Normative Reflectance and Transmittance Measurements on Healthy Newborn and 1-Month-Old Infants

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Normative reflectance and transmittance measurements on healthy newborn and one-month old infants

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Abstract

**Objective:** Ear-canal-based wide-band reflectance (WBR) measurements may provide objective measures to assess and monitor middle-ear status in young babies. This work presents WBR measurements of power reflectance and transmittance on populations of healthy newborn babies (3-5 days) and healthy one-month old babies (28-34 days). Thus, this work determines how power reflectance and transmittance vary between newborn and one-month old babies and characterizes the range of these measures in normal populations.

**Design:** Power reflectance and transmittance were calculated from pressure measurements made in the ear canals of seven newborn (12 ears) and eleven one-month old (19 ears) babies. Permutation tests, t-tests, and regression (random effects) models were used to test the effects of age (newborn vs. one month), gender, and ear side (right versus left).

**Results:** The power reflectance and transmittance did not differ significantly for the age comparison (newborn versus one month), although the results suggest a possible difference between newborn and one-month old ears near 2000 Hz. There were no differences between the male and female ears. There are small but significant differences between left and right ears in three frequency bands encompassing 500-4000 Hz, where the predicted power reflectance mean for the left ear differs from the right ear by 0.02 to -0.07, depending on the frequency band.

**Conclusions:** At most frequencies, power reflectance and transmittance are indistinguishable for newborn and one-month old healthy babies, with limited or no differences between the two age groups and the males and females. There were small differences in some frequency bands for left and right ears. The measurements made here are similar to other published results in some frequency ranges, but differ in other frequency ranges; differences among other studies from neonatal intensive care unit (NICU) babies, healthy newborn babies, and healthy one-month-old babies are discussed.

**Keywords:** middle ear; reflectance; newborn; hearing screening

**Abbreviations:** WBR Wide-band reflectance, NICU Neonatal intensive care unit, IRB Institutional Review Board, $R$ Power reflectance, $T$ Transmittance
1 Introduction

1.1 Overview and motivation for work

The diagnosis of middle-ear fluid in infants less than four to seven months of age can be difficult because 226 Hz tympanometry is inconsistent and unreliable in these young ears (e.g., Paradise, Smith, & Bluestone, 1976; Sprague, Wiley, & Goldstein, 1985; Holte, Margolis, & Cavanaugh, 1991). Both 1000 Hz tympanometry (e.g., Margolis, Bass-Ringdahl, Hanks, Holte, & Zapala, 2003; Kei et al., 2003; Calandruccio, Fitzgerald, & Prieve, 2006; Baldwin, 2006) and reflectance measures (e.g., Keefe et al., 2000; Keefe, Zhao, Neely, Gorga, & Vohr, 2003; Keefe, Gorga, Neely, Zhao, & Vohr, 2003; Shahnaz, 2008; Sanford & Feeney, 2008; Hunter, Bagger-Sjoback, & Lundberg, 2008; Hunter, Tubaugh, Jackson, & Propes, 2008; VanderWerff, Prieve, & Georgantas, 2007; Sanford et al., 2009) have been explored as possible alternatives for middle-ear assessment on these younger ears, and Sanford et al. (2009) found that, at birth, reflectance measures are better predictors of DPOAE outcomes than 1000 Hz tympanometry. The long-term goal of this work is to determine if wide-band reflectance (WBR) measures (e.g., Keefe, Bulen, Arehart, & Burns, 1993; Voss & Allen, 1994; Allen, Jeng, & Levitt, 2005; Sanford et al., 2009) can be used as objective measures to detect middle-ear fluid in infants, both at the time of newborn hearing screening and when middle-ear fluid is suspected in young ears. There are limited reports of WBR measures made on healthy, full-term, newborn babies (i.e., only measurements from Sanford et al., 2009) and normal hearing one-month old babies (Keefe et al., 1993; Sanford & Feeney, 2008); adding to these limited measurements is the topic of this work.

One to three percent of newborn babies are referred for further audiological assessment at the time of their newborn hearing screening. Of these referrals, 90 percent are false-positives that can occur as a result of transient fluid or debris within the external or middle ear (Thompson et al., 2001; Doyle, Rodgers, Fujikawa, & Newman, 2000). The differentiation between transient loss associated with middle-ear fluid or debris and permanent conductive
or sensorineural hearing loss is made via follow-up testing. In order to provide more complete audiological information starting at birth, a study funded jointly by the Centers for Disease Control and Prevention and the Association of Teachers of Preventative Medicine recommends the development of a screening tool for middle-ear function at the time of newborn screening (Gravel et al., 2005).

In addition to helping diagnose newborn babies who refer during newborn hearing screenings, WBR measurements may also help diagnose and manage young infants with otitis media. Acute otitis media and otitis media with effusion affect 91 percent of children by age two (Paradise & Rockette, 1997); medical management of children who suffer from recurrent otitis media includes substantial efforts to evaluate their middle-ear air space for fluid, as this fluid leads to conductive hearing loss and increased risk for developmental delays (Gravel & Ellis, 1992). To determine the extent of fluid in the middle ear, clinicians rely on a combination of otoscopy, pneumatic otoscopy (which introduces ear-canal static pressure for the subjective judgement of tympanic-membrane mobility), air-conduction and bone-conduction audiograms, and tympanometry (Nozza, Bluestone, Kardatzke, & Bachman, 1992, 1994). With this set of diagnostic tests, it can be difficult to diagnose middle-ear fluid in children under six months of age (Margolis et al., 2003). However, medical management of infants with middle-ear fluid is essential in order to ensure they develop language appropriately and don’t suffer from long-term effects of chronic otitis media. Thus, WBR based testing could be useful in following middle-ear fluid in babies under the age of six months, for which there currently exists no objective diagnostic test.

1.2 Wide band reflectance (WBR) measures

“Wide band reflectance” (WBR) measures refer to a group of quantities that can be used to represent the acoustic behavior of the ear. This term includes the related quantities: impedance, admittance, reflectance, transmittance, and power reflectance\(^1\). A method and

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\(^1\)Power reflectance is a preferred term over the commonly employed term of energy reflectance. Power is the energy transfer per unit of time, whereas energy is measured over a specific time period.
equipment to measure these quantities exists (Allen, 1986; Keefe, Ling, & Bulen, 1992; Keefe et al., 1993; Voss & Allen, 1994). With this method, the Thévenin equivalent of a sound source and microphone system is measured using the system’s acoustic responses made in a set of cavities or tubes. A single pressure measurement in a load such as an ear can then be used to calculate all of the WBR quantities.

For example, given the sound source’s Thévenin equivalent impedance \( Z_{TH}(f) \) and pressure \( P_{TH}(f) \), both functions of frequency \( f \), the impedance at the probe-tip location in the ear canal \( Z_{ear}(f) \) can be calculated via a pressure measurement \( P_{ear}(f) \) as

\[
Z_{ear}(f) = \frac{Z_{TH}(f)P_{ear}(f)}{P_{TH}(f) - P_{ear}(f)}.
\] (1)

Note, the admittance is the reciprocal of the impedance, and both the impedance and admittance are complex quantities with magnitudes and angles. From the impedance, the pressure reflectance is calculated as

\[
R(f) = \frac{Z_{N_{ear}}(f) - 1}{Z_{N_{ear}}(f) + 1},
\] (2)

where \( Z_{N_{ear}}(f) \) is the normalized impedance such that \( Z_{N_{ear}}(f) = \frac{Z_{ear}(f)}{\rho c A} \) where \( \rho \) is the density of air, \( c \) is the speed of sound in air, and \( A \) is the cross-sectional area of the ear canal. The pressure reflectance \( R(f) \) is a complex quantity that can be interpreted as the ratio between the reflected pressure wave and the incident pressure wave within the ear canal. Inherent in this interpretation and equation is that there are no losses along the ear canal; measurements made on cadaver ears support this assumption for adult ears (Voss, Horton, Woodbury, & Sheffield, 2008).

From the pressure reflectance \( R(f) \) we can compute a quantity called the power reflectance \( \mathcal{R} \), where

\[
\mathcal{R}(f) = |R(f)|^2.
\] (3)

The power reflectance is a real number between 0 and 1, with \( \mathcal{R}(f) = 0 \) representing all power transmitted to the ear and with \( \mathcal{R}(f) = 1 \) representing all power reflected at the tympanic
membrane back into the ear canal. Transmittance $T(f)$ in units of dB is calculated from the power reflectance $R(f)$ as

$$T(f) = 10 \log(1 - |R(f)|^2).$$

(4)

The transmittance is a useful quantity because its dB scale reduces the variability in power reflectance at the lower and higher frequencies and also provides a measure that might best relate to hearing levels (Allen et al., 2005), which would be useful and familiar to clinicians.

In this work, we present the WBR measures of power reflectance $R$ and transmittance $T$.

1.3 Brief literature review

Significant changes occur in newborn outer and middle ears during the first six months of life. This includes an increase in size of both the ear-canal diameter and length and the middle-ear cavities, a change in the orientation of the tympanic membrane, a tightening of the ossicular joints connecting the ossicles, the formation of the bony ear-canal wall, and a decrease in the overall mass of the middle ear due to changes in bone density and loss of mesenchyme (Qi, Lui, Lufty, Funnell, & Daniel, 2006; Keefe et al., 1993; Saunders, Kaltenback, & Relkin, 1983). The ways in which these changes in newborn- and infant-ear anatomy affect WBR measurements at any given age are not fully understood; below, we review the current work related to WBR measures on newborn and infant ears.

WBR measurements from a population of healthy, full-term normal hearing newborn babies have been reported in one article (Sanford et al., 2009), and prior WBR measurements have been made on neonatal intensive care unit (NICU) newborn babies and young healthy babies (Keefe et al., 1993, 2000; Shahnaz, 2008; Sanford & Feeney, 2008; Hunter et al., 2008).

Keefe et al. (1993) conducted a study of 78 healthy babies ages one to 24 months in which they found systematic changes in reflectance with increasing age. They also found that middle-ear compliance is lower and middle-ear resistance is higher in infants than in adults, leading them to suggest that a substantial increase in ear-canal wall motion occurs at lower frequencies in young infants, which may account for the unreliability of 226 Hz tympanograms.
Therefore, Keefe et al. (1993) recommends that impedance and reflectance measurements in the 2-4 kHz range could potentially be a useful clinical tool.

Keefe et al. (2000) conducted the first study of WBR measures in neonates. The study included 2081 neonates combined from three populations: neonates in neonatal intensive care units (NICU), neonates in well-baby nurseries, and neonates with one or more risk factors associated with hearing loss in well-baby nurseries. Keefe et al. (2000) found a median reflectance near 0.2 across all frequencies from 250 to 8000 Hz, and the middle 50 percent range varied with reflectance measurements from 0.1 to 0.3 with only modest variation with frequency. Keefe et al. (2000) also found significant differences between left and right ears and male and female ears for some frequency bands. They also found that changes as a function of conceptual age from 33 to 48 weeks were modest in comparison to age-related changes found by Keefe et al. (1993). Additionally, reflectance measurements were found to be inconsistent during the first 24 hours after birth; they suggested that the middle ear of one day olds might differ from two to four day olds, presumably due to the presence of vernix and other material in the external and middle ear that clears up in the first few days after birth. Keefe et al. (2000) also highlight that a leak-proof seal (no air space between the ear tip and the ear-canal wall) is vital in making accurate measurements.

Shahnaz (2008) conducted a study of 26 NICU newborn babies with a mean gestational age of 37.8 weeks and compared these data to power reflectance $R$ measurements taken from 56 adults and one-month old babies from Keefe and Levi (1996). Shahnaz (2008) found that there is a clear separation between NICU babies and adults below 727 Hz, with NICU babies having lower $R$ values than adults. The NICU newborn mean $R$ from Shahnaz (2008) is larger at all frequencies than the corresponding mean for one-month old babies from Keefe and Levi (1996).

Hunter et al. (2008) conducted a study on 159 ears from 81 children age 3 days to 47 months; within this population 138 ears were classified as normal and 21 as abnormal. Contrary to previous conclusions drawn by Keefe et al. (1993) and Keefe et al. (2000) regarding
systematic changes with age, Hunter et al. (2008) found no significant age effect with respect to reflectance measurements except at 6000 Hz. They also found no significant effects of ear or gender.

Sanford and Feeney (2008) report power reflectance $R$ from 60 healthy full-term infants, with 20 infants each aged 4 weeks, 12 weeks, and 24 weeks. These data were generally consistent with the infant data from the study of Keefe et al. (1993), with some differences below 2000 Hz. Sanford and Feeney (2008) attribute the differences to the variation of methods used to estimate infant cross-sectional ear-canal size, which is done through an acoustic estimate in the study conducted by Keefe et al. (1993) and calculated using a set value based on the diameter of calibration tubes (which were sized based on actual infant ear-canal diameters) by Sanford and Feeney (2008).

Sanford et al. (2009) report the first set of normative WBR data on healthy, full-term newborn ears that passed newborn DPOAE hearing screening. These measurements were generally made during the first two days of life (mean age 25.5 hours, standard deviation 8.0 hours). The results were compared with a group of ears that did not pass the DPOAE screening, and the findings are consistent with the hypothesis that WBR measures could be useful in detecting fluid in young ears.

VanderWerff et al. (2007) looked at test-retest reliability of wideband reflectance measures in 127 infants ages 2 weeks to 24 months. They demonstrated the importance of an adequate probe fit for newborn babies and found that compressible foam tips are significantly more effective than rubber probe tips in obtaining adequate test-retest reliability.

1.4 Goal of this work

This work characterizes the wide-band reflectance measures of power reflectance $R$ and transmittance $T$ of normal-hearing, healthy, full-term newborn and one-month old babies. Keefe et al. (1993) demonstrated that power reflectance changes systematically with age, from one month past the age of two years. Other work focuses on power reflectance in NICU babies
(e.g., Shahnaz, 2008; Keefe et al., 2000), babies one month and older (Sanford & Feeney, 2008; Keefe & Levi, 1996; Keefe et al., 1993), or groups that include a range of newborn to more than one month (Hunter et al., 2008). Here, we present WBR measures on normal-hearing, healthy newborn and one-month old babies. Ultimately, these types of normative measurements will be needed to develop a WBR metric to determine normal and abnormal WBR responses for different ages.

The specific goals of this study are: (1) To determine how WBR measures of power reflectance and transmittance vary as a function of age between newborn (age 3 to 5 days) and one-month old (age 28 to 34 days) infants, and (2) To characterize the normative range of power reflectance and transmittance in these populations.
2 Methods

2.1 Subjects and testing protocol

All measurements were approved by the Smith College Institutional Review Board (IRB), and all parents consented for their baby via an IRB approved consent form. The measurements reported here are from eight newborn (ages 3 to 5 days, 4 male and 4 female) and eleven one-month old (age 28 to 34 days, 7 male and 4 female) babies; one baby was included in both groups. Subjects were full-term (gestation age 40 ± 2 weeks), healthy babies who passed their newborn hearing screening. During a well-baby visit, each subject underwent an otoscopic examination to ensure a clear ear canal, and an ear-canal pressure measurement was made, from which WBR measures were calculated, and DPOAE measurements were made. Measurements were taken on both ears from 7 of 8 newborns and on 8 of 11 one-month olds, for a total of 34 ears. In cases where both ears were not measured, one ear was not measured due to excessive wax, and three were not measured due to fussiness of the baby. Parents held their babies, and if the baby cried, he or she was encouraged to suck on a pacifier or nurse. The cord of the probe tip was held by the experimenter in order to maximize its stability.

After the ear-canal pressure measurements used to calculate WBR were made, DPOAEs were recorded in all but two ears; in both cases the baby was crying and measurements were not feasible. DPOAEs were recorded at the $f_2$ frequencies of 2000, 3000, 4000, and 6000 Hz, with levels $L_1$ and $L_2$ at 65 and 55 dB SPL and a frequency ratio $f_2/f_1$ of 1.2. To pass, an ear had to meet DPOAE pass criteria at three of the four $f_2$ frequencies, and the DPOAE pass criteria were (1) a signal-to-noise ratio greater than 3 dB and (2) a DPOAE magnitude with a level of at least -6 dB SPL. Three ears did not meet these criteria and were eliminated from further analyses; these ears were 4, 5, and 5 days old. Four additional ears did not explicitly meet these criteria. In two of these four cases, the DPOAEs were not measured due to the baby crying. In the other two cases, the noise floors were greater than 10 dB SPL for at least one $f_2$, and it was assumed that the baby was noisy during the measurement. Analyses
were carried out both with and without these four ears, and the presence or absence of these four ears did not affect the frequency ranges where significant changes are reported via the regression model. Thus, these four ears were included and results are reported from a total of 31 ears.

2.2 Instrumentation

WBR measurements were made on newborn and one-month old babies using the FDA approved HearID system from Mimosa Acoustics (version 4.4.100.0) with an Etymotic ER-10c sound delivery system. To minimize acoustic leaks, foam tips (size 14B, Etymotic Research) were used (VanderWerff et al., 2007), and these tips were thinned out with scissors to allow them to fit into newborn ear canals. Two wideband sequential chirps stimuli at 70 dB SPL were produced from each of the two channels of the ER-10c, resulting in two consecutive and independent pressure measurements in each subject for each ear tested. For each channel, the average of \( N \) measurements is reported. Here the averaging time was 20.05 seconds (\( N = 470 \), with FFT length of 2048, a sampling rate of 48 kHz, and a frequency resolution of about 25 Hz). The artifact rejection was enabled so that bins measured in the presence of increased background noise were rejected. Up to 60.03 seconds of data were collected to obtain the desired 470 bins for averaging. The software did not report the actual number of measurements obtained, but our qualitative sense is that most measurements reached the goal of 470.

2.3 Determination of the Thévenin equivalent and calculation of WBR measures

“Calibration” of the system refers to the measurements of the Thévenin equivalent \( Z_{TH} \) and \( P_{TH} \) of the ER-10c system, as described in the HearID manual. The calibration procedure was completed before measurements were made on each subject. Small variations in \( Z_{TH} \) and \( P_{TH} \) occurred over measurement sessions. While not documented here, these variations appear to depend on the orientation of the tip in the calibration cavities and not on real
changes in the behavior of the system. An independent measurement of \( Z_{TH} \) and \( P_{TH} \) was made in a quiet laboratory setting. This independent measurement approximated the median of all individual calibration measurements of \( Z_{TH} \) and \( P_{TH} \) (Merchant, 2009, Fig. 2-5). In order to reduce variability in reflectance measurements that would be introduced from variations in the calibration measurements of \( Z_{TH} \) and \( P_{TH} \), we calculated all WBR measures using these median \( Z_{TH} \) and \( P_{TH} \) measures.

The Thévenin equivalent of the system depends on the cross-sectional area of the cavity (or ear canal) to which the system is coupled. This area also affects the calculation of the reflectance (Eq. 2). Ideally, the diameter of the calibration tubes should approximate the diameter of the ear canal (Huang, Rosowski, Puria, & Peake, 2000). The HearID system is not directly set up to calibrate with a pediatric foam tip trimmed to a size to couple to a newborn ear canal. Therefore, we calibrated the system with the newborn sized (\( d=4.5\)mm) rubber tip and the corresponding smallest diameter HearID cavity set. Ear-canal measurements were made with the trimmed pediatric foam tip, and reflectance measures were calculated assuming an ear-canal diameter of 4.5mm.

2.4 Data analysis

The pressure measurement recorded from one of the two channels was analyzed for each ear. Channel A was selected as the default channel to be analyzed when the two channels were similar (Merchant, 2009, Appendix B shows measurements on both channels). Channel A was measured first and was chosen somewhat arbitrarily, with the reasoning that the baby was generally quieter when the experimenter chose to begin a measurement. However, in 9 of the 31 ears, channel B was analyzed instead of channel A; in two of the 9 cases the sound tube associated with channel A was visually seen to be blocked with debris after the measurements were made, and in 7 of the 9 cases the phase response of the impedance calculated from channel B was substantially flatter with frequency at low frequencies than that calculated from channel A, suggesting a better acoustic seal on channel B. The observation that channel
B was often associated with a better acoustic seal makes sense because the measurement on channel B was made several seconds later than that on channel A, allowing for more time for the foam tip to expand. We note that 18 of the 31 measurements were assessed to be equivalent for the two channels, 9 measurements were assessed to be superior on channel B and 4 superior on channel A (one probe filled with debris, one response consistent with the probe against ear-canal wall, and two impedance phase responses that were flatter at low frequencies on channel A as compared to channel B).

Pressures measured in the ear canal were smoothed using a 7-point moving average filter prior to computing WBR measures.

The data were analyzed to determine if differences existed among the ears for three categories: age (newborn vs. one month), gender (male vs. female), and ear (left vs. right). Three different statistical analyses were applied to identify frequency ranges where potential differences might exist within these three categories: a t-test, a permutation test, and a linear regression model. At each of the 248 measurement frequencies, p values were computed using both the t-test and permutation test. The t-test was run using the Matlab function “ttest2”, with the option “unequal” so that the test assumed that the two samples came from normal distributions with unknown and unequal variances. The permutation test was 2-sided with 10,000 iterations (Efron & Tibshirani, 1993) and replacement. Both the t-test and the permutation test were carried out within Matlab version 7.6. In these tests, the exact numerical value of the p value is not intended to show definitively whether or not there is a statistical significance, as no adjustment for multiple comparisons was made for either test and p values were calculated at all 248 frequencies. Instead, these tests were meant to explore the entire frequency range for indications of where potential differences might occur.

We assessed whether there were statistically significant differences between groups using linear regression random effects models (Laird & Ware, 1982; Finucane, Samet, & Horton, 2007), which compared mean power reflectance between groups averaged over frequencies while accounting for clustering within repeated measurements (i.e., measurements taken on
each subject at multiple frequencies). A random intercept term was fit for each subject. Separate models were fit for each of four groups of frequencies (500-1000 Hz, 1000-2000 Hz, 2000-4000 Hz, and 4000-6000 Hz). Main effects terms for age (newborn vs. one month), gender (male vs. female), and ear (left vs. right) were included in the model. No adjustment for multiple comparisons was undertaken. Mean differences that were significant at the 0.01 level or smaller are reported, along with the corresponding predicted mean difference. One might argue that with four frequency bins, the 0.01 significance level would be equivalent to a 0.04 significance level with a Bonferroni correction for multiple comparisons (Abdi, 2007).

3 Results

Figure 1 plots power reflectance (upper row) and transmittance (lower row) measurements on both newborn babies (left column) and one-month old babies (right column). The general patterns are similar for the two age groups. In both age groups, the mean power reflectance is a maximum (near 0.6) at the lowest frequency plotted (500 Hz) and decreases with frequency until about 2000 Hz where it reaches a minimum that is near 0.18 for the newborn group and near 0.09 for the one-month old group. As frequency increases above 2000 Hz, the mean power reflectance generally increases with frequency. The individual measurements are mostly similar to the mean’s behavior with a few exceptions. In some cases there is more fine structure with additional minima and maxima across frequency. There is one right newborn ear that has a deep minimum near 900 Hz and a maximum just below 2000 Hz; there is one one-month old ear with a power reflectance that doesn’t decrease with frequency for frequencies below about 1500 Hz. Both age groups also have some ears with sharp maxima in the 4000-6000 Hz range. The transmittance is calculated directly from the power reflectance (Eq. 4), resulting in comparable similarities and differences between the age groups and the means and individual ears. In both age groups, the mean low-frequency transmittance increases with frequency from about -4 dB at 500 Hz up to a maximum value near 2000 Hz of -0.9 and -0.4 for the newborn
and one-month old groups respectively. Above 2000 Hz, both means generally decrease with increasing frequency. The transmittance shows the same outliers in the individual data as described above for the reflectance, highlighting that the mean measurements are not always an accurate description of the individual measurements.

Figure 2 compares the measurements made on the same subject as a newborn at three days old (both ears) and at 28 days (right ear only). The measurements made at birth appear similar with sharp maxima in power reflectance in the 4000 to 6000 Hz range. These maxima are not apparent in the measurement made on the right ear at one month of age.

Figure 3 provides a direct comparison between the power reflectance (upper plot) and transmittance (middle plot) for the three categories: newborn ears vs. one-month old ears (left column), female vs. male (middle column), and left vs right (right column). The means for each group are plotted along with the population’s 25 to 75 percent range. The p values calculated via the t-test and the permutation test are reported at each frequency in the lower plot. The random effects models tested for significant differences within the frequency ranges 500-1000 Hz, 1000-2000 Hz, 2000-4000 Hz, and 4000-6000 Hz, and the corresponding p values (when p < 0.01) are reported within Fig. 3 along with the $\beta_0$ coefficient for any significant differences, where $\beta_0$ is the difference in the model’s prediction for the mean of the data within a given group.

At most frequencies, the power reflectance and transmittance for the groups of newborn and one-month old babies appear similar, with the largest differences near 2000 Hz (Fig. 3, left). The p values computed at each frequency via a t-test and permutation test are generally greater than 0.05, and they only dip below 0.05 at 2000 Hz for the permutation test. The t-test accounts for the unequal variances that are apparent between the two groups (Fig. 3, upper left) and differs slightly here from the permutation test. The frequency groupings for the random effects model, which were determined a priori to data analysis, led to no significant differences between these two groups.

The power reflectance and transmittance for the groups of male and female ears appear
similar, with no significant differences (Fig. 3, center).

The means of the power reflectance for the groups of left and right ears appear significantly different for the three lower frequency ranges, with differences in power-reflectance means (left minus right) that are 0.02, -0.07, and -0.05 for the 500-1000 Hz, 1000-2000 Hz, and 2000-4000 Hz frequency ranges respectively (Fig. 3, upper right). Similarly, the means of the transmittance for the groups of left and right ears are significantly different in the two mid-frequency ranges of 1000-2000 Hz and 2000-4000 Hz, with mean transmittance differences (left minus right) of 0.41 and 0.28, respectively (Fig. 3, middle right). Thus, there appear to be small, albeit significant, differences between the mean measurements of the left and right ears. The $p$ values computed via the t-test and permutation test have values substantially greater than 0.01 (Fig. 3, lower right), consistent with relatively small differences between the means and not an adequate number of data points to provide the power needed to determine a more significant difference at each of the 248 frequencies.

4 Discussion

4.1 Summary of data

Power reflectance and transmittance were calculated from pressure measurements made in the ear canals of newborn (3-5 days) and one-month-old (28-34 days) babies. Comparisons were made between groups within the categories: age (newborn versus one month old), gender (female versus male), and ear side (left versus right). At most frequencies, there were no significant differences between the groups in any of the categories. For the age comparison, the unadjusted $p$ values from the t-test and the permutation test had minima near 0.05 for frequencies near 2000 Hz, suggesting a possible difference between the newborn and one-month old groups near 2000 Hz (Fig. 3, left), and there were no significant differences for the age comparison within the four frequency bands for which the random effects regression analysis was done. There were no differences between the male and female ears (Fig. 3, center). The
random effects regression analysis revealed small yet significant differences between the left and right ears for frequencies up to 4000 Hz (Fig. 3, right).

4.2 Comparison to other data

4.2.1 Other data

Figure 4 compares the power reflectance $R$ from this work to other measurements\textsuperscript{2}. For frequencies below 3000 Hz, the newborn mean $R$ from this work (black solid triangles) was similar to the mean $R$ measured on NICU babies from Shahnaz (2008) (black solid squares); above 3000 Hz the two data sets diverge with the Shahnaz (2008) $R$ approaching 0.6 and the $R$ measured here remaining below 0.4. The NICU median $R$ reported by Keefe et al. (2000) was substantially less than those from all other comparison measurements for frequencies at and below 1000 Hz; above 1000 Hz, the $R$ reported by Keefe et al. (2000) was generally similar to other measurements. This newborn population of Keefe et al. (2000) included NICU babies, healthy newborns, and newborns at risk for hearing loss. The relatively low $R$ at and below 1000 Hz might indicate poor acoustic seals in some ears. The mean power reflectance reported by Sanford et al. (2009) (black solid circles) from 375 newborn ears was greater than the other means reported in the literature for NICU and newborn ears; we hypothesize that the Sanford et al. (2009) ears might have had larger power reflectances than the ears reported in the work here as a result of transient ear-canal vernix that is shed over the first few days of life. This is discussed in more detail in the next section.

Within their newborn population, Keefe et al. (2000) found $R$ was larger in left ears as compared to right ears for frequencies below 1400 Hz, and $R$ was larger in right ears for higher frequencies. Our results agree with this finding of Keefe et al. (2000). In the frequency band 500-1000 Hz, our left ears had a significantly larger mean (0.02) power reflectance than the right ears. In the frequency bands of 1000-2000 Hz and 2000-4000 Hz, the left ears had a

\textsuperscript{2}Data from (Hunter et al., 2008) are not included because their youngest population included ages 3 days to 2 months grouped together
significantly smaller mean (0.07 and 0.05) power reflectance than the right ears. Keefe et al. (2000) also showed that below 2000 Hz, the male $R$ was larger than the female $R$. Our current study, which had both fewer ears and ears from only healthy babies, does not show these gender differences.

The mean one-month-old $R$ from this work (gray triangles) was generally similar to the other measurements made on one-month-old babies for frequencies from 1000 to 6000 Hz. Below 1000 Hz, the $R$ from this work was higher than that from the other two studies: Sanford and Feeney (2008) and Keefe et al. (1993).

Figure 4 also plots $R$ from a population of adult ears (Voss & Allen, 1994) in order to highlight the differences in $R$ between adult ears and young ears. With the exception of the (Sanford et al., 2009) newborn ears, adult ears have a larger $R$ at most frequencies below about 3000 Hz, and in particular, on average, the $R$ from adult ears is substantially larger than from infant ears at both the lowest frequencies (500 Hz here) and in a frequency band around 2000 Hz.

4.2.2 Methodological differences

Additional factors are possible explanations for differences in reflectance measurements between this study and other published work. With the exception of the Sanford et al. (2009) data, no other study included a population of healthy newborns, as age ranges were either grouped together over the first month or few months of life or healthy newborns were mixed with NICU and at-risk newborns. There is also a difference in the Sanford et al. (2009) age range, as those ears were roughly two to four days younger than the newborn ears measured as a part of the present work. As a result, no set of data exists for exact comparisons to this study, and population differences could account for some of the observed variations.

Methodological differences between the current study and other published data could also result in variations. VanderWerff et al. (2007) showed significant differences in test-retest reliability between rubber and foam probe tips, and they showed that rubber tips have poor
test-retest reliability in comparison to foam tips. During the methodological development of the current study, we also found that rubber tips had a tendency to fall out and that it was very difficult to obtain a leak-free seal using them. Rubber Etymotic tips were used in two of the published studies (Shahnaz, 2008; Hunter et al., 2008), foam tips were used by Keefe et al. (1993), GSI tympanometry tips were used by Sanford and Feeney (2008), GN Otometrics tips (made for the Madsen AccuScreen device) were use by Sanford et al. (2009), and the probe tips used by (Keefe et al., 2000) are unknown.

The calculation of power reflectance depends on the cross-sectional area of the ear-canal (Eq. 2). Huang et al. (2000) showed that accurate WBR measurements require that the Thévenin equivalents of the the acoustic measurement system be determined with loads that have diameters within 10-15% of the actual ear-canal diameter. The Mimosa Acoustics System [used in this study and also by Shahnaz (2008) and Hunter et al. (2008)] estimates the cross-sectional area of the ear-canal based on the probe tip diameter and the calibration cavity used during calibrations. With this system the cross-sectional area is either estimated to be 4.5mm (rubber-tip cavity) or 7.5mm (foam-tip cavity), as described in the Methods. Newborn ear-canal diameters have been found to have diameters of about 4.4 mm (Qi et al., 2006; Keefe et al., 1993), therefore calibrations using Mimosa’s “rubber-tip cavities” (used here) are appropriate. However, use of an adult sized foam tip and corresponding cavity during calibrations would result in a cavity diameter mismatch greater than the 10-15% recommended by Huang et al. (2000). Numerical simulations that explore the effects of variations in ear-canal cross-sectional area show that for our newborn and one-month-old ears, increases in the cross-sectional area increases $R$ at most frequencies. Thus, it is possible that differences between our measurements and those of others in Fig. 4 are partially a result of different definitions of ear-canal cross-sectional area. While Keefe et al. (1993), Keefe et al. (2000), Sanford and Feeney (2008), and Sanford et al. (2009) did not use the Mimosa System, estimations of the cross-sectional area of the ear canal from these studies could also result in variations. Sanford and Feeney (2008) and Sanford et al. (2009) both assumed the ear-canal diameter
doesn’t differ substantially from their calibration cavity dimensions of 4.8 mm. Keefe et al. (1993) and Keefe et al. (2000) used an acoustic estimate made from the measured impedance measurement, and this acoustic estimate has been shown to be inaccurate in some cadaver ears (Voss et al., 2008). Thus, variations in ear-canal cross-sectional area estimates may account for some of the variability among published reflectance measurements. In order to compare various studies, it is important to report the cross-sectional area used in calculating reflectance measures.

Finally, in the hours after birth, the reflectance can be influenced by “debris” – for example, the presence of vernix or amniotic fluid in the ear canal or mesenchyme or amniotic fluid in the middle ear. Keefe et al. (2000) made qualitative arguments based on their large data set that are consistent with ears less than 24 hours old differing from 24 to 72 hour-old ears in that the younger ears have, on average, somewhat higher reflectances. While the times at which various debris types disappear have not been clearly documented, the Keefe et al. (2000) interpretation suggests that there may be a significantly higher percentage of ears filled with debris within the first 24 hours of life than a few days later. Similarly, Doyle, Kong, Srobel, Dallaire, and Ray (2004) recommended performing newborn hearing screening as close as possible to the time of discharge so that the ears can be as free of vernix as possible. These arguments are consistent with the differences in power reflectance between the ears of Sanford et al. (2009) and the ears presented in this work. The Sanford et al. (2009) ears were on average 25.5 hours old (± 8 hours standard deviation), and while they had all passed DPOAE screening, it is likely that such young ears continue to shed ear-canal vernix over the period of several hours to a few days. Thus, it may be that the ears reported here in the present work, at ages 3 to 5 days, have a lower mean power reflectance due to the extra few days of age. Similarly, the ears on NICU babies from the other comparison studies were generally not measured within 25 hours of birth, and these ears too would have had more time to shed ear-canal vernix.
4.3 Clinical application and significance

Overall, this study has demonstrated that the WBR measures of power reflectance and transmittance are essentially the same in healthy, normal-hearing newborn (3-5 days) and one-month-old babies. While WBR measures could lead to a clinical tool for the assessment of middle-ear status and fluid in newborn and young babies, a normative data set showing changes (if any) across small age increments in normal populations is necessary. This work adds normative measurements on normal hearing newborn babies to the other normative measurements available and summarized in Fig. 4. Future work will need to provide both (1) more WBR measurements in healthy newborn and infant populations to improve the normative database and (2) WBR measurements on ears with fluid for comparison to normal ears. Ultimately, comparison between normal and fluid-filled ears will lead to determination of the efficacy of WBR measurements to monitor and detect fluid in newborn and infant ears.
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1. Power reflectance (upper row) and transmittance (lower row) measurements for left (solid) and right (dashed) ears, means (thick black), and 25 to 75 percent ranges (gray shaded region) measured on 7 newborn (left column) and 11 one-month old (right column) healthy babies. Measurements on both ears were obtained for 5 of the 7 newborns and for 8 of the 11 one-month olds.  

2. Power reflectance (upper row) and transmittance (lower row) measurements made on the same subject as a newborn at three days old (both ears) and at 28 days (right ear only).  

3. Summaries of the power reflectance (upper), transmittance (middle), and $p$ values at each frequency from the t-test and permutation test (lower) for the three tested comparisons: newborn ears vs. one-month old ears (left column), female vs. male (middle column), and left vs right (right column). Plotted for the power reflectance and transmittance are the means of the measurements for each category and the corresponding 25 to 75% range of the data. The regression model tested for significant differences within the frequency ranges distinguished by the vertical, gray, dotted lines; the corresponding $p$ values are reported within the power reflectance and transmittance plots for the frequency ranges with significant differences, along with the $\beta_0$ coefficient that represents the predicted difference in the means for that frequency range.  

4. The mean power reflectances measured on newborn (solid black triangle) and one-month old (open gray triangle) ears are plotted in comparison with data reported by Sanford et al. (2009) (mean healthy newborn ears, 25.5 ± 8 hours), Shahnaz (2008) (mean ears in a NICU), Keefe et al. (2000) (median data from NICU, healthy, and at-risk for hearing loss babies at 39 to 40 weeks conceptional age), Sanford and Feeney (2008) (healthy one-month olds), and Keefe et al. (1993) (healthy one-month olds). To increase visibility, measurements from this work and from Shahnaz (2008) have symbols spaced at every 15 data points, whereas the data from Keefe et al. (1993), Keefe et al., (2000), Sanford and Feeney (2008), and Sanford et al. (2009) have symbols at every data point. Also plotted is the mean from 10 adult ears measured by Voss and Allen (1994).
Newborn Ears

One-month Ears

- LEFT (N=5)
- RIGHT (N=7)

- LEFT (N=11)
- RIGHT (N=8)

Frequency (Hz)

Power Reflectance

Transmittance (dB)

25-75% Range

Mean
Transmittance (dB)

Frequency (Hz)

Power Reflectance

Newborn Left

Newborn Right

One-month Right