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8-2008

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Recommended Citation

Voss, Susan E.; Horton, Nicholas J.; Woodbury, Rebecca R.; and Sheffield, Kathryn N., "Sources of Variability in Reflectance Measurements on Normal Cadaver Ears" (2008). Engineering: Faculty Publications, Smith College, Northampton, MA. [https://scholarworks.smith.edu/egr_facpubs/147](https://scholarworks.smith.edu/egr_facpubs/147?utm_source=scholarworks.smith.edu%2Fegr_facpubs%2F147&utm_medium=PDF&utm_campaign=PDFCoverPages)

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Sources of variability in reflectance measurements on normal cadaver ears

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In Press Ear and Hearing

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This work was supported by grant NIH 1 R15 DC007615-01 from the NIDCD, National Institutes of Health.

Abstract

Objective: The development of acoustic reflectance measurements may lead to noninvasive tests that provide information currently unavailable from standard audiometric testing. One factor limiting the development of these tests is that normal-hearing human ears show substantial inter-subject variations. This work examines inter-subject variability that results from measurement location within the ear canal, estimates of ear-canal area, and variations in middle-ear cavity volume.

Design: Energy reflectance (ER) measurements were made on nine human-cadaver ears to study three variables. (1) ER was measured at multiple ear-canal locations. (2) The earcanal area at each measurement location was measured and the ER was calculated with the measured area, a constant area, and an acoustically estimated area. (3) The ER was measured with the middle-ear cavity in three conditions: (1) normal, (2) the mastoid widely opened (large air space), and (3) the mastoid closed off at the aditus ad antrum (small air space).

Results: Measurement-location effects are generally largest at frequencies below about 2000 Hz, where in some ears reflectance magnitudes tend to decrease systematically as the measurement location moves away from the tympanic membrane but in other ears the effects appear minimal. Intra-subject variations in reflectance due to changes in either measurement location within the ear canal or differences in the estimate of the ear canal area are smaller than variations produced by large variations in middle-ear cavity air volume or inter-subject differences. At frequencies below 2000 Hz, large increases in cavity volume systematically reduce the ER, with more variable changes above 2000 Hz.

Conclusions: ER measurements depend on all variables studied: measurement location, ear-canal cross-sectional area, and middle-ear cavity volume. Variations within an individual ear in either measurement location or ear-canal cross-sectional area result in relatively small effects on the ER, supporting the notion that diagnostic tests (1) need not control for measurement location and (2) can assume a constant ear-canal area across most subjects. Variations in cavity volume produce much larger effects in ER than measurement location or ear-canal area, possibly explaining some of the inter-subject variation in ER reported among normal ears.

Keywords: middle ear; reflectance; cadaver measurements

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I. Introduction

A. Overview

Objective tests do not exist for many middle-ear pathologies. When hearing problems appear to originate in the middle ear, diagnoses often depend on a combination of otoscopy, pneumatic otoscopy, air- and bone-conduction audiograms, and tympanometry. With this available set of diagnostic tests, many middle-ear lesions remain unconfirmed unless surgical intervention occurs. Additionally, it can be difficult to diagnose fluid in the middle-ear cavity in children under six months of age in whom tympanometry is not reliable (Holte, Margolis, & Cavanaugh, 1991). A related problem is the need to differentiate between middle-ear conductive loss and sensorineural hearing loss during newborn hearing screening. The measurement of energy reflectance (ER) has potential as an objective test of middle-ear function to (1) determine specific middle-ear lesions for all ages, (2) determine the duration of fluid in the ears of children prone to otitis media, and (3) differentiate between conductive and sensorineural hearing loss in newborn hearing screening programs (Gravel et al., 2005).

In order to differentiate between reflectance measurements on normal and abnormal middle ears, the reflectance in normal ears must be well defined and understood. This requires understanding the variability in reflectance measurements that occurs in normal ears. One factor limiting the development of diagnostic tests that rely on ear-canal reflectance measurements is that normal-hearing human ears show inter-subject variations of up to 10 dB between 100 and 4000 Hz and up to 25 dB between 4000 and 10000 Hz (Voss & Allen, 1994). To quantify how normal variations affect the reflectance of normal ears, the effects of three sources of variability are characterized in this work: (1) effect of ear-canal measurement location; (2) effect of ear-canal cross-sectional area; and (3) the effect of middle-ear cavity volume. These three topics need to be characterized, as they potentially contribute to the variability in reflectance among normal ears, which must be understood in order to describe the reflectance of a normal ear and to develop reflectance-based diagnostic tests.

B. Definition of reflectance and assumptions related to its measurement

When sound waves vibrate the tympanic membrane, some acoustic energy is reflected back into the ear canal, some is transmitted to the cochlea where it is transduced to a neural signal and sent to the brain, and some can be absorbed by the middle ear and shunted away from the cochlea. The ratio between the reflected pressure wave and the incident pressure wave is described quantitatively as the reflectance R, with $0 \leq |R| \leq 1$. Similarly, the fraction of energy that is reflected can be described quantitatively by the energy reflectance ER where $ER = |R|^2$, such that $ER = 1$ indicates all energy is reflected and $ER = 0$ indicates that no energy is reflected.

To measure reflectance, sounds are generated and sensed by an ear-canal probe system. The reflectance is commonly computed from measurements of the acoustic impedance in the ear canal Z_{EC} , where Z_{EC} can be computed from a pressure measurement in the ear canal and the Thévenin equivalent of the sound-delivery system (Allen, 1986; Keefe, Ling, $\&$ Bulen, 1992; Voss & Allen, 1994). Reflectance R is related to the impedance by the equation:

$$
R(f) = \frac{Z_{EC}^N(f) - 1}{Z_{EC}^N(f) + 1} \tag{1}
$$

where f is frequency and Z_{EC}^N is the ear-canal impedance normalized by its characteristic impedance z_o , with $z_o = \rho c/A$ where ρ is the density of air, c is the speed of sound in air, and A is the cross-sectional area of the ear canal in the plane of the measurement. Energy reflectance (ER) is calculated as $ER = |R(f)|^2$.

The reflectance and the ER have several advantages over the impedance when the goal is interpretation of ear-canal based measurements (Voss & Allen, 1994). Most importantly, the impedance depends on the location of measurement within the ear canal because it is sensitive to standing waves in the ear-canal pressure. In contrast, if the ear canal can be modeled as a loss-less cylindrical tube of constant cross section, then both the reflectance magnitude and the ER are independent of measurement location; this feature is crucial for a diagnostic test, as it is not practical to require the transducer to sit at a known distance from the tympanic membrane. The extent to which this assumption holds – that reflectance does not depend on measurement location – is tested in the work presented here by making measurements of reflectance at several locations in the cadaver ear canals.

Equation 1 also depends on the ear-canal cross sectional area A. When reflectance is calculated from an impedance measurement, the area A is typically assumed to be an average area of a human ear canal (e.g., on the order of 0.43cm³). Alternatively, a procedure has been suggested to estimate the area based on a pressure measurement in the ear canal (Keefe, Bulen, Arehart, & Burns, 1993; Huang, Rosowski, Puria, & Peake, 2000b). Either way, the effect of making estimates of the area A instead of using actual measurements of A has not been fully described. In the work presented here, we use molds of the ear canal to measure the area A and determine how sensitive the reflectance measurement is to the measured ear-canal cross-sectional area.

C. Middle-ear cavity

The acoustics of the human middle-ear cavity (or air space) are described in detail by Stepp and Voss (2005). Briefly, this air space consists of the tympanic cavity, aditus ad antrum, the antrum of the mastoid, and the mastoid air cells (e.g., p. 151 Donaldson, Duckert, Lambert, & Rube, 1992). The tympanic cavity houses the ossicular system and lies between the tympanic membrane and the inner ear. The posterior-superior portion of the tympanic cavity narrows into the passage called the aditus ad antrum which extends to the antrum. Attached to the antrum is a system of mastoid air cells that communicate with one another and vary in size (Donaldson et al., 1992). The total volume of the middle-ear cavity is highly variable among normal-hearing ears. The tympanic cavity has a volume ranging from 0.5 cm³ to 1 cm³ (e.g., Gyo, Goode, & Miller, 1986; Whittemore, Merchant, & Rosowski, 1998); the mastoid air cell system has a much wider volume range, reported¹ by Molvaer et al. (1978) to be about 1cm^3 to 21cm^3 and by Koç et al. (2003) to be 4cm^3 to 14cm³ .

¹The measurements of middle-ear volume by Molvaer, Vallersnes, and Kringlebotn (1978) include the tympanic cavity and the mastoid air cell system. We approximate the measurements of the mastoid air cell system to be approximately one cm^3 smaller than the reported measured volume of the entire space.

Stepp and Voss (2005) suggest that the middle-ear cavity in normal ears may play a role in some of the variability observed in ear-canal based acoustical measurements. In particular, the large (i.e., order of magnitude) variation in volume among normal ears coupled with the observation that the volume affects ear-canal measurements (Whittemore et al., 1998; Stepp & Voss, 2005) suggests that some of the variability among the reflectance of normal ears may result from middle-ear cavity volume variations. The measurements presented here will quantify the frequency ranges that are sensitive to variations in middle-ear cavity volume and determine how variations in volume affect measurements of ER.

D. Goals of this work

The goal of this work is to quantify how normal variations affect the reflectance of normal ears. Specifically, three sources of variability are characterized through controlled measurements made on cadaver ears: (1) effect of ear-canal measurement location; (2) effect of ear-canal cross-sectional area; and (3) the effect of middle-ear cavity volume. These three topics are crucial to characterize, as they potentially contribute to the variability in reflectance among normal ears, which must be understood in order to describe the reflectance of a normal ear.

II. Methods

A. Overview

This work assesses how energy reflectance (ER) measurements are affected in normal ears due to three factors: (1) measurement distance from the tympanic membrane, (2) crosssectional area of the ear canal at the measurement location, and (3) variations in middle-ear cavity volume. Using an Etymotic ER-10c sound-delivery system, ear-canal pressure measurements were made systematically at 6 to 11 ear-canal locations. ER was then calculated for each measurement location using the pressure measurements, a measurement of the earcanal cross-sectional area at that location, and the measured Thévenin equivalent of the ER-10c system (Allen, 1986; Keefe et al., 1992; Voss & Allen, 1994). To determine the effects of the ear-canal cross-sectional area on ER, the ER was calculated using the measured ear-canal area and a constant ear canal area; the ear-canal area was also estimated acoustically. To study the effects of varying middle-ear cavity volume, ER measurements were made systematically with the middle-ear cavity in three conditions: (1) normal, (2) the mastoid widely opened to approximate a large airspace, and (3) the mastoid closed off at the aditus ad antrum, in order to approximate a small air space of approximately 1cm^3 .

B. Cadaver ear acquisition and preparation

1. Subjects

Nine cadaver ears were obtained through the nonprofit group Life Legacy (LL), specifically the right and left ears of four subjects (LL5, LL7, LL8, LL9, ages 59, 84, 49, 41 respectively) and the left ear of subject LL4 (age 75). The ears included the complete outer ear, middle ear, and inner ear. The donors had no known history of ear disease, and each ear appeared normal when examined with an otologic operating microscope.

2. Cadaver ear preparation

The ears were shipped on dry ice and kept frozen until measurements were made. When present, the pinna was removed to allow access to the entire length of the ear canal. The middle ear cavity was vented with tygon tubing sealed with dental cement to the Eustachian tube's middle-ear entrance so that no static pressure difference would accumulate across the tympanic membrane. The tympanic membrane and middle ear were moistened periodically with saline, which was suctioned away before measurements were taken.

3. Transducer Positioning

The ER-10c transducer was first placed as close to the tympanic membrane as possible, and the first pressure measurement was taken at this location. The transducer was pulled away from the tympanic membrane in approximately 2mm increments using a measuring stick as a reference, and pressure measurements were made at each increment. Measurements continued along the ear canal until an air-tight seal could no longer be formed between the yellow foam tip and the ear canal. At each location, the distance from the lateral end of the yellow foam tip to the most lateral point of the anterior side of the ear canal was measured with a ruler. This distance was later compared to a mold of the ear canal to determine the corresponding distance from the end of the transducer to the posterior portion of the tympanic membrane.

4. Ear Canal Cross-Sectional Area

ER depends on the cross-sectional area of the ear canal, A (Eq. 1). To estimate the crosssectional area of the ear canal, a silicone-based mold of each ear canal was made. The mold was cut into cross-sections, each roughly perpendicular to the long axis of the canal, with a razor blade, and the cross-sectional area at each measurement location was determined using a microscope camera and digital analysis software (ImageJ). The estimate of the area found using this technique is referred to as the "measured area".

In order to determine the sensitivity of the ER to the estimate of the cross-sectional area, two other estimates of ear-canal area were used: acoustic and constant. The acoustic estimate of the area is based on expressing a time-domain response in terms of the inverse Fourier Transform of the impedance and assuming that at time zero there is no reflection so that the response at time zero depends on the characteristic impedance of the ear canal (Keefe et al., 1993; Huang et al., 2000b). This approach leads to an expression for the ear-canal cross-sectional area A

$$
A = \frac{\rho_o c}{(1/N)\sum_{i=1}^N \Re(Z(i))},\tag{2}
$$

where $\Re(Z)$ is the real part of the impedance measured in the ear canal at N frequencies, with i the frequency index, ρ_o is the density of air, and c is the speed of sound in air. The summation over the input resistance in Eq. 2 can vary with the upper cutoff frequency; here we use an upper cutoff frequency of 10.7 kHz, consistent with Keefe et al. (1992), as our comparisons of impedance measurements and theory made in control cavities suggest the impedance measurements are accurate to that frequency. We also eliminate any frequencies for which the measured real part of the impedance was negative (Huang et al., 2000b). Nonetheless, this truncation does introduce error into the area calculation (Keefe et al., 1992).

The constant cross-sectional area is 0.43 cm^3 at all positions in all ears (Voss & Allen, 1994). This area estimate is based on the assumption that the diameter of the average human ear canal is 0.75 cm (Shaw, 1978). This diameter is similar to the diameter of the cylindrical cavities (0.71 cm) used to determine the Thévenin equivalent of the transducer.

5. Variations in middle-ear cavity volume

To quantify the effects of varying middle-ear cavity volume, pressure measurements were made systematically on three ears² with the middle-ear cavity in three conditions: (1) normal, (2) the mastoid widely opened to approximate a large airspace, and (3) the mastoid closed off at the aditus ad antrum to approximate a small air space of approximately 1 cm^3 . To open the mastoid widely, a canal-wall-up mastoidectomy was performed on each ear so that the tympanic cavity could be viewed through the antrum. Measurements were made in this condition to approximate a large air space. Next, the antrum was plugged using acoustic clay and dental cement, and measurements were made in this condition of a small air space.

C. Measurement system

Ear-canal pressures were generated and sensed with an Etymotic ER-10c earphone/microphone system controlled by a computer running SysID and Matlab software. The sound stimulus was a broad-band chirp containing frequencies from 25 to 24,000 Hz and an input voltage of 0.1 V. Response magnitudes and angles were calculated from either a 1024- or 2048-point DFT of the time domain average of 500 responses sampled at 48 kHz.

The middle-ear input impedance at the measurement location, Z_{EC} , was calculated from the measured ear-canal pressure and the Thévenin equivalent of the transducer (Voss $\&$ Allen, 1994; Allen, 1986; Keefe et al., 1992; Neely & Gorga, 1998). Pressure measurements made in four cylindrical tubes provided four complex equations for the two unknown Thévenin

²Measurements on a fourth ear were made but are not reported because during the opening of the mastoid air space the posterior incudal ligament was damaged.

parameters, the Thévenin source and the Thévenin impedance. Acoustic estimates of the tube lengths were obtained by minimizing the error function in the over-determined system of four equations (Voss & Allen, 1994; Allen, 1986; Keefe et al., 1992), and the optimized lengths were used to compute the two unknown Thévenin parameters. Results were checked by comparing measured and theoretical impedances in five additional tubes. At frequencies in the range 0.2–6 kHz the measured impedances were within 1 dB in magnitude and 0.01 cycles in angle of the corresponding theoretical impedances, except at maxima and minima in the impedance, where the estimates depend heavily on the precise length of the tube; at these frequencies the differences approached 5 dB in magnitude and 0.05 cycles in angle.³ ER was calculated by squaring the magnitude of the pressure reflectance (Eq. 1).

Data where $|R| > 1$, corresponding to negative real parts of the impedance, are discarded from the analysis and are not plotted. This situation most often occurs at the lower frequencies where $|R|$ approaches one (typically below 500 Hz) and may result from small errors in the determination of the Thévenin equivalent of the transducer.

III. Results

A. Energy-reflectance measurements with the probe tip at a typical clinical position

Measurements made with insert earphones in the audiology clinic are typically 10-20 mm from the tympanic membrane (TM). For each of the nine ears, the measurement made at a location closest to 15 mm from the TM (range 11-16 mm) is plotted in Fig. 1 (A) along with the corresponding mean and range (25-75%). Consistent with other reports [Fig. 1] and Fig. 2], at the lowest frequencies the ER is nearly one. As frequency increases toward

³The optimized lengths of the four closed brass cylindrical tubes used to determine the Thévenin equivalent were 55.5, 30.6, 18.5, 10.7 mm, and the lengths of the six additional tubes used to check the Thévenin equivalent were 43.7, 37.5, 25.4, 20.8, 14.7, 10.0 mm. The lengths of these six additional tubes were determined using the same procedures used to estimate the Thévenin equivalent. The diameters of all tubes were 7.1 mm.

1000 Hz, the ER decreases to a mean of about 0.5. Above 1000 Hz, the mean ER measured on these nine ears is relatively flat, ranging from 0.54 to 0.67 between 1000 and 6000 Hz; however, individual measurements show far more fine structure and variability with frequency [Fig. 1 (A)]. The mean and middle 50% range of these measurements are compared to other measurements in Fig. 1 (B-D) and Fig. 2; the comparisons are developed further in the Discussion.

B. Effects of measurement location on energy reflectance

Figure 3 shows the ER measured in each of the nine ears with ear-canal location the parameter. The effect of measurement location appears generally small at most frequencies in most ears. Above 1000 Hz, within a given ear, the ER tends to follow the same trends regardless of measurement location, with the exceptions of two of the nine ears (LL5R and LL9L) where there is more variability in these higher frequency measurements. Below 1000 Hz, six of the nine ears show relatively little variability with measurement location (LL5L, LL5R, LL8L, LL8R, LL9L, and LL9R), but three ears show substantial variability (LL4L, LL7L, LL7R). In these three ears, the ER generally decreases with distance from the TM, consistent with loss in the ear canal.

To compare the effects of measurement location with inter-subject variability, Fig. 4 plots the ER as a function of measurement location for each ear at the discrete frequencies of 500, 1000, 2000, and 4000 Hz. The results from the left and the right ear of the same subject are generally more similar than the results between ears from different subjects. Additionally, the position-induced variations in ER within a given subject are generally smaller than the intersubject variations. Most of the plots indicate a trend that ER decreases as measurementlocation distance from the TM increases, especially as this distance exceeds 20 mm from the TM. In summary, Fig. 4 demonstrates that (1) variations in ER introduced by the measurement location are generally smaller than variations in ER within this population of ears, and (2) ER generally decreases as measurement location increases in distance from the TM.

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Figure 1: Comparisons of the mean and 25-75% ranges for energy reflectance (ER) measurements made in this and other work. A. ER measurements made on 9 cadaver ears in the normal state as part of this work. Within this population of 9 ears are 4 sets of left and right ears from the same donor and one additional ear from a fifth donor. Plotted here in thin black lines is one representative ER measurement from each of these 9 ears; the measurement made closest to 15 mm from the TM (range 11-16 mm) was selected (16 mm was selected when choice was 14 or 16 mm). B. ER calculated from the impedance measurements on cadaver ears published by Voss et al. (2000); measurements here were made within a few mm of the tympanic membrane (TM). The 12 ears were from 10 donors, with an age range of 43-103 (mean 74, median 77). C. The mean and 25-75% range of ER measurements on live ears from Feeney et al. (2003). D. In-progress ER measurements (Voss et al., 2007) on live ears from 25 individuals, using similar methods to those reported here.

Figure 2: Comparisons of the means from the measurements in Fig. 1 with additional published results. For the Kringlebotn (1988) model, the energy reflectance (ER) is calculated at the tympanic membrane (TM).

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Figure 3: Energy reflectance (ER) measurements from nine ears with ear-canal measurement location the parameter. Each of the nine displays shows measurements on a single preparation, indicated in the lower right corner. (For example, LL4L indicates the ear came from "Life Legacy" (LL) and it was ear number 4 and a left ear (L); an R at the end of this tag indicates a right ear. The measurement distance from the tympanic membrane is indicated in each legend.

Figure 4: Plots showing the ER at 500, 1000, 2000, and 4000 Hz for each of the nine ears (from five cadaver donors) at each measurement location. These plots show that the changes in ER within each ear due to measurement location are generally not as great as the inter-subject variability in ER for different measurement locations. In addition, the left and right ears from the same donor are generally more similar to each other than to ears from a different donor.

C. Effects of ear-canal cross-sectional area on energy reflectance: Measurements on cadaver ears

For each ear and each measurement location, the ER was calculated using three values for the cross-sectional area of the ear canal: (1) the area was measured using a silicone-based mold, (2) the area was estimated acoustically from an impedance measurement (Keefe et al., 1993; Huang et al., 2000b), and (3) a constant area of 0.43 cm² (Voss & Allen, 1994) was used. For all nine ears and all ear-canal measurement locations, the percent errors between (1) the acoustically measured and the physically measured area and (2) the constant and physically measured area are summarized in Table 1 and through histograms (Fig. 5). For the acoustic estimates, 50% of the estimates have percent errors relative to the measured area between -4% and 90%. In contrast, the constant estimate is within -23% to 8% of the measured area 50% of the time. Thus, the assumption of an area of 0.43 cm² appears to provide a more robust estimate of cross-sectional area than the use of an area algorithm (Eq. 2).

The mean of the 65 ear-canal area measurements from all ear canal locations (Table 1) is 0.49cm² (median 0.48cm²). Using the physical measurement of area at the ear-canal location where the probe tip most closely sits in the clinic (Section III-A), the mean ear-canal area is 0.48cm² (median 0.47cm²). These estimates are about 10% greater than the ear-canal area of 0.43cm² assumed in previous work (Voss & Allen, 1994).

In order to examine the sensitivity of the ER to cross-sectional area, ER was calculated for each ear using the measured and constant areas for measurements made closest and furthest from the tympanic membrane (Fig. 6). [Since the acoustic area estimates were determined to be less accurate than the constant area, the effects of the areas estimated acoustically were not explored here.] The effects of the cross-sectional area on the ER are generally smaller than the effects of measurement location, as the two ER plots at the closest location are generally distinct from the two ER plots at the furthest location (Fig. 6).

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Figure 5: Histograms comparing the percent error between the measured ear-canal areas and those estimated with the acoustic method (Upper) and the constant assumption (Lower). For each preparation, the ear-canal cross sectional area was measured at each measurement location (indicated in Fig. 2) for a total of 63 comparisons across the nine preparations.

Table 1: Estimates of the ear-canal cross sectional areas for all ears and all ear-canal locations. The ear (preparation) is indicated in the first column. The second column labeled "position" indicates the ear-canal location as the distance from the TM. The "measured" area is the measured area, as described in the text. The "acoustic" area is that estimated via Eq. 2 and the corresponding parentheses indicate the percent error between the measured and acoustically estimated areas. The "constant" area is set at 0.43 cm² and the corresponding parentheses indicate the percent error between the measured and the constant areas.

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Figure 6: Plots showing the effect of the cross-sectional area estimate on the ER. The measurements made closest to the tympanic membrane are plotted in black and those made furthest from the tympanic membrane are plotted in gray. The ER calculated using the measured area is plotted with a solid line and that calculated using the constant area of 0.43 cm^2 is plotted with a dashed line.

Figure 7: Plots showing the effect of the middle-ear cavity volume on the ER. The measurements made with the air space in its normal configuration are plotted in black solid lines; those made with the mastoid widely opened ("large cavity") are plotted in black dotted lines; and those with the antrum sealed ("small cavity") are plotted in gray dashed lines. The measurement distances from the TM were 16 mm (LL8R), 13 mm (LL8L), and 21 mm (LL9R). The break in data for LL8R for the mastoid open case appears as a result of the measured impedance having a negative real part, suggesting a measurement error at these frequencies.

D. Effect of middle-ear cavity volume

Figure 7 shows the ER for each volume state of the middle ear for the three ears on which this type of measurement was made. At frequencies below 1000 Hz, the ER generally decreases systematically as the volume of the middle-ear cavity increases. Above 1000 Hz, the ER from each ear includes local maxima and minima that are generally affected by changes in middle-ear cavity volume; there does not appear to be any definitive trend between cavity volume and ER at these frequencies above 1000 Hz. The extreme volume ranges introduced here lead to variations in ER that are larger than those seen with variations in measurement location or area (Figs. 3 and 6).

E. Comparisons of changes in ER

Figures 8 and 9 compare the relative effects on ER of changes in measurement position, differences in cross-sectional area, variations in the state of the middle-ear cavity and interindividual differences in reflectance. For each of the nine ears, percent differences that describe the effects of these parameters were calculated (Fig. 8). The 25 to 75% ranges of these differences are plotted in Fig. 9 for the comparisons between ears, ear-canal area, and measurement position.

To compare how inter-ear variations in ER compare with the other variations introduced (i.e., ear-canal measurement location, area, and cavity volume), we calculated the percent difference between each measurement made at the "clinical location" (Section A) to the mean of the population measurements made at this position. For each ear, this percent difference is plotted in Fig. 8 as a thick black line. The region shaded gray in Fig. 9 (A) shows the 25-75% range of this percentage difference, which is generally within $\pm 25\%$ across all nine ears. At most frequencies, inter-subject variation relative to the population mean exceeds the individual changes introduced by area Fig. 9 (B) and measurement location effects Fig. 9 $(C-D)$.

To quantify the effect of the ear-canal area, ER was calculated with both the constant area of 0.43 cm² and the measured area; the percent difference between the two ER calculations was determined for two measurement locations: closest and furthest from the TM in each ear (Figures 8 gray lines). [We note that the percent differences depend on the difference in area between the measured value and the constant value (Table 1).] A summary of the percent differences for the ear-canal areas is shown in Fig. 9 (B) where the shaded gray area indicates the 25 to 75% range of ER calculated from the constant area estimate relative to the measured area from locations both closest and furthest from the TM (18 comparisons for nine ears). In general, the differences introduced by ear-canal area effects are less than 10% and are the smallest of all differences analyzed here.

To quantify the effect of measurement position, for each ear the percent difference between the measurement made furthest from the TM and the measurement made closest to the TM was calculated and plotted. [We note that there are different ranges of measurement position for each ear.] These percent differences are shown in Fig. 8 as thin solid black lines and are labeled "position". Fig. 9 (C) shows the 25-75% range of this percentage difference introduced by measurement positions that span the entire measurement range along the ear canal. In general, the differences introduced by position effects are systematically negative at frequencies below 5000 Hz; thus, ER is reduced as measurement position moves away from the TM. These differences are generally less than 20% of the measurement made closest to the TM. Above 5000 Hz, position appears to introduce larger differences that are not systematic in direction. Fig. 9 (D) shows the 25-75% range of the percentage difference when measurements are limited to those made within 20mm of the TM. In this case, below 5000 Hz the systematic trend toward negative changes is reduced and the differences are smaller; above 5000 Hz the results are similar to those with the entire ear-canal length considered.

To illustrate the effect of the middle-ear cavity volume, the percent difference between the measurements made in the modified cavity states and the normal state are plotted for the three ears on which these measurements were made (LL8R, LL8L, and LL9R). These changes in area introduce the largest percent changes from normal among all modifications compared, with the changes ranging across frequency from nearly zero to in excess of 50% at some frequencies in all three ears.

IV. Discussion

A. Summary of results

Three sources of intra-subject variations in ER measurements on cadaver ears were studied: (1) effects of ear-canal measurement location, (2) effects of the ear-canal cross-sectional area, and (3) effects of middle-ear cavity volume. The results are summarized in Figs. 8 and 9. The effects of middle-ear cavity volume introduce the largest variations in ER (Fig. 8). The results in Fig. 9 demonstrate that intersubject variations in ER exceed variations from measurement location or ear-canal area.

The calculation of ER from the ear-canal pressure measurement and the source's Thévenin

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Figure 8: Percent differences calculated for each ear to demonstrate changes in ER that result from different area estimates, different measurement positions, middle-ear cavity volumes (three ears), and variations across ears. Gray lines show the percent difference between the ER calculated with a constant area of 0.43cm³ and the ER calculated with the measured area; thin gray lines are the comparison at the measurement location closest to the TM and dashed gray lines are the comparison at the measurement location furthest from the TM. Thin solid black lines show the percent difference between the ER measurement made furthest from the TM as compared to the one made closest to the TM. Thick black lines plot the percent difference between the ER measurement made at the "clinical location" relative to the mean ER measurement from all nine bones measured at the "clinical location" (from Fig. 1A). In three ears the percent difference was calculated for measurements of ER between (1) the mastoid open case (large middle-ear cavity volume) and the normal case and (2) the antrum sealed (small middle-ear cavity volume) and the normal case. In these ears, these measurements were made at 16 mm, 13 mm, and 21 mm for ears LL8R, LL8L, and LL9R, respectively.

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Figure 9: Summary of percent differences from the nine preparations of Fig. 8. In each case, the 25 to 75% range of percent differences across the nine preparations is plotted. A. The shaded region shows the range for the inter-subject comparisons where each individual's ER made at the "clinical location" is compared to the population mean (from Fig. $1A$). **B.** The shaded region shows the range of percent differences introduced through the calculation of ER from the constant area estimate relative to the measured area. The 25 to 75% range includes the measurement locations both closest and furthest from the TM (18 comparisons for nine ears). C. The shaded region shows the range of percent differences between the ER measurement made furthest from the TM as compared to the one made closest to the TM. D. The shaded region is similar to that shown in C (measurement location effect); here the range of percent differences is calculated between the measurement made furthest from the TM but also within 20 mm of the TM and the measurement made closest to the TM. 25

equivalent requires the ear-canal cross-sectional area. Here, physical measurements of the cross-sectional area compared with acoustical estimates and a constant area demonstrate that (1) the constant area estimates the measured area better than the acoustic-area estimate (Eq. 2) and (2) variations in the area lead to changes in ER that are generally smaller than changes in ER that result from measurement location (Fig. 9). Thus, it appears that a constant ear-canal area is reasonable for the calculation of ER, at least in normal adult ears.

B. Cadaver versus live-human ears

Historically, human-cadaver ears have been widely used to study middle-ear function (e.g., Helmholtz, 1869; Mach & Kessel, 1874; Békésy, 1960; Kirikae, 1960; Onchi, 1961; Zwislocki, 1962; Kringlebotn & Gundersen, 1985; Vlaming & Feenstra, 1986; Gyo, Aritomo, & Goode, 1987; Nishihara, Aritomo, & Goode, 1993; Kurokawa & Goode, 1995; Merchant, Ravicz, & Rosowski, 1996; Merchant et al., 1997; Merchant, Ravicz, & Rosowski, 1997; Voss et al., 2000; Voss, Rosowski, Merchant, & Peake, 2001b, 2001a; Puria, 2003; Ravicz, Rosowski, & Merchant, 2004; Nakajima, Ravicz, Merchant, Peake, & Rosowski, 2005; Gan, Dai, & Wood, 2006; Rosowski, Chien, Ravicz, & Merchant, 2007). One advantage of cadaver-earbased studies as compared to live-human-ear studies is the ability to determine how specific middle-ear anatomical variations and/or pathologies affect a specific measurement of middleear sound transmission (e.g., ER). The cadaver-ear preparation allows for measurements that can be made on a single ear when the ear is in the normal condition. Next a specific modification can be made to the ear and the same measurement repeated. In many cases, the modification can be reversed to allow repeated control measurements. This technique offers a substantial advantage over clinical populations in that inter-subject variability is avoided during comparisons between measurements on ears in different states. With the cadaver-ear preparation, the difference between the normal and the altered state of the ear is the only variable under study.

Comparisons between measurements on live and cadaver ears have demonstrated that the acoustical properties of populations of human cadaver ears and live ears are statistically indistinguishable from each other for ear-canal based measurements, specifically middle-ear input impedance (Rosowski, Davis, Merchant, Donahue, & Coltrera, 1990) and umbo velocity (Goode, Ball, & Nishihara, 1993). In contrast to these ear-canal based measurements, Huber et al. (2001) compared measurements of stapes velocity from live and cadaver preparations and concluded that response magnitudes were reduced in the live ears relative to the cadaver ears at lower frequencies. However, more recent work of Chien, Ravicz, Rosowski, and Merchant (2007) studied this difference in stapes velocity and shows that "much if not all of the differences in V_S (stapes velocity) measurements between live and cadaveric ears can be explained by the differences in measurement angle" (Chien et al., 2007). Thus, it appears that ER measurements made on cadaver ears should be representative of ER measurements on live human ears.

C. Comparisons to other ER measurements

Figure 2 compares ER measurements made on both live and cadaveric populations with the measurements presented here. In general, the measurements show similar trends. However, between 2000 and 4000 Hz, the mean ER measurements made on cadaver ears (black and gray lines in Fig. 2) appear to have higher values than the mean ER measurements made on live ears⁴. Another possibility for the differences in ER between the cadaver ears and the live ears is an age effect. Feeney and Sanford (2004) show that in the 2000-4000 range, ER is generally higher in older ears than in younger ears, consistent with the results shown here. The cadaver populations compared here have mean ages comparable to the older group of Feeney and Sanford (2004).

⁴There is a difference between the ER measurements made here (black dotted line in Fig. 2) and by Voss et al. (2000) (gray dotted line in Fig. 2); here the ears were frozen prior to measurements and those of Voss et al. (2000) were fresh (i.e., never frozen). Ravicz, Merchant, and Rosowski (2000) determined that freezing leads to changes in the stapes-cochlear impedance in some cadaver ears. In particular, Ravicz et al. (2000) found that the stapes-cochlear input impedance Z_{SC} sometimes increases in magnitude with freezing by a factor of 2 to 3. Comparison of ER from the model of Kringlebotn (1988) with the default Z_{SC} compared to a Z_{SC} increased by a factor of 3 shows an increase in ER in the 200-1200 Hz range; however, the effect of increased Z_{SC} does not have any effect on ER at frequencies above 1200, which are where the biggest variations appear in Fig. 2 that compares the fresh and frozen preparations. Thus, it appears that the differences in ER between the fresh and frozen cadaver ears are not entirely a result of changes in Z_{SC} .

D. Effects of measurement position and ear-canal losses

Figures 3 and 4 demonstrate a systematic trend of reductions in ER as the measurement position increases from the TM. The reductions are generally larger at lower frequencies and their size appears to depend on the preparation; similar trends were observed in cat ears by Huang, Rosowski, Puria, and Peake (2000a). We hypothesize that these reductions might result from losses in the ear canal that occur when the canal walls are not rigid. These losses might differ among preparations depending on the condition and makeup of the cartilage and skin lining the lateral half of the ear canal or the bone and skin lining the medial half of the ear canal.

In order to estimate how a nonrigid, lossy ear canal might affect ER, we analyze variations of an ear-canal model terminated by a middle-ear model. The ear canal is modeled as a cylinder of length 2 cm and a constant cross-sectional area of 0.43 cm³, and the models considered are (1) cylindrical rigid tube with no losses (e.g., Eq. 3 Voss & Shera, 2004); (2) cylindrical rigid tube with thermoviscous losses (e.g., Eq. 13 Keefe, 1984), and (3) cylindrical tube with thermoviscous losses and nonrigid walls that include the effects of resistive and mass components of the fleshy surfaces of the ear canal. The properties of the nonrigid walls are modeled with values estimated for the vocal tract (Stevens, 1998).

For the cases with losses, the 2 cm long ear canal is divided into 200 segments (each 0.01 cm) and each segment is modeled with a series and a shunt element (Fig. 10). The values of the series and shunt impedances differ for the loss conditions. For the "thermoviscous loss" these impedances are from Eq. 13 of Keefe (1984). For the cases in which the walls of the ear canal do not have an infinite impedance, the series impedance is the same as that from Keefe (1984) and the shunt impedance is the shunt impedance from Keefe (1984) plus an additional impedance that represents the nonrigid effects of the fleshy ear-canal surface. This additional impedance is estimated by Stevens (1998) for the vocal tract as

$$
Z_{wall} = R_{wall} + jX_{wall},\tag{3}
$$

with $R_{wall} \approx 1000$ and $X_{wall} \approx 2\pi f$ 2 dyne – s/cm³. Since the skin lining the ear canal

Figure 10: Transmission-line model representing the relationship between the pressure (P) and volume velocity (U) at the tympanic membrane (TM) and a point in the ear canal (EC) 2 cm away. Each single dashed box represents one 0.01 cm-long segment of the ear canal that is modeled by a series and shunt impedance. 200 of these segments are in series to represent the entire ear canal. In the case of thermoviscous losses only, the impedance in the ear canal $(Z_{EC} = P_{EC}/U_{EC})$ is determined from these 200 segments terminated by the impedance at the TM Z_{TM} from Kringlebotn (1988) with Z_{series} and Z_{shunt} from Eq. 13 of Keefe (1984). In the case of additional ear-canal wall losses, the model is the same as for thermoviscous losses with the Z_{shunt} also including Z_{wall} . (Eq. 3)

appears thinner than the flesh lining the vocal tract, we also estimate Z_{wall} as three (case "B" in Fig. 11) and ten (case "C" in Fig. 11) times the amount suggested by Stevens (1998) for the vocal tract. In these cases where Z_{wall} is included, the resistive component of the shunt impedance leads to energy loss to the wall of the ear canal.

Figure 11 compares model predictions for the different ear-canal conditions. The ER with the lossless ear canal (solid black line) is nearly identical to that with the thermoviscous loss. When losses within the ear-canal wall are included, there is a reduction in the ER, primarily at frequencies below 1000 Hz. The general trends of this model are consistent with the measurements; in particular, when the ear-canal wall impedance Z_{wall} is ten times that estimated for the vocal tract, the reduction in ER is similar to those ER measurements that show reductions in ER with measurement position. Thus, the hypothesis that low-frequency reductions in ER as measurement position increases from the TM result from loss in the ear-canal wall is consistent with both the measurements and model.

E. Implications for clinical ER measurements

ER measurements depend on measurement location, middle-ear cavity volume, and earcanal cross-sectional area (summarized in Figs 8 and 9). It appears that variations within an individual ear in either measurement location or ear-canal cross-sectional area estimates

Figure 11: Energy reflectance (ER) calculated using the models described in Fig. 10. In all cases, the ER is calculated at 2 cm from the tympanic membrane. The "thermoviscous losses" are calculated using the equations from Keefe (1984). The plots labeled "ear-canal wall loss" all use the model for wall losses proposed by Stevens (1998) (pp. 156-160) and described further in the text. Here, "wall loss A" is the impedance value used by Stevens (1998) for the vocal tract (Eq. 3), "wall loss B" is a factor of 3 greater than "wall loss A", and "wall loss C" is a factor of 10 greater than "wall loss A".

result in relatively small effects on the ER (Fig. 9). Thus, these two variables are not generally responsible for substantial differences in ER among ears within a clinical population. Practically speaking, this result suggests that knowledge of the exact ear-canal measurement location and the exact ear-canal cross sectional area is not crucial. Thus, the assumptions that diagnostic tests (1) need not control for measurement location and (2) may use a constant area both appear valid. The results here demonstrate an average ear-canal area of 0.48cm² instead of the 0.43cm² assumed in previous work (Voss & Allen, 1994); however, the 0.48cm² average comes from measurements on only nine ears (from five individuals) and more measurements would be helpful in defining the average ear-canal area.

Variations in middle-ear cavity volume produce larger effects in ER than measurement location or ear-canal area (Fig. 8). Most likely, the resonances of the middle-ear cavity affect the ER in highly individualistic ways: below 1000 Hz the effects are likely influenced by overall volume but at higher frequencies the individual anatomy will determine resonances that will differ from ear to ear (Stepp & Voss, 2005). It seems likely that some of the inter-subject variation in ER observed here and by others results from the geometry of the middle-ear cavity. These predictions might be further studied in a clinical population through CT scans of middle-ear cavities.

Acknowledgments

This work was supported by grant NIH 1 R15 DC007615-01 from the NIDCD, National Institutes of Health. Preliminary accounts of this work have been presented in parts by Woodbury, Horton, and Voss (2006), Voss et al. (2007) and Voss, Horton, Woodbury, and Sheffield (2007). We thank John Rosowski, Patrick Feeney, and an anonymous Ear and Hearing reviewer for helpful comments and suggestions on the manuscript. We also thank Patrick Feeney and Douglas Keefe for providing data from their reflectance studies and Christopher Shera for helpful discussions.

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