane responses to clicks preceding (left column) and following (right column) a 4-minute exposure to a 7-kHz, 100-dB SPL tone.** Each trace has been normalized to stimulus pressure (i.e., velocity divided by peak click pressure). Peak intensities of the click stimuli are indicated above the post-exposure traces. The gain scale (bottom left), which applies to all traces, has units of mm/s/Pa. The data were obtained in the same cochlea illustrated in Figure 1. The abscissa indicates time (in milliseconds) elapsed from onset of electrical input to earphone.

and that the recordings coincidentally improved after the exposure.]

The upper panels of Figure 9 display the magnitude frequency spectra for the responses to clicks of Figure 8. As in the case of cochlea L110 (Figure 7), the pre-exposure gain-vs.-frequency curves (upper left panel) grew nonlinearly at near-CF frequencies. As a result, sensitivity at CF decreased systematically with stimulus level, tuning became broader and the response peak shifted to frequencies (6–7 kHz) about 1/2 octave lower than CF (10 kHz) for intense stimulation. Nonlinear growth was largely eliminated by acoustic overstimulation (upper right panel): irrespective of click intensity, responses peaked at the same frequency, some 1/2 octave below the original CF. Such downward shift of CF due to overstimulation is similar to that seen for responses to tones in another cochlea (L83) in Figure 5, and was also demonstrated in a third cochlea (L84, not shown).

Effects of Overstimulation on the Phases of Basilar-Membrane Responses

The bottom panels of Figure 9 display phase-vs.-frequency curves, with intensity as the parameter, for the pre- and post-exposure responses to clicks. In relatively healthy cochleae the phase curves for responses to clicks exhibit intensity-dependent group delays (i.e., slopes; Ruggero *et al.*, 1992a) and may contain irregularities (lower left panel of Fig. 9), accompanied by notches in the magnitude spectra (upper left panel), at frequencies near CF. After overexposure, the phases and group delays near CF tended to become less dependent on stimulus intensity and the irregularities disappeared (lower right panel of Fig. 9). Figure 10 shows the net changes in phase spectra of responses to moderate-intensity clicks measured in 4 cochleae. After acoustic overexposure, response phases changed little at frequencies lower than CF/2 but responses at

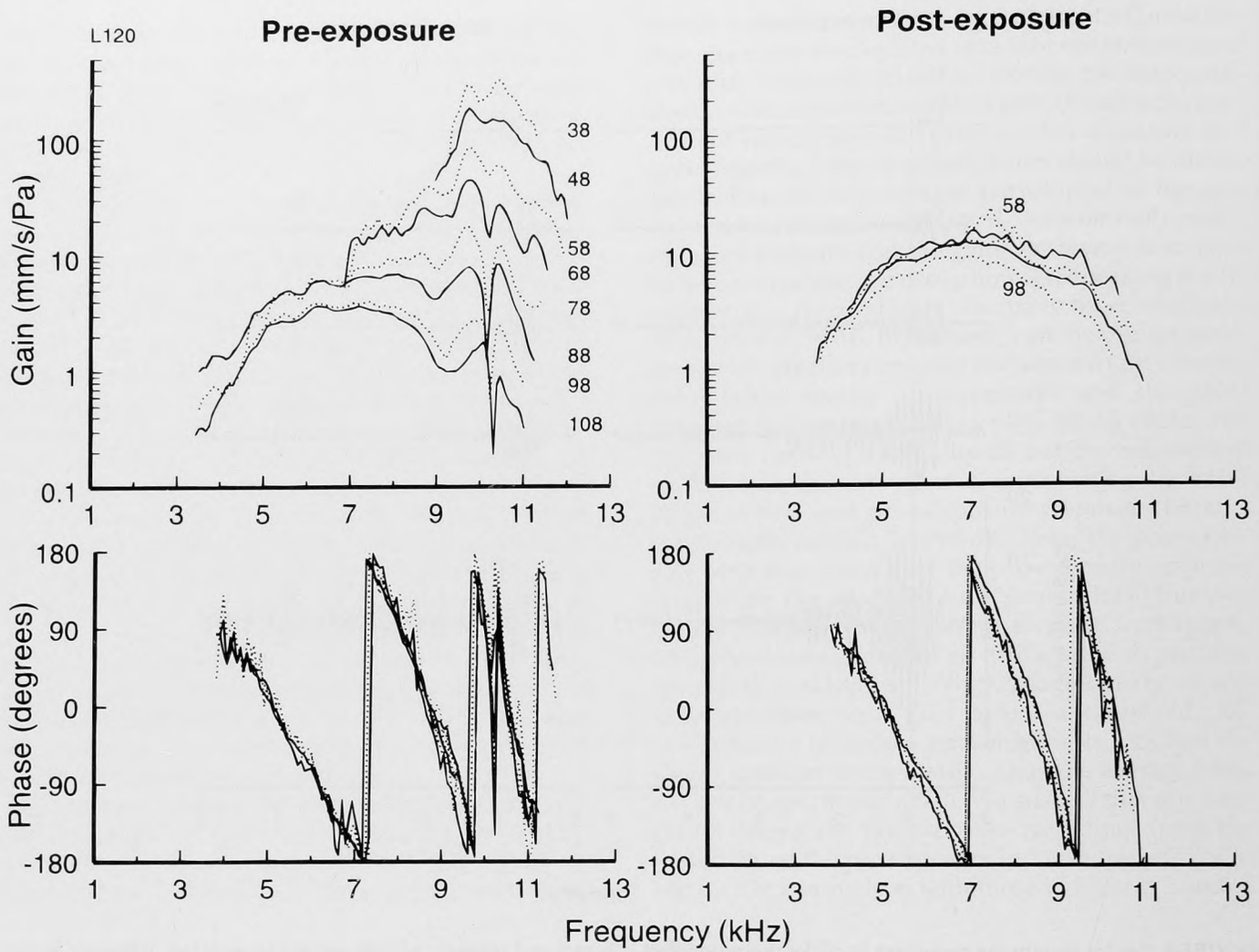

FIGURE 9 **Gain and phase frequency spectra of responses to clicks, computed by Fourier transformation of the time-domain traces of Figure 8.** Left panels: spectra for responses preceding a 4-minute exposure to a 7-kHz, 100 dB tone. Right panels: spectra for responses following overexposure. Each of the upper two panels consists of a family of curves representing the velocity gain (velocity normalized to sound pressure level) of basilar-membrane responses to clicks as a function of spectral frequency (abscissa) and intensity (parameter, in dB SPL). The lower panels display response phases as a function of frequency.

frequencies near CF underwent substantial phase lags. The net phase lags were largest (45–180 degrees) at frequencies close to or somewhat higher than CF.

The upper panels of Figure 11 show phase-vs.-intensity curves for pre- and post-exposure responses to CF tones in 4 cochleae. Pre-exposure (upper panel), all the phase curves exhibited some (non-monotonic) dependence on stimulus intensity: phase leads increased with intensity at low and moderate stimulus levels, but tended to decrease at intensities higher than 60–70 dB. The post-exposure curves (middle panel) became less intensity dependent and showed substantial phase lags relative to the pre-exposure curves.

The lower panel of Figure 11 shows the net phase effects of overexposure on responses to CF tones, as a function of stimulus intensity. In accordance with the magnitude effects of overexposure, which were largest at low stimulus levels (Figs. 1–5), overexposure induced phase lags that were generally largest (60–100 degrees) at CF tone intensities below 50–70 dB and tended to diminish at higher stimulus intensities.

For two of the 4 cochleae (L110 and L120), two pre-exposure phase curves are shown in the upper panel of Figure 11, one measured early in the experiment (dashed lines) and the other immediately preceding acoustic overstimulation (solid lines). These curves can be used to further explore the extent to which cochlear deterioration might have influenced the estimation of the effects of overstimulation. As noted above, cochlea L110 suffered a 7-dB loss of sensitivity at CF during the 3-hour interval required for the measurement of the pre-exposure frequency-response data of Figure 4 (left panel). Figure 11 shows that this sensitivity loss was accompanied by phase lags

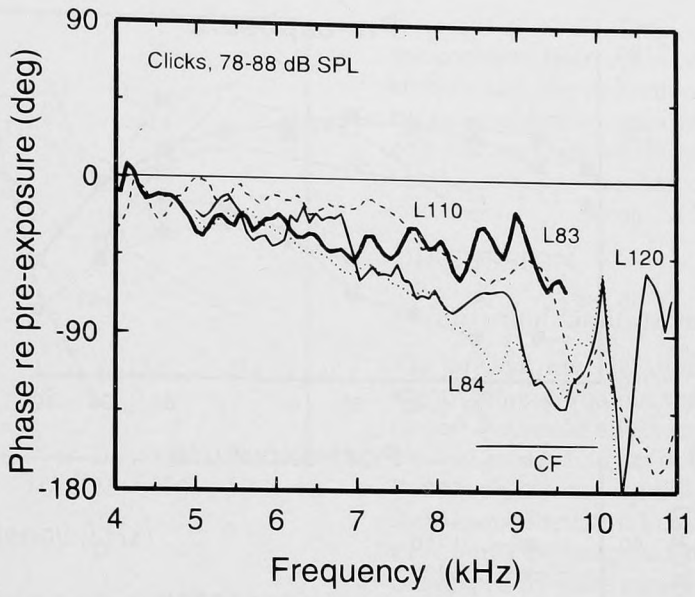

FIGURE 10 **Phase effects of acoustic overstimulation.** Net changes in phase spectra of responses to clicks are shown for 4 cochleae (including some of the responses of Fig. 9). Each cochlea is represented by a single curve computed from responses to clicks with peak level of 78–88 dB SPL. Negative phases represent relative lags induced by overexposure.

amounting to 27 degrees or less, depending on intensity. The post-exposure phase curve, measured some 81 minutes after obtaining the control pre-exposure curve, exhibits net lags (bottom panel) that are several-fold larger than those incurred during the 3-hour interval. The corresponding curves for cochlea L120 depict a similar contrast: pre-exposure phase changes were no larger than 23 degrees in a time span of 49 minutes but net post-exposure phase lags (bottom panel) amounted to 81–120 degrees, measured within 25 minutes of recording the pre-exposure control.

Effects of Overstimulation on Compound Action Potential Thresholds

In advance of attempting recordings from the basilar membrane, we used round-window recordings of compound action potentials (CAPs) to ascertain that in chinchillas 4-minute exposures to 7-kHz, 100-dB SPL tones produce threshold elevations which reach peaks at frequencies close to the CF (9–10 kHz) of our typical basilar membrane measurement site (Fig. 12). The patterns of threshold shift measured a few minutes after exposure have sharp and consistent low-frequency boundaries at the frequency of overstimulation, more variable and gradual high-frequency slopes and threshold-shift peaks of 30–42 dB at 8–10 kHz.

A confounding factor in interpreting the results of some previous studies of acoustic overstimulation is that it is not clear whether the exposures used produced solely transient hearing loss or actually could cause permanent damage. In the present investigation, the exposure intensity was designed to produce large, yet reversible, threshold shifts in normal cochleae. Figure 13 illustrates the recovery of CAP thresholds in two otherwise intact cochleae following 4-minute exposures to a 7 kHz, 100-dB SPL tone. This exposure caused immediate threshold elevations which were largest for 10 kHz stimuli. After an initial recovery of only about 6 dB during the first 1–2 hours post-exposure, thresholds declined steadily but very slowly. In one cochlea, complete recovery at 10 kHz was not reached until 30 hours had elapsed; in the other cochlea, a residual 9-dB elevation remained at 24 hours post-exposure.

Comparison of the Effects of Acoustic Overstimulation on Compound Action Potential Thresholds and Basilar-Membrane Responses

CAP thresholds were measured before and after overexposure (Fig. 12) in two of the cochleae (L83 and L84) for which basilar-membrane recordings are available (Fig. 2). CAP threshold measurements were performed using stimulus frequencies—8.4 and 10 kHz—closely flanking the basilar-membrane CF (9 kHz). Interpolation between these frequencies yields estimates of CAP threshold shifts at 9 kHz of 29 dB in cochlea L83 and 28 dB in L84. For stimulation with low-level CF tones, corresponding basilar-membrane response reductions were 17 and 25 dB. Thus, in these two cochleae most (59% and 89%) of the threshold shift measured in the auditory nerve could be accounted for by reductions in basilar-membrane responses to low-level (i.e., near-threshold) CF stimuli.

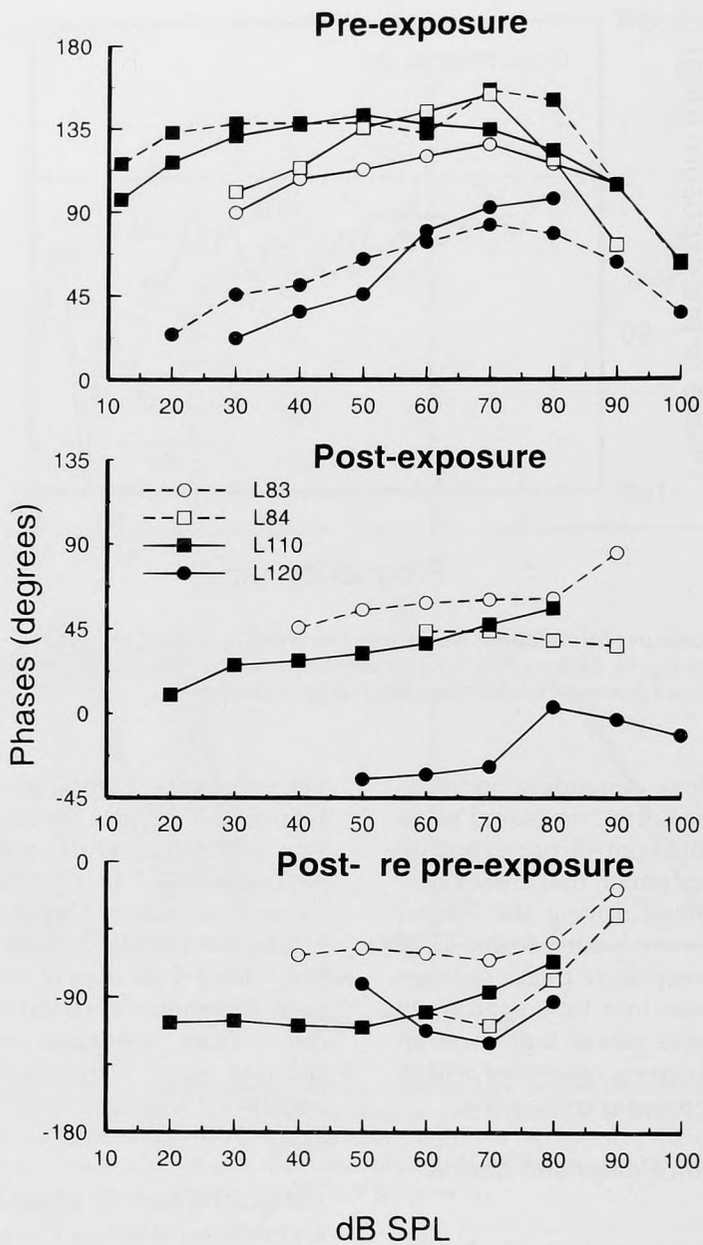

FIGURE 11 **Phase effects of acoustic overstimulation in responses to CF tones.** Upper panel: the phases for responses immediately preceding overstimulation plotted as a function of stimulus intensity. Also shown are phases for responses recorded early in the experiments in two cochleae (dashed lines). Middle panel: the phases for responses immediately following overstimulation. Lower panel: the net phase effects of overstimulation. Pre-exposure phases were subtracted from post-exposure phases so that negative phases represent relative lags induced by overstimulation.

Acoustically-induced CAP threshold shifts in intact cochleae (T3–T6 in Fig. 12) generally exceeded the magnitude of changes induced by the same exposures on basilar-membrane responses to CF tones. CAP threshold elevations in these intact cochleae reached peak values of 33–42 dB (average: 38 dB), whereas basilar-membrane response reductions at low stimulus levels ranged between 9 and 25 dB. It is likely that the discrepancy resulted, at least partly, from cochlear injury incurred in opening the otic capsule. In the four cochleae for which basilar-membrane recordings are illustrated in the figures, surgically-induced sensitivity losses at CF at the time of overexposure ranged between 8 and 31 dB, estimated using CAP thresholds and changes in mechanical sensitivity at CF. Table I lists these losses and also the losses due to the acoustic overexposure. There is a clear negative correlation ($r = -0.95$) between pre-exposure and post-exposure losses. A linear regression of post-exposure losses on pre-exposure losses has a slope of –0.74 dB/dB and an

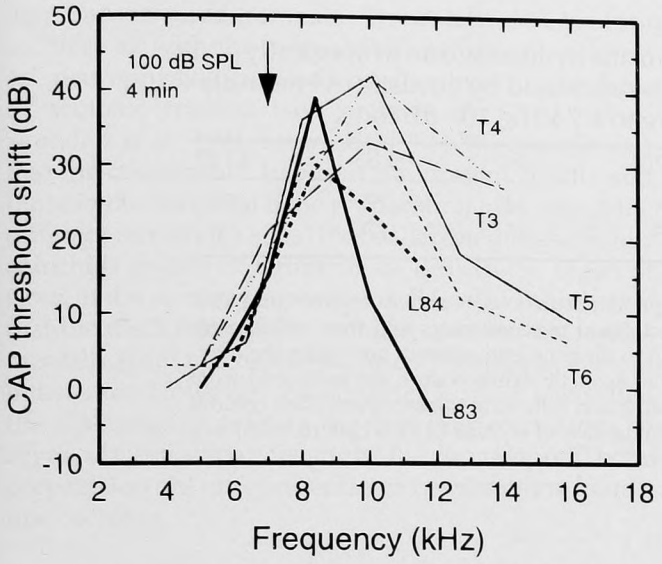

FIGURE 12 **Initial threshold elevations in chinchilla cochlear responses induced by exposures to intense tones.** Compound action potential (CAP) thresholds were measured in 6 chinchillas immediately preceding and following a single 4-minute exposure to a 7-kHz tone presented at 100 dB SPL. The ordinate indicates threshold shift (i.e., threshold after exposure minus threshold before exposure) in decibel units. The abscissa indicates the frequency of the test tone pips used to elicit CAPs. Thresholds were measured at 0.25–0.5 octave intervals. The measurements in two of the chinchillas (labeled L83 and L84) were obtained nearly concurrently with basilar-membrane recordings (see Fig. 2).

intercept of 32 dB. Thus, one may postulate that, had the cochleae been entirely normal at the time of overstimulation, the reduction in basilar membrane sensitivity would have amounted to 32 dB, i.e., 76–97% (or 84% on average) of the threshold elevations seen in CAP recordings in intact cochleae.

DISCUSSION

Validity and Limitations of the Present Findings

The present investigation shows that acoustic overstimulation with tones that cause large but temporary neural threshold shifts in intact cochleae induce substantial acute changes in basilar-membrane vibrations that can account, at least qualitatively, for the major features of the neural changes. This conclusion, however, must be tempered by the fact that the effects of overexposure were measured in cochleae that were not intact but had suffered surgically-induced trauma. Thus, our findings may not be fully applicable to overstimulation in normal cochleae. The most extreme and damaging possibility is that, had the cochleae been in fact totally normal, the effect of overstimulation on basilar-membrane vibrations would have been less traumatic than those we actually measured in surgically-traumatized cochleae. For example: the pre-existing surgical damage might have turned an otherwise temporary threshold shift into a permanent one. We believe that, in fact, pre-existing damage *reduces*, rather than enhances, the acute *net* effects (i.e., the post-exposure gains minus the pre-exposure gains) of overstimulation on basilar-membrane vibration. This belief is

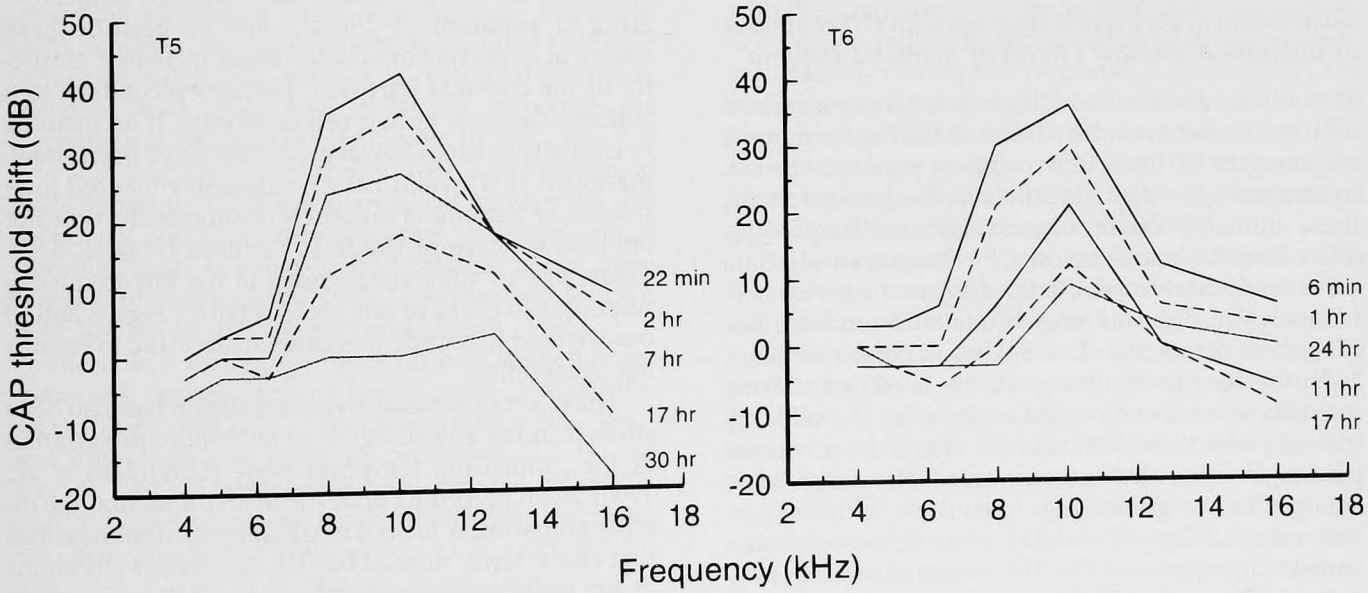

FIGURE 13 **Recovery of cochlear sensitivity following exposure to intense tones.** Two chinchilla cochleae were exposed for 4 minutes to a 7-kHz tone presented at 100 dB SPL. The two panels display CAP threshold shifts as a function of test frequency (abscissa) and time (minutes or hours) elapsed since overstimulation (parameter).

TABLE I
Comparison between pre-exposure sensitivity losses (due to surgically-induced trauma) and the net sensitivity losses caused by acoustic overstimulation using a 4-minute exposure to a 7-kHz, 100-dB tone.

	L120	L84	L83	L110
Pre-exposure sensitivity loss (dB)	8	14	17	31
Net effect of overstimulation (dB)	25	25	17	9

All sensitivity losses are expressed in decibels. The pre-exposure losses are derived from measurements of CAP thresholds preceding the initial mechanical measurements and from subsequent basilar-membrane sensitivity shifts at CF (if any) up to the time immediately preceding the overstimulation. The net basilar-membrane losses due to acoustic overexposure are measured from responses to low-level CF tones immediately preceding and following the exposure. Each column represents one cochlea, identified as in the figures. In the case of cochlea L110, which received two exposures, only the effect of the first exposure is given

based on the following facts. 1) Basilar-membrane vibration in damaged guinea pig cochleae are relatively invulnerable to acoustic overstimulation (Zhang *et al.*, 1994). 2) There is a clear negative correlation between the *net* effect of overexposure on chinchilla basilar membrane vibration and the extent of pre-exposure surgically-induced trauma (Table I). The loss of basilar-membrane sensitivity due to overstimulation approximates the CAP threshold elevation measured in intact cochleae if this negative correlation is taken into account. 3) The *net* effects of furosemide, an ototoxic diuretic whose effects on basilar-membrane vibration resemble those of overstimulation, are also negatively correlated with pre-existing surgically-induced trauma (Ruggero and Rich, 1991b, p. 1059).

Comparison of the Present Findings with Other Studies of Basilar-Membrane Effects of Acoustic Trauma

The only data in live animals available for comparison with the present results consist of basilar membrane responses to CF tones in two guinea pigs and one cat. In contrast with the chinchillas in the present study, these animals received overexposures at frequencies other than 0.5 octave below CF. The guinea-pig data were accidental byproducts of measuring velocity-intensity functions for long-duration CF tones (Sellick *et al.*, 1982; Patuzzi *et al.*, 1984). As in chinchilla (Figs. 1–3), the sensitivity changes were confined to low-intensity stimuli and tended to linearize the velocity-intensity functions. Patuzzi *et al.* (1984) described the phase changes that accompanied the magnitude changes in one guinea pig. In contrast with the present results (Figs. 10 and 11), acoustic overexposure caused large phase leads. The effects of overstimulation (4-kHz tone, 132 dB, 1-minute duration) were measured in one cat cochlea (Cooper and Rhode, 1992). Consistent with our findings in chinchilla, overstimulation caused reduction, linearization and large phase lags in basilar-membrane responses to CF (34.5 kHz) tones.

The changes in basilar-membrane response phases induced by acoustic overstimulation in chinchilla—reduction or abolition of intensity-dependent group delays and overall phase lags at frequencies near CF (Figs. 10–11)—resemble the effects of ototoxic drugs (Ruggero and Rich, 1991b; Recio and Ruggero, 1995; Ruggero *et al.*, 1996). From a theoretical perspective, relative phase lags at frequencies near the CF might be considered inappropriate counterparts of the reduction in sharpness of tuning since in many systems, including the normal basilar membrane, sharper tuning tends to be accompanied by larger group delays (Geisler and Rhode, 1982). The phase lags may be linked to the lowering of apparent CF (Figs. 5 and 9): both of these effects of overexposure could reflect increased elasticity of the cochlear partition, perhaps secondary to a stiffness decrease of hair cell stereocilia. If an increase in elasticity existed, however, it must have been small, inasmuch as it should have resulted in enhanced low-frequency responses but such enhancement was not obvious in three of the four cochleae (Figs. 4, 5, 7). There was an apparent increase in the low-frequency responses to clicks of one cochlea (L120; Figs. 8 and 9) but we tend to ascribe this to an artifact due to recording instabilities.

The effect of acoustic overstimulation has also been studied in the apical region of an *in-vitro* preparation of the guinea-pig temporal bone (Ulfendahl *et al.*, 1993). Even preceding acoustic overstimulation the *in-vitro* preparation lacks the CF-specific nonlinearities that characterize normal basilar-membrane vibrations at the cochlear base. Surprisingly, "following overstimulation . . . the vibration amplitude generally increased . . ." (Ulfendahl *et al.*, 1993). This unique finding is difficult to reconcile either with basilar-

membrane measurements in relatively healthy cochleae or with the extensive literature on anatomical, neurophysiological and psychophysical correlates of acoustic trauma (see reviews by Clark, 1991; Saunders *et al.*, 1991, Schmiedt, 1984). It is conceivable that the discrepancy between the present results and those in the temporal bone preparation may reflect the different recordings sites (the basilar-membrane in our chinchilla *in-vivo* experiments *vs.* cells in the organ of Corti in the *in-vitro* preparation). It is also conceivable that the mechanics of the cochlear apex differs fundamentally from mechanics at the cochlear base, even in intact ears. However, we consider it most likely that the discrepancy reflects the fact that recordings of organ of Corti vibrations in the *in-vitro* temporal bone preparation are unrepresentative of vibrations in normal cochleae.

Relation Between the Effects of Acoustic Overstimulation on Basilar-Membrane Vibration and on Auditory-Nerve Fibers and Inner Hair Cells

The effects of acoustic overexposure on basilar-membrane vibrations resemble and probably largely account, at least qualitatively, for the most prominent cochlear effects of acoustic overexposure, namely the CF-specific response reductions universally reported in studies of acoustic overstimulation in the auditory-nerve (e.g., Cody and Johnstone, 1980; Salvi *et al.*, 1983; Liberman and Dodds, 1987) and in inner and outer hair cells (Cody and Russell, 1988). Thus, the present findings tend to undermine theories of temporary threshold shift that would assign a central role to alterations of inner hair cells, notably Hunter-Duvar's (1977) proposal that floppy stereocilia constitute the reversible structural basis of temporary threshold shift. However, an involvement of the inner hair cells cannot be ruled out, since it is conceivable (albeit, unlikely) that inner hair cells can influence the mechanics of the basilar membrane. In any case, it is probable that exposures that induce acute threshold shifts substantially larger than 40 dB involve the disruption of cochlear processes other than mechanical vibration, such as synaptic transmission at the inner hair cells (e.g., Spoendlin, 1971; Liberman and Mulroy, 1982; Robertson, 1983; Henry and Mulroy, 1995).

The effects of intense tones on basilar-membrane responses cannot account for changes in auditory-nerve thresholds (e.g., Liberman and Dodds, 1987) and inner hair cell receptor potentials (Cody and Russell, 1988) at stimulus frequencies well below CF, since at these frequencies basilar-membrane responses are linear and rather invulnerable to acoustic trauma (Figs. 3–5, 7, and 9) or ototoxic drugs (Ruggero and Rich, 1991b; Recio and Ruggero, 1995; Ruggero *et al.*, 1996; however, see Murugasu and Russell, 1995). Auditory-nerve threshold elevations at "tail" frequencies, often paralleled by reduced spontaneous activity, have been reported to accompany temporary threshold shift (Liberman and Dodds, 1987). These elevations might reflect reductions in inner hair cell receptor potentials (Cody and Russell, 1988) and/or vacuolization, swelling or other alterations in afferent dendrites (Spoendlin, 1971; Liberman and Mulroy, 1982; Robertson, 1983; Henry and Mulroy, 1995). The response reductions in inner hair cell potentials may involve an intrinsic disruption of mechanical-to-electrical transduction, as discussed below in the context of outer hair cells. Paradoxically, hypersensitivity of tuning-curve tails and increases in spontaneous activity have been reported in auditory-nerve fibers as a result of acoustic overexposure (Liberman and Dodds, 1987). Again, such changes cannot be explained in any simple way by the present basilar-membrane data.

Cellular Mechanisms for the Effects of Overexposure on Basilar-Membrane Vibration

The alterations of basilar-membrane function induced by exposure to intense tones resemble changes following death or surgical damage (Rhode, 1973; Sellick *et al.*, 1982; Robles *et al.*, 1986) and intoxication with furosemide or quinine (Ruggero and Rich, 1991b; Recio and Ruggero, 1995). This resemblance is not unreasonable if one views CF-specific nonlinearities, sharp frequency tuning and high response sensitivity as a triad of features of basilar-membrane vibration that are inextricably interlinked (Ruggero *et al.*, 1996) and ultimately dependent on a feedback mechanism, presumably centered in the outer hair cells (Davis, 1983; Brownell *et al.*, 1985; Ruggero and Rich, 1991b; Murugasu and Russell, 1996). Whatever interferes with the components of the feedback mechanism will, of necessity, reduce or abolish all three features of normal basilar membrane response. Conceivably, the feedback mechanism could be disturbed in its reverse branch (electrical-to-mechanical transduction). For example, intense sounds might alter the turgor of the outer hair cell, thus changing its voltage-to-motion gain (Kakehata and Santos-Sacchi, 1995).

At present, however, the most likely hypothesis is that acoustic trauma primarily disrupts mechanical-to-electrical transduction in outer hair cells, reducing the receptor potential and, secondarily, also reducing the mechanical assist which outer hair cells, presumably, provide to basilar-membrane vibrations. Direct evidence for a primary effect on mechanical-to-electrical transduction comes from intracellular recordings from outer hair cells: acoustic overstimulation reduces the magnitude of receptor potentials elicited by low-frequency tones in higher-CF cochlear regions (Cody and Russell, 1988). Parallel results have been obtained in recordings of low-frequency cochlear microphonics at the base of the cochlea (Patuzzi *et al.*, 1989a). Since basi-

lar-membrane vibrations are linear and unaffected by acoustic overexposure at stimulus frequencies well below CF, the alteration of receptor potentials (or their reflection, cochlear microphonics) implies that overexposure acts at a stage of signal transformation located central to basilar-membrane vibration. It is not clear, however, whether the transduction conductances are themselves affected. Experiments using cochlear microphonics (Patuzzi *et al.*, 1989a, 1989b) support this notion, since they suggest that a certain percentage of transduction channels becomes nonfunctional following overexposure. On the other hand, intracellular recordings from outer hair cells (Cody and Russell, 1988, 1992) and Hensen's cells (whose AC electrical potentials probably follow the receptor potentials of outer hair cells; Zhang and Zwislocki, 1995) seem to contradict it, showing that maximal transduction currents can be elicited by sufficiently intense stimulation.

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