Loud Sound-Induced Changes in Cochlear Mechanics

ANDERS FRIDBERGER,5 JIEFU ZHENG,1,3 ANAND PARTHASARATHI,4 TIAN YING REN,1 AND ALFRED NUTTALL1,2
1Oregon Hearing Research Center, Department of Otolaryngology/Head and Neck Surgery, Oregon Health & Science University, Portland, Oregon 97239; 2Kresge Hearing Research Institute, The University of Michigan, Ann Arbor, Michigan, 48109-0506; 3Department of Otolaryngology, Chinese PLA General Hospital, Beijing 100083, P.R. China; 4Bose Corporation, Framingham, Massachusetts 01701; and 5Karolinska Institutet, Department of Physiology and Pharmacology, SE-171 77 Stockholm, Sweden

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Fridberger, Anders, Jiefu Zheng, Anand Parthasarathi, Tianying Ren, and Alfred Nuttall. Loud sound-induced changes in cochlear mechanics. *J Neurophysiol* 88: 2341–2348, 2002; 10.1152/jn.00192.2002. To investigate the inner ear response to intense sound and the mechanisms behind temporary threshold shifts, anesthetized guinea pigs were exposed to tones at 100–112 dB SPL. Basilar membrane vibration was measured using laser velocimetry, and the cochlear microphonic potential, compound action potential of the organ of Corti AC potentials were unaffected by loud sound, indicating that transducer channels remained intact. In most experiments, both the basilar membrane and the cochlear microphonic response to the 12-kHz overstimulation was constant throughout the duration of the intense stimulus, despite a large drop of cochlear sensitivity. It is concluded that the reduction of basilar membrane velocity that followed loud sound was caused by changes in cochlear amplification and that the cochlear response to intense stimulation is determined by the passive mechanical properties of the inner ear structures.

INTRODUCTION

Loud sound is an important part of our acoustic environment and prolonged exposure may cause substantial disability. However, we have very limited knowledge about the response of the hearing organ during sustained exposure to intense sound. Given the large number of studies dealing with the consequences of acoustic overstimulation, it is important to clarify how the inner ear responds to such stimulation.

If loud sound exposure is severe enough, a permanent threshold shift occurs. Generally, permanent threshold shifts are associated with damage to hair cells and the afferent dendrites contacting the inner hair cells. The underlying mechanisms may involve local metabolic factors, such as the generation of free radicals (Yamasoba et al. 1998), calcium concentration increase (Fridberger et al. 1998), and excitotoxicity caused by excessive glutamate release from the inner hair cells (Duan et al. 2000; Puel et al. 1998). For the most severe exposures, there is probably also a component of pure mechanical damage to the organ of Corti and the basilar membrane (for review, see Borg et al. 1995).

When less intense stimulation is used, a temporary threshold shift (TTS) may occur. The TTS recovers within seconds to 2–3 wk, depending on the exposure parameters. The mechanism behind TTS is unclear. Theories about its origin fall into two general categories. The first associates TTS with alterations of outer hair cell (OHC) stereocilia or transducer channels (Patuzzi et al. 1989a; Ruggero et al. 1996) and the second category implies alterations at the level of the OHC cell bodies such as depolarization (Cody and Russell 1985) or stiffness changes (Chan et al. 1998).

The goal of the present study is to describe alterations of cochlear function during loud sound exposure and to further investigate the origin of TTS. To accomplish this, we have used laser interferometry to measure basilar membrane (BM) vibrations in response to repeated 100–112 dB SPL tone bursts. These BM vibrations provide an excellent indicator of the functional state of the organ and show a high degree of correlation with the activity of the auditory nerve fibers. In addition, we used recordings of local electric AC potentials in the organ of Corti and the cochlear microphonic potential (CM) measured at the round window of the cochlea as indicators of OHC mechnoelectrical (“forward”) transduction.

METHODS

Animal preparation

Young pigmented guinea pigs weighing 200–400 g were anesthetized using a combination of ketamine (40 mg/kg im) and xylazine (10 mg/kg im) with supplemental doses given on a schedule or as needed, judging from the leg withdrawal response to a toe pinch. The rectal temperature and electrocardiogram were monitored. The animal temperature was kept at 37–38°C using a heating blanket. All animals were tracheotomized and, following application of local anesthetic, the head of the animal was fixed in a head holder that was heated to prevent cooling of the head and cochlea. Mechanical ventilation was

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not used. Part of the animal’s mandible was removed to expose the bulla, which was opened, and a silver wire recording electrode was placed in the round window niche. Using a chlorided ground electrode placed in the neck muscles, the $N_0$–potential threshold (10 $\mu$V at the round window) for the compound action potential (CAP) was determined. The CAP response to gated tone bursts near threshold is highly frequency specific (e.g., Dallos and Cheatham 1976a; Johnstone et al. 1979). The values for the CAP threshold given in the ensuing text refers to the average threshold at 16 and 18 kHz [the two frequencies closest to the characteristic frequency (CF) of the recording location on the BM]. Following the determination of the CAP threshold, the styloid process and soft tissues were removed to expose the bulla widely. The bulla was opened using a scalpel and dental burr used at low speeds, and the tendons of the middle ear muscles were transected. During surgery, the “simple” difference tone evoked by continuous tones at 18 and 18.9 kHz was recorded by the round window electrode (i.e., the round window signal at the frequency of 900 Hz) (Nuttall and Dolan 1993). When the amplitude of this potential decreased, surgery was halted to allow the ear to recover. The cochlea was opened by gently shaving the bone of the basal turn over the scala tympani, the size of the opening being approximately $300 \times 400$ $\mu$m, and a gold-coated glass bead (~20 $\mu$m diam) was placed on the BM. To avoid optical distortions and fluid vibrations affecting the optical path length, a piece of glass cover slip was subsequently placed over the opening.

**Sound stimulation and calibration**

Stimuli were generated through custom Labview programs that controlled the data acquisition and stimulus generation system described below. Stimuli were delivered to the ear either through a pair of Bruel and Kjaer 1/2-in. condenser microphones driven by custom power amplifiers or through a RadioShack Supertweeter speaker. The microphones were coupled to the external ear canal through a custom speculum that also housed a probe microphone (Etymotic 10 B+) for the monitoring of the sound pressure delivered to the ear. The tip of the probe microphone was approximately 8 mm back from the tip of the speculum. This system was capable of generating sound pressures $\leq 103$ dB SPL at 12 kHz. The Supertweeter speaker was capable of generating $\leq 125$ dB SPL at a distance of 11 cm from the ear canal; it was calibrated at the end of each experiment through placing a Bruel and Kjaer 1/8-in. condenser microphone at the entrance of the animal’s ear canal, taking care to ensure that none of the equipment surrounding the animal was moved.

For the acquisition of tuning curves, a pseudorandom noise waveform was synthesized by adding equal amplitude sinusoids with frequencies between 4 and 24 kHz, the phase of each frequency component determined by a random number generator. The intensity of the noise ranged from 10 to 90 dB SPL; it was presented 100–1,000 times at each intensity to average the data. Tuning curves were obtained by Fourier transformation of the averaged time waveforms.

**Overstimulation was delivered in two different ways**

In protocol 1 (Fig. 1), a 17-kHz tone at approximately 55 dB SPL was used to probe the response at the expected CF of the recording location on the BM. A 12-kHz tone at 100–112 dB SPL was introduced 25 ms after the start of the probe tone. This intense tone had a duration of 225 ms and, when it ended, the 17-kHz probe continued for 50 ms to allow tracking of CF response alterations caused by the loud stimulus. In some experiments, the 17-kHz probe tone was replaced by a 90-dB SPL probe tone at either 1 or 4 kHz. Following the end of the probe tone, there was a pause to allow the ear to recover completely from the effects of the loud tone. In early experiments, this pause was 500 ms long; later it was increased to 8 s. This mode of overstimulation usually produced no sustained shift of auditory sensitivity. The reason for choosing 12 kHz as the frequency for the overstimulation was that previous experiments have shown that maximum damage to a specific cochlear region occurs when overstimulation is delivered one-half octave below the best frequency of that location (e.g., Lonsbury-Martin and Meikle 1978). Also, preliminary experiments showed that the BM recording location that was used in these experiments had a best frequency close to 12 kHz when stimulated at intensities exceeding 90 dB SPL. Thus both the 17-kHz tone and the 12-kHz tone excited the same cochlear region despite the large frequency difference.

In protocol 2 (Fig. 1), overstimulation was delivered as 100-ms tone bursts at 12 kHz (100–112 dB SPL) separated by a 150-ms pause. This stimulus was repeated for up to 2 h and it produced a pronounced threshold shift that showed limited tendency to recover within the time frame of these experiments (in most experiments, the response of the ear was measured for about 20 min after terminating the overstimulation). The response to several presentations of the intense stimulus were averaged and saved to disk. In early experiments, 100 averages were used, but in later experiments the number of averages was decreased (down to a software-imposed limit of two) to observe changes of the response occurring during the first few presentations of the stimulus. In addition, each individual BM response was displayed on an oscilloscope. This type of overstimulation affected not only the measurement site on the BM, but also a broad range of frequencies covering a large section of the basal turn (as judged from the alteration of the CAP). Before and after each type of overstimulation, several sets of BM tuning curves and multiple determinations of the CAP threshold were performed.

**Interferometry**

The procedures for recording the BM velocity with a commercial laser doppler velocimeter have been described (Nuttall et al. 1991). In brief, a custom-built microscope with a $\times 20$, 0.4 NA objective lens (Mitutoyo, Japan) was used to visualize the BM. The microscope was connected to a Polytec OFV-1000 He-Ne laser velocimeter (Polytec, Waldbronn, Germany). The laser beam of the instrument was fed into a sideport on the microscope and, through careful alignment, the interferometer and the CCD camera attached to the microscope had identical focal planes. The output of the interferometer consisted of a voltage proportional to the velocity of the target bead on the basilar membrane. This voltage was sampled by a Tucker-Davis Technologies System II A/D converter connected to a PC. In later experiments, a Computer Boards CIO-DAS16/M116 A/D converter was utilized...
instead. The voltage from the interferometer was low-pass filtered by an 8-pole filter with a 40-kHz cutoff frequency before sampling at either 100, 125, or 250 kHz. Due to the breathing movements of the animal, the signal-to-noise ratio of the interferometer sometimes underwent cyclic variations that adversely affected the quality of the data. When the signal-to-noise ratio declined, the interferometer generated large amplitude noise uncorrelated to BM motion. To avoid sampling this signal, the software controlling the acquisition rejected all records where the “raw” BM velocity voltage signal exceeded a prespecified threshold level. In later experiments, the signal-to-noise ratio was monitored directly, and when it fell below a predefined level, sampling was temporarily halted.

**Cochlear microphonic potentials**

The silver wire placed in the round window niche was used to record the CM simultaneously with the BM response. The potentials were amplified 10 times by a preamplifier with a 0.3-Hz high-pass filter, and subsequently by a custom 100 times amplifier, low-pass filtered at 40 kHz, and sampled on the hardware described above. To guard against electrical cross talk, the RadioShack speaker was contained in a grounded aluminum box, leaving an aperture to allow the sound to escape. When using this arrangement, the level of the cross talk was 40 dB below the microphonic potentials recorded from the animal.

**Organ of Corti AC potentials**

Sharp microelectrodes with impedance in the range 13–27 MΩ were pulled on a Sutter 2000 puller using 1-mm-diam borosilicate glass capillaries. Following the recording of BM velocities, electrodes were filled with 2 M KCl solution and advanced toward the organ of Corti through the scala tympani opening. Potentials were recorded with an Axon Instruments (Union City, CA) Axoprobe 1A amplifier with a HS-2 headstage using a ground electrode placed in the animal’s neck muscles. All potentials were amplified 1,000 times and recorded by the data acquisition system used for the BM velocity measurements. The electrodes were advanced until the BM was contacted; it was penetrated using short current pulses from the amplifier, resulting in electrode locations within the tunnel of Corti or spaces of Nuel. The location of the electrode tip was indicated by the magnitudes of the AC and DC voltage recorded by the electrode. Penetration of the BM was apparent through a transient negative potential followed by an increase of the AC response to a continuous low-level 17-kHz tone presented as the electrode was advanced toward the BM. The main advantage with recording from within the organ of Corti is that potentials seen by the electrode originate from a small number of hair cells due to the limited space constant of the organ of Corti fluid spaces, thus avoiding the phase interaction problems that may occur when using electrodes on the round window. This spatial selectivity of AC potentials recorded within the organ of Corti was seen clearly in recordings using pseudorandom noise stimuli. Figure 2 shows potentials recorded in a sensitive ear in response to such stimuli (dashed line, left scale). A distinct peak was found at the frequency corresponding to the electrode location. In contrast, CM recordings from the round window showed a broad bandpass characteristic without apparent tuning (not shown). BM velocities (Fig. 2, solid line, right scale) recorded 0.5 h earlier from a bead located close to the electrode had peak velocity at the same frequency as the organ of Corti AC potential. BM responses were recorded both before and after completion of the microelectrode measurements.

**Signal analysis**

Data were analyzed off-line using Fourier transformation of the time waveforms. The short-time Fourier transform was used to examine time-dependent response changes at specific frequencies, using a sliding 512-point Hanning window with 256 points overlap. This resulted in a time resolution of 2 ms and a frequency resolution of 244 Hz.

**RESULTS**

**Basilar membrane vibration**

The general characteristics of the BM response to low-level stimulation was similar to those described previously from experiments in the basal turn (for review, see Ulfendahl 1997), i.e., sharply tuned responses at low stimulus levels and compressive nonlinearity in the region around the CF. As outlined in Fig. 1, a stimulus with a frequency of 12 kHz was always delivered at or above 100 dB SPL, whereas a stimulus frequency of 17 kHz was used to investigate the response to moderate stimulation levels. From the time domain records, it could be seen that the 17-kHz response was reduced immediately after the end of the loud tone and that some recovery usually took place. Using the short-time Fourier transform, the amplitude change of the two frequency components as functions of time were computed. Figure 3 shows the result of this analysis method as applied to a preparation in which both the BM velocity and the round window CM were recorded. Excluding the 5-ms rise time, the BM response amplitude at 17 kHz was constant, but, as the loud tone started, an almost immediate suppression occurred (Fig. 3, arrow labeled “S”); suppression onset speed was presumably influenced by the 5-ms risetime of the loud tone). Control experiments showed that no such significant interaction between the tones was present in the acoustic stimulus, and the suppression was also less pronounced in damaged ears. After the end of the 12-kHz loud tone, the BM response amplitude at 17 kHz had decreased (Fig. 3A, arrow labeled “TTS”). A gradual but fast initial recovery was seen, but a residual loss of vibration amplitude...
was often present at the end of the recording. During the pause that followed each repetition of the stimulus there was further recovery, evidenced as a higher initial amplitude at 17 kHz during the next stimulus presentation. These features of the BM response were seen in 13 of 14 preparations.

In the experiment shown in Fig. 3, the 12-kHz stimulus was applied at 100 dB SPL. Excluding the short rise time, the BM response to the 12-kHz tone was constant throughout the stimulus. This was the case in all animals studied, regardless of the sensitivity of the ear. Thus, although the response to 17 kHz stimulation at a lower level was reduced by 9.7 dB immediately after the end of the loud tone, this change was in no way reflected in the high level response. Rather, the 12-kHz high level response appeared to be independent of any change of the low level 17-kHz response. Also, there was no difference in the response to the loud tone in records acquired early in the experiment compared with those acquired late, implying that time-dependent changes were not important for the loud tone.

Round window cochlear microphonic data

In seven different preparations, the round window CM was recorded simultaneously with the BM velocity responses. Figure 3B shows the CM recording for the same preparation as in Fig. 3A. As for the velocity, the response at 17 kHz was stable before the loud tone was introduced and an immediate suppression occurred when turning on the 12-kHz stimulus (Fig. 3B, arrow labeled S). When the loud tone was turned off, there was an immediate recovery of the CM, and the amplitude remained constant until the 17-kHz tone ended. Thus the gradual initial recovery seen in the BM velocity records was not present in the CM. There was a 0.8-dB decrease of the CM amplitude, but this small loss did not recover in the same way as the BM velocity.

To show the difference between the BM and the round window CM response following the termination of the loud tone, these records were plotted together in Fig. 4 using data from different animals, omitting the response to the loud 12-kHz tone. Thus the figure only shows the response to the 17-kHz CF tone before and after the overstimulation. The animal in Fig. 4A had suffered <5 dB loss of the CAP threshold due to surgery; the CF was 16.8 kHz, with highly nonlinear velocity responses at the CF as the stimulus intensity increased. At the onset of the 17-kHz tone, the CM had an amplitude of 2.7 μV. Immediately after the end of the loud tone, the CM amplitude had decreased by 1.4 dB to 2.3 μV and it remained stable until the 17-kHz tone was turned off. The BM velocity also showed a decrease after the end of the loud tone, but the loss was much more pronounced, 15 dB (Fig. 4A, arrow labeled “TTS”). A rapid recovery occurred and, immediately before turning off the 17-kHz tone, the residual loss was 6.4 dB. Thus, over the course of 40 ms, the BM had recovered by approximately 9 dB despite a constant CM amplitude. In the seven experiments in which the velocity and the CM were simultaneously recorded, four showed the behavior described above. In the remaining three preparations, the CM showed a similar behavior as the velocity. An example of a preparation in which the CM and BM response recovered in parallel is shown in Fig. 4B. Both the velocity and the CM amplitude were lower in this preparation, probably due to the substantial loss of sensitivity that the preparation had suffered. Following the end of the loud tone, a decrease of both the BM velocity and the CM occurred and both recovered rapidly and with a similar time course, although the BM velocity showed a residual loss of amplitude at the end of the recording.

Organ of Corti AC potentials

The lack of CM change in some preparations raised the possibility that the hair cell transducer currents generating the CM were unaffected by the loud sound. However, there was a substantial variability and, since the CM originates from a number of hair cells, the measured amplitude may be the result of phase interactions occurring between neighboring cells. Therefore microelectrode recordings were performed in four different preparations at sites very close to beads used for recording BM velocities. Figure 5A shows voltage responses from the organ of Corti plotted together with BM velocities from the same preparation: an animal with no loss of the CAP threshold prior to the recording of BM velocities. In this preparation and in all others in this series, there was a close correspondence between BM vibration and organ of Corti electric AC potentials. Following the loud tone, both BM and
organ of Corti AC potentials showed a transient reduction followed by rapid recovery.

For stimulus frequencies close to the CF of the recording site, BM vibration is amplified through active mechanisms dependent on the voltage changes generated by the gating of the transducer channels (Ashmore 1987; Brownell et al. 1985). Therefore the changes shown in Fig. 5A could be the result of damage to transducer channels, changes in OHC motility, or both. For low stimulus frequencies, BM vibration is not affected by the active mechanisms that enhance sensitivity at CF (Patuzzi et al. 1989b) and thus low-frequency stimulation can be used to examine changes in the transducer currents isolated from changes in OHC motility. Figure 5B shows organ of Corti AC potentials and BM vibration for a 4-kHz probe in the same preparation as Fig. 5A. The loud tone caused no significant change of either BM vibration or electric AC potentials. Iden-
tical results were obtained from other animals and were also duplicated when a 976-Hz probe was used.

The organ of Corti AC response to the intense 12-kHz tone was constant throughout its presentation, much like the BM response shown in Fig. 3A.

Prolonged exposures

To further investigate the cochlear response to high-intensity sound, some animals were additionally exposed to 100-ms tone bursts at 12 kHz. These tone bursts were repeated every 150 ms for up to 2 h, with the object of producing a pronounced and long-lasting threshold shift. Figure 6 shows an example of BM velocity and CM responses for this type of overstimulation. In this animal, the BM velocity response amplitude was stable throughout the recording, which lasted for 70 min. At the onset, the velocity was 6,700 μm/s and after 70 min it was 7,700 μm/s, a 1.2-dB increase. In most preparations, there were alterations of response phase during the overstimulation, but the extent and direction of the change varied between different animals with no consistent pattern emerging. The round window CM amplitude was unchanged during the overstimulation (Fig. 6B), which was delivered at an intensity of 106 dB SPL. Despite the invariance of the 12-kHz responses, the exposure produced a large CAP threshold shift. At 16 and 18 kHz, the CAP threshold was elevated by 33 dB, and at 20 kHz the threshold was shifted by 35 dB.

In the seven animals in which both the round window CM and the velocity responses to the 12-kHz overstimulation were recorded, four showed a round window CM decrease < 0.5 dB, one animal displayed a CM increase of 0.6 dB, and two animals showed large CM decreases. These two animals had a substantial loss of the CAP threshold before the overstimulation (~20 and ~23 dB, respectively). The average change of the CAP threshold due to the overstimulation was 23 dB (range 10–33 dB). The BM velocity was constant or slightly increased in five of seven animals. One animal died during the course of the exposure and in this animal there was an abrupt decrease of the CM and a more gradual decrease of the BM velocity. Before the death of the animal, both the velocity and the CM were stable. Death was accompanied by a large change of the CM phase in addition to the amplitude changes.

DISCUSSION

The findings of this study can be summarized as follows.

1) After exposure to a 12-kHz intense tone, the BM vibration amplitude was transiently reduced, but rapid recovery was seen in most preparations. This behavior was also seen in recordings of organ of Corti local electric AC potentials, but not always reflected in the round window CM.

2) Recordings of low-frequency organ of Corti AC potentials showed that the transducer currents underlying these potentials were unaffected by the intense sound. This finding leads to the conclusion that the TTS seen in our preparations was due to an alteration of cochlear amplification.

3) The BM and organ of Corti AC response to the 12-kHz overstimulation was constant throughout the duration of the stimulation, despite a large loss of cochlear sensitivity at CF. This was also the case for the round window CM, but given the problems inherent to this method of recording the CM, the interpretation is uncertain.

Cochlear responses to high-intensity sound

Despite a large number of studies addressing the mechanisms behind the damage caused by loud sound, we have had very limited insight into how the cochlea handles such high-intensity stimulation. Many sensory systems show some form of adaptation during an intense stimulus, but this appears to be absent in the round window CM, the BM velocity response, and the organ of Corti AC response to the 12-kHz stimulus. The BM response to each stimulus presentation was essentially a mirror of the input waveform; there was no evidence for any protective mechanism acting to decrease the vibration amplitude during the stimulus. In the awake animal, the middle ear reflexes may have some protective role (Borg et al. 1995), and it has also been shown that activation of both the lateral and the medial components of the efferent system decrease the magnitude of TTS (Rajan 2000; Cody and Johnstone 1982). In the present set of experiments, the middle ear muscles were cut and the efferent system was inactivated due to the anesthesia (Brown 1989).

At high stimulus levels, the response of the BM is believed to be determined by “passive” mechanics (the stiffness, mass, and friction of the inner ear structures) (Lighthill 1991; de Boer and Nuttall 2000). Since the inner ear showed a large loss of sensitivity at CF following overstimulation yet had a constant response to the loud tones, this indeed implies that the vibration of the BM at high stimulus levels is determined by the
passive mechanical properties of the cochlear partition and is not affected by the active mechanisms that enhance sensitivity at low levels. Most likely, these passive mechanical properties were constant during the stimulation, or a change of the vibration amplitude would have occurred. There was no indication of an alteration of the stiffness of the OHCs, such as that described following impulse noise (Chan et al. 1998). This may be because the stimulation levels used in the present study were less intense compared with many other studies. In such a case, the physiological changes described here are likely to represent early events in the progression to a noise-induced hearing loss.

Electric responses of the cochlea

The CM is considered to be generated chiefly by the OHCs (Dallos and Cheatham 1976b) through the gating of their transducer channels in response to sound. Since it is an extracellular potential, a population of OHCs contribute to the measured response, the size of which is dependent on the stimulus level. For levels between 35 and 55 dB SPL, Russell and Nilsen (1997) showed that a length of the BM of approximately 1.25 mm was capable of responding to a 15-kHz stimulus, corresponding to about 440 OHCs. These OHCs presumably would be the same hair cells that contribute to the CM at these stimulus intensities. As the intensity of stimulation increases, larger sections of the BM begin to move, and for loud sounds of the intensity and frequency used in this study, a large part of the basal turn probably contributes to the measured response. The amplitude of the CM may be affected by phase cancellation effects between neighboring hair cells, producing complex patterns of reduction and enhancement of the CM amplitude when two tones are simultaneously presented (e.g., Nuttall and Dolan 1991). Such interactions probably also influence the CM amplitude during single frequency stimulation, and these interactions are a possible reason for the observed discrepancies between the round window CM and organ of Corti responses, since the latter represent localized responses of a very small number of cells around the electrode location. This improved spatial selectivity was evidenced by the close correspondence between organ of Corti tuning curves and the mechanical responses of the BM. Another possible reason for the discrepancy could be that hair cells close to the round window electrode contribute more to the measured response than the OHCs influenced by the loud sound. Such local weighting was observed by Patuzzi et al. (1989b).

The phase effects described above could be avoided through the use of intense low-frequency probe tones, since low frequency tones cause the BM to vibrate almost synchronously in the entire basal turn (Patuzzi et al. 1989b). The low frequency CM response may be affected by the response of hair cells from apical sites, but this potential problem was largely avoided here through the use of local recording of organ of Corti AC potentials. The fact that low frequency responses were unaltered despite the threshold loss that occurred is evidence that the transducer channels were not affected by the intense stimulation.

Cochlear mechanics during and following loud stimulation

In both the BM velocity and organ of Corti AC potentials, a reduction of the response amplitude at 17 kHz occurred during the presentation of the loud tone. Immediately following the end of the loud tone, the organ of Corti AC potentials and BM vibration were transiently reduced, but quickly recovered. Current theories of cochlear mechanics (e.g., Brownell et al. 1985; Nuttall and Ren 1995; Ruggero and Rich 1991) hold that the forward transduction of the OHCs (i.e., the OHC receptor potential that follows from stereocilia motion) drives the OHC motility. The OHC electromechanical ("reverse") transduction feeds energy back into the BM motion. The reduction of both BM velocity and organ of Corti AC potentials at 17 kHz is consistent with this general framework, but it is obvious that experiments using CF stimulation cannot answer the question whether the TTS was caused by alterations of forward or reverse transduction due to the fact that the two processes are linked so closely. The fact that organ of Corti low frequency AC potentials were unaltered in our study, however, is strong evidence that forward transduction was intact following the type of stimulation we used, since OHC reverse transduction does not influence the cochlear response at frequencies far away from CF (for review, see Robles and Ruggero 2001). Thus the data presented here suggest that an altered gain of the feedback path (OHC electromotility) was the mechanism behind the threshold shift seen in our preparations.

Several lines of evidence do indeed suggest that the gain of OHC electromotility can be modulated. Murugasu and Russell (1996) have shown that perfusion of the basal turn with acetylecholine (the efferent transmitter acting on the OHCs) causes a reduction of the BM vibration amplitude, probably through a calcium-dependent mechanism. Similarly, it has been shown that elevation of the intracellular calcium concentration in isolated OHCs causes an altered response to electrical stimulation (Dallos et al. 1997; Frolenkov et al. 2000).

Two previous electrophysiological studies have yielded results similar to those of the present study. Intracellular recording of receptor potentials from inner and outer hair cells during the presentation of 12.5-kHz loud tones showed that the AC potentials of both cell types had a constant amplitude despite a 23-dB reduction of CAP sensitivity at CF following the stimulus (Cody and Russell 1995). Also, Zhang and Zwislocki (1995) have shown that the AC potentials of Hensen cells (believed to closely reflect the potentials of OHCs in Mongolian gerbils) at low stimulus levels were much diminished after noise trauma. As the probe tone stimulus level increased, those potentials approached the amplitude seen before the exposure and, at approximately 70 dB SPL, pre- and postexposure amplitudes were equal. As noted by Zhang and Zwislocki, this result virtually rules out damage to the stereocilia or transducer channels. The threshold shift that occurred was thus attributed to damage to the active feedback mechanism inherent to the OHCs. In both these studies, as well as in the present study, the exposures were relatively short and generally at levels < 110 dB SPL. For exposures at higher intensities or for longer durations, it is likely that other pathological alterations occur (such as channel inactivation, destruction of stereocilia, synaptic damage, or elimination of hair cells).

Several studies have investigated the effects of acoustic overstimulation on the low level responses of the basilar membrane (Cooper and Rhode 1992; Patuzzi et al. 1984; Ruggero et al. 1996; Ulfendahl et al. 1993). In the basal turn, these studies showed reduction of the vibration amplitude, loss of compressive nonlinearity, and reduced best frequency. The reduction of
the vibration amplitude appeared to explain most of the TTS (Ruggero et al. 1996). The present study provides data to suggest that the gain of OHC reverse transduction is modulated by loud tones and that this mechanism is an early event that contributes to the development of TTS.

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