Middle Ear Forward and Reverse Transmission in Gerbil

Wei Dong and Elizabeth S. Olson

J Neurophysiol 95:2951-2961, 2006. First published Feb 15, 2006; doi:10.1152/jn.01214.2005

J Neurophysiol 95: 2951–2961, 2006.
First published February 15, 2006; doi:10.1152/jn.01214.2005.

Middle Ear Forward and Reverse Transmission in Gerbil

Wei Dong and Elizabeth S. Olson
Department of Otolaryngology, Head and Neck Surgery, Columbia University, New York, New York

Submitted 24 October 2005; accepted in final form 7 February 2006

Dong, Wei and Elizabeth S. Olson. Middle ear forward and reverse transmission in gerbil. *J Neurophysiol* 95: 2951–2961, 2006. First published February 15, 2006; doi:10.1152/jn.01214.2005. The middle ear transmits environmental sound to the inner ear. It also transmits acoustic energy sourced within the inner ear out to the ear canal, where it can be detected with a sensitive microphone as an otoacoustic emission. Otoacoustic emissions are an important noninvasive measure of the condition of sensory hair cells and to use them most effectively one must know how they are shaped by the middle ear. In this contribution, forward and reverse transmissions through the middle ear were studied by simultaneously measuring intracochlear pressure in scala vestibuli near the stapes and ear canal pressure. Measurements were made in gerbil, in vivo, with acoustic two-tone stimuli. The forward transmission pressure gain was about 20–25 dB, with a phase–frequency relationship that could be fit by a straight line, and was thus characteristic of a delay, over a wide frequency range. The forward delay was about 32 μs. The reverse transmission pressure loss was on average about 35 dB, and the phase–frequency relationship was again delaylike with a delay of about 38 μs. Therefore to a first approximation the middle ear operates similarly in the forward and reverse directions. The observation that the amount of pressure reduction in reverse transmission was greater than the amount of pressure gain in forward transmission suggests that complex motions of the tympanic membrane and ossicles affect reverse more than forward transmission.

INTRODUCTION

The middle ear is responsible for transmitting sound from the ear canal (EC) to the cochlea. The middle ear is a passive system (not expending energy in its basic operation) and because of the middle ear's ability to impedance match between the environment and the inner ear, a substantial fraction of the environmental acoustic energy at the ear canal is delivered to the inner ear, at least over a range of frequencies (Rosowski et al. 1986). Although all mammals have a similar middle ear—eardrum and three ossicles—there are also differences that correlate with the frequency range of hearing. For example, low-frequency hearing is correlated with a large middle ear space and relatively freely moving ossicles and high-frequency hearing with relatively secure ossicles and a smaller middle ear space (Rosowski 2003). Both the inner and middle ear influence a species' frequency range of hearing (Ruggero and Temchin 2002).

The ear canal pressure (ECP) is transmitted to the cochlea with substantial pressure gain that historically has been attributed to the workings of a piston system that leverages the pressure up by a factor roughly equal to the ratio of eardrum area to stapes footplate area (reviewed in Rosowski 2003). For gerbil, this ratio is about 17.24 mm^2/0.62 $mm^2 \cong 28$ (Lay 1972), quite close to our experimental value of pressure gain. However, the operation of the middle ear is not that of a simple piston system. In particular, the ossicles do not move as rigid bodies and the eardrum's response to sound is a complex, wavelike motion (Decraemer et al. 1989, 1991). In spite of this complexity, by recording stimulus sound pressure in the ear canal and either sound pressure in the cochlea or stapes velocity, the input–output function of the middle ear of common laboratory animals is known to be relatively simple, with a magnitude that can be quite flat with frequency throughout a wide frequency range (reviewed in Ruggero and Temchin 2002).

Otoacoustic emissions (OAEs) are sounds that are produced in the cochlea by a nonlinear mechanism associated with cochlear amplification (Avan et al. 2003) and emanate out of the cochlea and "backwards" through the middle ear to the ear canal. These emissions are not typically audible but can be detected with a sensitive microphone in the ear canal. OAEs are present in healthy ears and their absence is used to diagnose hair cell impairment. OAEs are primarily used to study and diagnose inner ear operation, but because they are transmitted through the middle ear, the middle ear will also influence them. Ever since the discovery of OAEs, their shaping by the middle ear has been explored and discussed (Kemp 1980). This study quantifies the reverse transmission of sound through the middle ear and compares forward and reverse transmission in gerbil.

Forward transmission through the middle ear has been measured by ear canal and cochlear pressure measurements in several labs and different animals (cat: Lynch et al. 1982; Nedzelnitsky 1980; guinea pig: Dancer and Franke 1980; Decory et al. 1990; gerbil: Olson 1998; Olson and Cooper 2000; and human temporal bone: Puria et al. 1997). Recent work has explored middle ear transmission in both directions: forward and reverse (human temporal bone: Puria 2003; Puria and Rosowski 1996; guinea pig: Avan et al. 1998; Magnan et al. 1997, 1999; cat: Voss and Shera 2004). To study the reverse transmission an intracochlear sound source is needed. Such a source is available in OAEs. In a healthy ear presented with two pure tones (f_1 and f_2) combination tones are produced at $2f_1$–f_2 and $2f_2$–f_1, as well as other combination frequencies. The combination tones are called distortion products (DPs), and they lead to distortion product OAEs (DPOAEs) that can be detected in the ear canal. Magnan et al. (1997) measured the $2f_1$–f_2 DP in the scala vestibuli (SV) and EC to characterize the middle ear's reverse transmission. Voss and Shera (2004) also used the $2f_1$–f_2 DP to study reverse transmission; in their measurements, stapes velocity was measured in place of SV

Address for reprint requests and other correspondence: W. Dong, Department of Otolaryngology, Head and Neck Surgery, Columbia University, P&S 11-452, 630 W. 168th Street, New York, NY 10032 (E-mail: wd2015@columbia.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

pressure (SVP). A submersible speaker was used to produce the intracochlear sound source in the measurements of Puria (2003) and Magnan et al. (1999).

In this contribution, we extend reverse transmission measurements to gerbil and to higher frequencies compared with previous measurements. Our strategy was to stimulate with two tones and compare the DPs in the SV pressure to the DPOAEs in the EC, as in several of the studies cited above. To summarize the results: Our measurements of forward transmission through the middle ear confirmed earlier measurements in gerbil, showing a magnitude that was quite flat with frequency and a delaylike linear phase with frequency. The forward gain in pressure ($|\,SVP/ECP\,|$) was approximately 20–25 dB, and the middle ear delay was about 32 μs. The reverse transmission magnitude ($|\,ECP/SVP\,|$) was also quite flat with frequency, with a value of about −30 to −40 dB. Therefore the pressure usually decreased when transmitted through the middle ear in the reverse direction about 10–15 dB more than the pressure increased in the forward direction. The phase–frequency relationship of reverse transmission was nearly delaylike, with a slope that indicated a delay time that was similar to, but in general a few microseconds longer than, the forward transmission delay. In magnitude, the results were similar to the results from guinea pig (Magnan et al. 1997). With respect to phase our conclusion is different from that of Magnan et al. who did not see a delay in reverse transmission, although the difference can be explained by the more extensive high-frequency region over which phase was measured in the gerbil. The forward and reverse middle ear transmissions were similar to the human temporal bone results (Puria 2003) in some ways and different in others.

METHODS

Animal preparation

All measurements were made in gerbils, 50–70 g in mass. Use of the animals was approved by the Institutional Animal Care and Use Committee of Columbia University. The gerbil was deeply anesthetized throughout the experiment and overdosed with anesthetic at the end. A tracheotomy was performed to maintain a patent airway. The animal core temperature was maintained at about 37°C using a thermostatically controlled heating blanket and the head holder was also maintained at about 37°C by running DC current through an attached high-power resistor. The left bulla was widely opened with great care. A small hole was hand-drilled through the bony wall of the cochlea just next to the stapes to introduce the SV pressure sensor into the cochlea. An electrode at the round window measured the compound action potential (CAP) response of the auditory nerve to tone pips of frequencies between 1 and 40 kHz, as a monitor of cochlear health (Johnstone et al. 1979). The CAP threshold was recorded several times during the experiment, in particular before and after making the SV hole. The middle ear muscles were not cut but were likely not active because of the deep pentobarbital anesthesia. Because the scala vestibuli pressure responses to single tone stimuli scaled linearly from 50 to 90 dB sound pressure level (SPL), middle ear muscle contractions at high sound levels did not appear to influence our results.

Stimulus presentation and response measurement

The animal was sound stimulated by a speaker coupled to the ear canal, as in the diagram in Fig. 1. A closed-field acoustic system was sealed into the ear canal after removal of the pinna. The system used a plastic T-tube of 1/8-in. diameter, with one branch of the T connected to the ear canal, the opposite branch providing access to the microphone's probe tube, which was sealed within the T branch, and the middle branch of the T connected to the speaker (a single Radio Shack 40–1377 tweeter) by 8 cm of Tygon tubing. The total tube length from the speaker to the ear canal was about 10 cm. Acoustic stimuli were generated digitally [Tucker Davis Technologies (TDT) System 3]. Acoustic distortion was checked in a cavity and was ≥70 dB beneath the level of the primaries when the primary level was 100 dB SPL, and smaller for lower primary levels (Dong and Olson 2005). The SPL was calibrated in the EC within 3 mm of the tympanic membrane (TM) using a Bruel & Kjær probe microphone system designed by Sokolich (1977). The transfer function of the probe tube was accounted for when setting the SPL and analyzing the data. This microphone also served as the receiver of the otoacoustic emission pressure. The noise level of the microphone is about 5–10 dB SPL up to 30 kHz, and slightly larger at higher frequencies.

The intracochlear pressure was measured by specialized fiber-optic pressure sensors inserted into the SV through a small hole that was hand-drilled next to the stapes. The sensor was normally inserted about 0.2 mm within the SV hole. The sensor position is illustrated in the *inset* of Fig. 1, which is based on a computed tomography (CT) scan of a cochlea with sensor (courtesy of W. Decraemer). The scan is oriented such that the round window was at the back. Much of the detail that was in the CT scan was excluded from the illustration, but a view of the basal turn organ of Corti is shown through an artificial "window" in the drawing. The pressure sensor was described previously (e.g., Olson 1998). It has a cylindrical shape, with outer diameter either 170 or 150 μm. The pressure sensors were calibrated individually before and after an experiment following the method by Schloss and Strassberg (1962), by submerging them a known distance beneath the surface of a vial of water that is shaken with a preset acceleration using a commercial shaker with built-in accelerometer (Bruel & Kjær model 4290). The acceleration was typically set to 0.2 m/s^2, so that when submerged 1 mm beneath the surface of water (density 1,000 kg/m^3) the pressure was 0.2 Pa (80 dB SPL). Standing waves in the vial were accounted for at the highest frequencies but their contribution is relatively small. The sensor sensitivity is nearly flat (within 3 or 4 dB) up to ≥40 kHz. Above 40 kHz the sensor's calibrated sensitivity drops off slightly, but because the shaker's frequency response is reliable up to only about 50 kHz we do not correct for the apparent drop off in sensor sensitivity, and use the sensor sensitivity from the frequency range <40 kHz in calculations. The sensitivity is usually about −30 dBV/80 dB SPL, but it varies with usable sensors from about −20 to −40 dBV/80 dB SPL. Changes in sensor sensitivity from day to day or after an experiment of about 10 dB are not uncommon. These changes are flat with frequency, but set a limit on the accuracy of the absolute pressure the sensors measure. The sensitivity changes are likely a result of the fragility of the sensor membrane. The noise floor of the pressure sensors corresponds to a pressure of about 60 dB SPL.

EC and SV pressure responses were recorded simultaneously. The TDT System 3 was operated with a sample period of 5 μs. The stimulus frequencies were adjusted so that the stimulus repeated every 4,096 points. Usually 51 repetitions were played. The first set of 4,096 points in the response (about 20 ms) was deleted to avoid the transient response and the remaining 50 time-locked segments were averaged and stored. The stored data were analyzed by Fourier transform, using MATLAB.

Experimental design

The EC and SV pressure were measured when two-tone stimuli were applied to the EC. The two primary tones produce DPs inside the cochlea when the cochlea is in good condition. A whole family of DPs is produced (Dong and Olson 2005); for this study we concentrated on the components that are usually the largest, $2f_1–f_2$ and $2f_2–f_1$. The two

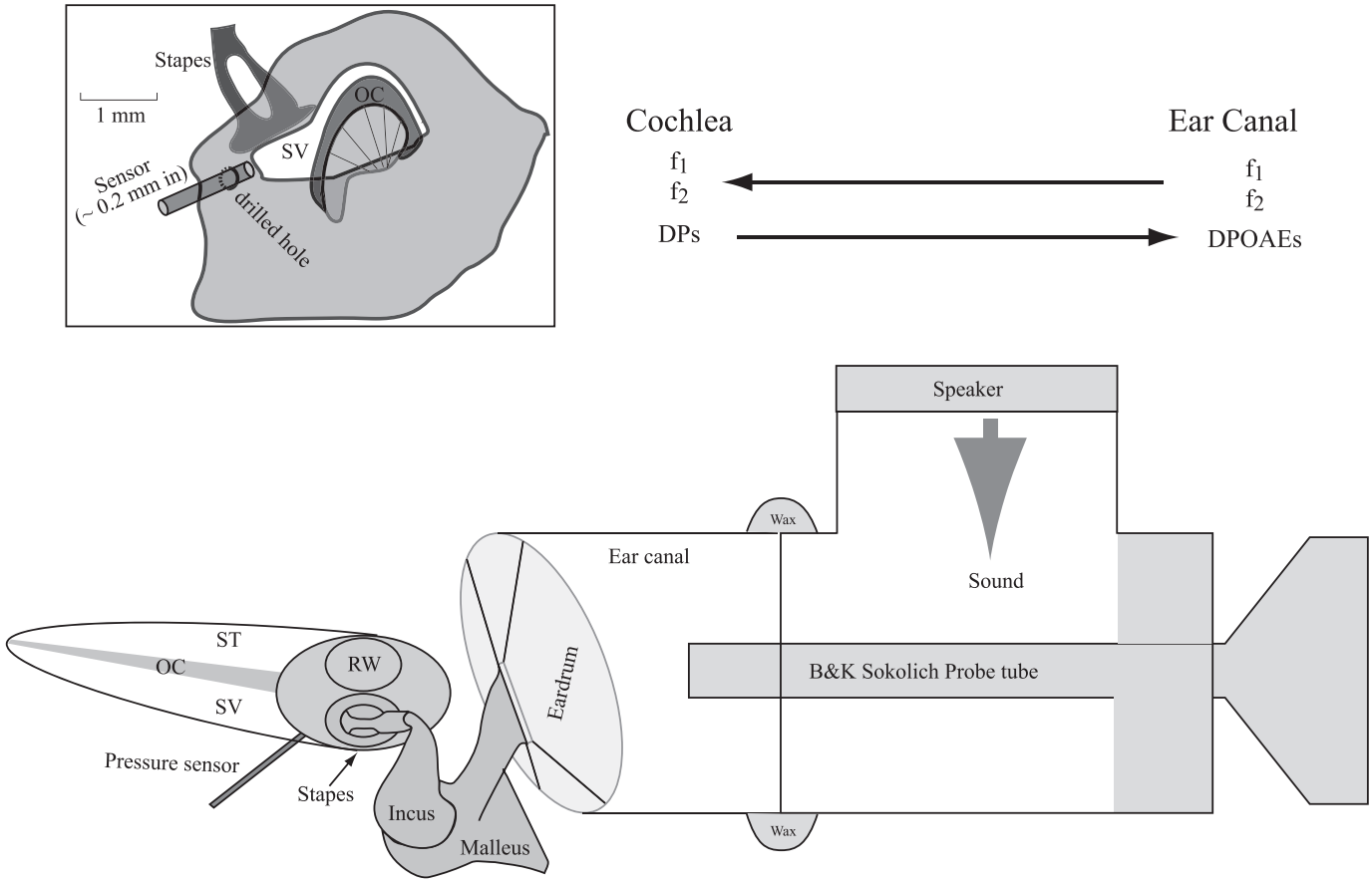

FIG. 1. Experimental approach for measuring middle ear forward and reverse transmission. A series of 2 tones with fixed f_2/f_1 ratio were used to drive the middle ear in the forward direction, whereas the distortion products that were generated within the cochlea drove the middle ear in the reverse direction. Pressure responses were measured simultaneously in the ear canal and scala vestibuli. Large diagram at the *bottom* shows the T-tube, to which ear canal, the speaker-coupling tube (8 cm Tygon tubing, not illustrated) and the microphone probe tube are attached. Fiber-optic pressure sensor was inserted about 0.2 mm into scala vestibuli in the region between the oval and round window, close to the stapes. *Inset*: based on a computed tomography (CT) scan of a cochlea and sensor, shows the position of the sensor with respect to the stapes and basal organ of Corti.

primary tones were of equal intensity and were varied in frequency while keeping the frequency ratio fixed at either 1.05 or 1.25. By comparing the primary (f_1 or f_2) SV pressure to the EC pressure, forward transmission was measured. By comparing the DPOAEs in EC pressure to the DPs in SV pressure, reverse transmission was measured. The highest frequency for which both DPs and DPOAEs could be detected was 20–30 kHz, and this set the upper frequency for which reverse transmission could be measured.

Definition of middle ear transfer functions

The forward and reverse transfer functions (FTFs and RTFs, respectively) were calculated using the SV pressure (SVP) as response and the EC pressure (ECP) as stimulus in the case of forward transmission, and using the ECP as response and the SVP as stimulus in the case of reverse transmission.

The middle ear FTF magnitude was obtained as the ratio of the SVP magnitude to the ECP magnitude of each of the primary tones

$$FTF_{mag} = SVP_{mag}/ECP_{mag} \text{ (at stimulus frequency)}$$

The FTF phase was obtained as the difference of the phase of the SVP and the ECP of each of the primary tones

$$FTF_{\phi} = SVP_{\phi} - ECP_{\phi}$$

The forward transmission group delay is defined as the negative derivative of the FTF phase as a function of frequency. In general, the group delay varies with frequency, but we will show that the slope is usually fairly constant within a data set and we do a linear least-squares fit to the data, using MATLAB

$$\text{Forward transmission group delay} = -dFTF_{\phi}/df$$

The middle ear RTF magnitude was obtained from the ratio of the ECP magnitude to the SVP magnitude of each of the $2f_1–f_2$ and $2f_2–f_1$ distortion components

$$RTF_{mag} = ECP_{mag}/SVP_{mag} \text{ (at a DP frequency)}$$

The RTF phase was obtained as the difference of the phase of the ECP and the SVP at the DP frequencies. The reverse transmission group delay is defined as the negative derivative of the RTF phase as a function of frequency

$$RTF_{\phi} = ECP_{\phi} - SVP_{\phi}$$

$$\text{Reverse transmission group delay} = -dRTF_{\phi}/df$$

The magnitude of the transfer functions is expressed in decibels, the phase in cycles, and the group delay in microseconds.

RESULTS

Results from four animals (wg62, wg67, wg68, and wg70) are reported herein and the results from other animals were consistent with those presented here. These four experiments

were chosen for several reasons: *1*) CAP threshold changes (1–30 kHz) were <10 dB before and after making the SV hole and introducing the sensor into the cochlea; *2*) the DPOAEs changed little before and after making the SV hole and introducing the sensor, and during several hours of measurements; and *3*) two-tone stimuli produced large and stable DPs up to or even above 20 kHz in both the EC and the SV.

Cochlear condition control

Both the CAP threshold and DPOAEs were used to monitor the cochlear condition. They were recorded at key steps in the procedure, especially before and after hand-drilling the SV hole next to the stapes. Figure 2 illustrates these responses.

The *top panels* of Fig. 2 show the individual CAP audiograms from 1 to 40 kHz. Thick lines represent the CAP audiograms measured after surgery, with the bulla widely open but before opening the cochlea. The CAP threshold was based on visual detection of the averaged CAP response, which usually corresponded to a peak-to-peak value of 5–10 μV. Thin lines show the CAP audiograms after making the SV hole next to the stapes and introducing the sensor into the cochlea. Usually the CAP threshold was >30 dB SPL at frequencies <1,000 Hz and then was quite flat ≤24 kHz, at a sound level of around 30 dB. Above this frequency, the CAP threshold usually started to climb quickly. After making the SV hole the CAP thresholds changed very little; they basically remained within 5 dB of the original measurements at frequencies <30 kHz. At frequencies >30 kHz, changes were likely caused by localized damage resulting from the basal location of the sensor.

Although the primary reason to show the CAP data is to illustrate that the CAP was changed little after introducing the sensor to the cochlea, the absolute CAP thresholds before making the small hole to the cochlea are also of interest; therefore we insert a brief discussion of these results here. The CAP thresholds were generally similar to the results of Muller (1996), but the increase in threshold at high frequency was not observed in another study (Overstreet et al. 2003). The Muller and Overstreet results are replotted together with our CAP threshold data in Fig. 2 (Muller: thick dashed-dotted line; Overstreet et al.: thin dashed–dotted line). The differences in threshold in the three studies likely have several causes. The vertical shifts in threshold were likely related to threshold criteria—in our case it was a visual detection of averaged data; this varies as a result of different noise levels. The differences in high-frequency cutoff might arise because of varying degrees of basal region damage and because of different methods for setting the sound stimulus. The gerbil has a large round window opening and, depending on the fluid level in the cochlea, the round window membrane can lie almost flat on top of the basilar membrane in the extreme basal region, which corresponds to frequencies of ≳30 kHz. On opening the bulla, it is very difficult to maintain healthy cochlear nonlinearity in the mechanical responses in this high-frequency region (even with care taken to open the bulla very quietly and use of a head heater), whereas the 20-kHz region just apical of the round window is relatively robust. If only a small hole were made in the bulla to access the round window for CAP electrode placement, as in the study by Overstreet, high-frequency thresholds would likely be better maintained than in our widely open bulla.

A second issue is the measurement of the sound within the ear canal at high frequencies. At frequencies >35 kHz the sound level varies by ≥5 dB between a position about a millimeter within the ear canal opening and a position close to the umbo, 2.5 mm deeper within the ear canal. The differences are about 15 dB at frequencies from 45 to 55 kHz. These unpublished data support previous reports of variations of sound pressure in the ear canal at frequencies that are high enough that the wavelengths are of comparable size to the ear canal (Khanna and Stinson 1985). Because of the difficulty of specifying the sound level in the ear canal at high frequencies, both the Overstreet and Muller reports cited above relied in part on sound measurements performed in a cavity rather than the ear canal to set the sound level in their experiments. Therefore variations in reported high-frequency responses are inevitable because of the different methods used to measure and set the sound stimulus level in the ear canal. This issue will

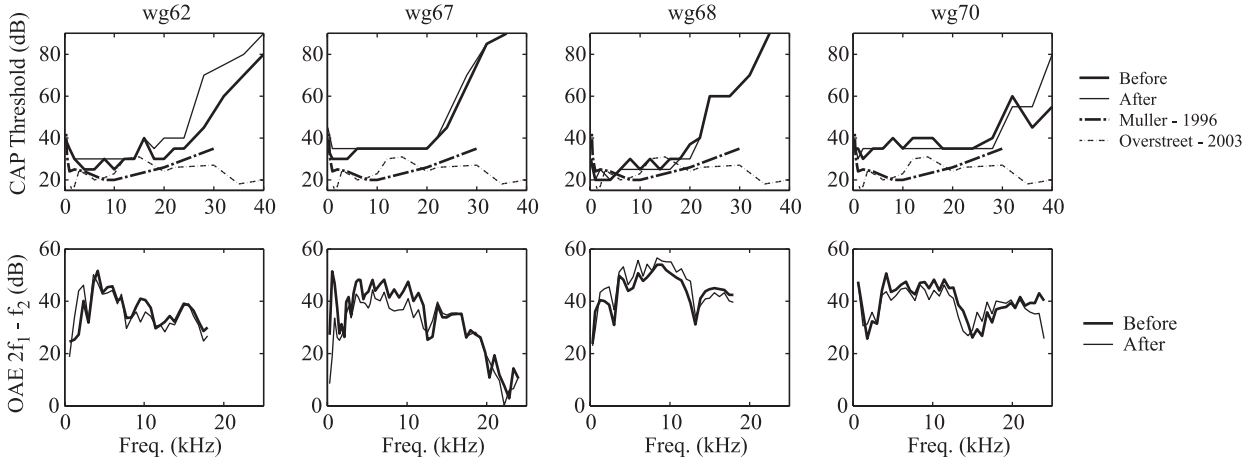

FIG. 2. Compound action potential (CAP) audiograms and otoacoustic emissions measured before and after making the scala vestibuli (SV) hole. *Top panels*: CAP audiograms measured from 1 to 40 kHz before (thick line) and after (thin line) making the SV hole in animals wg62, wg67, wg68, and wg70. Variation between before and after was within 5 dB for most of the frequencies <30 kHz. Muller (1996) and Overstreet et al. (2003) results are plotted as thick and thin dashed–dotted lines. *Bottom panels*: otoacoustic emission $2f_1$–f_2 plotted as a function of its own frequency before (thick line) and after (thin line) making the SV hole. Two primary tones were equal-intensity tones of 90 dB SPL, with a fixed f_2/f_1 ratio of 1.25. Variation between before and after was about 2 dB.

come up again below when reporting forward transmission gain.

The *bottom panels* of Fig. 2 show the DPOAE $2f_1–f_2$ amplitude plotted versus its own frequency before and after making the SV hole. The rest of the DPOAE family members (e.g., $2f_2–f_1$, $3f_1–2f_2$) showed similar results. The two primaries were equal-intensity tones of 90 dB SPL with a fixed f_2/f_1 ratio of 1.25. The 90 dB SPL data are shown because the $2f_1–f_2$ in the EC was well above the noise floor up to or even above 20 kHz. The thick and thin lines represent the $2f_1–f_2$ amplitude before and after making the SV hole, respectively. The change in DPOAE amplitude could be large (e.g., 2 kHz data, animal wg67), but in general the changes were only a few decibels. Both increases and decreases were observed, but did not change the basic shape of the DPOAE response. The relatively large low-frequency variations in evidence in wg62 and wg67 might have arisen from small changes in the coupling between the ear canal and ear canal tube, which was disconnected and reconnected between the two measurements.

The CAP response is a good indicator of cochlear condition and the stability of the CAP response throughout most of the frequency range confirms that making the small hole to the SV is experimentally practical and does not disturb the fragile physiological sound-processing mechanism except at the highest frequencies (Olson 2001). The observation that the DPOAE changed little after opening the cochlea indicates that the presence of the sensor within the cochlea did not substantially influence either the production of distortion products or their transmission out of the cochlea and through the middle ear. This study is concerned with middle ear transmission and the health of the cochlea is not expected to be critical to the characteristics or significance of our results. However, the health of the cochlea was of practical importance because even at high stimulus levels DP responses could not be detected >10 kHz in damaged cochleae. The fact that the cochleae were maintained in good physiological condition indicates that our findings are applicable to the study of emissions in an intact preparation.

Middle ear forward transmission

Figure 3 shows the middle ear FTF magnitude and phase (SV relative to EC pressure response) as a function of frequency for the four study animals. The stimuli were 80-dB SPL pure tones. The response of each individual is plotted as a thin line and the average is shown as the thick line. There is about 10 dB of spread between different animals. One contributor to this spread is uncertainty in sensor sensitivity. The FTF was quite flat with frequency from 1 to 40 kHz, with an average value of about 20–25 dB. The FTF phase varied approximately linearly as a function of increasing stimulus frequency, with an average slope corresponding to a group delay of 32 μs. The group delays were calculated by a linear least-squares fit to the data for frequencies from 1 to 50 kHz. The literature on the middle ear reports that it operates linearly up to high stimulus levels (e.g., Nedzelnitsky 1980) and in tests with single-tone acoustic stimuli we confirmed this at stimulus levels from 50 to 90 dB SPL (not shown).

The amplitude drop off >40 kHz is different from previous results (Olson 1998). This high-frequency difference is likely related to a technical difference in the present and previous studies. In previous studies a fiber-optic microsensor was used to measure ear-canal sound pressure close to the umbo (within about 1–2 mm). In the present study the larger and more sensitive Bruel & Kjær microphone measured the sound pressure by a probe tube about 1.5 mm inside the entrance of the bony ear canal, a distance of nearly 2 mm from the previous position. Our own ear canal pressure maps show spatial variations in pressure of up to 15 dB at frequencies >45 kHz (unpublished results), and the drive voltages to the speaker (which were set based on the ear canal pressure) show a pronounced dip at frequencies around 50 kHz in the present but not the previous set of measurements. Therefore the high-frequency difference in FTF magnitude is likely a matter of where the sound was measured within the ear canal. Another difference between the present and previous results is that the FTF magnitude was reported previously as about 30 dB, but here is 20–25 dB. Again the difference is likely explained by the fact that in the previous set of experiments the ear canal sound was measured with one of the fiber-optic microsensors. When used in fluid, the sensor sensitivity is uncertain at the 10-dB level, as noted above. In addition, the air/fluid difference of the fiber-optic sensor sensitivity tends to favor fluid sensitivity in sensors that have been used (perhaps because a film develops on the membrane that dissolves off in the water but not in the air) and on experiment days only water calibrations were performed. This would introduce a bias in the previous results toward larger FTF. Therefore we believe the 20- to

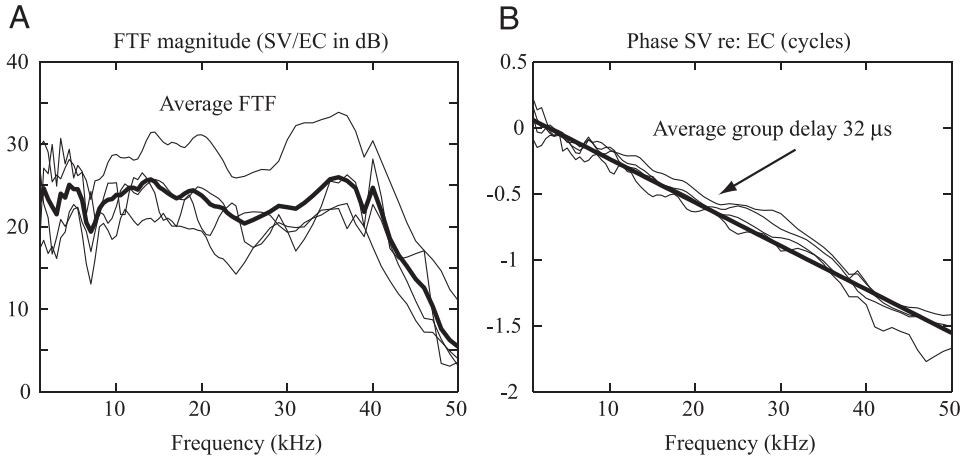

FIG. 3. Grouped middle ear forward transmission results from 4 animals, found with the scala vestibuli pressure (SVP) response to 80-dB SPL single-tone stimulation. *A*: individual middle ear forward transfer functions [(SVP)/ear canal pressure (ECP)] are plotted as thin lines, their average as the thick line. Below 40 kHz the forward transfer function (FTF) amplitudes were primarily flat, with mild peaks and valleys. Over this frequency range the average FTF amplitude was 23 ± 4 dB (thick line). *B*: SVP phase responses relative to the ECP phases. Individual animal responses are plotted as thin lines, their average as the thick line. Phase varied linearly with frequency with an average group delay of about 32 μs.

25-dB FTF gain of the present measurements is the more reliable number. Finally, the FTF group delay of 32 μs was longer than the nearly 25-μs delay reported previously. Again, this difference is likely related to the position within the ear canal where the sound stimulus was measured. It is also influenced by the frequency range used to calculate the delay.

Middle ear forward and reverse transmission

Figures 4 and 5 show forward and reverse transmission with f_2/f_1 ratios of 1.05 and 1.25, respectively. The frequency spacing between adjacent f_2 primaries (the frequency resolution) is not the same for the different animals. For wg62 it was 1 kHz, for wg67 it was 250 Hz, and for wg68 and wg70 it was 500 Hz. The finer frequency resolution was useful for exposing details in the reverse transmission. These data were gathered with primary levels set at 90 dB SPL. This relatively high sound level was used to produce a wide frequency range of detectable DPs and DPOAEs. The reverse transfer function found with 80-dB primaries was the same as with 90-dB primaries and we do not think the high stimulus level influenced the validity of the RTF results.

In the EC row (*row A* of Figs. 4 and 5), the two primaries are plotted as gray (f_1) and black (f_2) thin lines in the figure. The DPOAEs are shown as thick lines, $2f_1$–f_2 (gray) and $2f_2$–f_1 (black). With the f_2/f_1 ratio of 1.05 (*row A* in Fig. 4), the amplitudes of the $2f_1$–f_2 and $2f_2$–f_1 DPOAEs were nearly the same, and about 50 dB down compared with the level of the EC primaries. In SV (*row B* in Fig. 4), the primaries were at a level of about 110–120 dB SPL, and the DPs were about 30–40 dB smaller. With the increase of f_2/f_1 ratio to 1.25 (*rows A* and *B* in Fig. 5), the $2f_1$–f_2 DP was slightly greater than the $2f_2$–f_1 DP both in the SV and the EC. Also, more structure was apparent in the $2f_2$–f_1 component both in the SV and the EC: a pronounced dip at 7–8 kHz in wg67, wg68, and wg70 and at 13 kHz in wg62 (black arrowheads in Fig. 5). In both the EC and SV, the DPs were above the noise floor at frequencies $\leq$20–30 kHz. (The noise floor is indicated by the dotted line.)

The middle ear FTF and RTF magnitudes are shown in *row C* (forward: thin, reverse: thick). To see more detail in the TFs we plot −RTF (in dB) instead of RTF, then both the FTFs and −RTFs can be plotted on scales from 10 to 50 dB. The FTF magnitude was flat with frequency to about ±5 dB in all these animals. Its basic level varied from about 25 dB (wg62 and wg67) to about 20 dB (wg70) to $\lesssim$20 dB (wg68). The FTFs from wg67, wg68, and wg70 showed a dip at about 7 kHz (gray arrowheads in Figs. 4 and 5, *row C*). Structure in this region has been noted before (Olson 1998) but its cause is not known. It is notable in this regard that the RTFs did not show the 7-kHz dip, although it *was* present in the individual SV and EC DP pressures in wg67 and wg68. Therefore the 7- to 8-kHz structure seems to arise within the mechanics of the cochlea, or at the interface between the cochlea and the middle ear but not (for example) at the tympanic membrane.

In examining the RTFs from Figs. 4 and 5, what is most striking is that within a given animal, the RTFs were very similar even though the SV and EC results that went into these varied quite a bit. Each animal's RTF had frequency variations containing both large-frequency scale structure and fine-frequency scale structure. In terms of the large-scale structure, the RTF increased 5–10 dB (−RTF decreased) from 0 to 10 kHz and then flattened out in wg62, wg70, and wg67. Animal wg68's RTF had the most pronounced large-scale structure, with two lobes separated by a broad 10-dB peak (dip in −RTF)

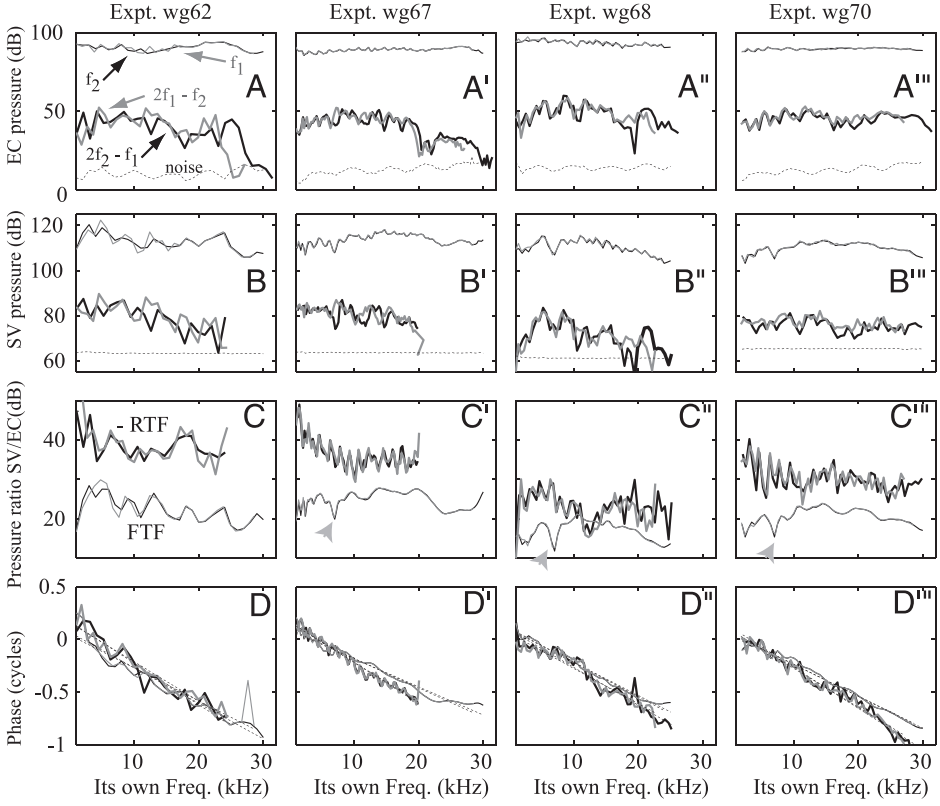

FIG. 4. Middle ear forward (FTFs) and reverse transfer functions (RTFs) calculated with fixed f_2/f_1 ratio of 1.05 and $L_1 = L_2 = 90$ dB SPL. Primary components, f_1 and f_2, are shown as gray and black thin lines; the distortion components, $2f_1$–f_2 and $2f_2$–f_1, are shown as gray and black thick lines. *Row A*: EC pressure. *Row B*: SV pressure. Dotted lines in *rows A* and *B* represent the amplitude noise floor. *Row C*: middle ear FTF (thin lines) and RTF (thick lines). *Row D*: primary SVP phase relative to ECP phase (thin lines) and distortion product otoacoustic emission (DPOAE) phase relative to SV distortion product (DP) phase (thick lines). Dotted lines show the linear fits to the phase data. Frequency spacing for animals wg62, wg67, wg68, and wg70 was 1,000, 250, 500, and 500 Hz, respectively. Gray arrowheads point at recurring features at about 7 kHz in FTF.

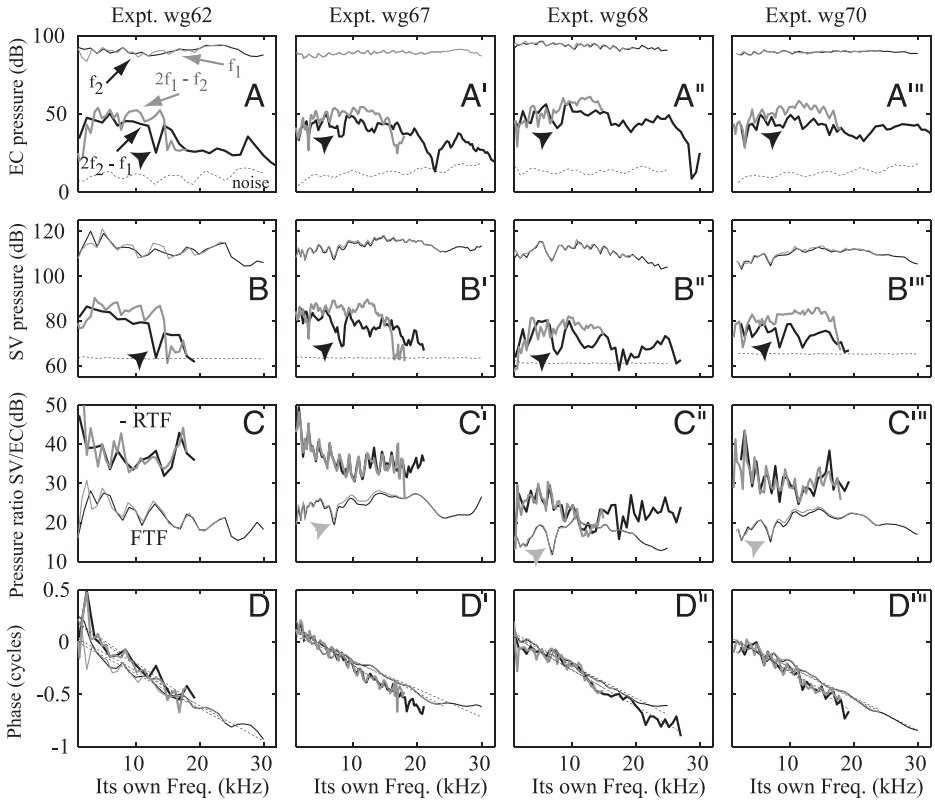

FIG. 5. Middle ear FTFs and RTFs calculated with fixed f_2/f_1 ratio of 1.25 and $L_1 = L_2 = 90$ dB SPL. Primary components, f_1 and f_2, are shown as gray and black thin lines; the distortion components, $2f_1–f_2$ and $2f_2–f_1$, are shown as gray and black thick lines. *Row A*: EC pressure. *Row B*: SV pressure. Dotted lines in *rows A* and *B* represent the amplitude noise floor. *Row C*: middle ear FTF (thin lines) and RTF (thick lines). *Row D*: primary SVP phase relative to ECP phase (thin lines) and DPOAE phase relative to SV DP phase (thick lines). Dotted lines show the linear fit to the data. Frequency spacing for animals wg62, wg67, wg68, and wg70 was 1,000, 250, 500, and 500 Hz, respectively. Black arrowheads indicate pronounced features in the DP and DPOAE that are not present in the RTF. Gray arrowheads indicate recurring features at approximately 7 kHz in FTF.

at about 12 kHz. In terms of the fine structure, all the animals have ±3- to 4-dB variations superimposed on the large-scale structure. In wg67, wg68, and wg70 these variations were very regular, with a frequency spacing of about 1.5 kHz. As discussed in the following text, this fine structure might be caused by reflections in the sound system. Such fine-scale structure might not appear in wg62 because the frequency resolution of the adjacent f_2 primaries, at 1 kHz, was not fine enough.

The most broad-brush description of the RTF magnitudes is that they were quite flat from about 2 kHz to the highest measurable frequency of 30 kHz. This echoes the broad-brush description of the FTF magnitude. It is notable that many of the pronounced features in the DP responses in SV and EC (black arrowheads in Fig. 5) disappeared when taking the ratio to find the RTF magnitude.

If the increase in pressure in forward transmission through the middle ear were balanced by an equal decrease in pressure in reverse transmission, the absolute values of FTF and RTF would be the same. However, this was not seen; instead the pressure decreased by about 10–15 dB more in the reverse direction than it increased in the forward direction. (It is worth noting that this difference between RTF and FTF magnitudes is not attributable to uncertainty of the fiber-optic pressure sensor sensitivity because a mistake in calibrated sensitivity would influence both RTF and FTF, and not influence this difference.)

The middle ear FTF and RTF phases, plotted in units of cycles, are shown in *row D* of Figs. 4 and 5. The FTF phases of the f_1 and f_2 primaries are plotted as thin gray (f_1) and black (f_2) lines. The RTF phases found with the DPOAEs and DPs are plotted as thick gray ($2f_1–f_2$) and black ($2f_2–f_1$) lines. In the phase, the RTF was similar to the FTF in being an approximately linearly decreasing function of frequency that can be described as a simple delay. Finely spaced (about 1.5 kHz) variations in the RTF phase go along with the regular variations in the amplitude in wg67, wg68, and wg70, but do not mask the overall linear progression. The average delay, found as the negative slope of the line, is 38 μs, which is longer than the 32-μs delay of forward transmission. wg68, the animal whose RTF amplitude had the most pronounced large-scale frequency response, also had a relatively pronounced large-scale frequency variation in its RTF phase. The RTF magnitude peak (−RTF dip) at about 12 kHz was accompanied by a region of increased phase slope and at frequencies preceding the peak the phase slope was relatively gradual compared with the FTF slope and the RTF slope of the other animals.

Figures 4 and 5 showed that, although the FTF and RTF magnitudes were both fairly flat and the phases were both fairly delaylike, fine structure existed in the RTF that did not exist in the FTF. This will be discussed further below. Here we show a final figure that demonstrates a complementary finding: sometimes variations appeared in FTF that were not in evidence in the RTF. Figure 6 contrasts results from wg67 early in the experiment (dotted lines) to results from 4 h later (solid lines), but postmortem and after the cochlea had been damaged by passage of DC current (as part of a study of electrically evoked emissions to be published later). The DPOAEs and DPs at frequencies $\gtrsim$10 kHz were reduced to the noise level, but at lower frequencies were well above the noise level. [Emissions in unhealthy cochlea at high stimulus levels are well known in the literature and are thought to be physiological in origin (Avan et al. 2003; Dorn et al. 2001).] Note that the SVP at the primary frequencies was reduced in the late measurement; thus FTF was decreased. A decrease in FTF at primary frequencies does not always occur postmortem, and we do not know

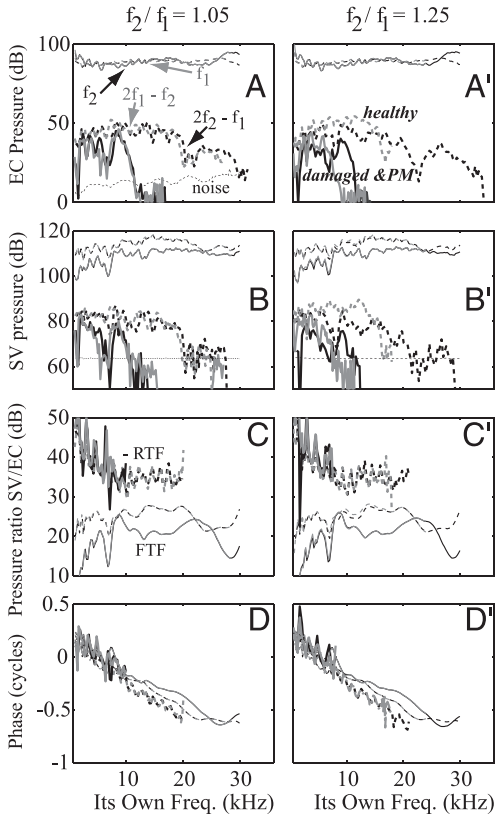

FIG. 6. FTF and RTF in damaged cochlea (animal wg67). *Left and right columns*: used f_2/f_1 ratios of 1.05 and 1.25, respectively ($L_1 = L_2 = 90$ dB SPL). Dotted lines show in vivo results as in Figs. 4 and 5; the solid lines show primary pressures late in the experiment, postdamage and postmortem. Thick lines show distortion pressures, postdamage and postmortem. Thin dotted lines show the noise floor and is excluded in the *right top* figure to reduce clutter. *Row A*: EC pressure. *Row B*: SV pressure. *Row C*: middle ear FTF (thin lines) and RTF (thick lines). *Row D*: primary SVP phase relative to ECP phase (thin lines) and DPOAE phase relative to SV DP phase (thick lines). SVP at the primary frequencies was reduced in the postdamage, postmortem condition at most frequencies; therefore the FTF changed in the late vs. early condition. DPs and DPOAEs were both smaller postdamage, postmortem and could be detected only through about 10 kHz. However, the level and large-scale frequency structure of the RTF did not change.

whether the decrease here is attributable to severe damage to the basal organ of Corti or some aspect of the postmortem condition, such as retraction of cochlear fluid level. What is important is that the FTF changed in the late versus early measurements. The dip at 8 kHz was deeper and the amplitude dropped by 5–10 dB at most frequencies and even more at the lowest frequencies. In contrast, the level and large-scale frequency structure of the RTF did not change, and the fine-frequency structure was nearly the same, although a little more pronounced (±5 dB rather than ±3–4 dB). This lack of change in RTF was observed, even though both DPs and DPOAEs were substantially reduced, and illustrates an important lack of sensitivity of the RTF to the cochlear condition. The late RTF phase was nearly identical to the early RTF phase through a frequency of 10 kHz where late data were out of the noise. Although the reduced-frequency region made the delaylike behavior less obvious in the late RTF, the late/early similarity in the phase–frequency relationship strongly suggests a delay-like relationship, with ECP following SVP, confirming that these robust postdamage, postmortem distortions were generated within the cochlea.

DISCUSSION

Otoacoustic emissions are used to explore the auditory system noninvasively, both with clinical objectives and to understand the basic mechanism of hearing. Because they are generated within the cochlea, OAEs are primarily used to explore the cochlea, rather than the middle ear. However, an understanding of how emissions are shaped by the middle ear is necessary to be able to parse the characteristics of emissions into middle and inner ear effects. For example, the frequency response of emissions might be determined by both the middle and inner ear. Moreover, the reverse transmission must be quantified to be able to work backward from emissions for the sizes of intracochlear pressures and forces. Several recent studies have measured the transmission of emissions out through the middle ear. This study extends the measurements to substantially higher frequencies than those of previous studies. This extension proved useful, particularly for characterizing the phase.

This study made use of cochlear nonlinearity to generate an intracochlear pressure to explore reverse transmission of emissions through the middle ear. In gerbil, in vivo, forward and reverse middle ear transfer function magnitudes were to a first approximation flat with frequency from about 2 kHz to the highest frequency for which they are measurable, about 30 kHz. In the forward direction the pressure gain was about 20–25 dB, whereas in the reverse direction the pressure loss was usually about 30–40 dB. The transfer function phases were linear with frequency, with slopes that indicated a forward group delay of about 32 μs and a reverse group delay of about 38 μs. The FTF departed from this simple description most prominently with a nearly 5-dB dip that often occurred at about 7 kHz. Structure in the RTF occurred on both large- and fine-frequency scales, introducing about ±6 dB of variation. At frequencies <10 kHz the RTF usually decreased by 5–10 dB as the frequency decreased.

Comparison with previous studies

Our results show both similarities and differences with previous middle ear reverse transmission studies. Our studies are most akin to those of Magnan et al. (1997). Magnan et al. (1997) directly measured the guinea pig forward and reverse middle ear functions in vivo, by comparing $2f_1$–f_2 pressure in EC and SV from 1.5 to 8 kHz ($L_1 = 50$ dB, $L_2 = 60$ dB, $f_2/f_1 = 1.2$). The forward and reverse transfer function magnitudes were found to be nearly flat, at levels of 25 and −35 dB, respectively. The difference between the forward and reverse transfer functions was suggested as being attributable to the different loads acting on the middle ear chain, which depends on the direction of the signal transmission. Phase was not presented in the 1997 paper, but in a subsequent study (Magnan et al. 1999) that used an intracochlear speaker to produce the intracochlear sound source, the phase of the RTF was reported to be about 0 cycles up to 2,000 Hz. Some of our data are not inconsistent with this (e.g., data <5 kHz in wg68 and wg70) but we do not have enough data points in the low-frequency region for a good comparison. By extending the

frequency range upward to include more of the response area of the gerbil, a delaylike phase was clear in our data. A delaylike phase *was* apparent in the FTF of Magnan et al.'s results, for which phase was reported up to 20 kHz. Puria (2003) characterized the forward and reverse transfer functions of the middle ear of human temporal bone. The middle ear was driven by a sound source in both the forward and reverse directions: for forward transmission the sound source was positioned in front of the tympanic membrane; for reverse transmission the sound source was coupled to the inner ear near the round window. The FTF and RTF magnitudes were not flat, but band-pass. Despite this basic difference, there are also similarities between the human and gerbil results. Between 2 and 8 kHz the RTF showed about 10–15 dB more loss than the FTF showed gain, and in this frequency region the phase was approximately delaylike, with the reverse group delay longer than the forward delay (111 vs. 83 μs, calculated from Fig. 2 of Puria 2003). Finally, Voss and Shera (2004) studied the cat's middle ear in forward and reverse directions by comparing EC pressure to stapes velocity from 100 Hz to 10 kHz with two-tone stimuli. Prominent in their results was a peak at about 1 kHz; at 10 kHz the forward response had settled down to a value about 10 dB less than the peak value. The forward phase-versus-frequency was approximately delaylike, with a value of about 50 μs. The RTF magnitude was not as simply related to the FTF magnitude as in the results of Magnan et al. (1997) and those reported here, and the RTF phase was not delaylike. Explorations of a wider frequency range would be useful in cat to better understand the species differences because cat shares similarities with human (prominent peak in response) and gerbil (wide frequency range of hearing).

Load effects

The primary purpose of the middle ear is to transmit sound efficiently from the relatively low impedance space of air to the relatively high impedance space of the cochlea. Experiments in which the cochlea was removed from the system show the importance of the cochlear load to the middle ear's normal forward transmission (e.g., Puria and Allen 1998). Forward transmission is relatively uninfluenced by ear canal load—by whether the sound is introduced to a foam-plugged ear canal by an earphone or to the open ear canal from a distant source. Conversely, reverse transmission from the cochlea into the ear canal is expected to be sensitive to the load at the ear canal, and the robustness of the reverse transmission result—how sensitive it is to particular measurement conditions—is largely a question of the effect of the termination of the ear canal. In human OAE studies the termination is typically a nonreflective foam ear plug with narrow speaker and microphone tubes threaded through. In measurements in animals, the termination is often an ear tube of nearly the same diameter as that of the ear canal, which is itself terminated some centimeters distant at the speaker, and through which a narrow probe tube is threaded to measure the sound at the ear canal. This is the way our system was, with an 8-cm Tygon tube attaching the speaker to a 2-cm-long ear tube. An open field measurement is another alternative, although it is not commonly used.

The effect of an open field on emissions was explored experimentally by Withnell and colleagues (1998) in guinea pig. They compared electrically evoked emissions in a sealed ear canal (similar to the usual condition in human studies) to the emissions in an open-field condition, in which the microphone probe tube was inserted some distance into the open ear canal. In those studies the effect of the open condition was a reduction in emission level at frequencies <5 kHz, and no change in emission level at frequencies >5 kHz. In the low-frequency region the reduction became larger as frequency was reduced, with a 7- to 8-dB reduction at 1 kHz. Magnan et al. (1999) also explored the influence of load in guinea pig by making ear canal recordings in both open and closed conditions. They found that the open condition decreased the emission size compared with the closed condition by 30 dB at 100 Hz, but the difference between the two conditions decreased steadily with an increase in frequency and was no longer influential above 1,000 Hz. The observation that the open condition does not reduce emission size at frequencies above a few kilohertz can be understood as a result of the sound wavelengths being small enough so that the emission pressure at the TM radiates into the air space of the ear canal as a largely unreflected sound wave, whether the ear canal is terminated with foam or simply left open. (In other words, the observed insensitivity to ear canal condition should not be interpreted as signifying that reverse transmission is insensitive to ear canal load, but rather that the ear canal load is not sensitive to whether the ear canal is open, at frequencies above a few kilohertz.) The literature on power flow through the middle and outer ear is relevant to this topic (Ravicz et al. 1996; Rosowski et al. 1986). Extending the results of Whitnell and Magnan to gerbil, we might expect that at frequencies above a few kilohertz the emissions would be fairly independent of ear canal termination (as long as it is not too reflective) and in that sense robust.

On the other hand, a perturbation arising from ear canal load is a reasonable explanation for the frequency spacing of the evenly spaced ripples we observed in the RTF of animals wg67, wg68, and wg70. A 10-cm tube has half-wavelength–resonant frequencies at multiples of about 1.7 kHz, close to the approximately 1.5-kHz spacing observed. The primaries also had ripples with this spacing (*row A* in Figs. 4 and 5), which themselves mirrored small ripples in the ear canal measurements used to set the sound level. The observation that the ripples appeared in the primaries suggests that the tube's reflective properties changed slightly after the initial ear canal measurements. This might occur, for example, as the result of a bend in the sound coupling tube or a small change in its length where tubes are coupled. Because the ripples in the primaries were small we did not work to eliminate them from our system. It is notable that the FTF in these animals did not contain ripples, thus supporting the validity of the expectation that the ear canal load will be more influential on the RTF than on the FTF. If the supposition that the small ripples arose from reflections in the sound tube is correct, this aspect of the results is dependent on the ear canal load.

The results just discussed above indicated that the ear canal load was more influential to the RTF than to the FTF. Figure 6 illustrates the complementary result, that the cochlear load was more influential to the FTF than to the RTF. The results of Fig. 6 are clinically important in demonstrating that cochlear changes that affect DPOAEs likely do not affect the transmission of the emissions out of the cochlea and through the middle ear. Another important aspect of the results of Fig. 6 is that the

direction of the phase delay—with DPs preceding DPOAEs even in the damaged, postmortem condition—indicates that even high stimulus level postmortem emissions arise within the cochlea.

Why was there more reverse transmission loss than forward transmission gain?

If the RTF and FTF were reciprocals of each other, their magnitudes would sum to zero when expressed in decibels, and we have noted that the observed RTF and FTF magnitudes did not. The technical concept of reciprocity has a specific meaning in engineering, which was not tested here. In particular, a system that is technically reciprocal would not in general have RTF and FTF magnitudes that were reciprocals because the relative magnitudes of the RTF and FTF values would depend on the loads to the system (ear canal and cochlea for the system of the middle ear). Recognizing that we are not addressing this technical concept of reciprocity, it is nevertheless instructive to consider why the magnitudes of FTF and RTF differ. One possibility for the difference in magnitude is that for forward transmission the sound pressure is likely fairly uniform as it travels down the sound tube and ear canal to the eardrum (in unpublished pressure maps, substantial ear canal variations in pressure occur only at frequencies >40 kHz, as noted above) but in reverse transmission the ear canal pressure might be spatially varying. That is because in reverse transmission the ear canal pressure is likely directly sourced by the motion of the TM, which is known to break up into a complex modal pattern at frequencies $\gtrsim$2 kHz when sound is delivered to the ear canal (Decraemer et al. 1989). This could be one factor in the relatively large pressure decrement of reverse transmission, and perhaps for the increased delay as well, because the eardrum's wavelike modal motion might be more important to transmitting sound out, than transmitting it in. In this regard, based on measurements in cat the forward delays that can be calculated from the wave patterns observed by Decraemer et al. (1989) and analyzed by Fay et al. (2005) are substantially longer than the forward transmission delay deduced by Puria and Allen (1998); this argues against a prominent role of TM waves in forward transmission. Another possibility is that the *intracochlear* pressure in the vestibule is spatially varying in the case of reverse transmission, in which case the additional delay we see in reverse transmission might be a cochlear delay. As Fig. 1 shows, our SV measurement was very close to the stapes and this explanation seems unlikely, but cannot be ruled out. In general, additional measurements are required to probe the physical basis for both forward and reverse transmission through the middle ear.

Finally, we end with the question that motivated the studies: What is the effect of the middle ear on OAEs? In gerbil, for measurements in a closed system that included a 10-cm length of tubing, and at frequencies $\gtrsim$1 kHz, the middle ear reduces the intracochlear pressure by about 35–40 dB and delays it by about 38 μs, across a wide range of frequencies. Therefore differences in different DPOAEs (which were notable here with a ratio of 1.25) and pronounced features (e.g., in the $2f_2$–f_1 components at a ratio of 1.25) are likely attributable to the cochlea. On the other hand, the RTF can introduce structure of its own—in particular, reflections in the ear canal load appeared to introduce evenly spaced variations into the RTF in some of our experiments. The nearly flat reverse transmission and its delaylike phase are likely closely coupled to the nearly flat magnitude and delaylike phase of forward transmission. Based on the results of others, in species for which forward transmission is peaked in frequency, reverse transmission can also be expected to be peaked.

ACKNOWLEDGMENTS

The authors are grateful to S. Khanna for the Sokolich microphone setup, to S. Khanna and O. de La Rochefoucauld for helpful comments, and to the JASA reviewers, J. Rosowski and a second reviewer who remained anonymous, for improvements to the manuscript.

GRANTS

This work was funded by National Institute of Deafness and Other Communications Disorders Grant R01 DC-03130.

REFERENCES

Avan P, Bonfils P, Gilain L, and Mom T. Physiopathological significance of distortion-product otoacoustic emissions at 2f1-f2 produced by high- versus low-level stimuli. *J Acoust Soc Am* 113: 430–441, 2003.

Avan P, Magnan P, Smurzynski J, Probst R, and Dancer A. Direct evidence of cubic difference tone propagation by intracochlear acoustic pressure measurements in the guinea-pig. *Eur J Neurosci* 10: 1764–1770, 1998.

Dancer A and Franke R. Intracochlear sound pressure measurements in guinea pigs. *Hear Res* 2: 191–205, 1980.

Decory L, Franke RB, and Dancer AL. Measurement of middle ear transfer function in cat, chinchilla and guinea pig. In: *The Mechanics and Biophysics of Hearing*, edited by Dallos P, Geisler CD, Matthews JW, Ruggero MA, and Steele CR. Berlin: Springer-Verlag, 1990, p. 270–277.

Decraemer WF, Khanna SM, and Funnell WR. Interferometric measurement of the amplitude and phase of tympanic membrane vibrations in cat. *Hear Res* 38: 1–17, 1989.

Decraemer WF, Khanna SM, and Funnell WR. Malleus vibration mode changes with frequency. *Hear Res* 54: 305–318, 1991.

Dong W and Olson ES. Two-tone distortion in intracochlear pressure. *J Acoust Soc Am* 117: 2999–3015, 2005.

Dorn PA, Konrad-Martin D, Neely ST, Keefe DH, Cyr E, and Gorga MP. Distortion product otoacoustic emission input/output functions in normal-hearing and hearing-impaired human ears. *J Acoust Soc Am* 110: 3119–3131, 2001.

Fay J, Puria S, Decraemer WF, and Steele C. Three approaches for estimating the elastic modulus of the tympanic membrane. *J Biomech* 38: 1807–1815, 2005.

Johnstone JR, Alder VA, Johnstone BM, Robertson D, and Yates GK. Cochlear action potential threshold and single unit thresholds. *J Acoust Soc Am* 65: 254–257, 1979.

Kemp DT. Towards a model for the origin of cochlear echoes. *Hear Res* 2: 533–548, 1980.

Khanna SM and Stinson MR. Specification of the acoustical input to the ear at high frequencies. *J Acoust Soc Am* 77: 577–589, 1985.

Lay DM. The anatomy, physiology, functional significance and evolution of specialized hearing organs of gerbilline rodents. *J Morphol* 138: 41–120, 1972.

Lynch TJ 3rd, Nedzelnitsky V, and Peake WT. Input impedance of the cochlea in cat. *J Acoust Soc Am* 72: 108–130, 1982.

Magnan P, Avan P, Dancer A, Smurzynski J, and Probst R. Reverse middle-ear transfer function in the guinea pig measured with cubic difference tones. *Hear Res* 107: 41–45, 1997.

Magnan P, Dancer A, Probst R, Smurzynski J, and Avan P. Intracochlear acoustic pressure measurements: transfer functions of the middle ear and cochlear mechanics. *Audiol Neurootol* 4: 123–128, 1999.

Muller M. The cochlear place-frequency map of the adult and developing Mongolian gerbil. *Hear Res* 94: 148–156, 1996.

Nedzelnitsky V. Sound pressures in the basal turn of the cat cochlea. *J Acoust Soc Am* 68: 1676–1689, 1980.

Olson ES. Observing middle and inner ear mechanics with novel intracochlear pressure sensors. *J Acoust Soc Am* 103: 3445–3463, 1998.

Olson ES. Intracochlear pressure measurements related to cochlear tuning. *J Acoust Soc Am* 110: 349–367, 2001.

Olson ES and Cooper NP. Stapes motion and scala vestibuli pressure in gerbil. *Proc 23th Ann MidWinter Mtg Assoc Res Otolaryngol, St. Petersburg Beach, FL*, 2000.

Overstreet EH 3rd, Richter CP, Temchin AN, Cheatham MA, and Ruggero MA. High-frequency sensitivity of the mature gerbil cochlea and its development. *Audiol Neurootol* 8: 19–27, 2003.

Puria S. Measurements of human middle ear forward and reverse acoustics: implications for otoacoustic emissions. *J Acoust Soc Am* 113: 2773–2789, 2003.

Puria S and Allen JB. Measurements and model of the cat middle ear: evidence of tympanic membrane acoustic delay. *J Acoust Soc Am* 104: 3463–3481, 1998.

Puria S, Peake WT, and Rosowski JJ. Sound-pressure measurements in the cochlear vestibule of human-cadaver ears. *J Acoust Soc Am* 101: 2754–2770, 1997.

Puria S and Rosowski JJ. *Measurement of Reverse Transmission in the Human Middle Ear: Preliminary Results*. Singapore: World Scientific, 1996.

Ravicz ME, Rosowski JJ, and Voigt HF. Sound-power collection by the auditory periphery of the mongolian gerbil *Meriones unguiculatus*. II. External-ear radiation impedance and power collection. *J Acoust Soc Am* 99: 3044–3063, 1996.

Rosowski JJ. The middle and external ears of terrestrial vertebrates as mechanical and acoustic transducers. In: *Sensors and Sensing in Biology and Engineering*, edited by Barth FG, Humphrey JA, and Secomb TW. New York: Springer-Verlag, 2003.

Rosowski JJ, Carney LH, Lynch TJ, and Peake WT. The effectiveness of external and middle ears in coupling acoustic power into the cochlea. In: *Peripheral Auditory Mechanisms*, edited by Allen JB, Hall JL, Hubbard AE, Neely ST, and Tubis A. New York: Springer-Verlag, 1986.

Ruggero MA and Temchin AN. The roles of the external, middle, and inner ears in determining the bandwidth of hearing. *Proc Natl Acad Sci USA* 99: 13206–13210, 2002.

Schloss F and Strasberg M. Hydrophone calibration in a vibrating column of liquid. *J Acoust Soc Am* 34: 958, 1962.

Sokolich WG. Improved acoustic system for auditory research. *J Acoust Soc Am* 62: s12, 1977.

Voss SE and Shera CA. Simultaneous measurement of middle-ear input impedance and forward/reverse transmission in cat. *J Acoust Soc Am* 116: 2187–2198, 2004.

Withnell RH, Kirk DL, and Yates GK. Otoacoustic emissions measured with a physically open recording system. *J Acoust Soc Am* 104: 350–355, 1998.