ISSN: 2083-389X
eISSN: 2084-3127

J Hear Sci, 2025; 15(4): 30–45
DOI: 10.17430/jhs/214368

Received:
May 23, 2025
Revision accepted:
November 17, 2025
Published online:
February 2, 2026

Contributions:
A Study design/planning
B Data collection/entry
C Data analysis/statistics
D Data interpretation
E Preparation of manuscript
F Literature analysis/search
G Funds collection

WIDEBAND ACOUSTIC IMMITTANCE (WAI) AND HISTOPATHOLOGY IN A MOUSE MODEL OF OTITIS MEDIA

Wafaa Kaf*[1A-G], Richard J. Salvi*[2C-F], Brian Faddis*[3A-G]

* These authors contributed equally.

[1] Audiology Program, Communication Sciences and Disorders, Missouri State University, Springfield, USA
[2] Communicative Disorders and Sciences, University at Buffalo, USA
[3] Department of Otolaryngology, Washington University School of Medicine, USA

Corresponding author: Wafaa Kaf, Audiology Program, Communication Sciences and Disorders, Missouri State University, 901 S. National Avenue, Springfield, MO 65897, USA; email: wafaakaf@missouristate.edu

Abstract

Introduction: This study aimed to: (1) quantify and compare middle ear status in two mice strains, C57BL/6J and CBA/CaJ, using wideband acoustic immittance (WAI) to measure wideband absorbance at ambient pressure (WBA) and at tympanometric peak pressures (WBT); (2) determine the percentage of mice with histologic evidence of otitis media (OM) after nasal inoculations with *Bordetella hinzii*; (3) assess how *B. hinzii* affects WBA and WBT; and (4) evaluate if antibiotic treatment reduces OM and restores absorbance to normal.

Material and methods: Eight C57BL/6J and eight CBA/CaJ mice were used in Experiment 1. WBA and WBT (averaged across 0.5–8 kHz) were measured at baseline and 3 and 6 weeks after *B. hinzii* inoculation. Middle ear histopathology was performed to confirm the presence of OM. In Experiment 2, ten C57BL/6J mice with OM received antibiotics, and absorbance was tracked at baseline, during OM, and post-treatment.

Results: (1) Baseline absorbance responses were reliably measured in both strains and both showed similar results with peak WBA (~0.4) near 1 kHz and maximal WBT at –50 daPa near 6–8 kHz; (2) None of the CBA/CaJ developed OM, whereas 13 of 16 C57BL/6J ears showed OM histologically; (3) WBA and WBT remained normal in CBA/CaJ mice post-inoculation. In C57BL/6J mice, WBA at ambient pressure was insensitive to OM, but WBT was significantly reduced at 3 and 6 weeks post-inoculation ($p = 0.001$); (4) Antibiotic-treated C57BL/6J mice showed WBT recovery as OM resolved histologically.

Conclusions: Wideband acoustic immittance provides reliable absorbance measures in mice. C57BL/6J mice are susceptible to OM induced by *B. hinzii*, whereas CBA/CaJ are resistant. WBT can be used to detect and monitor OM in mice. Limitations of the study include a modest sample size and relative rather than absolute values of WBA and WBT due to species differences in calibration.

Keywords: otitis media • histopathology • antibiotics • mouse model • wideband tympanometry • *B. hinzii*

SZEROKOPASMOWA IMPEDANCJA AKUSTYCZNA (WAI) I HISTOPATOLOGIA W MYSIM MODELU ZAPALENIA UCHA ŚRODKOWEGO

Streszczenie

Wprowadzenie: Celem niniejszego badania było: 1) ilościowe określenie i porównanie stanu ucha środkowego u dwóch szczepów myszy (C57BL/6J i CBA/CaJ) z wykorzystaniem szerokopasmowej impedancji akustycznej (WAI) do pomiaru szerokopasmowej absorbancji w naturalnych warunkach ciśnienia atmosferycznego (WBA) i przy ciśnieniu szczytowym (WBT); 2) określenie odsetka myszy objawami zapalenia ucha środkowego (OM), stwierdzonego w badaniu histologicznym, po donosowym podaniu bakterii *Bordetella hinzii*; 3) ocena wpływu *B. hinzii* na WBA i WBT; oraz 4) ocena, czy leczenie antybiotykami zmniejsza OM i przywraca absorbancję do normy.

Materiał i metody: W eksperymencie 1. wykorzystano osiem myszy C57BL/6J i osiem myszy CBA/CaJ. WBA i WBT (średnia dla zakresu 0,5–8 kHz) zmierzono w stanie wyjściowym oraz 3 i 6 tygodni po zaszczepieniu *B. hinzii*. W celu potwierdzenia OM wykonano histopatologię tkanek ucha środkowego. W eksperymencie 2. dziesięć myszy C57BL/6J z OM otrzymało antybiotyki, a absorbancję zmierzono: w stanie wyjściowym, podczas OM i po leczeniu.

Wyniki: 1) Uzyskano wiarygodne pomiary absorbancji u obu szczepów myszy, a wyniki były zbliżone: dla szczytu WBA (~0,4) przy 1 kHz oraz maksymalny WBT przy –50 daPa w zakresie 6–8 kHz; 2) u żadnej z myszy CBA/CaJ nie stwierdzono rozwoju zapalenia ucha środkowego, podczas gdy w 13 z 16 uszu myszy C57BL/6J stwierdzono OM w badaniu histologicznym; 3) WBA i WBT pozostały prawidłowe u myszy CBA/CaJ po inokulacji. Wartości WBA u myszy C57BL/6J przy ciśnieniu wyjściowym pozostawały podobne niezależnie od tego, czy ucho

było zdrowe, czy wykazujące OM, natomiast WBT w przypadku OM uległo istotnemu obniżeniu w 3. i 6. tygodniu po inokulacji ($p = 0{,}001$); 4) u myszy C57BL/6J leczonych antybiotykami wartości WBT wracały do normy równolegle z ustępowaniem zmian histopatologicznych OM.

Wnioski: Szerokopasmowa impedancja akustyczna zapewnia wiarygodny pomiar absorbancji u myszy. Szczep C57BL/6J myszy jest podatny na rozwój zapalenia ucha środkowego wywołanego przez *B. hinzii*, podczas gdy CBA/CaAJ jest odporny. WBT może być stosowane do wykrywania i monitorowania OM u myszy. Do ograniczeń niniejszego badania należą: niewielka liczebność próby oraz wykorzystanie wartości względnych, a nie bezwzględnych WBA i WBT, które to wartości wynikają z różnic gatunkowych w kalibracji.

Słowa kluczowe: zapalenie ucha • histopatologia • antybiotyki • model mysi • tympanometria szerokopasmowa • *B. hinzii*

Key to abbreviations	
3D	three-dimensional
ABR	auditory brainstem response
AOM	acute otitis media
CO	cochlea
DPOAE	distortion product otoacoustic emission
EAC	external auditory canal
EDTA	ethylenediaminetetraacetic acid
IP	intraperitoneal
ME	middle ear
OM	otitis media
OME	otitis media with effusion
SMTP	sulfamethoxazole and trimethoprim
SNR	signal to noise ratio
WAI	wideband acoustic immittance
WBA	wideband absorbance
WBT	wideband tympanometry

Introduction

Two-thirds of children experience at least one episode of acute otitis media (AOM) by the age of 5 [1], with some children prone to recurrent or chronic OM [2]. Chronic OM may lead to a permanent conductive or mixed hearing loss as a result of chronic inflammation and hyperplasia of middle ear mucosa and breakdown of its epithelial lining [2–4]. For OM to develop, the pathogens must adhere to the nasopharyngeal epithelium, enter the middle ear cavity intranasally or through the Eustachian tube, and overcome the immune and other natural defense mechanisms of the middle ear [5,6].

Many species have been used to investigate OM and changes in middle ear status following inoculation with experimental bacteria [7–15]. Mice provide many advantages for OM research including low cost, ease of care, availability of inbred and mutant strains that could provide insights on the genetics of OM relevant to humans [1,13]. Standard tympanometry performed on 61 inbred mouse strains [16] revealed 46 with normal type A tympanograms, 3 with type B, flat tympanograms, 3 with type C tympanograms with a negative middle ear peak pressure, and 9 unclassified. Mcph1-deficient mice, a model of microcephaly, spontaneously developed OM that increased in frequency and severity with age [17].

The etiology of acute and chronic OM has been investigated with different pathogens, treatments and diagnostic tools [13,18–20]. Using otomicroscopy, BALB/c mice were found to be much more susceptible to bacteria-induced OM than C57BL/6J and Swiss-Webster mice [14]. After inducing OM in C57BL/6J mice with *Bordetella pseudohinzii*, auditory brainstem response (ABR) thresholds increased and distortion product otoacoustic emission (DPOAE) amplitudes decreased across a broad range of frequencies [9]; however, these functional metrics failed to distinguish middle ear dysfunction from cochlear hearing loss that can rapidly develop in some strains [21,22].

Sound transmission from the middle ear to the cochlea is frequency dependent, varies across species, and can be disrupted by middle ear pathologies [23–25]. In contrast to traditional tympanometric measurements typically performed at a single frequency (adults, 226 Hz; children, 1000 Hz), wideband acoustic immittance (WAI) provides measurements of (a) wideband absorbance at ambient pressure (WBA) that assesses the proportion of sound energy absorbed into the middle ear across a wide frequency range (0.25–8 kHz) at ambient pressure, and (b) wideband tympanometry (WBT) that measures energy absorbance at various ear canal pressures, similar to traditional tympanometry, but across a wide frequency range (0.25–8 kHz). In contrast to traditional methods performed at a single frequency, WAI is highly effective for assessing middle ear function over a broad frequency range by computing the power absorbed by the middle ear relative to the incident power from 0.25 to 8 kHz [26,27]. Acoustic power absorbance values, which range from 1.0 (100%) to 0.0 (0%), have proved useful in assessing middle ear pathologies such as otosclerosis, ossicular discontinuity, OM with effusion, and tympanic membrane perforation in humans [28–30]. However, additional research is needed in both humans and animals to develop norms and detect various dysfunctions [31]. WAI was used to study OM in chinchillas inoculated with non-typeable *Haemophilus* influenza [11]. Reduced absorbance at 4 days post-inoculation was attributed mainly to buildup of middle ear pressure whereas reduced absorbance at 8 days post-inoculation was attributed to OM with effusion and structural changes in the middle ear; however, these results were not confirmed histologically [32]. Because the full resolution of OM can occur over several weeks or more, further changes in absorbance would likely have been missed [13]. Although WAI is well established in human research, this study is innovative in its application of WAI to a mouse model of otitis media, incorporating longitudinal monitoring and pressure manipulation.

Table 1. Summary of the histology results including: tissue ID, strain of mouse, survival time (in weeks) post inoculation of *B. hinzii* and the status of the middle ear cavity. Results shows 13 of the 16 ears of all C57BL/J6 strains ($n = 8$) got infected, whereas none of the CBA/CaJ got infected with *B. hinzii*

Tissue ID	Strain	Survival time post inoculation [weeks]	Status of middle ear cavity
11WB03	C57BL/6J	3	Severe bilateral OM
11WB04	C57BL/6J	3	Moderate unilateral OM
11WB05	C57BL/6J	3	Severe bilateral OM
11WB06	C57BL/6J	3	Severe bilateral OM
11WB07	C57BL/6J	6	Clear
11WB08	C57BL/6J	6	Severe bilateral OM
11WB09	C57BL/6J	6	Severe bilateral OM
11WB10	C57BL/6J	6	Severe bilateral OM
11WB13	CBA/CaJ	6	Clear
11WB14	CBA/CaJ	6	Clear
11WB15	CBA/CaJ	6	Clear
11WB16	CBA/CaJ	6	Clear
11WB17	CBA/CaJ	3	Clear
11WB18	CBA/CaJ	3	Clear
11WB19	CBA/CaJ	3	Clear
11WB20	CBA/CaJ	3	Clear

WAI could be extremely useful for identifying middle ear dysfunction in murine models of human disorders such as Downs syndrome [33], genes that contribute to the development of OM [16], and assessing the development and resolution of OM and antibiotic treatment. In Experiment 1, we tested for differences in the development of OM between two widely used murine strains, C57BL/6J and CBA/CaJ mice, by inoculating them with *B. hinzii*, a human pathogen associated with OM. WAI was conducted pre- and post-inoculation and WBA and WBT were measured to compare the frequency- and tympanometric pressure-dependent changes in absorbance caused by OM in these two strains. OM was confirmed by histological assessment of the middle ear. In Experiment 2, C57BL/6J mice with OM induced by *B. hinzii* received a 4-week course of oral combination antibiotic treatment (sulfamethoxazole and trimethoprim) often used to treat bacterial middle ear infections in humans [34]. Following antibiotic treatment, the mice were evaluated by WBT and histology to assess the resolution of OM.

Material and methods

Experiment 1: WAI and histology pre/post *B. hinzii* inoculation

Animals

Eight male CBA/CaJ mice and eight male C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) were used in Experiment 1. The 16 mice (8 per strain) were inoculated with *B. hinzii*. **Table 1** shows the strains, timeline of sacrifice, and status of the middle ear cavity based on histological examination at time of sacrifice (see Results). Mice were 6 weeks of age at the start of the study and were housed in groups of 3 or 4 in autoclaved, static micro-isolator cages with food and water available *ad lib*. Irradiated gel packs (Hydrogel, Clear H_2O, Portland, ME) were used as water source to avoid potential cross-contamination between cages. Ambient temperature and relative humidity were maintained at 21–22°C and 55–60% respectively and a 12 : 12 light : dark cycle.

B. hinzii preparation

The two experimental groups ($n = 8$ per strain) were bi-nasally inoculated with 25 µL of a suspension of 2.45×10^3 cfu/mL of *B. hinzii* (isolated and characterized from a spontaneous occurrence within our animal facility) [15]. The isolate was maintained in tryptic soy broth with 5% glycerol and stored at –80°C. Inoculation medium was prepared by culturing bacteria on 5% sheep blood agar for 24 h at 37°C. Bacteria were then collected with a sterile swab and suspended in sterile phosphate buffered saline to the desired concentration.

WAI at both ambient pressure (WBA) and tympanometric pressure (WBT)

The Interacoustics wideband tympanometry system (research version 3.2.1) with Interacoustics probe assembly was used in this study. The equipment was calibrated

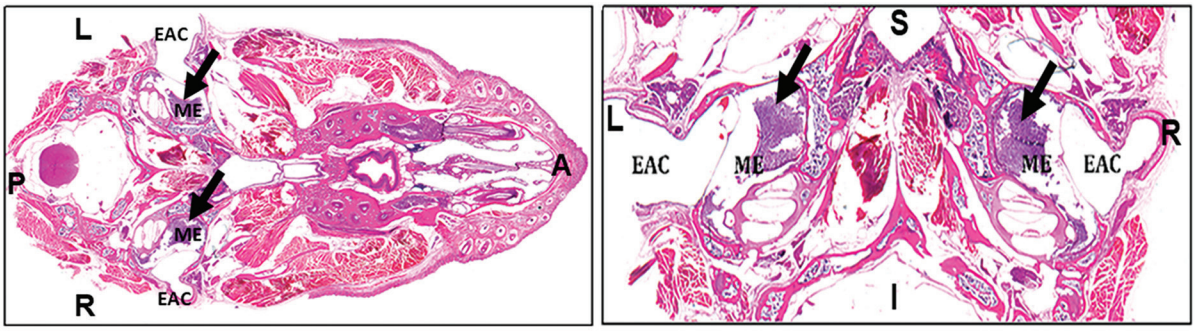

Figure 1. Standard histological preparation used in the present study. Upper panel shows a horizontal whole head section from a *B. hinzii*-exposed mouse; the temporal bone region is enlarged in the lower panel. In this bilaterally affected sample, note the accumulation of inflammatory exudate (black arrows) in the middle ear space (ME) and lack of exudate in the external auditory canal (EAC). Eustachian tubes, not shown in this section, were also always free of inflammation. All infected ears were graded as moderate or severe regardless of post-inoculation interval, consistent with the chronic nature of OM. Middle ear space (ME), external auditory canal (EAC), cochlea (CO), L (left), R (right), S (superior), I (inferior), A (anterior), and P (posterior)

according to the manufacturer's specification and a daily verification procedure was performed to ensure probe integrity and stimulus accuracy. In the absence of mouse species-specific calibration and given its smaller ear canal, we employed the 'newborn' calibration setting as a practical approximation [35]. Calibration was conducted by using the two tubes of the two sets of calibration tubes (Reflwin Interacoustics) at the beginning of each test day. Once successful tube calibration was obtained, WBA and WBT measurements were obtained from both ears of each mouse. Thirty-two click responses were acquired and valid sweeps averaged and analyzed.

All measurements were obtained from mice that had been lightly anesthetized in an isoflurane by chamber induction. Testing was completed in a quiet laboratory setting to minimize external noise. During absorbance testing, the anesthetized mouse was placed in the prone position on a flat surface. A sterilized probe ear tip, with a flanged flexible 3–5 mm diameter eartip was securely placed in the mouse ear canal (with practice, the investigators became adept at probe placement). Absorbance measures were obtained from both ears in response to clicks as pressure was slowly swept from +200 daPa to –300 daPa at a slow pump speed of 100 daPa/sec. The responses to the 32 click stimuli were recorded across 1/8 octave bands from 0.25 to 8 kHz, yielding 31 data points. Of the 31 recorded data points in the spectral analysis, 26 frequencies were analyzed; the six lowest frequencies (0.25–0.45 kHz) were excluded due to extremely low absorbance, possibly caused by the high stiffness of the mouse middle ear at low frequencies [36], and/or minor changes in the ear-probe calibration. Absorbance was measured five times during each test and the average was calculated. To ensure measurement reliability, the probe was removed and repositioned between recordings. WAI is an objective assessment of middle-ear status; however, the examiner was blinded to the experimental condition of each animal during data collection.

Histopathology

Mice were sacrificed either 3 weeks (half the mice) or 6 weeks (other half) post-inoculation. Mice were sacrificed with an overdose of pentobarbital (200 mg/mL, IP) and transcardially perfused with 45 mL of fixative containing 4% paraformaldehyde and 0.1% glutaraldehyde in Sorensen's phosphate buffer. Following perfusion, the lower jaw and scalp were removed, and the head immersed in fixative for several days. Heads were then rinsed free of fixative and placed in 14% acid free ethylenediaminetetraacetic acid (EDTA) for decalcification at 4°C for 2 weeks. Tissues were processed and embedded in paraffin and sectioned in the horizontal plane at a thickness of 4 µm. Ten sequential sections were saved at 100 µm intervals throughout the ventral–dorsal aspect of the middle ear space. Sections were stained with hematoxylin and eosin and cover slipped with Permount. All tissues were examined under an Olympus BH-2 upright light microscope for evidence of infection (inflammatory cells, bacterial biofilm, and debris) in both left and right middle ear spaces, external auditory canals, and Eustachian tubes, as well as the nasopharynx and sinus spaces. Histologically, the incidence of OM (present or absent) was classified as clear, unilateral, or bilateral. Earlier pilot studies had attempted to grade severity of the infection using a semi-quantitative scale (0–4) but showed infections to be robust or absent. For that reason, efforts to grade severity were not used in the present study.

Statistical analyses of Experiment 1

Test–retest reliability of baseline WBA as a function of frequency (0.5–8 kHz) and average WBT as a function of tympanometric pressure were analyzed to assess intra-session reliability and consistency of the five runs of each mouse strain using independent sample *t*-tests, and results were considered significantly different when Cohen's $d < 0.05$. Paired samples *t*-tests of the baseline WBA were conducted to test the mean differences between the right and left ears of each mouse strain. If results were not significantly different, data from both ears were averaged. Absorbance data were summarized as means and standard deviations.

Data analyses using parametric tests included both within- and between-mouse strain comparisons to identify differences in mean WBA versus frequency (0.5, 1, 2, 4, 6, 7, and 8 kHz) and mean WBT absorbance (averaged over 0.5 to

Table 2. Test–retest reliability between the five runs of baseline wideband absorbance at ambient pressure (WBA) in each of the C57 ($n = 8$) and CBA mice ($n = 8$). Results showed no significant differences between the five runs in both mouse strains at baseline

Frequency [kHz]	Mouse strain	Cronbach's α	95% CI Lower bound	95% CI Upper bound
0.5	C57	0.785	0.708	0.846
	CBA	0.729	0.636	0.803
1	C57	0.760	0.706	0.809
	CBA	0.717	0.610	0.800
2	C57	0.819	0.784	0.851
	CBA	0.801	0.734	0.855
4	C57	0.712	0.696	0.734
	CBA	0.839	0.813	0.865
6	C57	0.778	0.710	0.833
	CBA	0.839	0.813	0.865
7	C57	0.762	0.649	0.734
	CBA	0.805	0.754	0.849
8	C57	0.748	0.656	0.819
	CBA	0.809	0.762	0.851

8 kHz frequencies) at five tympanometric pressures (–200, –100, 0, +100, and +200 daPa) at baseline, 3 weeks post-inoculation, and 6 weeks post-inoculation. Within and between-strain comparisons were conducted using a series of one-way repeated measures ANOVA at three time points. If a main effect was significant, Bonferroni *post hoc* testing was performed with significance of $p < 0.05$.

Experiment 2: Recovery of WBA after antibiotics

For Experiment 2, 10 male C57BL/6J mice infected with *B. hinzii* were used to test the recovery of WBA and WBT absorbance following treatment of OM with antibiotics. The ten C57BL/6J mice were 6 weeks old at the onset of testing. Mice, lightly anesthetized with isoflurane, were inoculated intranasally with 25 μL of 1.95×10^4 cfu/mL suspension of *B. hinzii* and subsequently allowed to recover in their home cage. Four weeks after inoculation, a time sufficient to allow for development of OME, all mice were placed on a 4-week course of sulfamethoxazole (320 mg/L) and trimethoprim (64 mg/L) oral antibiotic suspension. Serial WBA and WBT absorbance measurements were conducted at baseline, after inoculation of *B. hinzii*, and following a 4-week antibiotic treatment to test for recovery of normal middle ear status.

Statistical analyses of Experiment 2

Recovery of absorbance was assessed in 10 C57BL/6J mice infected with *B. hinzii* following antibiotics treatment. Descriptive statistics were used to show the temporal changes in the WBT profiles measured at baseline, 1 week, and 4 weeks post-inoculation and then after 1 month of continuous antibiotic treatment (4 weeks post-antibiotic). To assess the recovery of absorbance following antibiotic treatment, a one-way repeated-measures, within-subject ANOVA was conducted, followed by multiple comparisons against the baseline values of the ten C57BL/6J mice infected with *B. hinzii*. Statistically significant differences were considered at a $p < 0.05$. All parametric statistical analyses were conducted using JASP software (version 0.18.3).

Ethical approval

All aspects of the care and use of these rodents in Experiment 1 and 2 were carried out in accordance with the Care and Use of Laboratory Animals of the National Institutes of Health, and the study protocol was approved by the Washington University in St. Louis, Missouri Animal Studies Committee (Protocol No. 20090049).

Results

Experiment 1: Histology and WBA pre/post *B. hinzii*

Histological phenotype

Histological examination of tissues from *B. hinzii* inoculated mice revealed inflammatory cells, acellular matrices, and bacterial biofilms localized to the middle ear. Although the suspension of *B. hinzii* was inoculated through the nasal cavity, the infection was limited to the middle ear cavity. **Table 1** illustrates the status of the middle ear cavity categorized by strain, group, and time of sacrifice. As shown in **Table 1** and **Figure 1**, histological examination of the eight C57BL/6J mice showed that 7 of 8 mice had

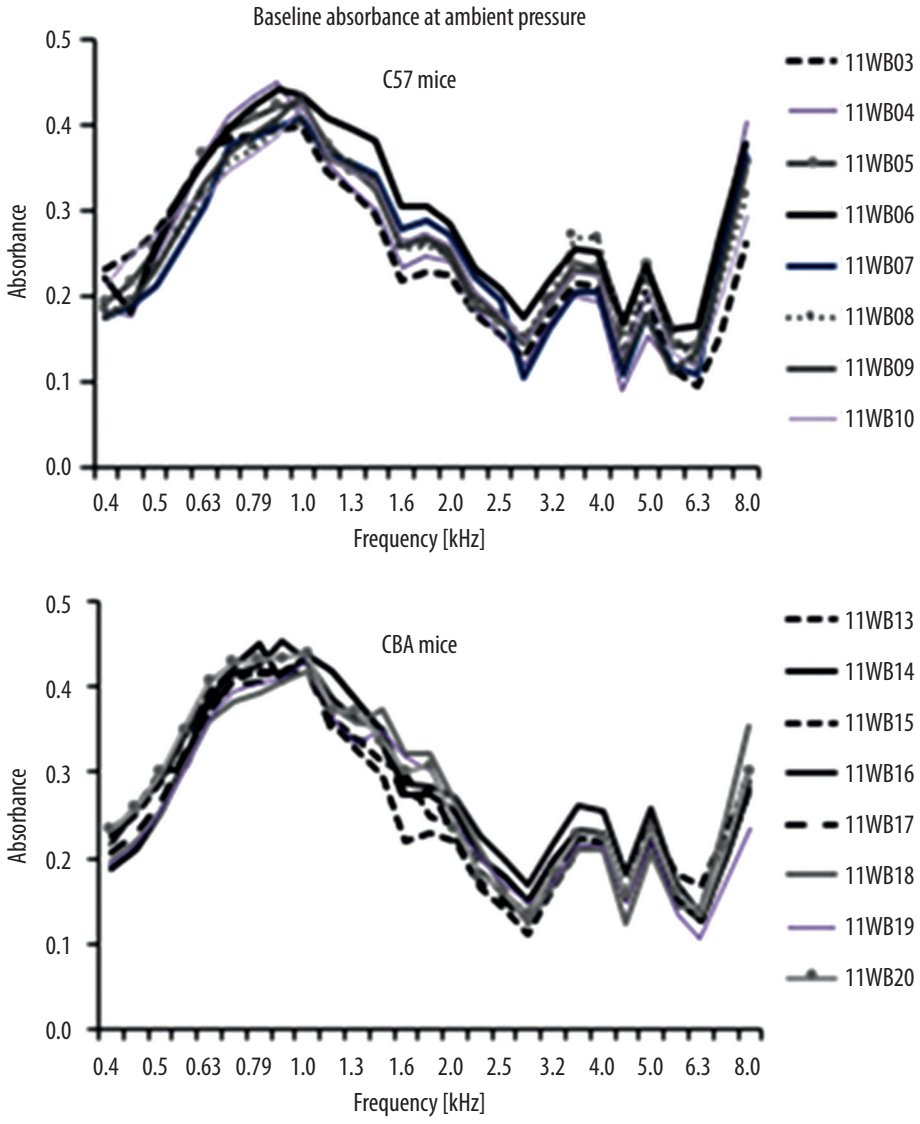

Figure 2. Baseline wideband absorbance versus frequency measured at ambient pressure (WBA). Mean data from both ears of each of the eight C57BL/6J mice (top panel) and each of the eight CBA/CaJ mice (bottom, $n = 8$). Both strains exhibited similar absorbance values with the highest peak around 1 kHz, a second peak near 8 kHz, and a characteristic M-shape absorbance profile between 2.8 and 6.3 kHz. The absorbance profiles within and between strains are similar, suggesting similar probe placement and middle ear properties in both strains

positive histology for OM. These seven C57BL/6J mice developed severe OM bilaterally ($n = 6$, 12 ears) and a moderate OM unilaterally ($n = 1$, 1 ear of 11WB04 mouse). Only one inoculated C57BL/6J mouse (11WB07) failed to develop signs of OM in either ear. Among the OM positive mice (**Figure 1**), infection was limited to the middle ear, causing acute and chronic suppurative OM (black arrows), without affecting the ear canal or inner ear. In contrast, none of the eight CBA/CaJ mice ($n = 8$, 16 ears) showed signs of OM in either ear at either 3 weeks or 6 weeks post inoculation, indicating that they were resistant to *B. hinzii* infection.

WBA post-inoculation

Based on histological findings of 16 C57BL/6J ears, 13 ears developed AOM bilaterally. Only three ears of two mice (two ears of 11WB07 and the left ear of 11WB4) were clear of infection; therefore, data from these three ears were not included in the analysis. **Table 2** shows the results of the test–retest reliability of the five WBA runs of each C57BL/6J and CBA/CaJ mouse. Cronbach's α correlation agreement was good to strong (0.712–0.839), with minimal variability between the confidence limits among the five WBAs recorded in each animal of the C57BL/6J and CBA/CaJ mice, suggesting stability of the recorded absorbance. Paired-samples *t*-test analysis of the baseline WBA from

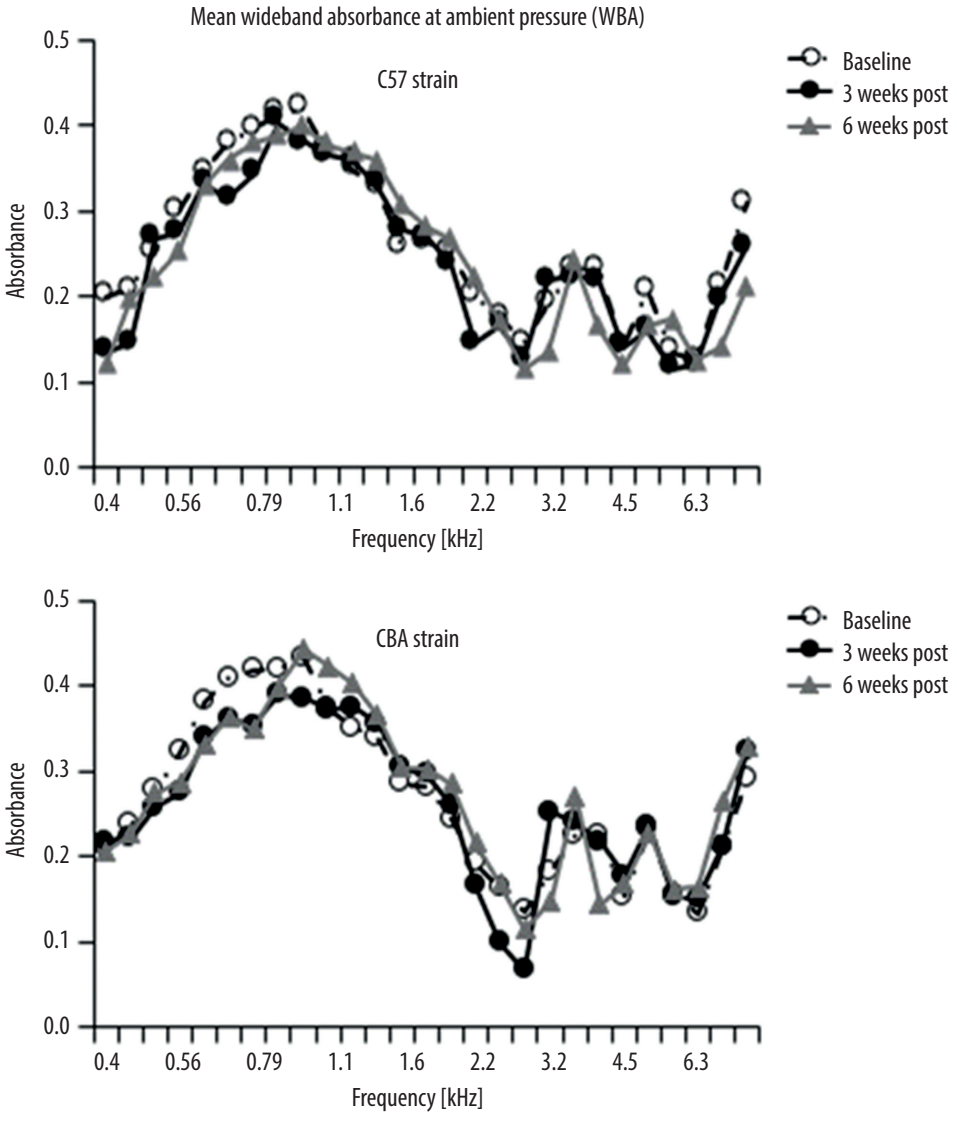

Figure 3. Mean wideband absorbance at ambient pressure versus frequency (WBA) in C57BL/6J (top, $n = 7$) and CBA/CaJ (bottom, $n = 8$) at baseline, 3 weeks post-inoculation, and 6 weeks post-inoculation with *B. hinzii*. Mean WBA in both mouse strains at 3 weeks and 6 weeks post-inoculation were unaltered and not significantly different from baseline ($p < 0.05$), suggesting that WBA lacks sensitivity in detecting histologically-verified OM

the right and left ears of each mouse strain did not show statistically significant differences between ears from 0.5 to 8 kHz for C57BL/6J strain, $t(7) = 0.812$, $p = 0.312$, nor for the CBA/CaJ strain, $t(9) = 0.914$, $p = 0.432$. Therefore, data from both ears were averaged for further analyses.

Figure 2 presents the baseline WBA versus frequency plot for each C57BL/6J infected mouse (top panel: $n = 7$, 13 ears) and CBA/CaJ mouse (bottom panel: $n = 8$, 16 ears). WBA from each mouse of the two strains exhibited similar absorbance values and patterns with the highest peak at an absorbance value of approximately 0.4 near 1 kHz, the second highest peak near 8 kHz, a slight dip at 1.6 kHz, and an M-shape between 3.2 and 6.3 kHz.

Figure 3 compares the mean WBA results from 0.5 to 8 kHz in C57BL/6J (top panel) and CBA/CaJ (bottom panel) mice at baseline, 3 weeks post-inoculation, and 6 weeks post-inoculation. The mean absorbance values and patterns follow those of the individual responses shown in **Figure 2**. Mean absorbance at 3 weeks or 6 weeks post-inoculation were not significantly different from baseline ($p < 0.05$). In both strains (C57BL/6J, top panel; CBA/CaJ, bottom panel), mean baseline WBA values were similar for the two strains at the three test times, suggesting no evidence of maturation. Repeated measures ANOVA of mean WBA were conducted for the two strains. Greenhouse–Geisser was used for sphericity correction. The C57BL/6J strain showed no statistically significant changes in mean absorbance at 3 weeks

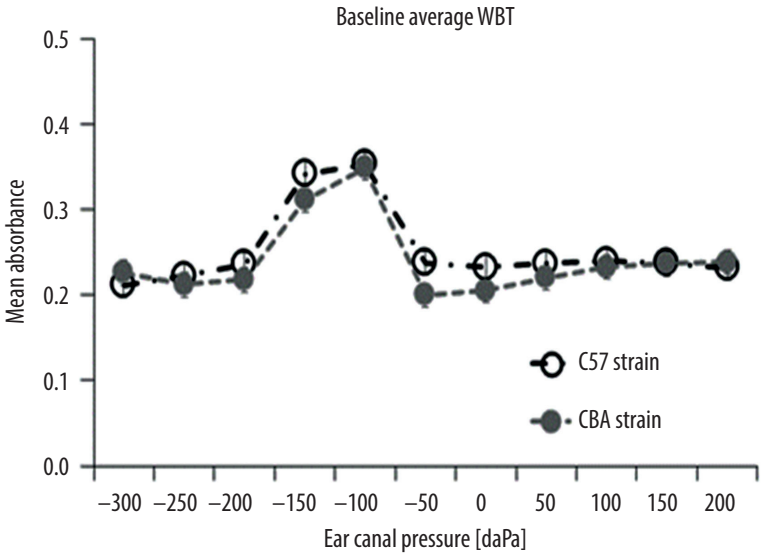

Figure 4. Mean baseline wideband tympanometry (WBT) for C57BL/6J and CBA/CaJ strains averaged across frequency for each mouse strain. WBT is averaged from 0.5 to 8 kHz and is plotted as a function of tympanometric pressure from +200 to –300 daPa. There is a single peak with highest absorbance around 0.38 at –50 daPa. Baseline WBT absorbance profiles are similar in both strains

(0.252 ± 0.034) and 6 weeks (0.251 ± 0.006) post-inoculation compared to baseline absorbance (0.265 ± 0.018) ($F = 0.696$, df = 1.257, 7.540, $p = 0.463$, $\eta_p^2 = 0.104$). The CBA/CaJ strain also showed no statistically significant changes in mean absorbance at 3 weeks (0.265 ± 0.009) and 6 weeks (0.271 ± 0.009) post-inoculation compared to baseline mean absorbance (0.273 ± 0.009) ($F = 1.740$, df = 1.914, $p = 0.213$, $\eta_p^2 = 0.199$). The lack of statistically significant changes in WBA in the C57BL/6J strain post-inoculation, despite the presence of moderate to severe OM documented histologically in the C57BL/6J group, suggests low sensitivity of WBA.

Average WBT absorbance and post-inoculations

The mean baseline WBT for C57BL/6J and CBA/CaJ strains averaged both across frequency and mice are show in **Figure 4**. The WBT is single-peaked; at baseline, the mean absorbance in both mouse strains shows a peak of approximately 4.1 around –50 daPa, with a similar absorbance profile. **Figure 5** presents the average WBT absorbance in C57BL/6J (top panel) and CBA/CaJ mice (bottom panel) averaged from 0.5 to 8 kHz and plotted as a function of tympanometric pressure (daPa) at baseline, 3 weeks post-inoculation, and 6 weeks post-inoculation with *B. hinzii*. As shown in **Figure 5**, mean WBT absorbance at 3 weeks and 6 weeks post-inoculation showed a marked reduction in peak absorbance to a nearly flat WBT profile in the C57BL/6J mice compared to baseline absorbance, suggesting the presence of OM with effusion. In contrast, the CBA/CaJ mice showed no changes in absorbance profile at 3 weeks and 6 weeks post-inoculation compared to baseline absorbance. These results indicate that C57BL/6J mice's middle ears are extremely susceptible to *B. hinzii* infection.

Table 3 presents the statistical analyses and significant post-inoculation differences of the average WBT in both strains. C57BL/6J mice showed a main effect across the three test times (baseline, 3 weeks post, and 6 weeks post *B. hinzii* inoculation) at all tympanometric pressures ($p < 0.05$). Bonferroni *post hoc* analysis revealed a significant reduction in absorbance at 3 weeks and 6 weeks post-inoculation compared to baseline absorbance ($p = 0.001$). There were no significant changes in absorbance between the 3-week and 6-week post-inoculation at –300 daPa ($p = 1.000$), –200 daPa ($p = 0.176$), –50 daPa ($p = 0.796$), and 0 daPa ($p = 0.483$), with slightly larger absorbance at 6 weeks than at 3 weeks post-inoculation at +100 daPa ($p = 0.015$) and +200 daPa ($p = 0.001$), possibly indicating an early resolution of OM. CBA/CaJ mice showed no statistically significant changes at 3 weeks and 6 weeks post-inoculation compared to baseline ($p > 0.05$). These findings suggest that C57BL/6J mice are more sensitive to *B. hinzii* infection than CBA/CaJ mice, consistent with the histological findings.

WBT absorbance in C57BL/6J mice following antibiotics

Figure 6 illustrates the temporal changes in WBT absorbance profiles in a typical C57BL/6J mouse from baseline (0 week) out to 1 week and 4 weeks post inoculation, and then during and after one month of antibiotic treatment; WBT evaluations were performed at 7, 8, and 9 weeks post-inoculation or 3, 4, and 5 weeks after the start of antibiotic treatment. At baseline (0 week), and consistent with the WBT absorbance profile of C57BL/6J mice from Experiment 1, the WBT plot shows two absorbance peaks: one at 1 kHz at –100–0 daPa and a second larger peak between 6 and 8 kHz at –300 daPa. At 1 week post-inoculation, the peak absorbance decreased at 1 kHz but increased significantly at 6–8 kHz at negative pressures compared to baseline value, followed by significant reduction of absorbance 4 weeks post-inoculation. The initial increase in absorbance at 1 week post-inoculation

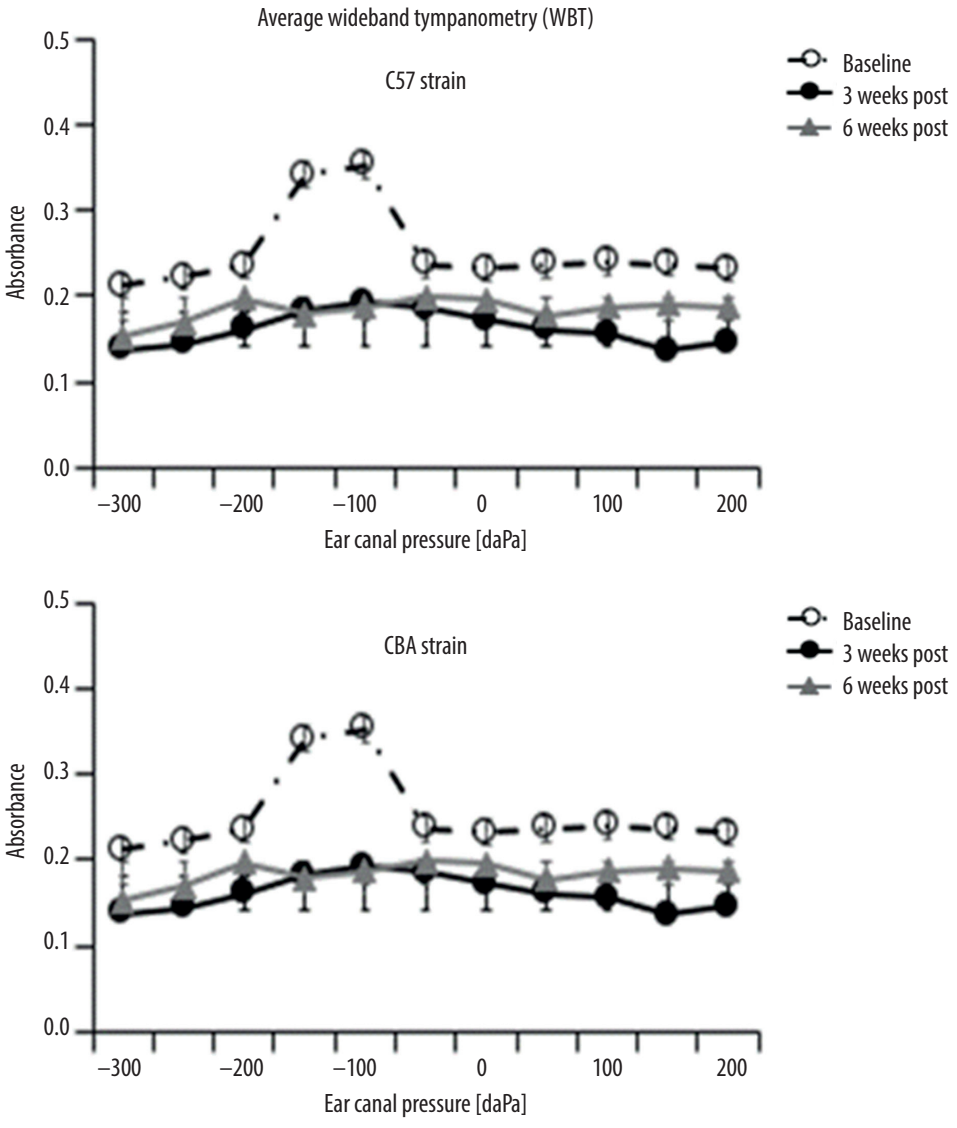

Figure 5. Mean wideband tympanogram (WBT) for two mouse strains, averaged across frequency (0.5 to 8 kHz) and plotted as a function of tympanometric pressure (+200 to –300 daPa) at baseline, 3 weeks post-inoculation, and 6 weeks post-inoculation with *B. hinzii*. The C57BL/6J strain is shown in top panel and CBA/CaJ strain at bottom. Mean WBT absorbance in C57BL/6J at 3 weeks and 6 weeks post-inoculation shows significantly reduced absorbance, resulting in a flat WBT indicative of OM. CBA/CaJ mice show no statistically significant changes in WBT absorbance at 3 weeks and 6 weeks post-inoculation compared to baseline absorbance, ruling out OM. Results indicate that C57BL/6J mice have succumbed to *B. hinzii* infection at 3 weeks post-inoculation and infection persists at 6 weeks

is consistent with negative middle ear pressure due to Eustachian tube dysfunction. This change was followed by severely reduced absorbance peaks to a nearly flat WBT profile at 4 weeks post-inoculation, indicative of the development of OM in C57BL/6J mice. After antibiotic treatment, absorbance recovered gradually over the course of antibiotic treatment and returned to baseline values at 8 and 9 weeks after antibiotic treatment. ANOVA showed a significant difference in absorbance across the four test times (baseline, 4 weeks post-inoculation, and after antibiotic treatment) ($F(2,36) = 6.231$, $p = 0.005$, $\eta_p^2 = 0.211$. Bonferroni *post hoc* analysis revealed significantly smaller absorbance at 4 weeks post inoculation compared to baseline ($p < 0.05$), and no significant difference post treatment compared to baseline ($p < 0.05$).

Histology in *B. hinzii* inoculated mice before/after antibiotics

The left panel of **Figure 7** illustrates typical histological findings in one *B. hinzii*-inoculated C57BL/6J mouse prepared for histological evaluation at a time when absorbance was reduced. The middle ear in this mouse was packed full of inflammatory material (see black arrow). The right panel of **Figure 7** illustrates the typical results in another *B. hinzii*-inoculated C57BL/6J mouse prepared for histologic evaluation 4 weeks after the start of antibiotic treatment (note: this mouse also showed a reduction of WBT at 4 weeks post-*B. hinzii* inoculation). After antibiotic treatment, the middle ear in this mouse shows a relatively clear middle ear space, with some

Table 3. One-way ANOVA comparing average WBT at different ear canal tympanometric pressure (daPa) for the C57BL/6J ($n = 13$ ears) and CBA/CaJ ($n = 16$ ears) mouse strains at three testing periods (baseline, 3 weeks post-inoculation and 6 weeks post-inoculation of *B. hinzii*

Canal pressure [daPa]	Mouse strain	*F*	*P*	η_p^2
–300	C57	5.725	0.018*	0.488
	CBA	1.217	0.328	0.158
–200	C57	17.745	<.001***	0.747
	CBA	0.032	0.968	0.005
–100	C57	37.476	<.001***	0.862
	CBA	3.437	0.066	0.923
0	C57	19.452	<.001***	0.764
	CBA	3.972	0.091	0.379
+100	C57	5.106	0.025*	0.460
	CBA	3.628	0.056	0.358
+200	C57	4.699	0.031*	0.439
	CBA	0.477	0.631	0.068

Note: Three ears from two C57BL/6J mice were excluded from the analysis due to lack of developing OM infection post-inoculation of *B. hinzii*

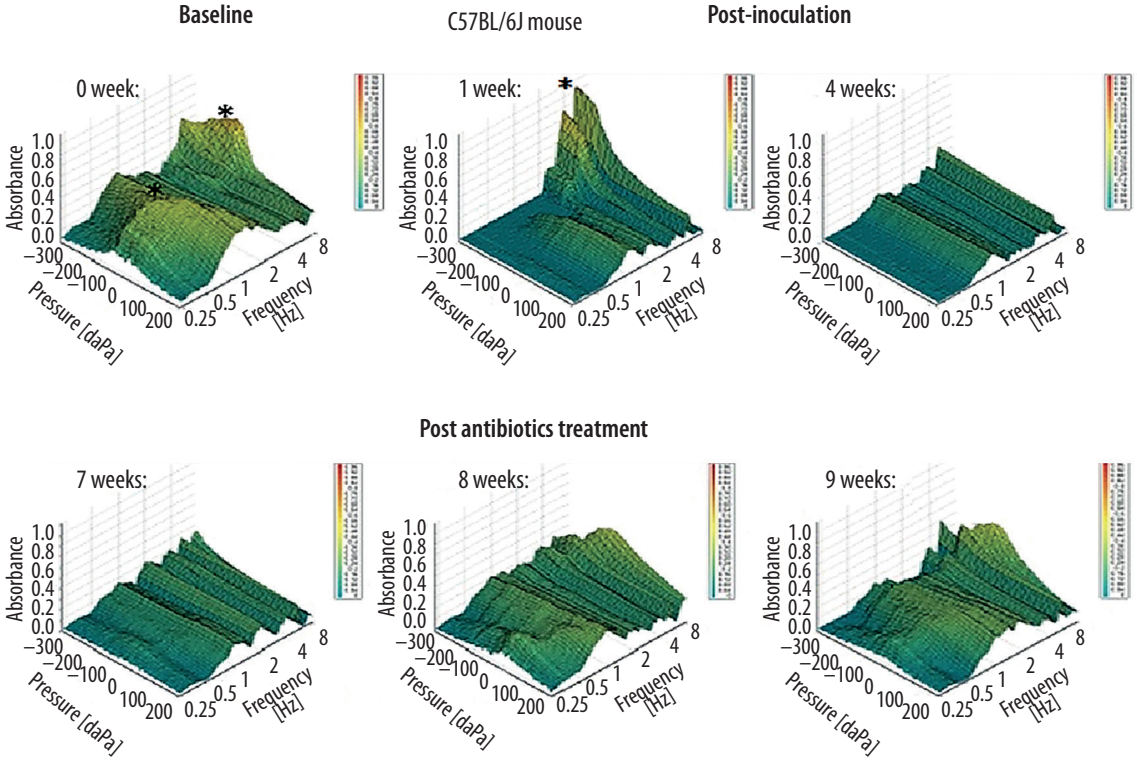

Figure 6. Changes in WBT absorbance over time from baseline (0 week), post inoculation with *B. hinzii* (1 and 4 weeks), and during continuous antibiotic treatment (7–9 weeks) in a treated C57BL/6J mouse. At baseline, two WBT absorbance peaks are present around 1 and 8 kHz (marked with *). At 1 week post-inoculation, the 8 kHz peak shows an increase in absorbance, but by 4 weeks post-inoculation, there is decreased absorbance at both regions. At 7 and 8 weeks post-antibiotic treatment, there is a gradual recovery (increase) of absorbance returning to near baseline values at 9 weeks of treatment

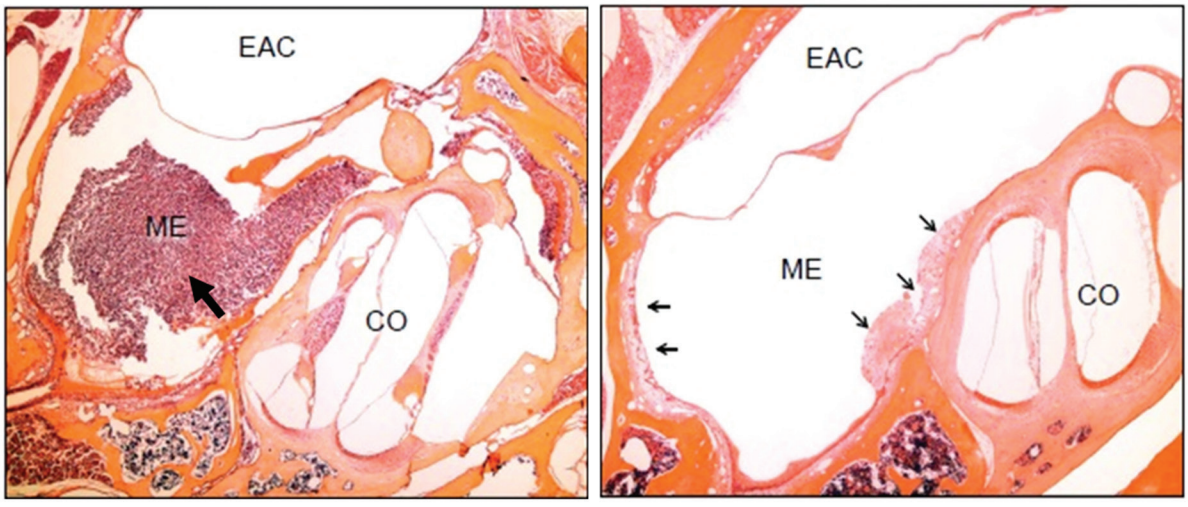

Figure 7. Histological profiles of the temporal bone region of two mice inoculated with *B. hinzii* before (left) and after (right) antibiotic treatment. Inoculation reduced wideband absorbance 4 weeks later. The mouse on the left, sacrificed and prepared for histology 4 weeks post-inoculation, shows a middle ear full of inflammatory material. The mouse on the right underwent a 4-week course of sulfamethoxazole and trimethoprim (SMTP) antibiotic treatment and was then sacrificed and prepared for histology. This mouse shows a relatively clear middle ear space, with only minor evidence of epithelial hyperplasia (arrows). Middle ear space (ME), external auditory canal (EAC), and cochlea (CO)

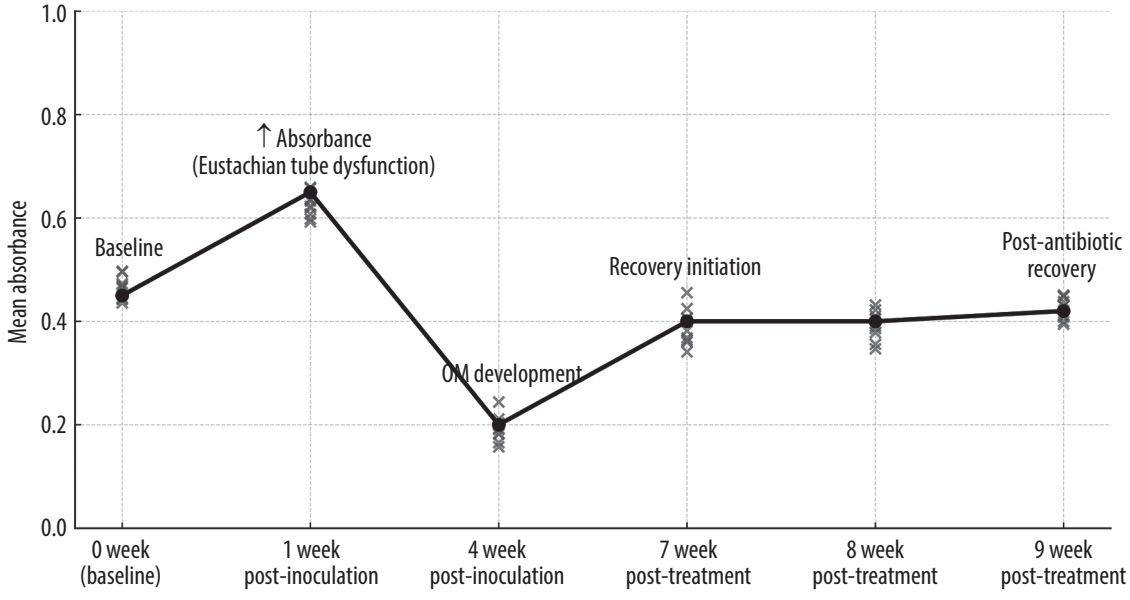

Figure 8. Time-course of wideband tympanometry (WBT) in C57BL/6J mice after inoculation with *B. hinzii*. Each gray dot represents data from an individual mouse ($n = 10$) and the black line denotes the mean absorbance at each time point. Mean absorbance increased at 1 week post-inoculation (↑ Absorbance), primarily reflecting Eustachian tube dysfunction, and decreased markedly at 4 weeks post-inoculation, consistent with the development of OM. Absorbance values progressively returned to near-baseline levels during the post-treatment period (7–9 weeks), indicating recovery and histologically confirmed resolution of OM

residual evidence of epithelial hyperplasia (black arrows). **Figure 8** illustrates the time-course of mean WBT absorbance across six measurement intervals in C57BL/6J mice. Individual data points from 10 mice are displayed to illustrate within-group variability across time. At baseline (0 week), mean absorbance was approximately 0.45. A significant increase in absorbance was observed at 1 week post-inoculation, likely reflecting altered Eustachian tube function. By 4 weeks post-inoculation, mean absorbance markedly decreased, consistent with OM development. Following antibiotic treatment, absorbance values at 7, 8, and 9 weeks post-treatment progressively improved and returned to near-baseline levels remained relatively stable, suggesting recovery and histologically confirmed resolution of OM. These findings are also consistent with the histopathological findings shown in **Figure 7**.

Discussion

Baseline WBA and WBT in young adult mice

This study is one of the first to use WAI to assess middle ear function in normal mice – before, during the development, and following the resolution of OM. WBA values provide estimates of absorbance as function of frequency at ambient pressure, whereas WBT values provide estimates of the average absorbance between 0.5 to 8 kHz at several tympanometric pressures from +200 to –300 daPa. Normal baseline measures were obtained from C57BL/6J and CBA/CaJ mice to determine if there were any significant differences in absorbance between these two widely used strains. Intra-session absorbance measured across five runs in which the probe was removed and replaced during each measurement showed good intra-session reliability, suggesting that WAI measure provides reasonably reliable estimates of absorbance across a broad range of frequencies and tympanometric pressures in mice – even though the device was not designed for species other than humans. Baseline WBA revealed two peaks, one peak near 1 kHz and a second around 8 kHz, in both C57BL/6J and CBA/CaJ mice.

Baseline WBT absorbance values, averaged from 0.5 to 8 kHz, were highest with a peak value around 0.41 in both C57BL/6J and CBA/CaJ mice when the ear canal pressure was –50 daPa. The general absorbance pattern in these two mouse strains is much different from humans. Peak absorbance in humans, which occurs near 4 kHz at 0 daPa, is approximately 0.8, a value twice that of mice [27,30]. The low absorbance values in mice likely reflects the fact that murine middle ear impedance is dominated by stiffness at frequencies below 6–8 kHz, ostensibly reducing the sensitivity of the Interacoustics device [18,36]. The Interacoustics device provides absorbance measures that cover much of the human clinical audiometric range (0.25 to 8 kHz). By contrast, the hearing range in mice ranges from approximately 1 to 80 kHz with maximum sensitivity around 20 kHz [37,38]. Given the limitations of using the Interacoustics WBT device in mice, future studies with this device might be better suited to the chinchilla, with an audiometric range similar to humans and an ear canal and middle ear space much larger than that of mice [11,39–41]. Laser doppler vibrometers are capable of measuring middle ear motion up to at least 20 kHz, making it more suitable for studies in mice with excellent high-frequency hearing [36]; however, these instruments are not readily available or suitable for most research clinicians.

Changes in WBA and WBT after inoculation of *B. hinzii*

Of the eight CBA/CaJ mice (16 ears) inoculated with *B. hinzii*, no statistically significant changes in WBA or WBT absorbance were noted at 3 weeks and 6 weeks post-inoculation compared to baseline as shown in **Figure 3** and **Figure 5**. Given that the middle ears in all CBA/CaJ mice were histologically clear of infection, the lack of change in absorbance accurately reflects the normal status of the middle ear and absence of OM in this strain following *B. hinzii* inoculation. Among the eight C57BL/6J mice inoculated with *B. hinzii*, seven developed histological evidence of OM, six had bilateral OM, and one had unilateral OM. This lack of sensitivity of WBA at ambient pressure could reflect a genuine, strain-specific biological response in which mild to moderate middle-ear changes do not substantially alter ambient absorbance across the 0.5–8 kHz band in mice. This null result could also be caused partly by using equipment mainly designed for testing human middle ears, and partly by the high stiffness of the mouse middle ear at low frequencies (stiffness dominated), making it difficult to detect absorbance changes cause by OM. Changes in absorbance might be detected in mice above 8 kHz where stiffness declines [36]. Therefore, the lack of significant WBA changes at ambient pressure should be interpreted with caution.

In contrast to our WBA results in mice, others have reported significant reductions in WBA among children with conductive hearing loss [27], Eustachian tube dysfunction [42], middle ear effusion, and tympanic membrane perforations [30]. The reductions in WBA in humans with conductive hearing loss were frequency-dependent. Decreased absorbance began around 1 kHz, increased up to approximately 4 kHz, and then rapidly decreased by 8 kHz. Thus, WBA appears to be more sensitive at detecting conductive hearing losses in humans than in mice. Possible reasons for these species difference are unclear but are likely related to species differences in ear canal and middle ear anatomy and acoustic properties, the use of the newborn calibration for the Interacoustics WBT device versus device calibration specifically optimized for mice, and possible differences in the duration and nature of the middle ear pathologies.

The average WBT revealed a significant reduction of absorbance at all tympanometric pressures at both 3 and 6 weeks post-inoculation (see **Figure 5**). The high sensitivity and specificity of WBT in detecting *B. hinzii*-induced OM in mouse is consistent with absorbance findings in patients with OM that showed 95% sensitivity and 88% specificity [43]. Notably, WBT detected OM in all mouse ears with documented OM, whereas WBT remained normal in mice with histologically normal middle ears, indicating high sensitivity and specificity. Our finding of reduced average WBT in confirmed cases of OM was noticeable at all tympanometric pressures. However, we unexpectedly observed an increase in absorbance around 6–8 kHz at negative tympanometric pressure after 1 week of infection, suggesting that C57BL/6J mice had developed negative middle ear pressure (**Figure 6**) during the early stage of infection prior to full blown OM. This finding is similar to results obtained in humans with Eustachian tube dysfunction which showed an increase in absorbance around 2–4 kHz, a result attributed to negative middle ear pressure [42]. These results suggest that WBT might be useful for detecting the negative middle ear pressure in mice associated with the early stages of OM infection.

Strain differences in OM susceptibility

C57BL/6J mice were highly susceptible to *B. hinzii*-induced OM whereas CBA/CaJ mice were highly resistant to infection for reasons that are poorly understood. Strain differences in susceptibility to various types of infections and viral-mediated cancers have been reported in the literature [44,45].

The factors that make C57BL/6J highly susceptible to *B. hinzii* are not fully understood, but some reports indicate that infected mice deficient in B cells are burdened by a greater number of bacteria [46]. Another factor that could contribute to the susceptibility of C57BL/6J mice to OM is the ability of certain bacteria to ascend the Eustachian tube into the middle ear [47]. C57BL/6J mice with compromised mucociliary transport appear to be susceptible to OM infection [48]. Other factors in C57BL/6J mice that could enhance susceptibility to pathogen-mediated infections include differences in cytokines and chemokines expression levels [49]. In addition, C57BL/6J mice have a mutation of a cadherin gene that is not only associated with early age-related hearing loss but is also known to mediate host–pathogen interactions [50]. The mutation of the cadherin 23 gene in C57BL/6J mice could allow for an opportunistic interaction with *B. hinzii* that is not present with the wild type cadherin present in CBA/CaJ mice [51]. If OM from *B. hinzii*-mediated infection is associated with this cadherin mutation, this would represent an important finding.

Recovery of WBT following antibiotic treatment

Our WBT absorbance data showed a significant reduction of absorbance 4 weeks following *B. hinzii* inoculation that recovered to baseline following the full course of antibiotic treatment. This recovery in absorbance was associated with virtually complete histological resolution of middle ear infection. These results indicate that combination antibiotic treatment (sulfamethoxazole and trimethoprim) often used to treat middle ear infections in humans was highly effective at treating OM from *B. hinzii*-mediated infection in C57BL/6J mice [34]. Because of the strong correlation with the histological findings, these results suggest that WBT measure is a reliable and highly sensitive research tool that can be used to study the development and resolution of OM and other middle ear pathologies in different murine strains infected with various pathogens. From a scientific and clinical perspective, WBT could be used to determine the susceptibility of mice with specific genetic mutations that contribute to the development of OM and other middle ear pathologies [21,44,45,50,51]. It could also be used to determine what drugs are most effective at resolving OM [52,53] or other middle ear pathologies induced by specific pathogens [4,9,54]. Our histopathological analysis following *B. hinzii* inoculation and antibiotic treatment also revealed evidence of ossicular chain remodeling in several cases, as well as minor residual infiltrates in some ears consistent with prior reports [55–57]. It is unclear if these subtle residual histopathologies would resolve with longer recovery times, longer treatments, or other antibiotics. Another related question that requires further investigation is whether the OM in the C57BL/6J mice would naturally resolve without antibiotic treatment. The outcome of such an experiment would likely depend on the sterility of the environment in which the mice were housed.

Conclusions

WAI, a recently developed technique to assess middle ear function in humans, can be used to monitor middle ear status and pathology in mice – one of the most widely used species in auditory research because of a plethora of genetic variants. The presence of *B. hinzii* bacteria in a mouse colony is a particularly serious problem for auditory researchers, because they cannot be easily eradicated by standard antibiotic administration, and because they can evade host immune responses. Our results show that WBT can be used to monitor the progression of *B. hinzii*-induced OM in mice and that a 4-week course of oral treatment with sulfamethoxazole and trimethoprim can effectively eradicate OM in C57BL/6J mice. Notably, C57BL/6J mice are highly susceptible to developing OM, in contrast to CBA/CaJ mice which are highly resistant to this form of bacterial infection.

Histological assessment of OM has confirmed the reliability and specificity of using WBT to assess the development and resolution of OM, but further research is needed to confirm the generality of these findings in other murine models, different species, and other pathologies. Although the trends in Experiment 1 and 2 were consistent and clear, a limitation of the study from a statistical standpoint was the relatively modest sample size. These statistical concerns could be overcome by increasing the numbers of mice used in Experiment 1 and 2 to enhance the statistical power of the analysis. However, the sample sizes we employed are typical of animal studies and reflect the practical concern of the large time, effort, and costs associated with performing serial measurements over an extended period. The sample sizes also reflect the ethical concern of not using an excessive number of animals for studies in which the subjects are sacrificed at the conclusion of the study. Notwithstanding statistical concerns, we believe the consistent trends observed across Experiments 1 and 2 support the general conclusions.

Another limitation of the method is that the instrument software assumes characteristic impedance values based on human ear-canal cross-sectional areas, rather than those of mice [58], so the absolute absorbance values used here need to be interpreted with caution [59]. However, this limitation is not unique to small animals; it reflects a broader, inherent constraint of WBT systems used in both human and non-human ears (IEC 60318-4, 2010). Nevertheless, although we did not calculate the mouse ear-canal equivalent volume or impedance using an in situ characteristic impedance, our findings remain valid for within-species comparisons. The use of a consistent measurement setup across all animals ensures that relative absorbance trends are meaningful and interpretable. Future studies should aim to establish the acoustically equivalent cross-sectional area of the mouse ear canal in situ in order to improve calibration accuracy and the reliability of absolute absorbance measurements. Future studies of WAI would benefit from the use of Titan instrumentation with enhanced features (e.g., having a probe with different dimensions optimized for mice and accurately measuring the ear-canal characteristic impedance and equivalent volume of mice) to test the generalizability of the current findings [30,60].

Acknowledgements

The authors thank Suellen Greco for preparation of *B. hinzii* inoculates, Kelly G. Lytte for data collection, and Pat Keller for histology. We also thank the RStat Institute at Missouri State University for helping with data analysis.

Funding

This research was supported by the National Institute on Deafness and Other Communication Disorders (NIDCD) grants T35DC008765 (PI, William W. Clark) and P30DC004665 (PI, Richard A. Chole) and funding from the Graduate College of Missouri State University.

References

1. Bhutta MF, Hedge EA, Parker A, Cheeseman MT, Brown SD. Oto-endoscopy: a reliable and validated technique for phenotyping otitis media in the mouse. Hear Res, 2011; 272(1–2): 5–12. https://doi.org/10.1016/j.heares.2010.09.002
2. MacArthur CJ, Pillers DA, Pang J, Kempton JB, Trune DR. Altered expression of middle and inner ear cytokines in mouse otitis media. Laryngoscope, 2011; 121(2): 365–71. https://doi.org/10.1002/lary.21349
3. Leichtle A, Lai Y, Wollenberg B, Wasserman SI, Ryan AF. Innate signaling in otitis media: pathogenesis and recovery. Curr Allergy Asthma Rep, 2011; 11(1): 78–84. https://doi.org/10.1007/s11882-010-0158-3
4. Ryan AF, Ebmeyer J, Furukawa M, Pak K, Melhus A, Wasserman SI, Chung WH. Mouse models of induced otitis media. Brain Res, 2006; 1091(1): 3–8. https://doi.org/10.1016/j.brainres.2006.02.004
5. Lanphear BP, Byrd RS, Auinger P, Hall CB. Increasing prevalence of recurrent otitis media among children in the United States. Pediatrics, 1997; 99(3): E1. https://doi.org/10.1542/peds.99.3.e1
6. Lim DJ, Chun YM, Lee HY, Moon SK, Chang KH, Li JD, Andalibi A. Cell biology of tubotympanum in relation to pathogenesis of otitis media: a review. Vaccine, 2000; 19 (Suppl. 1): S17–25. https://doi.org/10.1016/s0264-410x(00)00273-5
7. Bodet E, Romeu C, Gomez X, Manonellas A, Palomar V. [Experimental model of recurrent acute otitis media: morphological study of the cochlear damage using scanning electron microscopy]. Acta Otorrinolaringol Esp, 2005; 56(4): 139–42. https://doi.org/10.1016/s0001-6519(05)78588-5
8. Clark JM, Brinson G, Newman MK, Jewett BS, Sartor BR, Prazma J, Pillsbury HC. An animal model for the study of genetic predisposition in the pathogenesis of middle ear inflammation. Laryngoscope, 2000; 110(9): 1511–5. https://doi.org/10.1097/00005537-200009000-00019
9. Dewan KK, Taylor-Mulneix DL, Campos LL, Skarlupka AL, Wagner SM, Ryman VE, et al. A model of chronic, transmissible otitis media in mice. PLoS Pathog, 2019; 15(4): e1007696. https://doi.org/10.1371/journal.ppat.1007696
10. Giebink GS. Otitis media: the chinchilla model. Microb Drug Resist, 1999; 5(1): 57–72. https://doi.org/10.1089/mdr.1999.5.57
11. Guan X, Seale TW, Gan RZ. Factors affecting sound energy absorbance in acute otitis media model of chinchilla. Hear Res, 2017; 350: 22–31. https://doi.org/10.1016/j.heares.2017.04.003
12. Kariya S, Okano M, Fukushima K, Nomiya S, Kataoka Y, Nomiya R, Akagi H, Nishizaki K. Expression of inflammatory mediators in the otitis media induced by *Helicobacter pylori* antigen in mice. Clin Exp Immunol, 2008; 154(1): 134–40. https://doi.org/10.1111/j.1365-2249.2008.03740.x
13. MacArthur CJ, Trune DR. Mouse models of otitis media. Curr Opin Otolaryngol Head Neck Surg, 2006; 14(5): 341–6. https://doi.org/10.1097/01.moo.0000244193.97301.d7
14. Melhus A, Ryan AF. A mouse model for acute otitis media. APMIS, 2003; 111(10): 989–94. https://doi.org/10.1034/j.1600-0463.2003.1111012.x
15. Mitchell CR, Kempton JB, Scott-Tyler B, Trune DR. Otitis media incidence and impact on the auditory brain stem response in lipopolysaccharide-nonresponsive C3H/HeJ mice. Otolaryngol Head Neck Surg, 1997; 117(5): 459–64. https://doi.org/10.1016/S0194-59989770014-7
16. Zheng QY, Tong YC, Alagramam KN, Yu H. Tympanometry assessment of 61 inbred strains of mice. Hear Res, 2007; 231(1–2): 23–31. https://doi.org/10.1016/j.heares.2007.05.011
17. Chen J, Ingham N, Clare S, Raisen C, Vancollie VE, Ismail O, et al. *Mcph1*-deficient mice reveal a role for MCPH1 in otitis media. PLoS One, 2013; 8(3): e58156. https://doi.org/10.1371/journal.pone.0058156
18. Rosowski JJ, Brinsko KM, Tempel BI, Kujawa SG. The aging of the middle ear in 129S6/SvEvTac and CBA/CaJ mice: measurements of umbo velocity, hearing function, and the incidence of pathology. J Assoc Res Otolaryngol, 2003; 4(3): 371–83. https://doi.org/10.1007/s10162-002-3047-1
19. Rye MS, Bhutta MF, Cheeseman MT, Burgner D, Blackwell JM, Brown SD, et al. Unraveling the genetics of otitis media: from mouse to human and back again. Mamm Genome, 2011; 22(1–2): 66–82. https://doi.org/10.1007/s00335-010-9295-1
20. Trune DR, Zheng QY. Mouse models for human otitis media. Brain Res, 2009; 1277: 90–103. https://doi.org/10.1016/j.brainres.2009.02.047
21. Zheng QY, Johnson KR, Erway LC. Assessment of hearing in 80 inbred strains of mice by ABR threshold analyses. Hear Res, 1999; 130(1–2): 94–107. https://doi.org/10.1016/s0378-5955(99)00003-9
22. Zheng T, Huang W, Yu H, Hu BH, Song P, McCarty CM, et al. gom1 mutant mice as a model of otitis media. J Assoc Res Otolaryngol, 2022; 23(2): 213–23. https://doi.org/10.1007/s10162-022-00838-2
23. Margolis RH, Paul S, Saly GL, Schachern PA, Keefe DH. Wideband reflectance tympanometry in chinchillas and human. J Acoust Soc Am, 2001; 110(3 Pt 1): 1453–64. https://doi.org/10.1121/1.1394219
24. Peake WT, Rosowski JJ. Impedance matching, optimum velocity, and ideal middle ears. Hear Res, 1991; 53(1): 1–6. https://doi.org/10.1016/0378-5955(91)90208-q
25. Voss SE, Rosowski JJ, Merchant SN, Peake WT. How do tympanic-membrane perforations affect human middle-ear sound transmission? Acta Otolaryngol, 2001; 121(2): 169–73. https://doi.org/10.1080/000164801300043343
26. AlMakadma H, Kei J, Yeager D, Feeney MP. Fundamental concepts for assessment and interpretation of wideband acoustic immittance measurements. Semin Hear, 2023; 44(1): 17–28. https://doi.org/10.1055/s-0043-1763293
27. Keefe DH, Sanford CA, Ellison JC, Fitzpatrick DF, Gorga MP. Wideband aural acoustic absorbance predicts conductive hearing loss in children. Int J Audiol, 2012; 51(12): 880–91. https://doi.org/10.3109/14992027.2012.721936
28. Karuppannan A, Barman A. Wideband absorbance pattern in adults with otosclerosis and ossicular chain discontinuity. Auris Nasus Larynx, 2021; 48(4): 583–9. https://doi.org/10.1016/j.anl.2020.10.019

29. Karuppannan A, Barman A. Wideband absorbance tympanometry: a novel method in identifying otosclerosis. Eur Arch Otorhinolaryngol, 2021; 278(11): 4305–14. https://doi.org/10.1007/s00405-020-06571-x
30. Karuppannan A, Barman A, Mamatha NM. Wideband absorbance pattern and its diagnostic value in adults with middle ear effusions and tympanic membrane perforation. J Int Adv Otol, 2024; 20(2): 158–63. https://doi.org/10.5152/iao.2024.231048
31. Feeney MP, Hunter LL, Kei J, Lilly DJ, Margolis RH, Nakajima HH, et al. Consensus statement: Eriksholm workshop on wideband absorbance measures of the middle ear. Ear Hear, 2013; 34 (Suppl 1): 78S–79S. https://doi.org/10.1097/AUD.0b013e31829c726b
32. Guan X, Jiang S, Seale TW, Hitt BM, Gan RZ. Morphological changes in the tympanic membrane associated with *Haemophilus influenzae*-induced acute otitis media in the chinchilla. Int J Pediatr Otorhinolaryngol, 2015; 79(9): 1462–71. https://doi.org/10.1016/j.ijporl.2015.06.030
33. Chen GD, Li L, McCall A, Ding D, Xing Z, Yu YE, Salvi R. Hearing impairment in murine model of Down syndrome. Front Genet, 2022; 13: 936128. https://doi.org/10.3389/fgene.2022.936128
34. Leiberman A, Leibovitz E, Piglansky L, Raiz S, Press J, Yagupsky P, et al. Bacteriologic and clinical efficacy of trimethoprim–sulfamethoxazole for treatment of acute otitis media. Pediatr Infect Dis J, 2001; 20(3): 260–4. https://doi.org/10.1097/00006454-200103000-00009
35. Sanford CA, Keefe DH, Liu YW, Fitzpatrick D, McCreery RW, Lewis DE, et al. Sound-conduction effects on distortion-product otoacoustic emission screening outcomes in newborn infants: test performance of wideband acoustic transfer functions and 1-kHz tympanometry. Ear Hear, 2009; 30(6): 635–52. https://doi.org/10.1097/AUD.0b013e3181b61cdc
36. Rosowski JJ, Mehta RP, Merchant SN. Diagnostic utility of laser-Doppler vibrometry in conductive hearing loss with normal tympanic membrane. Otol Neurotol, 2003; 24(2): 165–75. https://doi.org/10.1097/00129492-200303000-00008
37. Koay G, Heffner R, Heffner H. Behavioral audiograms of homozygous med(J) mutant mice with sodium channel deficiency and unaffected controls. Hear Res, 2002; 171(1–2): 111–8. https://doi.org/10.1016/s0378-5955(02)00492-6
38. Radziwon KE, June KM, Stolzberg DJ, Xu-Friedman MA, Salvi RJ, Dent ML. Behaviorally measured audiograms and gap detection thresholds in CBA/CaJ mice. J Comp Physiol A Neuroethol Sens Neural Behav Physiol, 2009; 195(10): 961–9. https://doi.org/10.1007/s00359-009-0472-1
39. Clark WW, Clark CS, Moody DB, Stebbins WC. Noise-induced hearing loss in the chinchilla, as determined by a positive-reinforcement technique. J Acoust Soc Am, 1974; 56(4): 1202–9. https://doi.org/10.1121/1.1903409
40. Salvi RJ, Hamernik RP, Henderson D. Auditory nerve activity and cochlear morphology after noise exposure. Arch Otorhinolaryngol, 1979; 224(1–2): 111–6. https://doi.org/10.1007/BF00455233
41. Vrettakos PA, Dear SP, Saunders C. Middle ear structure in the chinchilla: a quantitative study. Am J Otolaryngol, 1988; 9(2): 58–67. https://doi.org/10.1016/s0196-0709(88)80009-7
42. Aithal S, Aithal V, Kei J, Anderson S, Liebenberg S. Eustachian tube dysfunction and wideband absorbance measurements at tympanometric peak Pressure and 0 daPa. J Am Acad Audiol, 2019; 30(9): 781–91. https://doi.org/10.3766/jaaa.18002
43. Pan JL, Yang J. [The clinical value of wideband tympanometry in the diagnosis of otitis media with effusion]. Lin Chuang Er Bi Yan Hou Tou Jing Wai Ke Za Zhi, 2018; 32(17): 1309–15. https://doi.org/10.13201/j.issn.1001-1781.2018.17.005 [in Chinese]
44. Ahlstedt S. Experimental *Escherichia coli* 06 infection in mice. 1. Effect of immunisation on resistance in relation to 06 antibody levels in mice of different strains. Acta Pathol Microbiol Scand C, 1980; 88(1): 39–45.
45. Rowe WP, Hartley JW. Genes affecting mink cell focus-inducing (MCF) murine leukemia virus infection and spontaneous lymphoma in AKR F1 hybrids. J Exp Med, 1983; 158(2): 353–64. https://doi.org/10.1084/jem.158.2.353
46. Dewan KK, Taylor-Mulneix DL, Campos LL, Skarlupka AL, Wagner SM, Ryman VE, et al. A model of chronic, transmissible otitis media in mice. PLoS Pathog, 2019; 15(4): e1007696. https://doi.org/10.1371/journal.ppat.1007696
47. Dewan KK, Sedney C, Caulfield AD, Su Y, Ma L, Blas-Machado U, et al. Probing immune-mediated clearance of acute middle ear infection in mice. Front Cell Infect Microbiol, 2021; 11: 815627. https://doi.org/10.3389/fcimb.2021.815627
48. Perniss A, Schmidt N, Gurtner C, Dietert K, Schwengers O, Weigel M, et al. *Bordetella pseudohinzii* targets cilia and impairs tracheal cilia-driven transport in naturally acquired infection in mice. Sci Rep, 2018; 8: 5681. https://doi.org/10.1038/s41598-018-23830-4
49. Gautam A, Dixit S, Embers M, Gautam R, Philipp MT, Singh SR, et al. Different patterns of expression and of IL-10 modulation of inflammatory mediators from macrophages of Lyme disease-resistant and -susceptible mice. PLoS One, 2012; 7(9): e43860. https://doi.org/10.1371/journal.pone.0043860
50. Zhao T, Ma P, Zhao F, Zheng T, Yan B, Zhang Q, et al. Phenotypic differences in the inner ears of CBA/CaJ and C57BL/6J mice carrying missense and single base pair deletion mutations in the Cdh23 gene. J Neurosci Res, 2021; 99(10): 2743–58. https://doi.org/10.1002/jnr.24905
51. Mateo M, Generous A, Sinn PL, Cattaneo R. Connections matter: how viruses use cell–cell adhesion components. J Cell Sci, 2015; 128(3): 431–9. https://doi.org/10.1242/jcs.159400
52. Ikeda K, Takasaka T. In vitro activity of ototopical drops against middle ear pathogens. Am J Otol, 1993; 14(2): 170–1.
53. Tong MC, Woo JK, van Hasselt CA. A double-blind comparative study of ofloxacin otic drops versus neomycin–polymyxin B–hydrocortisone otic drops in the medical treatment of chronic suppurative otitis media. J Laryngol Otol, 1996; 110(4): 309–14. https://doi.org/10.1017/s0022215100133523
54. Kristjansson S, Skuladottir HE, Sturludottir M, Wennergren G. Increased prevalence of otitis media following respiratory syncytial virus infection. Acta Paediatr, 2010; 99(6): 867–70. https://doi.org/10.1111/j.1651-2227.2009.01637.x
55. Post JC. Direct evidence of bacterial biofilms in otitis media. Laryngoscope, 2001; 111(12): 2083–94. https://doi.org/10.1097/00005537-200112000-00001
56. Quaranta A, Bartoli R, Resta L, Lozupone E. Candida and stapedial otosclerosis: histopathological findings. ORL J Otorhinolaryngol Relat Spec, 1992; 54(6): 334–6. https://doi.org/10.1159/000276327
57. Thornton JL, Chevallier KM, Koka K, Gabbard SA, Tollin DJ. Conductive hearing loss induced by experimental middle-ear effusion in a chinchilla model reveals impaired tympanic membrane-coupled ossicular chain movement. J Assoc Res Otolaryngol, 2013; 14(4): 451–64. https://doi.org/10.1007/s10162-013-0388-x

58. Norgaard KM, Fernandez-Grande E, Schmuck C, Laugesen S. Reproducing ear-canal reflectance using two measurement techniques in adult ears. J Acoust Soc Am, 2020; 147(4): 2334. https://doi.org/10.1121/10.0001094
59. Keefe DH, Bulen JC, Arehart KH, Burns EM. Ear-canal impedance and reflection coefficient in human infants and adults. J Acoust Soc Am, 1993; 94(5): 2617–38. https://doi.org/10.1121/1.407347
60. Aithal S, Kei J, Aithal V, Manuel A, Myers J, Driscoll C, Khan A. Normative study of wideband acoustic immittance measures in newborn infants. J Speech Lang Hear Res, 2017; 60(5): 1417–26. https://doi.org/10.1044/2016_JSLHR-H-16-0237

Wafaa Kaf, email: wafaakaf@missouristate.edu • 0000-0002-4391-4959
Richard J. Salvi, email: salvi@buffalo.edu • 0000-0001-9061-8602
Brian Faddis, email: btfaddis@wustl.edu • 0009-0000-1062-6208