JARO

Spontaneous Basilar Membrane Oscillation and Otoacoustic Emission at 15 kHz in a Guinea Pig

A.L. Nuttall,[1,4] K. Grosh,[2] J. Zheng,[1] E. de Boer,[3] Y. Zou,[1] and T. Ren[1]

[1]*Oregon Hearing Research Center, Department of Otolaryngology and Head & Neck Surgery, Oregon Health & Science University, Portland, OR 97239-3098, USA*
[2]*Department of Mechanical Engineering and Applied Mechanics, The University of Michigan, Ann Arbor, MI 48109-2125, USA*
[3]*Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands*
[4]*Kresge Hearing Research Institute, The University of Michigan, Ann Arbor, Michigan 48109-0506, USA*

ABSTRACT

A spontaneous otoacoustic emission (SOAE) measured in the ear canal of a guinea pig was found to have a counterpart in spontaneous mechanical vibration of the basilar membrane (BM). A spontaneous 15-kHz BM velocity signal was measured from the 18-kHz tonotopic location and had a level close to that evoked by a 14-kHz, 15-dB SPL tone given to the ear. Lower-frequency pure-tone acoustic excitation was found to reduce the spontaneous BM oscillation (SBMO) while higher-frequency sound could entrain the SBMO. Octave-band noise centered near the emission frequency showed an increased narrowband response in that frequency range. Applied pulses of current enhanced or suppressed the oscillation, depending on polarity of the current. The compound action potential (CAP) audiogram demonstrated a frequency-specific loss at 8 and 12 kHz in this animal. We conclude that a relatively high-frequency spontaneous oscillation of 15 kHz originated near the 15-kHz tonotopic place and appeared at the measured BM location as a mechanical oscillation. The oscillation gave rise to a SOAE in the ear canal. Electric current can modulate level and frequency of the otoacoustic emission in a pattern similar to that for the observed mechanical oscillation of the BM.

INTRODUCTION

To the best of our knowledge, detailed measurements of a spontaneous vibration of the basilar membrane (BM) in a mammalian ear have not been reported. Since one hypothesis for the generation of spontaneous emissions (SOAEs) involves ''uncontrolled or under-damped'' oscillations of the cochlear amplification mechanism(s) [an idea that originated with Gold (1948)], such measurements could provide valuable clues about the amplification process. Perhaps this would enable one to distinguish spontaneous emissions linked to the somatic mechanics of the outer hair cells (OHCs) (He and Dallos 2000) from those that might arise from spontaneous motion of the stereocilia (Martin et al. 2003). In this report we describe a relatively high-frequency SOAE from a guinea pig ear, which had a counterpart as a basilar membrane spontaneous oscillation (SBMO).

Spontaneous otoacoustic emissions (SOAEs) are sounds that are emitted from a human or animal ear. They are believed to originate from oscillations of the organ of Corti, in particular, the basilar membrane. Starting with Kemp (1979a,b) and Wil-

---
Correspondence to: A.L. Nuttall · Oregon Hearing Research Center · Oregon Health & Science University · 3181 SW Sam Jackson Park Road, NRC04 · Portland, OR 97239-3098. Telephone: (503) 494-8032; Fax: (503) 494-5656; email: nuttall@ohsu.edu

son (1980), who reported that subjects with normal hearing produced them, SOAEs were systematically studied as being characteristic of a normal functioning cochlea (Zenner and Ernst 1995). The healthy organ of Corti possibly possesses a positive feedback mechanism to amplify sound-evoked vibrations; this mechanism may have an innate tendency toward spontaneous oscillation, e.g., by pathology (Ruggero et al. 1983).

It has also been proposed that SOAEs are due to inhomogeneities of the organ of Corti, causing multiple internal reflections in the cochlea (Kemp 1979a, b; Shera 2003). These inhomogeneities may be morphological variations such as the presence or absence of OHCs (Lonsbury–Martin et al. 1988; Hilger et al. 1995), or more subtle variations in the mechanoelectric properties of individual cells. Such variations could be involved when spontaneous emissions are ''provoked'' by loud sound exposure (Clark et al 1984; Powers et al. 1995). The proposed mechanism of multiple reflection requires the presence of intracochlear amplification. Discovery of an SBMO with an accompanying SOAE would provide the ''missing link'' between cause and effect.

The SOAEs of many animals have been studied and characterized. Humans tend to have a large number of relatively low-frequency pure-tone-like SOAEs, the frequencies of which have a nearly constant frequency spacing (Zweig and Shera 1995). The SOAE levels and frequencies are functions of the mechanical status of the cochlea such as lymphatic pressure (Wilson 1980) or circadian and menstrual rhythms (Velenovsky and Glattke 2002; Bell 1992) and can be suppressed by activity of the olivocochlear efferent system (Collet et al. 1990; Velenovsky and Glattke 2002).

Animal models of SOAEs to allow for mechanical studies have not been found. Evans et al. (1981) report on aged guinea pig with an SOAE at about 1 kHz. Ohyama et al. (1991) found a high incidence of low-frequency SOAEs in awake guinea pigs. However, in our experience an anesthetized guinea pig will rarely evidence a SOAE, and, when it occurs, the frequency is about 1 kHz. In 20 years of cochlear physiological studies (by author ALN), the total number of such animals is about six. It is probable that anesthesia greatly reduces the level of guinea pig SOAEs (Brown et al. 1990; Ohyama et al. 1991). Low-frequency SOAEs in the guinea pig have been used to study the effect of heartbeat on cochlear mechanics (Talmadge et al. 1993; Ren et al. 1995).

Low-frequency vibrations in the apical cochlear turn of the guinea pig have been recorded *in vivo* (Keilson et al. 1993). These vibrations would not be efficiently detected in the basal turn where the char-

acteristic frequencies are above 12 kHz. Teich et al. (1994) have reported the only other example of a high-frequency SBMO of which we are aware. In their study, the magnitude spectrum of the BM velocity (from an observation made through the cat round window membrane) showed ''spurious'' pure-tone motion. There was no measurement of ear canal sound to demonstrate that the SBMO had a SOAE counterpart.

MATERIALS AND METHODS

Animal preparation

The subject was a pigmented guinea pig (strain 2NCR, obtained from the Charles River Laboratory) weighing 300 g. Until the day of the experiment, it was housed in facilities approved by the American Association for Accreditation of Laboratory Animal Care at the Oregon Health & Science University. The experimental protocols described in this report were approved by the Committee on the Use and Care of Animals, Oregon Health & Science University. The animal was anesthetized using ketamine (40 mg/kg i.m.) and xylazine (10 mg/kg i.m.). Supplemental doses of ketamine and xylazine were given on a schedule or as needed, judging by leg withdrawal to a toe pinch.

Rectal temperature of the animal was maintained at $38 \pm 1°C$ with a servoregulated heating blanket. Cochlear temperature was additionally controlled by a lamp and a heated headholder. The electrocardiogram and heart rate were continuously monitored as measures of anesthesia level and general condition of the animal. A tracheotomy was performed and a ventilation tube was inserted into the trachea to insure free breathing. A ventral and postauricular combined approach was used to expose the left auditory bulla with a large part of the external ear being removed to facilitate placement of the acoustic speculum. The bulla was opened wide to expose the cochlea. The middle-ear muscle tendons were sectioned.

Cochlear electrophysiology

The compound action potential (CAP) was measured from a ball electrode made of Teflon-coated silver wire (75 μm in diameter) placed in the round-window niche. An Ag/AgCl wire was inserted into the neck medial to the exposed bulla to serve as the ground electrode. A plastic coupler with a loudspeaker (made of a 0.5-in. B&K microphone) and an Etymotic Research ER−10B[+] microphone was connected to the ear canal to deliver acoustic stimuli and to record the otoacoustic emissions (OAEs). Tones (10 ms in
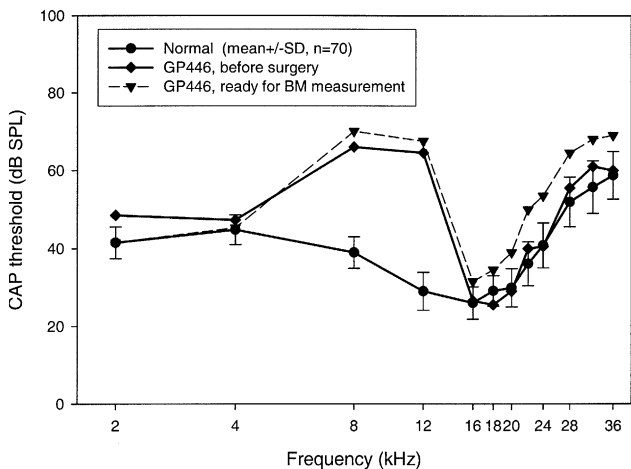
FIG. 1. Compound action potential (CAP) thresholds recorded from the round-window membrane of GP 466 (the guinea pig with spontaneous otoacoustic emission) compared to the mean threshold of normal animals measured in this laboratory. Before opening the cochlea (diamond symbols with solid line), there was a pronounced loss of sensitivity at the 8- and 12-kHz test frequencies. At the time of BM velocity recording (cochlea open and reflective beads placed), a small additional loss of sensitivity was evident for frequencies from 8 to 36 kHz.

duration, 1-ms rise/fall times) were generated using a 16-bit D/A converter (System II, Tucker Davis Technologies) and delivered to the ear canal as acoustic stimuli to evoke the CAP. The round-window signal was amplified 1000 times by AC preamplifiers (Grass Instrument Co. Model P15 and a custom-designed AC amplifier). The amplified signal was saved to hard disk and displayed on an oscilloscope for CAP threshold assessment. Detection of an N_1 component of 10 μV without averaging was used as the CAP threshold criterion. Figure 1 (to be described in more detail later) shows the CAP thresholds for the animal described in this article.

Electric current stimulation

Electric current was passed across the cochlear duct from bipolar wire electrodes constructed from Teflon-insulated platinum–iridium wire (50 μm in diameter). The bare end (<100 μm long) of one wire was placed in a small hole drilled into the scala vestibuli (SV) in the first cochlear turn (approximately at the 18-kHz tonotopic location along the length of the cochlea), while a similar bare end of the other wire was placed in a hole drilled into the scala tympani (ST). The current was delivered by a custom-made, opto-isolated constant-current stimulator.

To manipulate the SOAE and SBMO, a specialized current sequence was used. It consisted of a repeating pattern of a positive 100-μA current pulse (''positive''
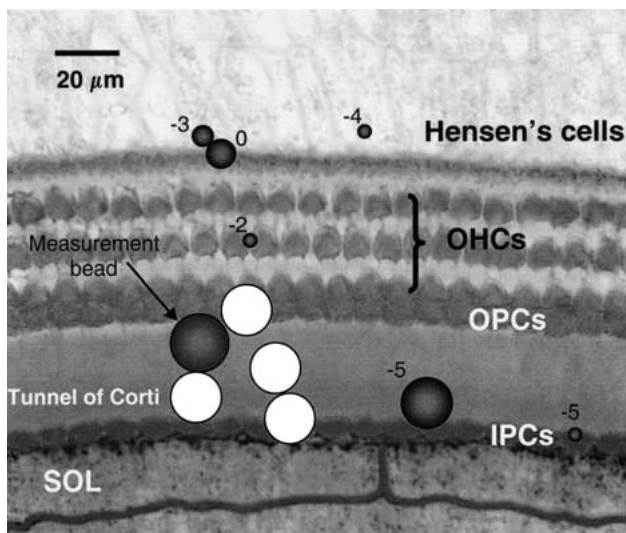

FIG. 2. A drawing of the basilar membrane (BM) area where measurements of the BM velocity were made. Depicted are the positions of gold-coated glass beads on the BM. Numbers beside six of the beads indicate the maximum BM velocity in dB of the spontaneous oscillation, relative to the one having the highest velocity (a bead at the boundary of the outer hair cells and Hensen's cells, marked ''zero''). The data were taken from the real-time spectrum analyzer, which computed 10 averages of the velocity magnitude spectrum. The approximate size and relative locations of the beads are given. White-colored beads were not measured. All data described in the following figures was obtained from the bead labeled ''measurement bead.'' OHCs: outer hair cells; OPCs: outer pillar cells; IPCs: inner pillar cells; SOL: spiral osseous lamina. Scale bar = 20 μm.

defined as SV electrode relative to ST), zero current (labeled ''control'' in figures to be described later on), a negative 100-μA current pulse, and zero current again. A rise-fall time (0.15 ms) was chosen to avoid transient BM velocity responses to the current pulses. One period of this pattern lasted 26.8 ms. See figures to be described later for a diagram of the current application.

Measurement of basilar membrane velocity

Basilar membrane (BM) velocity was measured at the 18-kHz best-frequency location. A small opening was made in the first-turn scala tympani bony wall of the cochlea. Glass beads ($\sim$20-μm diameter gold-coated and $\sim$3-μm diameter uncoated) were placed onto the BM to serve as reflective objects. See the placement of the beads for this experiment in Figure 2. The laser beam of a Doppler velocimeter (Polytec Corp. OFV 1102) was focused on one of the beads (Nuttall et al. 1991). The signal output of the velocimeter was digitized and averaged using custom-written software. BM velocities were determined and processed using Matlab[®] software.

Sound stimulation and otoacoustic emission measurement

Sounds were always given synchronously with the current, both being synthesized by custom-written software. In one paradigm, tones were 30 ms in length, shaped with 1-ms rise fall times, separated by 20 ms, and sampled at a rate of 250 k samples/s. They were presented as above and below SBMO-frequency "probe tones" and caused alterations of the SOAE and the SBMO.

In addition to tones, noise signals were used, coincident with the current pulses. The noise signal was played continuously through the speaker with a period of 26.8 ms. Each of the four 6.7-ms noise segments (corresponding to the current pulses, see above) had a bandwidth of 6–24 kHz.

Fast Fourier transforms of the four segments of the recorded signal (500–3000 averages) were computed, excluding the initial 1.5 ms of each current segment to avoid transients. Therefore, the measured portion of each segment was 5.1 ms long corresponding to a frequency resolution of 196 Hz.

The response of the BM to noise stimulation was measured in a second paradigm as described in de Boer and Nuttall (1997). Briefly, a pseudorandom noise stimulus was constructed in a 4096-point array and delivered at the rate of 208 k samples/s. This stimulus signal—with a period of 19.7 ms—was delivered repetitively and continuously (i.e., without gaps) for 1000 times or more. Synchronously acquired velocity signals were averaged and a cross-correlation function was computed between the averaged signal and the 4096-point stimulus array. The Fourier transform of the cross-correlation function was analyzed resulting in a spectrum with a resolution of 50.8 Hz. For this particular setup, the level of the sound stimulus is given in dB SPL per third octave.

All OAEs were recorded with a microphone (Etymotic Research ER–10B[+]) from the ear canal. A Stanford Research Systems (SR770) real-time FFT analyzer was used to observe and monitor the signals from the Etymotic microphone or the velocimeter.

RESULTS AND DISCUSSION

We have observed spontaneous pure-tone vibration of the BM and recorded and heard pure-tone sound from the ear canal of a guinea pig. For simplicity, we term these signals spontaneous BM oscillation (SBMO) and spontaneous otoacoustic emission (SOAE), respectively. The guinea pig was anesthetized and had undergone our routine surgical procedure to record the velocity response of the BM. In every way, the experimental conditions were unremarkable, however, the CAP thresholds recorded before and after the emission was detected were unusual. As Figure 1 shows, the CAP functions indicate a pronounced dip in the "audiogram" at 8 and 12 kHz. CAP threshold sensitivity at 16 kHz (near the emission frequency range around 15 kHz) was near normal, while thresholds at 18 kHz (slightly above the best frequency of the measured BM location) and higher were elevated (by 5–10 dB). A small loss of cochlear sensitivity such as this at 18 kHz and above is common following surgery, but a midfrequency loss at 8 and 12 kHz of the size shown by Figure 1 is rare.

The animal had undergone 1.5 h of data recording from beads placed on the BM (see Fig. 2) before our attention was directed to the spontaneous oscillation. The latter was first evidenced as a single peak in the real-time magnitude spectrum of BM velocity displayed by the FFT analyzer. The frequency of the SBMO, when first observed, fluctuated between 14.2 and 14.6 kHz, while the measured BFs of the beads on the BM were about 17 kHz. It is possible that the frequency shifts of the SBMO were related to natural variations of physiological parameters such as cerebrospinal fluid (CSF) pressure (de Kleine et al. 2000) and middle-ear stiffness (Hauser et al. 1993). Note that the real-time FFT analyzer performs an exponential running average (10 averages) over the magnitude of the frequency spectrum. No synchronizing signal was used. Any spontaneous constant-frequency oscillation should show up as a spectral peak.

Later, at the time of most data recording, the oscillation frequency stabilized near 15 kHz. We know from experience that at the bead location the BF for acoustic stimuli is 18 kHz in the normally sensitive animal. The BM response in this animal had the slightly downshifted BF of 17 kHz, reflecting the small loss of CAP sensitivity in this frequency range. The SBMO magnitude also varied somewhat during the time of measurement. It was stable during the time we recorded the data described below. The oscillation gradually declined in magnitude (but did not vary in frequency) and disappeared after having been observable for about 2 h.

At first we did not realize that the observed 15-kHz peak in the frequency spectrum displayed by the FFT analyzer was an SBMO. We observed that it disappeared when the door of the sound isolation chamber was opened, an effect probably due to external sound of which the main component was a 15.8-kHz "contamination" signal originating from the horizontal oscillator of the cathode ray display in the FFT analyzer. When the sound isolation booth door was opened, the peak at 15 kHz was "replaced" by one at 15.8 kHz. We could not investigate whether this disappearance of the emission was suppression or
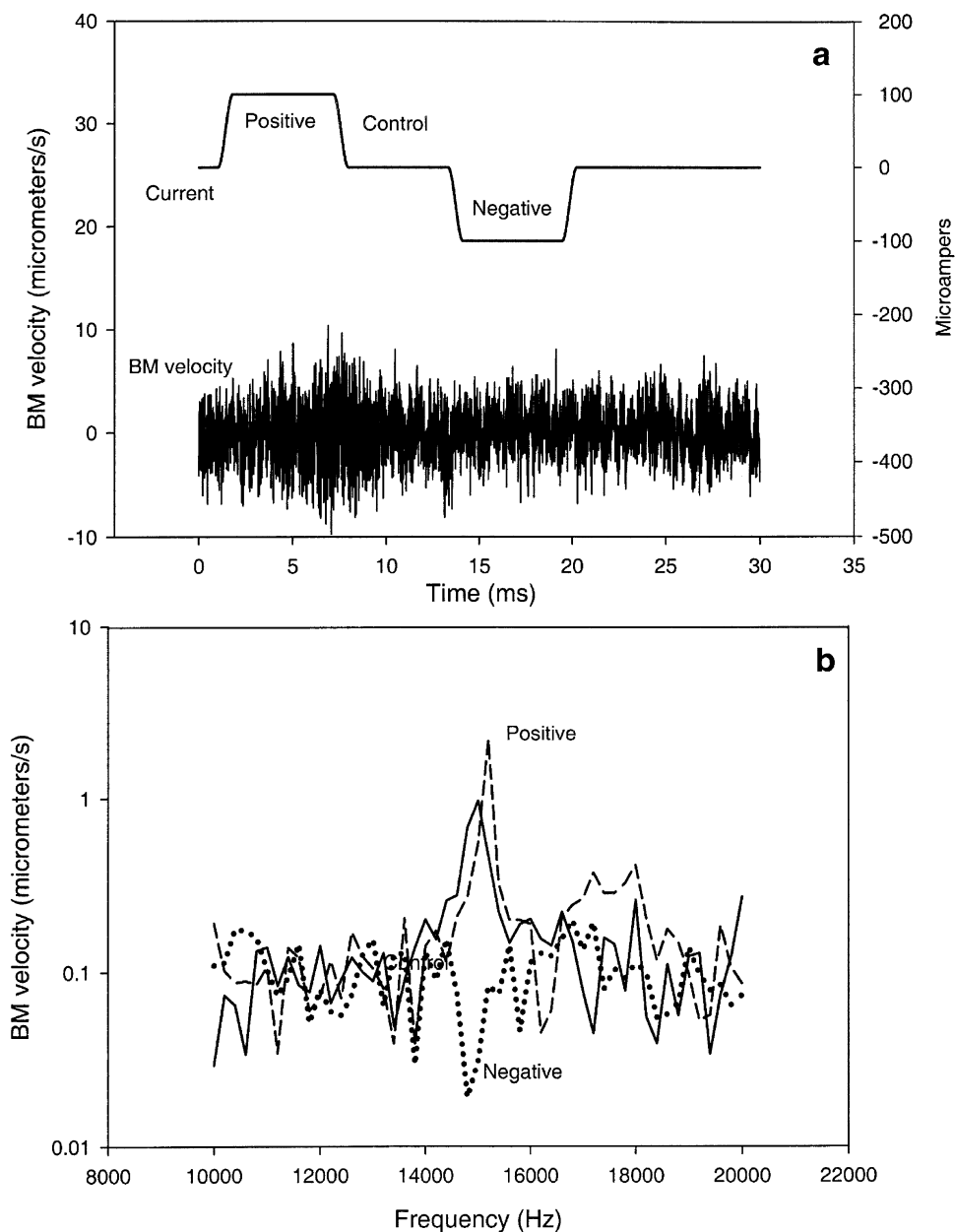
FIG. 3. Basilar membrane oscillations as modified by electric current applied to the cochlea. **a**. The upper waveform shows the pattern of the current applied across the cochlear duct. The lower waveform is the averaged time waveform of the BM velocity (500 averages). No external acoustic stimulation was used. **b**. The synchronous magnitude spectra of the BM velocity waveform from three time periods labeled "positive," "control," and "negative" in **a**. Positive current enhanced the oscillation and shifted its frequency upward while negative current reduced the oscillation.

entrainment. Below, we present the observation that a tone having a frequency higher than the emission frequency tone may entrain the emission.

Standing inside the sound booth with the door closed, the SOAE from the GP was clearly audible to a very sensitive listener (KG) when he held his ear about 5 cm from the bulla. This suggests strongly that an SOAE was associated with the spontaneous BM oscillation SBMO. Detailed measurements of the SOAE are reported below. Here we continue with mechanical data.

The frequency of the SBMO did not vary among the six beads on the BM (see Fig. 2) but its amplitude varied by about 5 dB. The highest SBMO level was observed from beads located near the OHCs and at

the boundary of the OHCs and Hensen's cells. The lowest level was at the edge of the spiral osseous lamina.

Level and frequency of the oscillation were influenced by current applied to the cochlea. The upper curve of Figure 3a shows the time course of the current application. Note that the current steps have onset and offset shaping to reduce generation of a local transient response at the characteristic frequency of the measured BM place (Nuttall et al. 1995; Parthasarathi et al. 2003). The lower panel of Figure 3a shows the time waveform of the averaged BM velocity. In this experiment, no acoustical stimulus was given. The averaging program computes the average of the input signal over a window that is
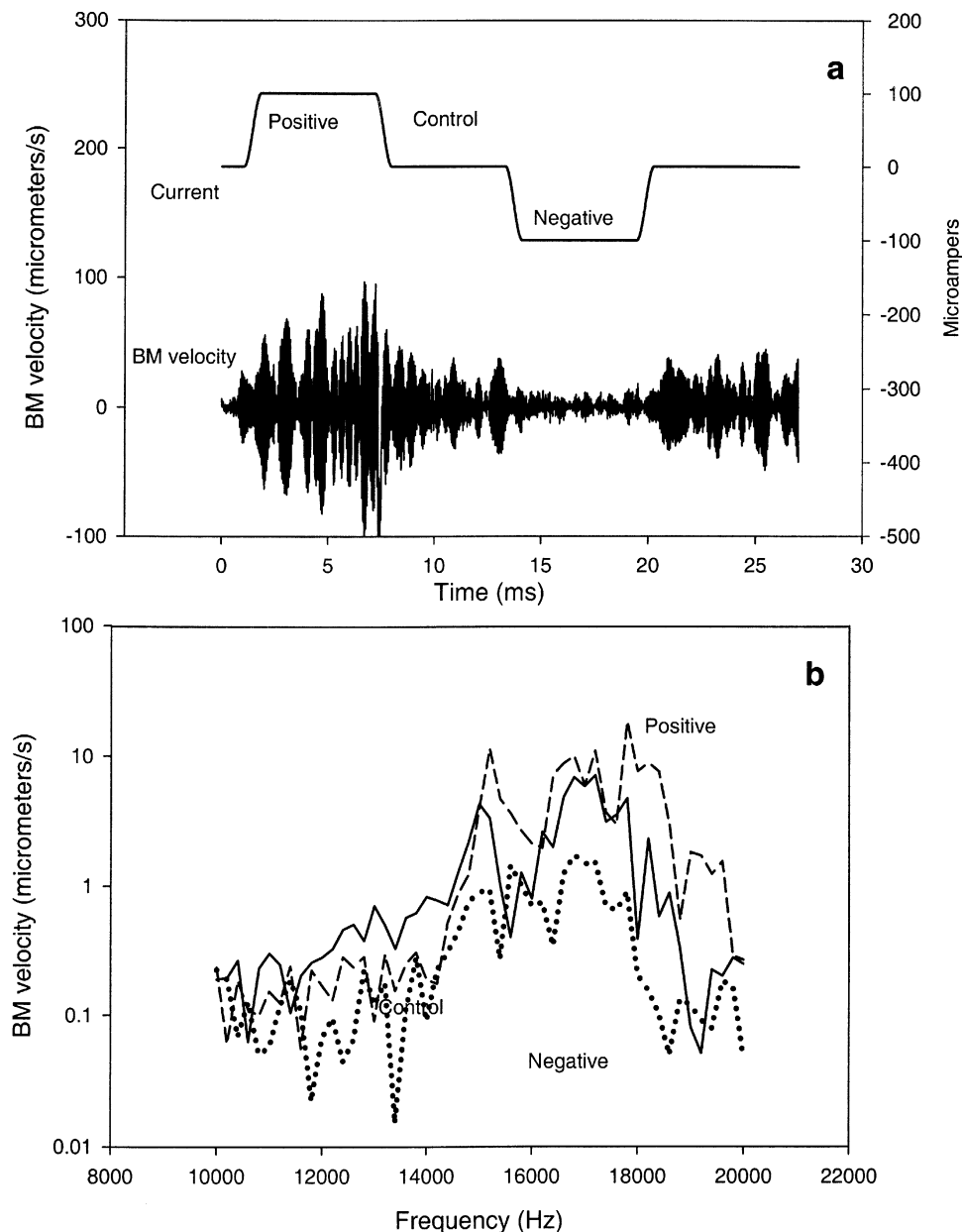
FIG. 4. Basilar membrane velocity responses to sound modified by electric current. **a**. The upper waveform shows the pattern of the current applied across the cochlear duct. The lower waveform is the averaged time waveform of the BM velocity produced by applying a pseudo-random noise to the ear (30 dB SPL per third octave, 500 averages). **b**. The synchronous magnitude spectra of the BM velocity waveform from three time periods are labeled "positive," "control," and "negative" in **a**. Positive current enhanced the response while negative current reduced it. The "control" (solid line) and "positive" spectra (dashed line) show two main peaks, the spontaneous oscillation peak at 15 kHz and the wide peak produced by the applied noise stimulus (6–24 kHz).

synchronous with the current pulse. All FFT spectra to be shown in what follows are "synchronous spectra" obtained from synchronously averaged signals. Since the spontaneous oscillation is not synchronized with this time window, it should ideally average to zero. In fact, we did observe only a very small average value (data not shown) when no acoustic or electrical stimulus was used. With a current or sound stimulus, however, we did observe a measurable oscillatory response at a frequency corresponding to the SBMO described before.

Figure 3b shows the magnitude spectrum for three time regions of the acquired waveform. In two of these a clear peak is seen. The positive current (+100 μA scala vestibuli SV re scala tympani ST) more than doubled the control (no current) SBMO and shifted its frequency upward from 15 to 15.2 kHz. Negative current (-100 μA SV re ST) reduced the SBMO to the noise floor. These effects of current are very similar to what has been observed for the local BF for sound stimulation when current was applied across the cochlear duct (Nuttall et al. 1995; Parthasarathi et al. 2003). The "gain" of the BM vibration increased and the BF shifted higher for positive current, whereas negative current decreased gain and reduced BF.

Note also the apparent delay in the enhanced BM velocity response (the lower waveform in Fig. 3a) caused by current. A similar delay was seen for the "buildup" of a transient response, as it is modified by current (See Figure 7 in Parthasarathi et al. 2003).

Generally, when a current was applied across the cochlear duct, BM motion had a very short delay (<100 μs, Nuttall et al. 1999). An appreciably longer delay can be attributed to the slow response buildup of a sharply tuned system. Since the initial 1.5 ms was omitted from the analysis, most of this transient buildup region is not represented in the spectra shown in Figure 3b.

Figure 4 illustrates the effect of electric current on the BM response to an acoustic noise stimulus (6–24 kHz). We have previously shown that positive current increased the BM velocity response and shifted the BF upward (Parthasarathi et al. 2003), whereas negative current had the opposite effect. Figure 4a shows the current stimulus and the BM velocity time waveform. Figure 4b shows that with positive current the SBMO shifted from 15 to 15.2 kHz, while the local BF shifted from 17.2 to 17.8 kHz. However, analysis was limited by the 200-Hz resolution of the FFT in this case. Negative current reduced the response. Note that the control level at the SBMO frequency (4.3 μm/s) was larger than that in Figure 3 (0.97 μm/s). Noise stimulation was more effective than current alone in causing the emission to be observed.

If the sound stimulus is a pure tone instead of a noise, there will be a different effect on the SBMO component and the response component to the tone. Figure 5 shows synchronous BM response spectra for three different current conditions, the "probe" tone's frequency was 14 kHz and the level was 15 dB SPL. It is clear that positive and negative currents had little effect on the BM velocity response to the "probe" stimulus, but they altered the SBMO as expected. Note that the suppression effect of the "probe" was small; in the control condition the oscillation had an amplitude of 0.8 μm/s with "probe" in Figure 5 versus 0.97 μm/s in Figure 3 without "probe." Figure 6 shows results of acoustic measurements (recording of OAEs) in the ear canal. No sound was presented but current was applied as described before. With this figure we show that frequency and level shifts of the ear canal SOAE produced by current application were completely consistent with those found in the BM velocity. In short, positive current enhanced and upward frequency shifted the emission as it did the mechanical SBMO. The results shown in Figures 3 and 6 confirm the hypothesis that an SOAE originates as a spontaneous vibration of the BM. This is a key finding in this serendipitous study.

We return to mechanical measurements. We tested the effect of pure tones above and below the SBMO frequency on the mechanical SBMO. In Figure 7a, the solid curve shows that a 14-kHz tone of 15 dB SPL produced about the same BM velocity as the SBMO
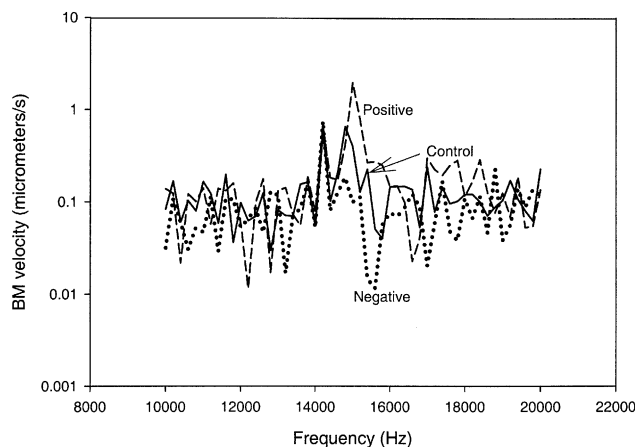

FIG. 5. The (synchronous) magnitude spectra of the BM velocity for acoustic stimulation of the ear with a 14-kHz pure tone at 15 dB SPL (500 averages). Current was applied as depicted in Figures 3 and 4. Positive current increased the oscillation in level and frequency but did not alter the response to the 14-kHz tone. Negative current reduced the oscillation without affecting the probe tone response.
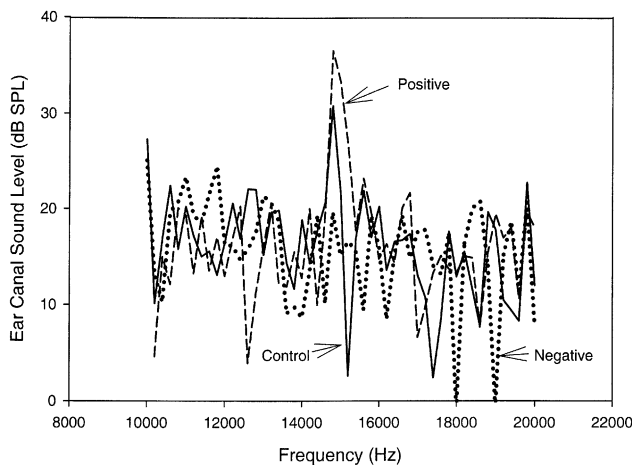

FIG. 6. Otoacoustic emission (OAE) associated with the spontaneous oscillation of the BM (500 averages) is illustrated. No sound was given. The ear canal sound level of the emission was modified by electric current (applied as depicted in Fig. 3 and 4). Positive current increased the emission level and its frequency. Negative current reduced the emission to the noise floor.

(which was at 15 kHz). Raising the tone level to 35 dB SPL (dotted curve) reduced the oscillation level by about 50% (dotted curve). In Figure 7b, we show the opposite case, using an above-SBMO frequency "probe". At the time of this experiment the SBMO had drifted to 14.7 kHz. A probe tone of 15 kHz could enhance or suppress the oscillation (Fig. 7b, inset). At probe levels of 45 dB SPL and higher the oscillation was no longer apparent, only the response to the probe. This effect could be due to entrainment of the oscillation to the frequency of the probe. These results appear to be consistent with "classical" sup-
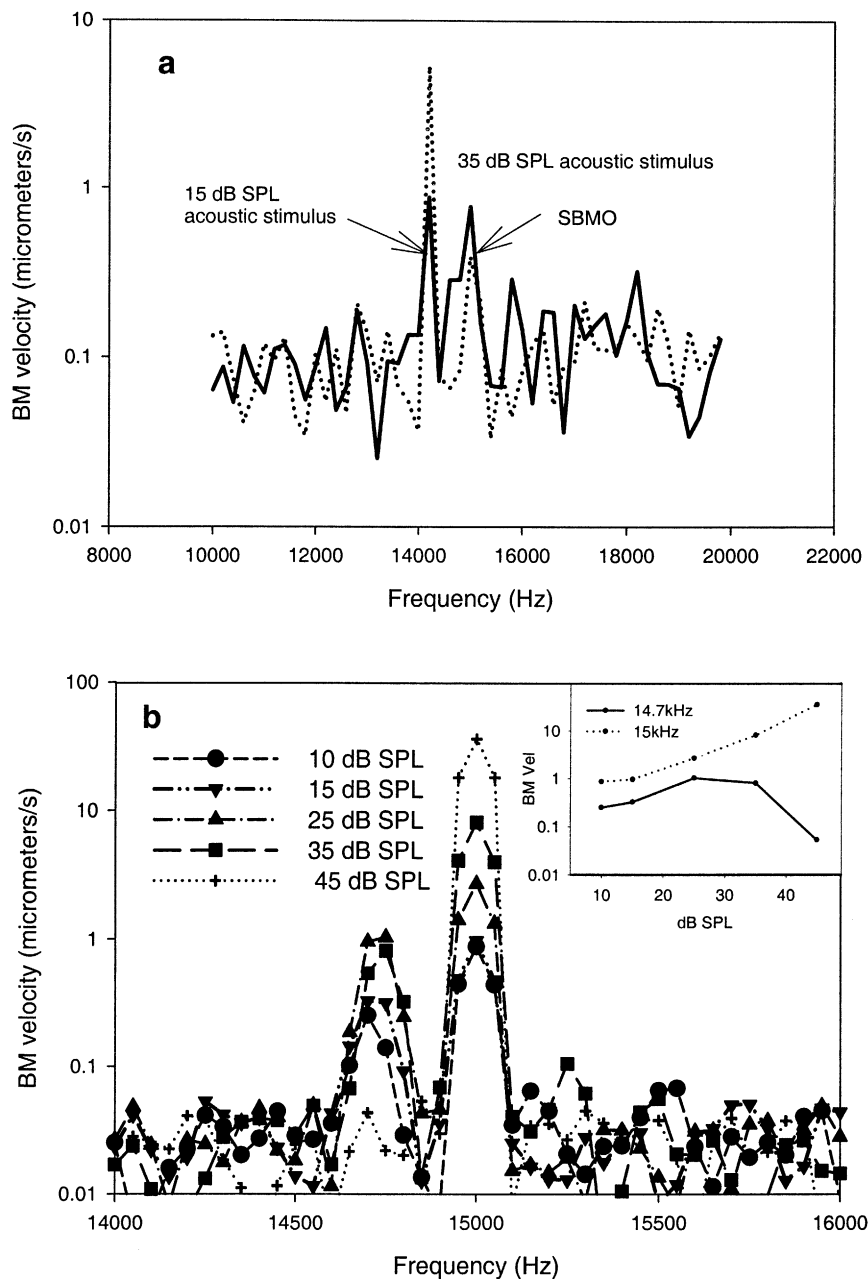
FIG. 7. The effect of pure tones below and above the spontaneous oscillation frequency on the BM response. **a.** A tone at 14 kHz had no effect at 15 dB SPL (solid line) but reduced the oscillation to near the noise floor at 35 dB SPL (dotted line). **b.** A tone at 15 kHz (probe tone) enhanced the oscillation level and shifted its frequency upward from 14.7 kHz. At 45 dB SPL the oscillation became entrained with the 15-kHz tone. The inset graph in **b** shows the level change of the BM velocity at the frequency of the spontaneous BM oscillation and at the 15-kHz probe tone frequency, as a function of the sound level of the probe tone. All data were averaged over 500 presentations.

pression and entrainment reported for human SO-AEs affected by pure tones (Wilson 1980; Wilson and Sutton 1981; Long et al. 1991). It is interesting to note that the 15-kHz responses to 10-dB and 15-dB tones are almost the same while the SBMO increases in amplitude. Apparently, there is a nonlinear interaction between the 15-kHz probe tone and the SBMO at 14.7 kHz at the lower probe levels.

In contrast to the enhancement, suppression, and entrainment seen for pure tones, we observed only enhancement when using a noise-band stimulus. In fact, the use of noise facilitated observation of the SBMO at an early time in the experiment. Figure 8a and b shows the BM velocity (synchronous) spectra

from stimulation with octave-band noise centered at 17 kHz, presented at 10 and 30 dB SPL per third octave, when no SOAE was apparent, compared to a recording made later when the spontaneous oscillation was present (as both a SBMO and a SOAE). Apparently, the cochlea had lost some sensitivity at this time, since the peak of the spectrum was reduced. In Figure 8a (solid line) there is no peak near 14.6 kHz for 10 dB per third-octave noise, while in Figure 8b (solid line) a SBMO at 14.6 kHz was "evoked" by 30 dB per third-octave noise before it was observed in the absence of noise stimulation. This evoked response was lower in level and wider in bandwidth than observed later at a time when the
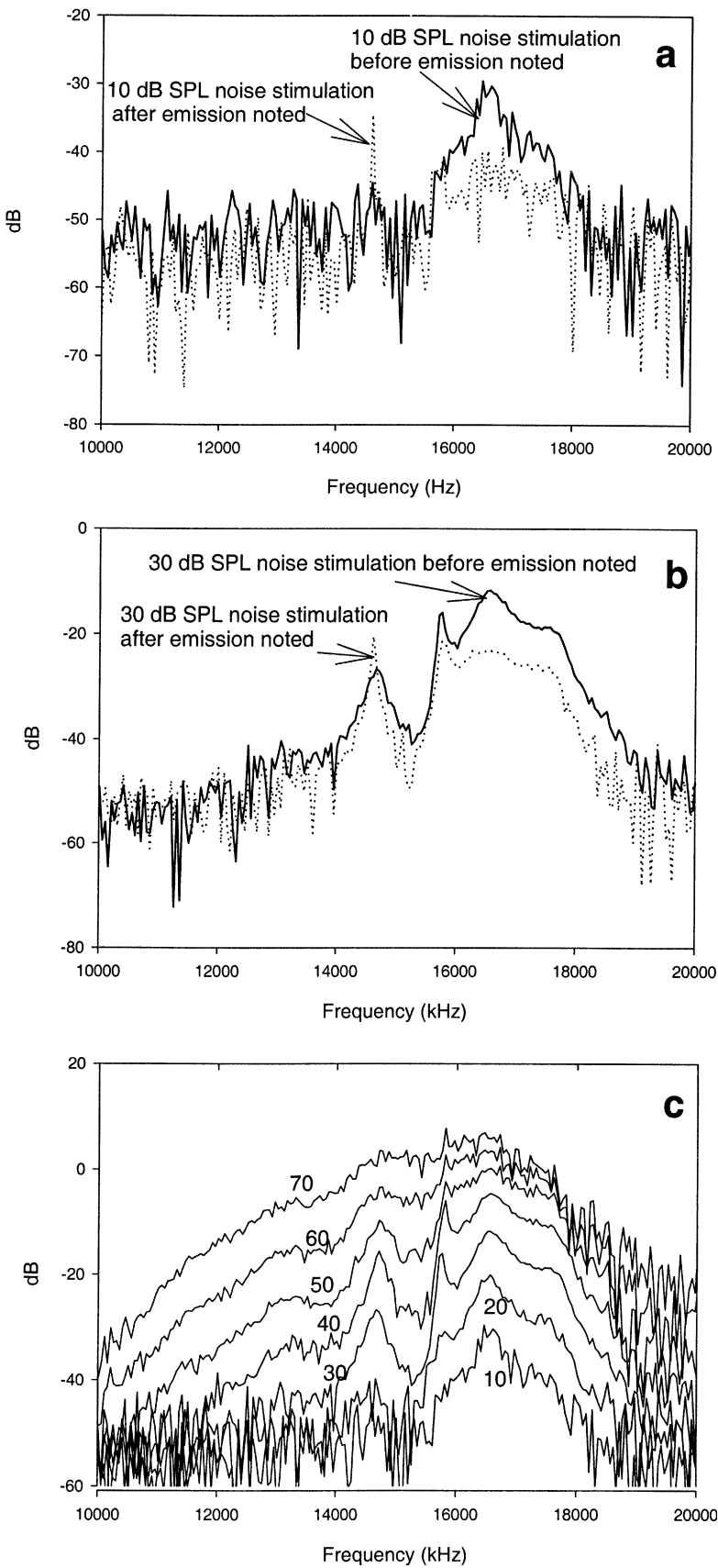
FIG. 8. Acoustic noise, 1 octave wide, given to the ear could enhance the mechanical oscillation but did not shift its frequency. **a**. At a time before the spontaneous oscillation was obvious, a pseudorandom acoustic noise of 10 dB SPL per third octave produced a standard type of response. Later the BM velocity spectrum of the same noise signal showed a clear oscillation peak at 14.6 kHz (dotted curve). **b**. The oscillation was still visible at a higher sound level, 30 dB SPL per third octave. Initially, the spectrum associated with the oscillation was broader and the maximum level at 14.6 kHz was lower than later when the oscillation became evident as a narrow peak (dotted curve). A second peak at higher frequency but below the BF could also be seen. **c**. As the sound level of the noise was raised more, the oscillation component increased, too, but demonstrated compressive nonlinearity. All spectra shown are synchronous spectra, averaged over 3000 periods.

SBMO and SOAE were clearly evident. Figure 8a shows also the robust SBMO (dotted line) present with 10 dB SPL per third-octave noise at a time when we had just noted the spontaneous oscillation. Then, the 30 dB per third-octave noise enhanced the SBMO (Fig. 8b, dotted line) and the SBMO had a narrower bandwidth than seen earlier. In Figure 8c, the SBMO is seen to increase with noise level. This increase was nonlinearly compressive, and the peak frequency did not shift with level. On closer inspection, the rise in level and the variation in bandwidth of the SBMO component were similar in character to the rise and variation of the acoustical response curves. Also apparent in Figure 8b and c is a second peak at about 15.65 kHz. This peak also increased in a nonlinear compressive fashion and its frequency did not shift with noise level. Although we did not direct particular attention to it, we interpret it as a second spontaneous oscillation; actually, there are more peaks that could be interpreted in this way.

QUESTIONS AND PROBLEMS

Origin of effects of electric current

The question arises as to whether electric current is causing the oscillation or the local gain to change (or both). Our hypothesis is that the SBMO near 15 kHz seen at the 17-kHz BF place is the local response to a reverse-traveling wave that goes from the 15-kHz location to the stapes. Since both the 15-kHz and 17-kHz locations are within the spread of current from the bipolar electrodes (those locations are less than 0.5 mm apical from the electrodes), the current can modify the organ of Corti at both locations. We hypothesize that the changes of the SBMO induced by the current arise mainly from effects at the 15-kHz and not the 17-kHz location. This hypothesis is supported by data showing that current affects mainly acoustical responses to BF and above-BF frequencies (Nuttall 1985; Parthasarathi et al. 2003). Figure 5 gives further support for this hypothesis.

Why do we observe the SBMO and SOAE in time-averaged responses from the cochlea?

As mentioned above, all signals were recorded by averaging time waveforms synchronously. One expects that the SOAE or SBMO would be averaged to zero by synchronous averaging. Indeed, we found that that appeared to be the case when there was no external stimulus, no current, and no sound. However, the phase of a SOAE is known to be easily synchronized to an acoustic click (Wilson and Sutton 1981). The acoustic and electric stimuli used in this work may have served the synchronizing function in

this study. It is stressed that all these stimuli were periodic and were used to define the analysis window. Although we used rise-fall time shaping of the electric stimulus to minimize BM velocity transients, there apparently was still sufficient synchronizing capacity. This is confirmed by the ''control'' condition in Figure 3a, where the current is (momentarily) zero but there is a substantial SBMO signal. In the case where acoustic stimulus signals are used, these stimuli are tone bursts or pseudorandom noise sequences of 20–25-ms duration and again can provide synchronization.

An alternative explanation of the family of peaks seen in Figure 8b and c is that they result from the oscillator mechanism being suppressed, but still remaining substantially more frequency-selective than the normal cochlear filter around 15 kHz. In this view the peaks should be interpreted as driven responses.

What is the origin and production mechanism of the SBMO?

SOAEs are proposed to originate from either point source oscillators within the cochlea or as standing waves supported by energy gain from cochlear amplification (Shera 2003). Guinea pigs do not normally have SOAEs as high as 15 kHz, although they do have noise in the BM motion that is present without intentional stimulation. This noise motion has a spectrum with a peak at the BF (Nuttall et al. 1997). Therefore, one is drawn to take into account the observation that this particular animal had a midfrequency CAP threshold elevation (see Fig. 1). This loss of cochlear amplification could have produced a more or less defined area of impedance variation that could serve as the preferred boundary region of wave reflection. Alternatively, it could have been responsible for ''rogue oscillation'' of outer hair cells at the 15-kHz tonotopic location. If the emission is the result of coherent reflection (Shera and Zweig 1993), i.e., due to a standing wave between the stapes and the 15-kHz cochlear location, then it is likely that the oscillation frequency is one of many frequencies that would naturally be supported by the cochlea. The presence of secondary peaks in the spectra of Figure 8 lends support to this possibility.

Otoacoustic emissions are typically observed at multiple frequencies having a characteristic average spacing. The standing-wave model of SOAEs predicts these frequency spacings as intervals over which the phase changes by full cycles. In animal ears SOAE spacing (normalized to a particular emission frequency) has been found to be larger than for human ears, perhaps by a factor of 2 (Long et al. 2000; Shera 2003). At 15 kHz, this predicts an average emission frequency spacing of about 1500 Hz. Thus, the SBMO

at 15.65 kHz in Figure 8 (about 1 kHz above the 14.6-kHz main emission) would be consistent with the standing-wave model.

We have observed that the SBMO can be suppressed, enhanced, and frequency shifted, just as acoustic pure tones can enhance, suppress, and entrain the frequency of a SOAE (e.g., Long et al. 1991). Acoustic noise may enhance oscillations as we show in Figures 4 and 8 (Maat et al. 2000). Note that, theoretically, we can expect to find all these properties no matter whether the origin of a SBMO lies in a ''rogue'' oscillator or is greatly due to coherent reflection. One unexpected finding is the strength of the enhancement of the SBMO by noise (20 dB at the lowest sound levels) coupled with the nonlinear growth and saturation over a 50-dB level range. The level dependence of the SBMO is, as noted earlier, similar to the compressive nonlinearity that acoustic responses show. The response peak may then be explained as due to the (nonlinear) response to the acoustic stimulus by a system that shows a pronounced narrowband resonance—what remains of the oscillator when it is suppressed. Incidentally, this feature would also explain the ''slow'' response to current transients in Figure 3a.

Our noise stimuli did not shift the frequency of the SBMO. This is likely because the stimulus bandwidth was centered near the spontaneous frequency. In the stimulus signal there was no dominant component to affect the oscillation conditions of ''rogue'' hair cells or of neighboring cells to influence wave propagation or coherent reflection. Therefore, the frequency component that was dominant in the response remained determined by the nature of the oscillator. Even if the spontaneous component were suppressed, the system would still respond as a frequency-selective system.

ACKNOWLEDGMENTS

REFERENCES

BELL A. Circadian and menstrual rhythms in frequency variations of spontaneous otoacoustic emissions from human ears. Hear Res. 58:91–100, 1992.

BROWN AM, WOODWARD S, GASKILL SA. Frequency variation in spontaneous sound emissions from guinea pig and human ears. Eur. Arch. Otorhinolaryngol. 247:24–28, 1990.

CLARK WW, KIM DO, ZUREK PM, BOHNE BA. Spontaneous otoacoustic emissions in chinchilla ear canals: correlation with histopathology and suppression by external tones. Hear Res. 16:299–314, 1984.

COLLET L, KEMP DT, VEUILLET E, DUCLAUX R, MOULIN A, MORGON A. Effect of contralateral auditory stimuli on active cochlear micromechanical properties in human subjects. Hear Res. 43:251–261, 1990.

DE BOER E, NUTTALL AL. The mechanical waveform of the basilar membrane. I. Frequency modulations (glides) in impulse responses and cross-correlation functions. J. Acoust. Soc. Am. 101:3583–3592, 1997.

DE KLEINE E, WIT HP, VAN DIJK P, AVAN P. The behavior of spontaneous otoacoustic emissions during and after postural changes. J. Acoust. Soc. Am. 107:3308–3316, 2000.

EVANS EF, WILSON JP, BORERWE TA. Animal models of tinnitus. In: EVERED D, LAWRENSON G (eds) Ciba Foundation Symposium 85—Tinnitus. Pitman Medical, London, pp 108–138, 1981.

GOLD T. Hearing II. The physical basis of the action of the cochlea. Proc. R. Soc. Lond. Ser. B Biol. Sci. 135:492–498, 1948.

HAUSER R, PROBST R, HARRIS FP. Effects of atmospheric pressure variation on spontaneous, transiently evoked, and distortion product otoacoustic emissions in normal human ears. Hear Res. 69:133–145, 1993.

HE D, DALLOS P. Properties of voltage-dependent somatic stiffness of cochlear outer hair cells. J. Assoc. Res. Otolaryngol. 1:64–81, 2000.

HILGER AW, FURNESS DN, WILSON JP. The possible relationship between transient evoked otoacoustic emissions and organ of Corti irregularities in the guinea pig. Hear Res. 84:1–11, 1995.

KEILSON SE, KHANNA SM, ULFENDAHL M, TEICH MC. Spontaneous cellular vibrations in the guinea-pig cochlea. Acta Otolaryngol. (Stockh.). 113:591–597, 1993.

KEMP DT. Evidence of mechanical nonlinearity and frequency selective wave amplification in the cochlea. Arch. Otorhinolaryngol. 224:37–45, 1979a.

KEMP DT. The evoked cochlear mechanical response and the auditory microstructure-evidence for a new element in cochlear mechanics. Scand. Audiol. Suppl. 9:35–47, 1979b.

LONG GR, TUBIS A, JONES KL. Modeling synchronization and suppression of spontaneous otoacoustic emissions using Van der Pol oscillators: Effects of aspirin administration. J. Acoust. Soc. Am. 89:1201–1212, 1991.

LONG GR, SHAFFER LA, DHAR S, TALMADGE CL. Cross species comparison of otoacoustic fine-structure. In: WADA H, TAKASAKA T, IKEDA K, OHYAMA K, KOIKE T (eds) Recent Developments in Auditory Mechanics. World Scientific, Sendai, Japan, pp 367–373, 2000.

LONSBURY–MARTIN BL, MARTIN GK, PROBST R, COATS AC. Spontaneous otoacoustic emissions in a nonhuman primate. II. Cochlear anatomy. Hear Res. 33:69–93, 1988.

MAAT B, WIT HP, VAN DIJK P. Noise-evoked otoacoustic emissions in humans. J. Acoust. Soc. Am. 108:2272–2280, 2000.

MARTIN P, BOZOVIC D, CHOE Y, HUDSPETH AJ. Spontaneous oscillation by hair bundles of the bullfrog's sacculus. J. Neurosci. 23:4533–4548, 2003.

NUTTALL AL. Influence of direct current on dc receptor potentials from cochlear inner hair cells in the guinea pig. J. Acoust. Soc. Am. 77:165–175, 1985.

NUTTALL AL, DOLAN DF, AVINASH G. Laser Doppler velocimetry of basilar membrane vibration. Hear Res. 51:203–213, 1991.

NUTTALL AL, KONG WJ, REN T, DOLAN DF. Basilar membrane motion and position changes induced by direct current stimulation. In: FLOCK A, OTTOSON D, ULFENDAHL M (eds) Active hearing. Pergamon Press, London, pp 283–294, 1995.

NUTTALL AL, GUO M, REN T, DOLAN DF. Basilar membrane velocity noise. Hear Res. 114:35–42, 1997.

Nuttall AL, Guo M, Ren T. The radial pattern of basilar membrane motion evoked by electric stimulation of the cochlea. Hear Res. 131:39–46, 1999.

Ohyama K, Wada H, Kobayashi T, Takasaka T. Spontaneous otoacoustic emissions in the guinea pig. Hear Res. 56:111–121, 1991.

Parthasarathi A, Grosh K, Zheng J, Nuttall AL. Effect of current stimulus on in vivo cochlear mechanics. J. Acoust. Soc. Am. 113:442–453, 2003.

Powers NL, Salvi RJ, Wang J, Spongr V, Qiu CX. Elevation of auditory thresholds by spontaneous cochlear oscillations. Nature 375:585–587, 1995.

Ren T, Zhang M, Nuttall AL, Miller JM. Heart beat modulation of spontaneous otoacoustic emissions in guinea pig. Acta Otolaryngol. (Stockh.) 115:725–731, 1995.

Ruggero MA, Rich NC, Freyman R. Spontaneous and impulsively evoked otoacoustic emissions: Indicators of cochlear pathology? Hear Res. 10:283–300, 1983.

Shera CA. Mammalian spontaneous otoacoustic emissions are amplitude-stabilized cochlear standing waves. J. Acoust. Soc. Am. 114:244–262, 2003.

Shera CA, Zweig G. Order from chaos: Resolving the paradox of periodicity in evoked otoacoustic emission. In: Duifhuis H, Horst JW, van Dijk P, van Netten SM (eds) Biophysics of Hair Cell Sensory Systems. World Scientific, Paterswolde, The Netherlands, pp 54–63, 1993.

Talmadge CL, Long GR, Tubis A. Classification and small scale structure of spontaneous otoacoustic emissions (abstr. 389). Sixteenth Midwinter Research Meeting of the Association for Research in Otolaryngology, St. Petersburg Beach, FL, 1993.

Teich MC, Heneghan C, Khanna SM. Spontaneous cellular vibrations in the basal turn of the living cat cochlea (abstr. 352). Seventeenth Midwinter Research Meeting of the Association for Research in Otolaryngology St. Petersburg Beach, FL, 1994.

Velenovsky DS, Glattke TJ. The effect of noise bandwidth on the contralateral suppression of transient evoked otoacoustic emissions. Hear Res. 164:39–48, 2002.

Wilson JP. Evidence for a cochlear origin for acoustic re-emissions, threshold fine-structure and tonal tinnitus. Hear Res. 2:233–252, 1980.

Wilson JP, Sutton GJ. Acoustic correlates of tonal tinnitus. In: Evered D, Lawrenson G (eds) Ciba Foundation Symposium 85—Tinnitus. Pitman Medical, London, pp 82–107, 1981.

Zenner HP, Ernst A. Cochlear motor tinnitus, transduction tinnitus, and signal transfer tinnitus: Three models of cochlear tinnitus. In: Vernon J, Moller AR (eds) Mechanisms of Tinnitus. Allyn and Bacon, Boston, pp 237–254, 1995.

Zweig G, Shera CA. The origin of periodicity in the spectrum of evoked otoacoustic emissions. J. Acoust. Soc. Am. 98:2018–2047, 1995.