Human Inner Ear Mechanics Studied With Experimental, Anatomical, and Computational Approaches

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Human Inner Ear Mechanics Studied with Experimental, Anatomical, and Computational Approaches

A dissertation presented

by

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to

The Division of Medical Sciences

in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

in the subject of

Speech and Hearing Bioscience and Technology

Harvard University

Cambridge, Massachusetts

(June 2019)
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Human Inner Ear Mechanics Studied with Experimental, Anatomical, and Computational Approaches

Abstract

Our understanding of human inner ear mechanics is mostly based on laboratory-animal studies. This thesis presents new findings specific to human hearing. Our methodological approach was threefold: We performed experiments in fresh human cadaveric temporal bones and live patients, carried out anatomical studies, and used mathematical models to advance our understanding of human inner ear mechanics.

In Chapter 1, we investigate the impedance and stiffness of the human basilar membrane (BM) in the cochlear base and show that previous studies either underestimated or overestimated the BM stiffness by up to one order of magnitude. Chapter 2 looks beyond the BM and investigates the motion across the entire width of the cochlear partition (CP). We identify a soft-tissue structure in the CP in human—the “bridge”—that connects the BM and osseous spiral lamina (OSL) and has approximately the same width as the BM. We show that the bridge as well as the OSL moves considerably in humans. The motion of the bridge and OSL questions the applicability of the classic mammalian hearing model, based on laboratory-animal data, to humans. In Chapter 3, we characterize the anatomical microstructure of the bridge and OSL.
Fibers traversing the bridge and the high porosity of the OSL could explain the nature of the bridge and OSL motion.

Chapters 4 and 5 investigate the propagation of low-frequency sound and infrasound through the human middle ear and inner ear. In Chapter 4, we show that the middle ear limits sound energy propagated to the inner ear at low frequencies. A perturbation of the inner ear impedance by means of opening the semicircular canal changes the sound flow and sensitivity of the ear to low-frequency sound. We also characterize the impedance of semicircular canal dehiscence. Chapter 5 ties together experimental, clinical, and computational modeling results to propose how hearing-threshold shifts at low frequencies may be exploited to diagnose patients with a pathological defect of the semicircular canal.

The thesis offers a comprehensive understanding of human passive cochlear mechanics in the cochlear base, as well as a comprehensive understanding of low-frequency sound propagation in the human inner ear.
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Acknowledgments

I would like to thank Heidi Nakajima for four incredibly fulfilling years in the Ear Mechanics Lab. Through Heidi’s supervision and mentorship I grew as a scientist and as a person. Further, I would like to thank members of my dissertation advisory committee, John Guinan, Denny Freeman, and Sunil Puria. My DAC members were wonderful mentors during the second half of my PhD, shaped my thinking and thesis in important ways, and were important advocates for my work. The DAC meetings have truly been the highlight of my PhD. I would also like to thank my exam committee, John Rosowski, Lisa Olson, M. Charles Liberman, and the exam chair Dennis Freeman for critical review of this thesis.

I would like to thank members of the lab: Xiying Guan and Song Cheng for teaching me how to prepare temporal bones; Salwa Masud for being a wonderful lab partner for so many years; Darcy Frear for showing us how to successfully navigate a PhD; Peter Bowers for preparing several Acoustics and Bootcamp lectures for younger students; Mike Ravicz for his superb and continuous technical support.

I would like to thank Joseph Nadol and the Otopathology laboratory for giving us unrestricted access to the temporal bone collection. Chapter 3 of this thesis is a direct result of this opportunity. Especially, I would like to thank Barbara Burgess who taught me and helped us cut cochlea microsections. Cornelia Idoff analyzed cochlea microsections and deserves credit for most figures in Chapter 3. Giacomo Marino made preliminary measurements that initiated Chapter 3. I would like to thank Diane Jones for teaching me how to remove temporal bones and going out of her ways to obtain specimens used in all of the Chapters. Meng Yu Zhu and Jennifer O’Malley are acknowledged for fruitful discussions and anatomy lessons over the course of the
last four years. Garyfalia Pagonis is acknowledged for help in designing the outline of the anatomical photographs in Chapters 2 and 3.

I would like to thank Haobing Wang, Leslie Liberman, and Ishmael Stefanov-Wagner for technical support on countless occasions during my PhD. I would like to thank Bertrand Delgutte for being such a dedicated concentration area advisor and for serving on my qualifying exam committee. I would also like to thank Tao Cheng for serving on my qualifying exam committee. John Rosowski is acknowledged for his mentorship over the years and feedback on Chapter 4. I would also like to thank Christopher Shera for his support that started months before the program. I would like to thank Jennifer Melcher and Satra Gosh for being my academic advisors and empowering me to look beyond the core curriculum of the program.

I would like to thank Aleks Zosuls, Irvin Bigio, and Nathan Blanke from Boston University who helped with the optical system used in Chapter 3. Aleks and I conducted several other experiments in 2017-2018 that are unfortunately not included in this thesis.

I would like to thank Jessica Sagers for her years-long commitment to making me a better scientific writer and the SHBT class of 2014 for being important support pillars.

Lastly, I would like to thank my family and partner for their support.

This thesis would not be the same without the dedication, hard work, and creativity of all the people mentioned. The work was supported by the NIH/NIDCD R01DC013303, a fellowship from the German National Academic Foundation and the German Ministry of Economic Affairs, a research grant from the American Otological Society, and an Amelia-Peabody scholarship from Massachusetts Eye and Ear.
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FIGURE 5.2: CHANGE IN $P_{diff}$ IN TEMPORAL BONES DUE TO SCD. EACH COLORED LINE REPRESENTS THE EFFECT OF SCD ON A TEMPORAL BONE SPECIMEN. THE GREY SHADED AREA REPRESENTS THE 95% CI ABOVE AND BELOW THE MEAN OF ALL EXPERIMENTS. THE EFFECT OF SCD IS MORE SEVERE AS THE FREQUENCY DECREASES, BUT THERE IS INTER-EAR VARIABILITY IN THE EFFECT OF SCD.


FIGURE 5.4: COMPARISON OF EFFECTS DUE TO SUPERIOR CANAL DEHISCENCE (SCD) ON $P_{diff}$ IN TEMPORAL BONE SPECIMENS AND ON AIR-CONDUCTED (AC) HEARING THRESHOLDS IN PATIENTS. THE GREY SHADEd REGION (95% CI AROUND THE AVERAGE) PLOTS THE SCD-INDUCED CHANGE IN $P_{diff}$. THE RED DATA SHOW THE DIFFERENCE BETWEEN AC HEARING THRESHOLDS OF SCD AFFECTED EAR AND CONTRALATERAL UNAFFECTED EAR IN PATIENTS WITH UNILATERAL SCD.
FIGURE 5.5: MODEL FOR SCD: A) THE MODEL INCLUDES THE PRESSURE IN SCALA VESTIBULI ($P_{SV}$) AND SCALA TYMPANI ($P_{ST}$) AND THE EFFECT OF THE SCD (CHANGE IN IMPEDANCE). B) A RELATIVELY LARGE SCD COULD AFFECT HEARING THRESHOLDS MORE AND AT HIGHER FREQUENCIES (RED), WHEREAS A SMALL SCD MAY AFFECT HEARING THRESHOLDS LESS AND OUTSIDE AUDIOMETRIC TEST FREQUENCIES (BLUE). (DETAILS OF THE MODEL IN (RAUFER, MASUD AND NAKAJIMA, 2018) WITH PARAMETERS $Z_{DIFF}$: $R_{DIFF} = 3.04 \times 10^{10}$ NS$^{-2}$ M$^{-4}$, $L_{DIFF} = 6.46 \times 10^{7}$ NS$^{2}$ M$^{-5}$; $Z_{RW}$: $R_{RW} = 1.47 \times 10^{10}$ NS$^{-2}$ M$^{-5}$, $L_{RW} = 7.76 \times 10^{5}$ NS$^{-2}$ M$^{-5}$, $C_{RW} = 3.59 \times 10^{-14}$ N$^{-1}$ M$^{5}$. YELLOW: $R_{SCD} = 1.7 \times 10^{10}$ NS$^{-2}$ M$^{-5}$, $L_{SCD} = 2.0 \times 10^{7}$ NS$^{2}$ M$^{-5}$. BLUE: $R_{SCD} = 4.5 \times 10^{10}$ NS$^{-2}$ M$^{-5}$, $L_{SCD} = 4.0 \times 10^{7}$ NS$^{2}$ M$^{-5}$. RED: $R_{SCD} = 0.6 \times 10^{10}$ NS$^{-2}$ M$^{-5}$, $L_{SCD} = 0.8 \times 10^{7}$ NS$^{2}$ M$^{-5}$.)

FIGURE 5.6: PREDICTED AIR-CONDUCTION HEARING LEVELS FROM PURE-TONE AUDIOMETRIC TESTING AT FREQUENCIES BELOW 250 HZ (DASHED LINES FOR PREDICTION). THE DATA FOR THE NORMAL AND SCD CONDITION WERE EXTRAPOLATED WITH A STRAIGHT LINE (ON A LOG-AXIS) FOR LOW FREQUENCIES FROM AVERAGE HEARING THRESHOLDS BETWEEN 250 HZ AND 1 KHZ.

FIGURE 6.1: (TOP) OCT IMAGE OBTAINED THROUGH THE ROUND WINDOW AND SCALA TYMPANI OF A FRESH HUMAN TEMPORAL BONE. (BOTTOM) HISTOLOGY OF A REPRESENTATIVE COCHLEA (DIFFERENT COCHLEA FROM OCT) NEAR THE BASE FOR REFERENCE. L=LIMBUS, B=BRIDGE, SL=SPIRAL LIGAMENT.

FIGURE 6.2: CP VELOCITY RE MAX VERSUS CP LOCATION FOR (A) OCT AND (B) LDV FOR TWO DIFFERENT EARS. COLORED LINES FOR FREQUENCIES.


FIGURE 6.4: MAGNITUDE RATIOS OF THE BASILAR MEMBRANE (BM) AND RETICULAR LAMINA (RL) AT DIFFERENT RADIAL LOCATIONS.

FIGURE 6.5: HIGH MAGNIFICATION BRIGHTNESS SCAN OF ORGAN OF CORTI IMAGED IN SITU THROUGH THE RW MEMBRANE. ABBREVIATIONS IN MAIN TEXT.
FIGURE 6.6: ILLUSTRATION OF THE HUMAN COCHLEAR PARTITION CONSISTING OF THE BONY OSSEOUS SPIRAL LAMINA, SOFT TISSUE (INCLUDING NERVE FIBERS AND BRIDGE), AND THE BM. ABBREVIATIONS: BM = BASILAR MEMBRANE; BC = BASILAR CREST; SL = SPIRAL LIGAMENT.

FIGURE 6.7: MESHED FINITE ELEMENT MODEL AND SENSITIVITY ANALYSES OF DIFFERENT PARAMETERS. SIMILAR MOTION PROFILES AS REPORTED IN CHAPTER 2 ARE POSSIBLE BY CHANGING THE YOUNG’S MODULUS OF THE BM, SOFT TISSUE, OR OSL BY ONE TO TWO ORDERS OF MAGNITUDE. BASE PARAMETERS (BLACK LINES) ARE: $E_{BM} = 10 \text{MPa}; E_{SOFTTISSUE} = 1 \text{MPa}; E_{BONE} = 1 \text{GPa}$. 

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Introduction

When sound waves enter the outer ear, they displace the tympanic membrane. The middle ear, coupled to the tympanic membrane, transmits the vibrations to the inner ear, where mechanosensitive cells convert mechanical waves into electrical signals of the auditory nerve. The auditory periphery has been studied in great detail in laboratory animals. However, the study of human inner-ear mechanics has not progressed as rapidly as in animal models, leaving fundamental questions concerning human hearing mechanisms unresolved. The experimental work on animal models has provided us with in-depth insights into the functioning of the inner ear and computational models based on these experiments have proven to be a powerful tool to quantitatively understand the behavior of the normal and pathological ear. This thesis presents new findings of the mechanics, acoustics, and anatomy specific to human hearing, achieved by utilizing experimental, anatomical, and computational studies.

In Chapter 1 we describe the acoustic properties of the human basilar membrane (BM) in the cochlear base using simultaneous velocity measurements of the BM and intracochlear pressure measurements in scala vestibuli. We show that assumptions of the BM impedance and stiffness based on past experimental findings likely need to be revised for humans. Furthermore we found that the largest motion on the BM is not near the bottom of the outer pillar as in the classic view of BM motion derived from laboratory animals, but close to the bottom of the inner pillar. To investigate this surprising finding further, in Chapter 2, we looked beyond BM motion and measured the motion across the entire width of the cochlear partition in humans. We describe a new soft-tissue region of the human cochlear partition which connects the BM to the osseous spiral lamina. We named this structure the bridge, which has approximately the same
width as the BM. We show that the newly-described bridge moves as much as the BM and contributes to sound analysis of the ear. We also found that the osseous spiral lamina, the bony part of the cochlear partition, has significant motion across its width. These findings don’t fit the classic view of mammalian cochlear mechanics, which does not take into account motion of the osseous spiral lamina and/or the bridge. Chapter 3 looks at the microanatomy of the osseous spiral lamina and bridge and offers an anatomy-based explanation as to why the motion of the human cochlear partition is so vastly different from the classic view. The osseous spiral lamina is thin and porous, allowing it to move, and collagen fibers of the BM traverse through the bridge, which could explain the BM-like motion of the bridge. In summary, we combined mechanical and anatomical studies of the human cochlea and found that the classic view of mammalian cochlear mechanics does not apply to humans.

We also studied the transmission of infrasound through the human middle ear and inner ear in Chapter 4. By measuring the motion of the stapes and round window membrane down to 1 Hz, we found that the middle ear limits the sound energy propagated to the inner ear at low frequencies. A mechanical perturbation, a hole in the bony wall of the semicircular canal (semicircular canal dehiscence), lead to a further decrease in low-frequency sound transmission to the cochlea. We determined the acoustic properties of the dehiscence using a computational model. Chapter 5 ties together results from experimental, clinical, and modeling work to propose how hearing-threshold shifts at low frequencies may be utilized in clinical settings to diagnose patients with inner ear dehiscences.

The methodological approach to our questions was fourfold. We conducted experiments in fresh human temporal bone cadaveric specimens (Chapters 1, 2, 4, and 5), related temporal bone data to clinical data (Chapter 5), computationally modeled the experimental data (Chapters
2 and 4), and detailed the anatomy related to the mechanics (Chapter 3). Methods included measuring the motion of the middle ear ossicles and CP with laser-Doppler vibrometry (Chapter 1, 2, 4, and 5), measuring pressures in cochlear scalae with fiber-optic pressure sensors (Chapter 1, 2, and 5), collecting audiometric data from patients (Chapter 5), and using conventional light microscopy as well as polarized light microscopy on histological cochlear sections (Chapter 3).

Each chapter of this thesis has been prepared for publication and may be read independently from the other chapters.
CHAPTER 1. The human cochlear partition: Impedance measurements of the basilar membrane


SR and HHN designed and performed research, analyzed data and wrote the paper.
ABSTRACT

The cochlea is a mechanical frequency analyzer, owing its characteristics to the impedance of the cochlear partition. In humans, the impedance of the partition has not been measured directly, and estimates of the stiffness (a principal component of the impedance) are based on loose assumptions. In this study, we examine not only the stiffness of the basilar membrane (BM), but also the osseous spiral lamina (OSL), which, in human, vibrates substantially. We hypothesize that the OSL contributes significantly to the volume stiffness of the cochlear partition (CP). We measured velocities of the BM and OSL at different radial locations 1 mm from the base of the cochlea in a fresh human cadaveric specimen. Simultaneously, we measured intracochlear pressures on the other side of the partition, in scala vestibuli. With the velocity and pressure measurements we can estimate the specific acoustic impedance of the BM and OSL \((Z = p/v)\). At frequencies well below the resonant frequency, the stiffness of these structures can be extracted by multiplying the impedance by the radian frequency. The specific acoustic stiffness was found to be 1.2 GPa/m on the BM, 6 GPa/m at the juncture where the BM attaches to the OSL, and 10 GPa/m at the midpoint of the OSL. A beam model, appropriate to model the radial motion of the BM in guinea pig or gerbil, cannot describe the displacement of the human CP in the base. Instead, we find that the OSL is hinged near the modiolus and vibrates significantly near the connection to the more compliant BM, contributing greatly the volume compliance of the CP.
INTRODUCTION

Cochlear models continue to rely on the assumption that the resonant frequency of the cochlear partition (CP) is proportional to the square root of the stiffness (H. Duifhuis et al., 1985; Epp, Verhey and Mauermann, 2010). This assumption, however, is not supported by experimental data from gerbils (Naidu and Mountain, 1998; Emadi, Richter and Dallos, 2004). For the human cochlea, there are no reliable data to verify this assumption. Thus, in order to understand the mechanisms of sound transmission in the human ear and develop more realistic cochlear models, it is important to determine the stiffness of the human CP experimentally.

The first attempt to measure the stiffness of the human CP was undertaken by von Bekesy in a series of experiments in the 1940s. In these measurements, however, the cochlea had been drilled open, leaving bone dust on the BM, and the force probe von Bekesy used (fine hairs) were not calibrated. More detailed and controlled animal experiments were performed on excised guinea pig and gerbil cochleae, and in live gerbils (Gummer, Johnstone and Armstrong, 1981; Olson and Mountain, 1991; Naidu and Mountain, 1998, 2001). These studies measured the mechanical stiffness of the CP at several longitudinal locations by contacting the basilar membrane with a force probe. Across the gerbil frequency range, which spans three orders of magnitude, CP stiffness changes by about two orders of magnitude (Naidu and Mountain, 1998).

Because there are a number of concerns with direct force probe measurements (see Kapuria et al. (2017) for a review), Dong and Olson measured the specific acoustic impedance $Z_A^S$ of the gerbil basilar membrane (BM), avoiding direct contact with the partition and displacement beyond physiological boundaries (Dong and Olson, 2009). This was achieved by simultaneously measuring intracochlear pressures and the velocity of the BM. At low frequencies, where $Z_A^S$ is stiffness-dominated, the stiffness can be extracted by multiplying $Z_A^S$ by
the radian frequency. We use a similar approach to determine the stiffness of the CP in humans, and calculate $Z_A^S$ by simultaneously measuring the velocity of the BM and the pressure in the vestibule during sound stimulation. The specific acoustic impedance is obtained by dividing the pressure in scala vestibuli $P_{SV}$ by the velocity of the BM $v_{BM}$, yields

$$Z_A^S = P_{SV} / v_{BM} \quad (1)$$

In the complex domain, the impedance of a simple mass-spring system can be represented as:

$$Z_A^S = \frac{P_{SV}}{v_{BM}} \propto R + j(M\omega - K/\omega) \quad (2)$$

where $\omega$ is the radian frequency, $R$ the resistance (or damping), $M$ the mass, and $K$ the stiffness of the system. Although the impedance of the BM cannot be represented by the impedance of a simple harmonic oscillator, Eq. 2 is a good approximation for frequencies about one octave below the resonant frequency of the CP.

To calculate the volume stiffness of a cross-sectional segment (as opposed to the point stiffness), fine-grained radial measurements or a mathematical model are necessary. In guinea pig, for example, a simple beam model can describe the radial deflection of the BM (Homer et al., 2004); the measurement from one single location is then sufficient to calculate the volume stiffness. But unlike in rodents, the human BM is not supported by a static osseous spiral lamina (OSL) (Stenfelt et al., 2003). The OSL, a hollow bony plate that has soft nerve fibers within it, occupies 85% of the radial length of the CP in the base of humans. We show that the human OSL moves considerably, and therefore, the BM motion cannot be modeled with a simple beam equation.
Figure 1.1: Experimental view of the specimen (a) and mid-modiolar cross section of basal turn of a human cochlea with detailed illustration of the cochlear partition (b). BM = basilar membrane; SL = spiral ligament.
MATERIALS AND METHODS

Temporal bones. Here, we present results from one representative experiment among several that we performed. For the general procedure of temporal bone removal and preparation see (J. Nadol, 1996; Nakajima et al., 2009). The velocity recordings of the BM and OSL were made through the round window membrane (RWM), which was removed to provide an unobscured view of the BM and OSL. Initial measurements of stapes and RWM velocities were performed to check no air entered the cochlea in the preparation process. A phase difference of one-half cycle between stapes and RWM velocities is a good indicator that no air is enclosed in the inner ear.

Figure 1.1a shows the specimen with stapes, pressure sensor, and RWM area. The RWM was removed to enable velocity recordings from the BM and the OSL. The dashed line refers to the location of radial measurements. Figure 1.1b shows a mid-modiolar cross section of a human temporal bone to highlight the anatomy of the human CP. The OSL occupies 80-85% of the space between modiolus and lateral wall of the cochlea in the base.

Sound stimulation. Acoustic pure tones between 50 Hz and 24 kHz (10 per octave) were generated by a Radio Shack driver (400-1377) and delivered to the ear canal through flexible tubing. The sound pressure level in the ear canal was monitored with a Knowles EK-23103-000 broadband microphone near the umbo and held constant at 94 (±2) dB SPL. A metal sleeve was cemented into the ear canal to position the microphone 1 mm from the umbo.

Velocity measurements. A laser Doppler vibrometer (Polytech CLV 700) was used to measure velocities of the stapes footplate, RWM, BM, and OSL. Reflective polystyrene microbeads (50 µm diameter) were used to enhance the signal-to-noise ratio for stapes and RWM measurements.
No reflectors were used for BM or OSL recordings. The temporal bone was oriented with the RWM parallel to the table; the laser beam was perpendicular to the BM and OSL.

**Pressure measurements.** Fiber-optic pressure sensors (Olson, 1998) were used to record pressures in the vestibule. For details regarding calibration and placement of the sensor see (Nakajima et al., 2009). The RWM was removed and the fluid was lowered to the level of the CP (yet preventing tissue from drying). As a result, the pressure in scala tympani is equal to atmospheric pressure, thus the pressure in scala vestibuli represents the pressure driving the CP. The pressure measured in scala vestibuli is far from the BM and does not reflect the local pressure near the CP for frequencies close to and above the characteristic frequency (CF) of the CP. Our impedance measurements are only valid for frequencies lower than one octave below the CF, where the spatial variation of the pressure can be neglected.
RESULTS AND DISCUSSION

Stapes velocity and intracochlear pressures

Figure 1.2a shows stapes velocity ($v_{\text{Stapes}}$) referenced to ear canal pressure ($P_{\text{EC}}$) and Figure 1.2b shows $P_{\text{SV}}$ referenced to $P_{\text{EC}}$. In Figure 1.2, we would like to highlight (i) the change in cochlear input drive due to the removal of the RWM, (ii) stability concerns of the pressure sensor, and (iii) the pristine condition of the middle ear.

(i) A critical step in our experiments is to remove the RWM. We are thus concerned about how the input to the cochlea changes due to this intervention. Because the RWM is a compliant, low-impedance termination of scala tympani, $v_{\text{Stapes}}$ and $P_{\text{SV}}$ are only slightly affected. In the mid- to high-frequency region, we observe an increase of up to 3 dB in $v_{\text{Stapes}}$ (continuous vs. dashed line in Figure 1.2a), but the phase shows no systematic change. Intracochlear pressures are, as far as we can assess, only affected for frequencies below 200-300 Hz, where both the magnitude and phase increase slightly. We may suspect the same mid-to-high frequency changes as observed in $v_{\text{Stapes}}$ but $P_{\text{SV}}$ remained stable. At frequencies above 1-2 kHz, the stapes motion is known to be more complex than a simple piston motion, and the one-point laser measurement may be catching a slight modal change in motion after we removed the RWM, also consistent with the slight change in phase in the mid frequency region.

(ii) Our custom-built pressure sensors can experience sensitivity drifts when used over an extended period of time. Although we evaluate and minimize the risk by going through an extensive calibration procedure, we cannot exclude sensitivity drifts. The data in Figure 1.2b are thus used to assess the stability of the sensor throughout the experiment. Above 500 Hz,
intracochlear pressures are stable (within 3 dB) throughout the experiment; only below 300 Hz do we observe a systematic change, presumably due to the removal of the RWM.

(iii) It is desired that the middle ear conducts high frequencies (above 10 kHz) well for this experiment. To be able to measure the impedance up to and above the resonant frequency (~14 kHz in the base of a passive human cochlea), the specimen must have a pristine middle ear and good conductivity at high frequencies. Figure 1.2 shows that the stapes response of this bone remained well above the noise floor at frequencies up to 15 kHz; intracochlear pressures are also recorded with high signal to noise ratio in that frequency range (noise floor outside visible range of graph).
Figure 1.2: (a) Stapes velocity ($v_{\text{Stapes}}$) referenced to ear canal pressure ($P_{\text{EC}}$) before (continuous line) and after removal of the round-window membrane (dashed line). (b) Intracochlear pressure ($P_{\text{SV}}$) referenced to $P_{\text{EC}}$ at several stages of the experiment.
Specific Acoustic Impedance of the Human BM and OSL in the Cochlear Base

The specific acoustic impedance is obtained by dividing the pressure $P_{SV}$ by the velocity $v_{BM}$ (see Eq. 1). In Figure 1.3a, the magnitude and phase of $Z_A^S$ are shown for four different radial measurement locations (indicated by arrows in the illustration).

At low frequencies, the decrease in magnitude of $Z_A^S$ is approximately 6 dB/octave. The negative phase at frequencies below 10 kHz is ascribed to a reactance in the form of a stiffness element. The frequency range where the CP is stiffness-dominated (phase near -0.25 cycles) is about one-half octave to an octave below the resonant frequency of approximately 14 kHz. Near and above the resonant frequency our pressure measurements do not reflect the pressure near the BM (Olson, 1999). Equation 2 is thus only valid for frequencies one octave below the resonant frequency.

Our measurements from multiple radial locations show the same resonant frequency of the OSL and BM. The impedance of the OSL is much higher than that of the BM, and the notch of the OSL impedance is not as pronounced as that of the BM. The phase of the BM shows rapid phase accumulation close to and above the resonant frequency and suggests a traveling wave on the BM. On the OSL, the findings are not consistent: some measurement locations suggest a traveling wave on the OSL (such as seen in (Stenfelt et al., 2003)), but other locations have a much shallower phase transition.
Figure 1.3: (a) Magnitude and phase of the specific acoustic impedance $Z_A^S$ of the cochlear partition at four radial measurement locations, indicated in the illustration. The dashed line indicates a 6 dB/octave decrease. (b) Specific acoustic stiffness of the same locations.
Specific Acoustic Stiffness of the Human BM and OSL in the Cochlear Base

From Figure 1.3a, the specific acoustic stiffness $K_A^S$ can be calculated by multiplying $Z_A^S$ by the radian frequency. The results are valid for frequencies where $Z_A^S$ is purely reactive (phase close to -$90^\circ$). The frequency range for which this is the case is 500 Hz to 7 kHz, approximately one octave below the resonant frequency. The $K$, shown in Figure 1.3b, amounts to 1.2 GPa/m on the BM, 6 GPa/m where the BM attaches to the OSL, 10 GPa/m at the midpoint of the OSL and 30 GPa/m at the medial end of the OSL.

Bekesy's volume compliance in the base, converted to $K_A^S$, results in a stiffness about one order of magnitude below what we measure (Olson, Duifhuis and Steele, 2012). Duifhuis et al. in one of their first cochlear models assumed a stiffness in the base of $s_0 = 10$ GPa/m for humans (H. Duifhuis et al., 1985). This is eight times greater than we determined experimentally. Dong and Olson (2009) report an average stiffness of the gerbil BM of 7 GPa/m (CF=40 kHz), which is closer to Duifhius et al.'s assumption, but incorrect for humans.

Regarding the accuracy of our postmortem data, there is evidence that the stiffness of the BM does not change significantly a few hours after death (Emadi, Richter and Dallos, 2004; Dong and Olson, 2009). Given that the specimens we use are immediately processed (48 hours postmortem) and never frozen, our stiffness data also apply to live humans. With more experiments, we will determine the range across ears.

Radial Displacement Profile of the Human BM and OSL

Figure 1.4 shows the displacement per unit pressure (referenced to the pressure in the vestibule) from 27 radial measurement locations. The separate lines in Figure 1.4a represent data from different stimulation frequencies, all well below the resonant frequency. The continuous
vertical line at \( x=0 \) \( \mu \text{m} \) indicates where the bony part of the OSL ends and the dashed vertical line indicates where the BM begins (cf. Figure 1.1).

An important observation made from Figure 1.4a is, that the OSL is not static, but is considerably deformed when pressure is applied; it appears to be pivoting at \( x=-600 \) \( \mu \text{m} \). Consequently, the attachment of the BM is not fixed but depends on the motion of the OSL. Furthermore, the maximum deflection is not observed at the center of the basilar membrane, but rather medially, close to the inner hair cell. The displacement of the medial anchor point of the BM has been observed in a number of species and cochlea models have considered the displacement of the OSL (Rhode, 1971; Taber and Steele, 1981; William S Rhode, 2007). However, unlike in other species, the human OSL occupies more than 80% of the radial extent of the CP in the base. Thus, ignoring the displacement of the OSL underestimates the volume stiffness of the CP by a factor of two.

Figure 1.4b shows how the stiffness of the BM changes due to drying effects. The continuous line is the average from Figure 1.4a and the dashed line represents repeated measurements after the CP was exposed to air for about 1 hour. Drying affects deflection of the BM, but not the OSL, which is why we prioritize measuring the velocity of the BM after we remove the RWM and lower the fluid over the BM.

In Figure 1.4c, we show the radial displacement profile at the resonant frequency (or characteristic frequency, CF) and how it compares to the low frequency data. At the CF, a maximum deflection of 3.5 nm per Pascal is observed, which is 3-4 times larger than in the sub-CF case. The deflection of the OSL does not depend on the input frequency, but because the deformation of the BM is increased by a factor of 3-4, the BM behaves more like a simple beam in that frequency range.
What distinguishes our measurements from those conducted by other groups (Homer et al., 2004) is that we measure the displacement of the BM while simultaneously measuring the pressures in the inner ear. We can thus normalize our data to the actual pressure causing the deflection. In the future, this will be valuable for developing mathematical models to describe the radial deflection pattern of the CP in humans.
Figure 1.4: (a) Displacement per unit pressure for 27 radial locations at quasi-static frequencies. (b) Comparison of radial displacement in fresh and dried tissue. (c) Comparison of radial displacement profile at quasi-static frequencies (average from panel a) and the characteristic frequency (CF). The vertical line at 0 µm indicate the beginning/end of the bony part of the OSL (negative values = OSL); the dashed vertical line indicates the beginning of the BM.
CONCLUSIONS

In this preliminary work, we measure the stiffness of the CP in the base of the human cochlea. Our two main findings are as follows: (i) The impedance of the CP is stiffness-dominated at frequencies well below the CF, which allows calculation of the stiffness of the BM and OSL. For our representative specimen, the specific acoustic stiffness of the human BM in the hook region is 1.2 GPa/m and thus considerably less than assumed in classical cochlear models (but an order of magnitude above von Bekesy's estimates). (ii) We show that in the base of the human cochlea, the OSL moves considerably, unlike in animals. At low frequencies, a simple beam model cannot account for the movement of the CP and therefore a more sophisticated mathematical model is needed to calculate the volume stiffness from single-point measurements.

UPDATES ON RESULTS AND DISCUSSION

Since the publication of this conference manuscript, we have conducted more experiments (data also included in Chapters 2). Figure 1.5 shows a summary of the results of the specific acoustic impedance and stiffness for six specimens. On average, the specific acoustic stiffness of the cochlear partition in the base is 0.85 GPa/m at its softest radial place.
Figure 1.5: (a) Magnitude and phase of the specific acoustic impedance of six specimens. (b) Real (solid lines) and imaginary part (dashed lines) of the impedance. (c) Specific acoustic stiffness derived from the imaginary part of the impedance. The measurement location was near the medial end of the basilar membrane.
In Figure 1.6, we compare our experimental findings to findings in gerbil, which have been converted from point-stiffness mechanical stiffnesses to specific acoustic impedances (Olson and Mountain, 1991, 1994; Naidu and Mountain, 1998; Olson, 2001; Emadi, Richter and Dallos, 2004; Dong and Olson, 2009). Furthermore, our results are compared to experimental findings of the static stiffness by von Bekesy and previous and current model assumptions of the human BM stiffness in the base as used in one-dimensional cochlear models.

Most of the studies that investigated the gerbil BM stiffness have relied on using a force probe that contacts the basilar membrane. In order to compare these results to estimates of the specific acoustic impedance, the mechanical point-stiffness measurements have to be converted using a beam model of the cochlear partition (Gummer, Johnstone and Armstrong, 1981; Miller, 1985; Olson and Mountain, 1994; Olson, Duifhuis and Steele, 2012). In Figure 1.6, the estimates of the acoustic stiffness of the gerbil BM are taken from (Dong and Olson, 2009) and the estimates of the acoustic stiffness of von Bekesy are taken from (Olson, Duifhuis and Steele, 2012). The most direct comparison of our data can be made with Dong and Olson’s (2009) data, as we use the very same technique. Compared to Dong and Olson’s (2009) average value of the specific acoustic stiffness of 5.5 GPa/m in the gerbil base (CF ~33 kHz), our human estimates are about six times smaller. We expect a lower stiffness in the human BM compared to the gerbil, as the human is a lower-frequency hearing mammal.

Bekesy’s static volume compliance experiments on human temporal bones, converted to the specific acoustic impedance, results in a value of the BM stiffness in the base of about 0.1 GPa/m (v. Bekesy, 1960; Olson, Duifhuis and Steele, 2012). For one-dimensional auditory models to produce the desired results, researchers needed to increase the basal stiffness of the human BM markedly. In one of the earliest models of the cochlea, Peterson and Bogert (1950)
assumed a stiffness in the base about two orders of magnitudes larger than what von Bekesy measured in experiments. Allen (1977) assumed a similar value for the basal stiffness in the range of 10 GPa/m. A recent study assumes a smaller values for the basal stiffness, in the range of $\sim 2$GPa/m (Neely & Rasetshwane 2017). These assumptions are closer to what we measured in our experiments.

Figure 1.6: Comparison of specific acoustic stiffnesses of the basilar membrane (BM) in gerbil (blue) and human (orange/red) as obtained from experiments or assumed in models.
CHAPTER 2. The human cochlear partition: Motion measurements across the entire cochlear partition width

This work is under review as: Stefan Raufer, John J. Guinan Jr., Hideko H. Nakajima. Cochlear partition anatomy and motion in humans differ from the classic view of mammals.

SR and HHN designed and performed research, SR, J.J. and H.H. analyzed data; SR, J.J. and H.H. interpreted the findings and wrote the paper.
ABSTRACT

Mammals detect sound through mechanosensitive cells of the cochlear organ of Corti that rest on the basilar membrane (BM). Motions of the BM and organ of Corti have been studied at the cochlear base in various laboratory animals, and the assumption has been that the cochleas of all mammals work similarly. In the classic view, the BM attaches to a stationary osseous spiral lamina (OSL), the tectorial membrane (TM) attaches to the limbus above the stationary OSL, and the BM is the major moving element with a peak displacement near its center. Here, we measured the motion and studied the anatomy of the human cochlear partition (CP) at the cochlear base of fresh human cadaveric specimens. Unlike the classic view, we identified a soft-tissue structure between the BM and OSL in humans, which we name the CP “bridge”. We measured CP transverse motion in humans and found that the OSL moved like a plate hinged near the modiolus with motion increasing from the modiolus to the bridge. The bridge moved almost as much as the BM, with the maximum CP motion near the bridge-BM connection. BM motion accounts for 100% of CP volume displacement in the classic view, but accounts for only ~27% - 43% in the base of humans. In humans, the TM-limbus attachment is above the moving bridge, not above a fixed structure. These results challenge long-held assumptions about cochlear mechanics in humans. In addition, animal apical anatomy doesn’t always fit the classic view.
INTRODUCTION

Our understanding of the mechanics of mammalian hearing is founded largely on measurements from the cochlear base of laboratory animals such as mouse, gerbil, guinea pig, chinchilla and cat. The results on humans presented here overturn the widely held belief that the pattern of motion of the cochlear partition is similar across mammals.

The cochlea is divided into two fluid-filled compartments by the cochlear partition (CP), which in the generalized classic view consists of a rigid osseous spiral lamina (OSL) and an elastic basilar membrane (BM). The classic view is that the frequency-dependent response of the cochlea mostly relies on the vibration of the BM, a thin collagenous membrane that is tuned to high-frequency sounds in the cochlear base and low-frequency sounds in the cochlear apex.

Sitting on the BM is the organ of Corti (OoC), which translates BM vibrations into deflections of the stereocilia (or “hairs”) of hair cells that amplify BM motion and transduce the motion into the firing of auditory-nerve fibers. The classic view has been that BM motion is translated into a shearing motion at the top of the OoC between the reticular lamina and the tectorial membrane (TM), and this shearing deflects hair-cell stereocilia (ter Kuile, 1900). Recent studies that were able to measure motion near the reticular lamina, TM, and hair cells, have shown that OoC motion is more complex (Lee et al., 2015, 2016; Ren, He and Kemp, 2016; Warren et al., 2016; Recio-Spinoso and Oghalai, 2017; Cooper, Vavakou and Heijden, 2018; Dong et al., 2018).

Despite the lack of a full detailed understanding of cochlear micromechanics, the belief persists that the basic pattern of CP motion is universal across mammalian species because BM and OoC anatomy are similar across mammals (Narayan et al., 1998).

It is commonly thought that OSL motion in response to sound is negligible, and this is reflected in classic cochlear models that assume there is no OSL motion (Boer, Nuttall and
Additionally, the attachment of the OSL to the BM and the attachment of the TM to the spiral limbus (which sits above the OSL), are also considered stationary. In contrast to this view, there have been reports of sound-induced OSL movement, but these reports have been largely ignored in overviews of cochlear mechanics and the formation of cochlear models (Boer, Nuttall and Shera, 2007; Steele, de Monvel and Puria, 2009; Meaud and Grosh, 2010; Zweig, 2016). Von Békésy (v. Bekesy, 1960), using static pressure, found that the cochlear partition “bent like an elastic rod that was free at one end and fixed at the other”. Kohlloeffel (Kohlloeffel, 1983) described the OSL as short and stiff in cat, rat, guinea pig, gerbil, squirrel monkey and rabbits, but as fragile and flexible in pig, cow, mole, and that in humans the OSL can deflect as much as the BM in response to low-frequency sound. Most of the early papers that measured BM responses to sound in live animals using quantitative measuring techniques made control measurements of nearby OSL motion and found that OSL motion was small compared to BM motion at best frequency (BF), although below BF OSL motion was sometimes said to be only 5 dB less than BM motion (e.g. Rhode, 1971; William S. Rhode, 2007). Recent measurements in a human temporal bone found that in response to air-conducted sound, the OSL vibrated transversely with amplitudes comparable to those of the BM (Stenfelt et al., 2003), but these measurements were only made at two points on the OSL for one specimen, and were not intended to establish the overall motion of the OSL or its consequences. The finding of substantial OSL motion in live animals and in a human cadaver conflicts with the generalized classic view that the OSL and the structures that attach above it are basically stationary. Although there have been a number of reports of OSL motion, there has not been a systematic
study of the motion of various points across the human cochlear partition, or across the OSL in any species.

To better understand the mechanics of hearing in humans, we measured sound-induced motion throughout the CP width in the base of fresh human temporal bones and compared the results to the classic view of cochlear motion. To determine human CP motion, we viewed the CP scala-tympani surface through the round window and measured transverse motion at many points across the CP width with laser-Doppler vibrometry. To interpret these experiments, we examined CP anatomy from histological sections.
RESULTS

Human cochlear partition anatomy differs from the anatomy in the base of laboratory animals

In the base of most laboratory animals much of cochlear anatomy is similar, in particular the anatomy of the cochlear partition. The bony plates of the OSL extend laterally (toward the spiral ligament) to come close to, or overlap with, the medial edge of the BM, approximately in the region of the inner pillars under the inner hair cells. Cochlear anatomy is shown for the base of guinea pig in Figure 2.1A, and additionally for four other commonly used laboratory animals in Figure 2.2 (below). In the cochlear base of laboratory animals such as the guinea pig (Figure 2.1A), the spiral limbus and its attachment point to the TM rest above the bone of the OSL. In Figure 2.1A, the BM occupies ~34% and the bony OSL occupies ~66% of the CP width.

In contrast, in the base of the human cochlea, the BM occupies ~15% and the bony OSL occupies ~70% of the CP width (Figure 2.1B). In humans, the bony part of the OSL does not come close to the BM. Instead, between the lateral edge of the OSL bone and the medial edge of the BM, there is non-bony tissue that forms a third CP region in addition to the OSL and the BM, which has not been anatomically delineated and which we name the CP bridge (Figures 2.1B, 2.1C). In humans, the attachment point of the TM on the spiral limbus does not rest above the bone of the OSL (as in the base of laboratory animals and in cochlear models), but instead rests on bridge tissue between the OSL bone and the BM. A magnified view of the CP bridge is shown in Figure 2.1C. We define the boundaries of the bridge as the bony OSL (medially), the limbus (toward scala vestibuli), the BM (laterally), and the abutting fluid (toward scala tympani). Within the bridge region are connective tissue and auditory-nerve fibers. The boundary between
the bridge, the limbus and the inner sulcus needs further examination and definition. The name “tympanic lip” has been used for the lower border of the inner sulcus (Merchant and Nadol, 2010) without regard to whether it is above bone or not. The bridge spans a substantial width. In the cochlear base the width of the bridge was 83% ±12% standard deviation (SD) of the BM width across six histologically-prepared temporal bones. The bridge is present in all turns of the human cochlea and has approximately the same width as the BM (both become wider in the apex).
Figure 2.1: Cross-sectional anatomy of guinea pig and human cochlear partitions (CP). Colored bars show the radial extents of the osseous spiral lamina (OSL), the basilar membrane (BM), and, in humans, the soft tissue “bridge” between the OSL and BM. (A) Guinea pig ~10 kHz place (5.5 mm from the base) and (B) human ~9 kHz place (6 mm from the base). (C) Magnified view showing the bridge region outlined with a dashed blue line. Other abbreviations are: tectorial membrane (TM), inner sulcus (IS), organ of Corti (OoC), and spiral ligament (SL).
Cochlear partition anatomy of humans and various laboratory-animal species

To provide perspective on the anatomy of humans versus laboratory animals, we present the cochlear anatomy of five species and of humans. The anatomy and motion in the base of these laboratory animals served as the main source for the generalized classic view of mammalian cochlear mechanics. The cross-sectional anatomy is shown for the basal part of the cochleas in Figure 2.2 and for the apical part in Figure 2.3. In each of the five laboratory species, cochleas of at least three different animals were studied, and because the images within a species were all similar, a representative image of each species and location is shown.

There are two major differences between the basal cochlear partition (CP) anatomy of humans and the classic view from laboratory animals: the presence of soft tissue between the osseous spiral lamina (OSL) and basilar membrane (BM) (i.e. a “bridge” region), and the positioning of the TM-limbus attachment above soft tissue as opposed to above OSL bone. Although we have found a bridge-like region in the apex of some animals (see Figure 2.3), an unresolved question is how similar these regions are to the human bridge, i.e. do these regions have the same anatomical characteristics as the bridge in humans, such as radial collagen fibers (Raufer et al., 2019). The second difference, the position of the TM-limbus attachment, is important because this position has implications for whether the medial end of the TM moves, or whether it is stationary as is assumed in classic cochlear models. If the TM-limbus attachment is over a bridge-like region with no bone, then it is much more likely that the TM attachment point moves in response to sound than if the TM-limbus attachment is over the bony OSL. The images in Figures 2.2 and 2.3 shed light on these questions, but much more detailed anatomical and physiological work is needed to fully answer them.
The images in Figures 2.2 and 2.3 are of histologically prepared sections stained with Hematoxylin and Eosin from the Otopathology Laboratory at our institution. To estimate the locations along the cochlea, we measured the BM widths from the sections shown in the figures, and translated BM width to cochlear location using published data: for human (Merchant and Nadol, 2010), guinea pig (Fernández, 1952), mouse (Ehret and Frankenreiter, 1977), gerbil (Plassmann, Peetz and Schmidt, 1987), cat (Cabezudo, 1978), and chinchilla (Bohne and Carr, 1979). Greenwood maps of these species were used to translate cochlear location to best-frequency (BF) (Greenwood, 1990).

The basal-turn locations in Figure 2.2 are approximately one-quarter to one-half cochlear turn from the basal end of the cochlea (distance from base labeled above each panel). Some general observations from Figure 2.2 are: (i) the OSL bone comes close to or overlaps with the inner pillar of the organ of Corti (OoC) in all species but human (the lateral extent of OSL indicated by upward pointing arrows); (ii) the TM-limbus attachment (downward pointing arrows) is above the bridge in humans but above the bone of the OSL in all other species.

The cochlear anatomies of the apical-turn are shown in Figure 2.3. They are 80-90% of the total cochlea length from the base. Some observations from Figure 2.3 are: (i) the apical OSL appears shorter compared to the basal OSL in Figure 2.2 (varying across species); (ii) the lateral edge of the OSL bone (upward pointing arrows) overlaps with the inner pillar of the OoC only in gerbil. In the other species, a soft-tissue region, similar to a bridge, exists between the OSL and BM (the guinea pig shows virtually no OSL bone in the apex, which was also noted by Fernandez (Fernández, 1952)); (iii) the TM-limbus attachment (downward pointing arrows) is over the bridge in humans and guinea pigs, but over the OSL bone in other species.
Figure 2.2: Basal-turn cross-sectional cochlear anatomy of six species used in cochlear mechanical measurements: (A) Human, (B) guinea pig, (C) mouse, (D) gerbil, (E) cat, and (F) chinchilla. These sections are near the regions where many CP motion measurements have been made. In each, an up-arrow points up to the most lateral extent of bone of the osseous spiral lamina (OSL), and a down-arrow points to the attachment of tectorial membrane (TM) to the limbus. In all species but human, the bone of the OSL extends near to, or underneath, the inner pillar cells of the organ of Corti. In all species but human, the TM-limbus attachment point sits on top of OSL bone (compare the medial/lateral locations of the down-arrows with the up-arrows). Above each panel: the width of the basilar membrane (BM), the best frequency (BF) of the section, and the distance of the section from the basal end of the cochlea.
Figure 2.3: Apical-turn cross-sectional cochlear anatomy of the six species of Figure 2.2. The lateral edge of the OSL bone (upward pointing arrow) extends to underneath the inner pillar cells only in gerbil. A soft-tissue bridge between the OSL and BM is seen in all species except gerbil. Across species, these soft-tissue regions vary in width with respect to the BM. In chinchilla, the soft-tissue region is short, while in the guinea pig there is virtually no bony OSL in the apical turn. The spiral limbus (including the limbal attachment of the tectorial membrane (TM)) sits on the soft-tissue CP bridge in human and guinea pig, and on the bony OSL in the other species. Above each panel: the width of the basilar membrane (BM), the best frequency (BF) of the section, and the distance of the section from the basal end of the cochlea.
The observation of a CP bridge-like region in the apex of guinea pigs led us to reexamine motion measurements in the guinea pig apex to look for possible motion consequences of the soft-tissue region. Warren et al. (Warren et al., 2016) presented motion measurements said to be from across the width of the BM in the guinea pig apex. In their figure 3E, they show “BM” motion measurements over a radial distance of ~300 μm (if the exponential fit to their data is extrapolated, “BM” motion would be over a ~400 μm range). However, the guinea pig BM shown in their figure 4B has a width of only ~200 μm. One explanation of these results is that Warren et al. measured motion over both the BM and a mobile CP bridge, but they attributed all of the measurements to the BM because they thought that only the BM had significant transverse motion. If so, the data of Warren et al. would be evidence for motion in a CP bridge region in a live animal. These measurements of cochlear motion, and many others, have not coordinated the motion measurements with the corresponding anatomy.

**Human cochlear partition motion is substantially different from the classic view of CP motion.**

A representative example of the normalized velocity of the human CP, measured at 34 radial locations across the CP tympanic surface, for frequencies below and near the BF, is shown in Figure 2.4. The measurements were normalized to the maximum velocity of the CP at each frequency (Figure 2.4A) or to the intracochlear pressure measured in the vestibule (Figure 2.4B-D). The normalized CP responses were independent of the tested stimulus levels of 80-120 dB sound pressure level (SPL) at the ear-canal.
In response to sound, the human OSL was not stationary (as in classic models), but moved almost as much as the BM. The displacement of the OSL increased linearly with radial distance from a pivot point near the modiolus (at a radial location of -600 μm from the OSL-bridge boundary in Figure 2.4A). In the CP bridge region medial to the inner sulcus (IS in Figure 2.1C), the motion of the bridge was continuous with that of the OSL, meaning that the velocity continued to increase linearly with the distance from the modiolus. In the more lateral bridge region near the inner sulcus (between the limbus and BM), the motion usually became greater than a simple extension of the OSL pivoting motion (Figure 2.4A). The largest transverse CP motion was generally on the BM near the BM-bridge boundary, close to where the inner pillars and inner hair cells are located (IP in Figure 2.4A). Lateral to the CP-motion peak, the motion decreased to a stationary point where the BM attached to the spiral ligament (SL in Figure 2.1A).
Figure 2.4: Cochlear partition (CP) cross-sectional motion profiles and tuning curves. (A) Normalized CP transverse velocity magnitude versus radial location in response to tones over a wide range of frequencies (see frequency color key) for a representative temporal bone (#16). Velocity was normalized by the maximum velocity at each frequency. Upward arrows indicate the lateral edge of the osseous spiral lamina (OSL) at 0 µm and the lateral edge of the basilar membrane (BM) at 230 µm. The thick line between A and B estimates the widths of CP structures; the orange arrows indicate the estimated locations of the bottoms of the inner pillar (IP) and outer pillar (OP). (B) CP transverse-velocity phase referenced to the intracochlear vestibule pressure phase. (C) CP-motion tuning-curve magnitudes and (D) phases referenced to vestibule pressure, at different radial locations (location color key shown on diagram in C). The best frequency of the BM was 14.4 kHz. Data recorded for ear-canal sound pressure levels of 108 dB SPL.
Figure 2.5 shows CP motion in all six temporal bones. In all, the OSL and bridge moved in response to sound, including the regions of the BM-bridge attachment and underneath the TM-limbus attachment. On average across the six temporal bones, the BM accounted for 27.2 ± 7.7% SD of the total transverse-area displacement of the CP for frequencies below BF, and 42.7 ± 26.6% SD of the CP area displacement at the BF. This contrasts with classic models in which BM motion is ~100% of CP area displacement. Overall, in all six human temporal bones, OSL and bridge motion accounted for a substantial fraction of the CP area displacement at all tested frequencies.

At frequencies below BF, the whole cochlear partition vibrated in phase with deviations of only a few degrees (pink and purple lines in Figures 2.4B, 2.6). At frequencies near BF, and particularly above BF, the phase in the bridge and BM regions sometimes lagged the phase in the OSL region (black and green lines in Figures 2.4B, 2.6). These data suggest that near and above the BF there can be phase differences between the OSL, bridge, and BM. CP phase changed with frequency, as shown by the separation of lines in Figure 2.4B; this phase-frequency relationship is examined in the next section.
Figure 2.5: Motion of the cochlear partition (CP) referenced to maximum motion. (A) Average velocity below best frequency (BF) for each specimen (n=6). (B) Average velocity near BF from each specimen (n=5, see Figure 2.6 for more data.) The overall CP width was normalized to be from 0 to 1. Orange arrows indicate the estimated locations of the bottoms of the inner pillar (IP) and outer pillar (OP). Ear-canal sound between 98-110 dB SPL.
Similar tuning characteristics of the OSL, bridge, and BM

The OSL, CP bridge, and BM all had similar frequency-response tuning (Figures 2.4C, 2.6). Across temporal bones the BFs of the BM ranged from 9.5 kHz to 14.4 kHz. On average, the passive human BM tuning sharpness, measured by $Q_{10}$ ($Q_{10}$ is defined as the BF divided by the bandwidth at which the peak sensitivity decreased by 10 dB), was 1.6 ±0.5 SD, which is similar to the $Q_{10}$ of passive BM motion in other species, including guinea pig: $Q_{10}$=1.4 at BF ~25 kHz (Cooper and Rhode, 1992), gerbil: $Q_{10}$=2.1 at BF ~33 kHz (Dong and Olson, 2009), chinchilla: $Q_{10}$=1.3-1.6 at BF ~6 kHz (Recio et al., 1998), and mouse: $Q_{10}$=1.1-1.8 at BF ~3-4.4 kHz (Lee et al., 2015, 2016; Ren, He and Kemp, 2016). Our basal BM tuning is also similar to more apical (12 mm from the base) human passive BM tuning sharpness as reported by Stenfelt et al. $Q_{10}$=1.0 (Stenfelt et al., 2003) and Gundersen et al. $Q_{10}$=2.44 (Gundersen, Skarstein and Sikkeland, 1978). In our data, the bridge $Q_{10}$ was 1.16 ±0.34 (SD), and the OSL $Q_{10}$ was 1.10 ±0.27 (SD). Although across specimens, there was a trend that tuning slightly sharpened from OSL, to bridge and BM, none of these changes reached statistical significance at $p<0.05$ (t-test) in our small population from different BFs.

For all locations across the CP, the response phase became more delayed as stimulus frequency increased. At the highest frequencies, sometimes there’s a hint that the phase plateaued (Figure 2.4D, 2.6). This pattern is consistent with that of a traveling wave, i.e., as frequency increased, phase delays increased slowly at low frequencies and fast near BF frequencies. This is evidence for traveling waves on the BM, and also on the OSL and the bridge.

Cochlear partition motion from six human temporal bones

Figure 2.6 presents radial velocity profiles and tuning curves for all six specimens tested.
Figure 2.6: Radial profiles and tuning curves of cochlear partition (CP) transverse motion from six human temporal bones. (A-F) Each panel is similar to Figure 2.4 and is from a location ~1 mm from the basal end of the cochlea. In panel F, only low-frequency data are shown for velocity versus radial location because high frequency data were only obtained for two BM locations.
DISCUSSION

Our findings provide a picture of the human cochlear partition that is substantially different from the classic view of the cochlear partition derived from the base of laboratory animals and accepted as almost universal among mammals. First, our observation of human CP anatomy in terms of its effect on function has led us to distinguish a third CP region between OSL and BM, the CP bridge. The bridge might have been considered as part of the OSL, but unlike the OSL (*osseous or bony* spiral lamina), it contains no bone. Most small laboratory animals do not show a bridge-like region in the cochlear base (Figure 2.2) and such a region is not included in classic cochlear models. Although classic models are assumed to apply to the apex as well as the base, even in laboratory animals there can be a bridge-like region in the apical CP (Figure 2.3). Second, since the BM traveling wave depends on its coupling with cochlear fluid motion, the additional fluid displacement by the OSL and bridge motion might have substantial impact on cochlear tuning and amplification, both of which involve the traveling wave. Finally, and perhaps most importantly, the radial profile of BM transverse motion in humans is different in key ways from the classic view. In particular, the regions where the BM attaches to the bridge and the TM attaches to the limbus move in humans but are considered stationary in classic cochlear models. Our data show that in this aspect of cochlear mechanics, and perhaps in other aspects such as tuning, the classic view of cochlear mechanics does not apply to the base of humans. Figure 2.3 shows that the classic view is also inconsistent with the apical anatomy of a number of small laboratory animals. Whether anatomy and cochlear motions in the base of larger mammals, or mammals more closely related to humans (e.g. primates), are similar to humans remains to be determined (for related work see (Rhode, 1971; Joris *et al.*, 2011; Sumner *et al.*, 2018)). Further, the sharper cochlear tuning found in humans compared to
laboratory animals (Shera, Guinan and Oxenham, 2002; Raufer and Verhulst, 2016; Sumner et al., 2018) may be related to our findings, but this remains to be determined.

Our CP motion measurements were made in the base of the cochlea, which is tuned to high frequencies. Our anatomical study on histological sections from 21 human temporal bones show that the bridge is present in all specimens. Also, the BM and bridge width are approximately the same and increase from base to apex. Additionally, the human OSL movement reported by Stenfelt et al. was from the 2 kHz cochlear region, which shows that human OSL movement is not restricted to the cochlear base (Stenfelt et al., 2003). Considering these observations, the overall pattern of OSL and bridge motion we have seen in the base is likely present throughout the human cochlea. This does not rule out there being differences in cochlear motions between base and apex as has been found in laboratory animals (Warren et al., 2016; Recio-Spinoso and Oghalai, 2017; Dong et al., 2018).

Comparison to other studies

Previous studies of human CP anatomy have described aspects of the CP bridge, but these studies were not focused on structural relevance to dynamics and did not clearly delineate the CP bridge from other structures (Corti, 1854; Neubert, 1950; Merchant and Nadol, 2010). The first reported quantitative measurements of OSL motion in a human temporal bone was by Stenfelt and coworkers (Stenfelt et al., 2003). In one specimen, they measured at points near the modiolus (OSL1), near the lateral edge of the OSL (OSL2), and on the BM. However, they did not distinguish a CP bridge region and made no mention of how they decided where their measurement points were relative to the detailed anatomy (Stenfelt et al., 2003). Stenfelt’s measurements were near the 2 kHz BF place while ours were near the 9-15 kHz BF places.
These factors prevent us from being able to make a detailed comparison of their results and ours. However, Stenfelt’s measurements are consistent with ours in that the magnitude of motion of the OSL was comparable to that of the BM, and that both BM and OSL phase patterns were appropriate for these structures to be carrying traveling waves.

Several studies in animals have reported measurements of sound-induced motion at different radial positions across the BM and sometimes from the OSL (reviewed in the Introduction). Reports from most animal experiments found minimal BM motion at its attachment to the OSL and the largest transverse motion in the region from the outer pillars to the center of the BM with only small differences in the phase seen at different BM locations (Cooper, 2000; Rhode and Recio, 2000; Warren et al., 2016). One exception is the studies of Nilsen and Russell (Nilsen and Russell, 2000) who made measurements with a self-mixing laser and reported the largest motions were near the outer pillars, but they also reported large phase differences (up to 180 degrees) for small variations in radial position. The different results of Nilsen and Russell can be accounted for by the possibility that their method included motion of deep structures of the organ of Corti, rather than just the surface motion of the BM. For a more detailed discussion, see (Guinan, 2018).

**Implications of measurements from a fresh cadaveric, passive preparation**

Passive-cochlea data are important for understanding human CP motion at high sound levels, or when active processes cease to work (e.g., in sensorineural hearing loss), and as an important foundation to understand CP motion with cochlear amplification.

Most animal studies have found no differences in the shape of the radial profile of BM transverse motion between active and passive cochleas (Cooper, 2000), but whether this holds
for humans is unknown. In an active cochlea, outer hair cells (OHCs) produce cochlear amplification by adding energy to the traveling wave (de Boer and Nuttall, 1999). In the long-wave region of the traveling wave, which is basal to the BF region, the sound pressure is close to uniform in a transverse section across the cochlear scalae and would affect the OSL, bridge and BM regions similarly. However, in the short-wave region near the BF, experiments and models of cochlear mechanics suggest that the sound pressure spread away from the BM may be spatially limited (Olson, 2001; Zweig, 2016), which may cause the OSL and bridge regions to move differently than the BM.

Another possible limitation of our measurements is that CP tissue properties may change substantially after death. Although we have seen no indication of this from our measurements over hours if the CP was kept moist, time effects cannot be ruled out because our earliest measurements were 47 hours post mortem. Many animal studies of BM motion before and shortly after death have shown a large initial motion decrease near BF (and a change in BF) from the loss of cochlear amplification, but over the next few hours only small changes occurred. Over longer periods after death, BM stiffness decreased (Kohlloeffel, 1972; Naidu and Mountain, 1998) and low-frequency BM motion increased (Rhode, 1973), but at 16 hours post mortem the shape of the cross-sectional motion profile was not affected (Cooper, 2000). In mice, TM material properties and TM traveling waves were similar at 1 versus 48 hours post mortem, and are similar to those of post-mortem human TMs (Farrahi et al., 2016). These experiments used cold, moist (never frozen) storage, as was done in our present study. Human cochlear input impedance from intraoperative measurements in live cochleas was similar to that in cadaveric temporal bones, which suggests that overall CP properties did not grossly change after death.
(Chien et al., 2009). Our measurements of CP motion in passive human cochleas are the freshest yet made, but may not be the same as just after death.

**Modeling human CP motion**

We found that the low-frequency cross-sectional motion of the CP can be described by a composite beam model with two elements: A rigid rod hinged near the modiolus (emulating the OSL and the medial half of the CP bridge), and a flexible beam with constant bending stiffness, simply supported at both ends (emulating the lateral part of the CP bridge and the BM). This model, described below, captures the overall properties of our CP motion measurements: (i) The OSL moves as a stiff plate hinged near the modiolus, (ii) the motion is large in the bridge region under the attachment of the TM to the limbus and where the BM attaches to the bridge, and (iii) the maximum CP motion occurs near the BM-bridge connection.

There are two other modeling studies that are relevant in that their CPs included both a rigid part and a flexible part (Kapuria et al. (Kapuria, Steele and Puria, 2017) and Taber and Steele (Taber and Steele, 1981); see below). In these models the volume displacement of the near-rigid region was large compared to that of the flexible BM region, but the near-rigid region had relatively little influence on the shape and BF of the traveling wave except for delaying the phase. These results may imply that the presence of a large fluid-volume displacement by the OSL and bridge in humans may have little effect on BF characteristics set by the BM. However, these models did not include cochlear micromechanics, in particular the mechanical drives to hair-cell stereocilia, where major effects due to a mobile OSL and bridge might be expected.
Methods for modeling human CP movement

In classic cochlear models, CP transverse motion was assumed to be restricted to BM motion, even though there had been evidence for some OSL transverse motion (see main-text Introduction). In classic models the BM was considered fixed at both ends. When systematic measurements of BM transverse motion as a function of radial distance across the BM became available, these provided a basis for modeling BM motion. Guinea-pig BM motion can be accurately modeled with a simple beam representing the BM as shown in Figure 2.7A (Cooper, 2000; Homer et al., 2004). In contrast, the CP of the human involves motion of multiple structures, such as the OSL, bridge, and BM, and is not well represented by a simple beam model. Human CP motions, for below-BF frequencies, can be adequately captured by a composite beam model.

A composite beam model was fit to averaged human CP motion data. The model is based on CP measurements for below-BF frequencies, where the whole CP moves in phase. Near and above BF, some BM phase measurements lagged the OSL (Figures 2.4, 2.6) and the beam equations, which apply only to unimodal motion, may not accurately capture the more complex motion at higher frequencies. The data in Figure 2.7B (below-BF data from Figure 2.5A) were loess-smoothed (locally weighted average (Cleveland, 1993)), yielding the blue line in Figure 2.7B. To account for varying widths across temporal bones, we normalized the x-coordinates of each cross-sectional motion profile by linear scaling of the measurements in each CP region. Scaling was performed so that the BM of each temporal bone was 13.3% of the CP width, the CP bridge was 11.5% of the CP width (83% of the BM) and the OSL was 75.2% of the CP width.

We characterized our measurements of CP motion with a mechanical model of the CP that included a composite beam of two elements: (i) a rigid rod hinged near the modiolus –
which emulated the OSL and the medial half of the CP bridge, and (ii) a flexible beam with constant bending stiffness, simply supported at both ends – which emulated the lateral part of the CP bridge and the BM. The rigid rod was hinged with a rotational spring at \( x = 0 \) (the medial attachment of the OSL) and a hinge near the mid-point of the bridge, which was assumed to have no torsional stiffness (the second derivative with respect to \( x \) is continuous). We used similar computations as in Homer et al. (Homer et al., 2004) (Figure 5d in Section IV and the Appendix in (Homer et al., 2004)) and adjusted the ratio of the stiffnesses of the two beams (\( \kappa \) in Homer et al.) to best match the averaged data (blue line in Figure 2.7B). Our parameters, defined in Homer et al., are: \( x_t = 0.81; x_1 = 0:0.01:x_t; x_2 = x_t:0.01:1; \kappa = 10^{-4.95}; q = 1; EI = 1 \). The resulting composite beam model in Figure 2.7B shows the overall properties of our CP motion measurements, including the OSL moving as a stiff plate hinged at the modiolus, and the maximum CP motion occurring near the BM-bridge connection (Figure 2.7B).

Several features of the model are worthy of comment: First, it may seem surprising that the OSL moved as if hinged near its connection with the modiolus. This might be because the OSL bone is porous in that region (Corti, 1854; Neubert, 1950; Fleischer, 1973; Küçük et al., 1991; Shepherd and Colreavy, 2004), which makes it more flexible and allows a discrete pivot point. Second, a hinge in the middle of the bridge was not expected. A comparison of the model hinge location to anatomical measurements indicates that the hinge is near the lateral end of the limbus and the medial edge of the inner sulcus. The medial region of the bridge parallels the tissue of the limbus, whereas the lateral region of the bridge parallels the inner sulcus and corresponds to an area of low tissue density, and presumably, less torsional stiffness.
Figure 2.7: Plots of model fits to cochlear partition (CP) motion in laboratory animals (A) and humans (B), each normalized by its peak value. (A) In a laboratory animal (guinea pig), basilar membrane (BM) motion was approximated by a mechanical beam model in which the BM was simply supported at the osseous spiral lamina (OSL) (x=0) and clamped at the spiral ligament (x=1) (from Homer et al. (Homer et al., 2004)). (B) In humans, CP motion was fit by a composite beam model consisting of a rigid rod hinged to a flexible beam. The thin black lines plot the average of below BF measurements (as in Figure 2.5A) for each human specimen. The blue line is a loess curve (a locally weighted averaging) of the measurements from all six ears. The thick, black line represents the motion of the composite beam model. Orange arrows point to estimated locations of the bottoms of the inner pillar (IP) and outer pillar (OP).
Two other modeling studies are relevant. First, Kapuria et al. (Kapuria, Steele and Puria, 2017) published a cross-sectional model of the gerbil BM. The gerbil has a very particular BM anatomy with a flexible BM arcuate zone and a stiff BM pectinate zone. Thus, the gerbil has both stiff and flexible parts of the CP, just as are present in humans (although at different structures and locations). The gerbil modeling results suggest that the BF is set mainly by the properties of the more flexible part of the CP (in humans, the BM) with relatively little influence from the stiff part (in humans, the OSL). If this concept from the gerbil is translated to our findings, the relatively large volume displacement of the OSL may play only a minor role in setting the BF of the human cochlea. Second, a cross-sectional model by Taber and Steele (Taber and Steele, 1981) included three CP regions, namely the OSL, BM arcuate zone (stiffened by the arches of Corti), and the BM pectinate zone. This model compared the BM motion with a very stiff OSL (typical of most mammals) to the BM motion with a flexible OSL (said to be typical of primates) and found that the model with the flexible OSL produced longer phase delays, as observed in squirrel monkey BM recordings (Rhode, 1971). In Kapuria et al.’s and Taber and Steele’s models, even though the volume displacement of the near-rigid OSL (or near-rigid BM region) was large compared to the flexible BM region, the properties of the near-rigid region had relatively little effect on the traveling wave other than its phase and did not substantially change the BF.
Implications of the human cochlear-partition motion profile

To understand how CP motion may relate to the motion of structures within the OoC, we consider simplified cross-sectional diagrams of the anatomy and low-frequency motion of the CP and OoC (Figure 2.8). The classic view of BM and OoC motion is shown in Figure 2.8A. As the BM moves up (toward scala vestibuli), the OoC rotates counter-clockwise about the bottom of the inner-pillars with BM motion greatest around the outer-pillars, OHCs, and the center of the BM. The resulting rotation of the TM about a stationary origin at the attachment to the limbus produces little radial motion of the TM at the TM attachment to OHC stereocilia. It has been hypothesized that the TM has substantial radial motion due to a TM mass-stiffness resonance (e.g. (Allen, 1980; Zwislocki, 1980)). However, measurements show that the TM is viscoelastic with large damping without sharp resonance, and the TM can carry longitudinally propagating traveling waves of radial motion (Ghaffari, Aranyosi and Freeman, 2007). Theoretical studies implicate the importance of both the TM propagation and heavily damped TM resonance (Meaud and Grosh, 2010). The longitudinally carried TM radial motion may be more important than TM resonances, but in-vivo TM motion is poorly understood and is likely different from the classic view. In addition, recent experiments show that the reticular lamina moves much more than the BM and there is differential motion between structures at the top of the OoC such as rotation of the reticular lamina (Nowotny and Gummer, 2006; Lee et al., 2015, 2016; Ren, He and Kemp, 2016; Warren et al., 2016; Recio-Spinoso and Oghalai, 2017; Cooper, Vavakou and Heijden, 2018; Dong et al., 2018). All of the above indicate that the classic view in Figure 2.8A needs modification and some models do incorporate more complex motions at the top of the OoC. Nonetheless, no new view has been widely accepted that replaces the classic view (Figure 2.8A) that the counter-clockwise rotation of the OoC moves the reticular lamina (at the OHC.
stereocilia) radially toward the modiolus, which deflects OHC stereocilia in the *excitatory* direction. Whatever the variations at the top of the OoC, most models (except (Taber and Steele, 1981)) assume that the edges of the BM are stationary and that the attachment of the TM at the limbus is stationary.

Figure 2.8B shows a recasting of the diagram of Figure 2.8A for the anatomy of the human CP and for low-frequency motion. The CP motion facing scala tympani is based on our measurements and model (Figure 2.7). The illustrated motion of the structures around the OoC is a simple extrapolation from our measured CP motion. In contrast to the classic view of CP motion, in humans the CP has substantial transverse motion in the region under the TM-limbus attachment and at the BM-bridge attachment, and has the greatest transverse motion near the inner pillar *medial* to the outer-pillar/OHCs. In upward BM motion, the BM-bridge attachment moves upward so that the OoC rotates *clockwise* (Figure 2.8B) – opposite to the rotation of the classic view (Figure 2.8A). This opposite rotation moves the reticular lamina radially away from the modiolus, which would deflect OHC stereocilia in the *inhibitory* direction. Furthermore, in humans the TM-limbus attachment, which is stationary in the classic view, is expected to move because it sits on a CP bridge region that moves. If the limbus rotates in a counter-clockwise direction for upward BM movement (as in Figure 2.8B), it would produce radial TM motion towards the modiolus, and this radial TM motion would further deflect OHC stereocilia in the *inhibitory* direction. Figure 2.8 is for low-frequency motion, but for frequencies near BF, fluid and tissue inertia will change the motion. As noted in the previous paragraph, the radial motion of the TM is unknown and the motions at the top of the OoC are likely to be more complicated than in the classic view. Nonetheless, the contrast between the CP motions in the classic view

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(Figure 2.8A) versus the simple extrapolation of our human CP measurements (Figure 2.8B) shows the highly consequential functional implications of the differences in CP motion.
Figure 2.8: Simplified diagrams of cochlear partition (CP) anatomy and motion for low frequencies. (A) In the classic model, the osseous spiral lamina and the limbus are stationary while the basilar membrane (BM) and organ of Corti (structures above the BM) move in response to a sound-pressure difference across the partition (depicted by +/-). (B) In the human cochlear base the BM and OoC move, and also, the OSL and bridge (including the attachments of the TM to the limbus) move in response to sound.
One major difference at the top of the OoC between the classic view and our findings in humans is in TM motion (Figure 2.8). In the classic view, the TM-limbus attachment is stationary and motion of the TM is produced only by the motion of OHC stereocilia and fluid in the subtectorial space. In humans, the CP limbus region has transverse movement (due to motion of the OSL and bridge), which implies that the TM-limbus attachment point moves, so that in humans both ends of the TM are attached to moving structures. Vibration of one end of the TM can be carried to the other radial end, and also carried longitudinally along the TM (Ghaffari, Aranyosi and Freeman, 2007). Thus, TM motion in humans appears to be more complex than in the classic view of cochlear mechanics. An attractive hypothesis is that the different gross OoC motions in Figure 2.8A vs 2.8B are at least partly compensated by different TM motion so that for upward BM motion OHC deflections are similar in humans and other species. This speculative hypothesis needs testing.

Cochlear mechanics is the origin of important basic properties of hearing such as hearing sensitivity and frequency tuning. The classic view of cochlear anatomy and mechanics was derived from measurements in the base of extensively-studied small animals. Here we have shown that both the anatomy and sound-evoked motion of the human CP differ in crucial ways from the classic view. Our results provide a new perspective on cochlear mechanics in humans. In addition, Figure 2.3 suggests that the classic view may also not apply in the apex of some laboratory animals. Understanding the mechanical differences between the human cochlea and those of other species will aid in interpreting results from laboratory animals and properly using them to understand human hearing.
METHODS

Detailed methods are described below. In brief, the CP motion measurements were from six human (53 to 78 years old, mean = 60.2 years) cadaveric temporal bone specimens, 47 to 108 hours post mortem. The CP was viewed through the round window after removing its membrane. The fluid in scala tympani was drained to a thin film over the CP. Measurements used a laser Doppler vibrometer focused directly on the CP, using the tissue’s reflected light. Acoustic pure tones between 100 Hz and 24 kHz were delivered to the ear canal. Fiber-optic pressure sensors were used to record sound pressure in the vestibule (Olson, 2001; Nakajima et al., 2009). Anatomy was quantified from histological preparations of human specimens and several animal specimens from the Otopathology Laboratory at our institution.

Temporal bones. We used seventeen unidentified fresh human cadaveric temporal bone specimens from the consented donor program at Massachusetts General Hospital. The first eleven specimens were used to perfect the technique. We report here results from six ears, 53 to 78 years old (mean = 60.2 years). All were left ears (to keep the measuring set-up consistent) and four were male. Details regarding the removal and initial preparation of the temporal bones, as well as our criteria to assess the condition of the bone are described in Nakajima et al. and Frear et al. (Nakajima et al., 2009; Frear, Guan, Stieger, Rosowski, et al., 2018). Immediately after extraction, specimens were kept in 0.9% saline and refrigerated at 0-4 degrees Celsius. After drilling and preparing a fresh (never frozen) specimen for measurement, we made reference measurements of stapes velocity, RW membrane velocity, and intracochlear pressures. Experiments were performed at room temperature, 47 to 108 hours post mortem. Post mortem times are defined as the time from death to the beginning of CP motion measurements.
To prepare for CP motion measurements, we removed the semi-opaque round window (RW) membrane with the sharp edge of a hypodermic needle to provide an unobstructed view of the cochlear partition. To prevent fluid leakage from the cochlea, and air entering through the open RW, the temporal bone was oriented with the CP at the RW region in a horizontal plane facing upward. The fluid level was controlled with absorbent tips (#503, Henry Schein) so that over the CP there was only a thin fluid film to avoid possible measurement artifact due to motion of the fluid-air boundary that differed from the motion of the CP (Cooper and Rhode, 1992). The removal of fluid in scala tympani reduced the mass seen by the CP. However, at low frequencies where the effect of mass is negligible, CP motion would not be affected by this reduction in mass. At high frequencies (>5-10 kHz) the fluid loading may change the response of the CP.

**Sound stimulation.** Acoustic pure tones between 100 Hz and 24 kHz (10 per octave) were generated by a Radio Shack driver (400-1377) and delivered to the ear canal with a flexible polyethylene tube. The sound pressure level in the ear canal 1 mm from the tympanic membrane was monitored with a calibrated probe-tube microphone (Knowles EK-23103-000, calibrated against a Larsen Davis 2541), which was fed through a metal sleeve that penetrated the bony ear canal. Sound pressure level was kept constant as a function of frequency in each bone (iso-input), but was varied across experiments (from 94-110 dB SPL) to produce adequate signal strengths for the laser Doppler vibrometer.

**Laser Doppler velocity measurements.** A laser Doppler vibrometer (LDV) (Polytec CLV 700) was used to measure the velocities of the posterior crus or footplate of the stapes, the center of
the RW membrane, and along the scala tympani surface of the CP. Reflective polystyrene microbeads (50 μm diameter, ~0.07 μg each) were used to enhance the signal-to-noise ratio for stapes and RW measurements. For CP measurements, the laser beam was oriented perpendicular to and focused on the scala-tympani surface of the CP. This provided adequate reflections without microbeads (e.g. as in (Dong and Olson, 2009)). The radial position of the CP measurement point was controlled with a micromanipulator. We measured up to 34 radial locations on the CP using 5 μm radial-position steps on the BM and bridge, and 50 μm steps on the OSL, skipping points where the reflectivity was too low. Thus, we depended on CP reflectivity, resulting in varied measurement locations across specimens. In our system the laser-beam spot size on the tissue can be as large as 37 μm (as indicated by Polytec specifications), but the actual spot size was visually smaller, and 5 μm movements often produced large changes in reflectivity. Thus, we think that the spot size did not produce substantial spatial smoothing in our data. The depth of field of the laser Doppler measurements (according to Polytec specification) is theoretically 2 mm, however, in this passive preparation the deeper structures are expected to vibrate the same as the surface CP. In several early experiments, we measured the motion of reflective microbeads (5-20 μm diameter) on the CP surface, washed them away, measured at the same spot and found similar velocities, i.e. motions within 2 dB and phases within 15 degrees, with similar CP velocity measurements with and without beads.

In five temporal bones we obtained data up to one-half octave above the BF. In one temporal bone, low laser signals prevented obtaining reliable near-BF data. For temporal bone #16 (used for Figure 2.4), we excluded the CP motion at f=4700 Hz because an anti-resonance in stapes velocity decreased the sound pressure in scala vestibuli by 40 dB. In one temporal bone (#15), during removal of the RW membrane, the OSL was punctured ~1 mm apical to the
measurement location. This caused stapes velocity and intracochlear sound pressure to change in a small frequency band centered around 2 kHz by less than 5 dB, but the measurements were unchanged for frequencies below 1.5 kHz and above 3 kHz, thus these data were used in Figures 2.5, 2.6, and 2.7. To determine possible vibrational coupling of the ear-canal sound to the temporal bone, we measured the velocity of the cochlear promontory during acoustic stimulation; all CP motion presented was ~10-20 dB larger than cochlear promontory motion.

**Intracochlear pressure measurements.** Fiber-optic pressure sensors developed by Olson (Olson, 1999) were used to record sound pressure in the vestibule (Olson, 2001; Nakajima *et al.*, 2009). Details regarding the calibration and placement of a sensor in the vestibule are given elsewhere (Nakajima *et al.*, 2009; Stieger, Rosowski and Nakajima, 2013; Frear, Guan, Stieger, Rosowski, *et al.*, 2018). Initial intracochlear pressure was measured with the RW membrane intact and then again after the RW was removed. Removing the RW caused minimal changes (<3 dB) in intracochlear pressures for all tested frequencies.

**Anatomical measurements.** During motion measurements, the anatomical boundaries of the OSL, CP bridge and BM were based on (i) the lateral edge of the bony OSL (clearly visible under the microscope used during the experiment), (ii) the edge of the spiral ligament (also visible under the microscope, and experimentally defined as the most lateral CP place where the velocity was close to the noise floor), and (iii) the width-ratio of the BM and CP bridge (bridge width/BM width = 0.83 ± 0.12 standard deviation, in the cochlear base as measured in histology).
In six histologically-prepared temporal bones stained with Hematoxylin and Eosin from the Otopathology Laboratory at our institution, we estimated the bridge/BM width ratios in the base. In additional 15 specimens we confirmed the presence of the bridge throughout the whole cochlear length. BM width was defined as the width between the basilar crest and the inner-sulcus cell that is adjacent to the bottom of the inner hair cells and close to the habenula perforata (Bhatt, Liberman and Nadol, 2001; Liu et al., 2015). Bridge width was the width between the lateral edge of the bony OSL and the BM. These widths were measured from 20 μm, mid-modiolar sectioned slides cut in the vertical plane (Merchant and Nadol, 2010). The locations examined were ~6 mm from the cochlear base and thus 4-5 mm apical from our motion-measurement location. Sections that are perpendicular to the CP at the motion-measurement location in the cochlear hook require an unusual cutting plane and were not available. However, since there was little difference in the bridge-width/BM-width ratio at other cochlear locations throughout the mid-modiolar sections, we expect that there are no substantial differences in this ratio from the basal anatomical measurement location to the motion-measurement location. The image in Figure 2.1B was chosen as a representative example from the six human temporal bones that we analyzed. The images in Figures 2.1A, 2.2, and 2.3 were from the basal and apical turns of a guinea pig, mouse, gerbil, cat, and chinchilla cochleas, also from the Otopathology Laboratory collection. Cochleas of at least three different animals from each species were studied and a representative section was chosen for the image of each species.

**Calculating Q values.** We quantified tuning sharpness by the quality factor, \( Q_{10} = f/\Delta f \), where \( f=BF \) and \( \Delta f \) is the bandwidth between the frequencies at which the response decreased by 10 dB from the maximum. For our human data, the sparse frequency sampling of 10 points per octave
affected the accuracy of measuring both BF and bandwidth. Thus the $Q_{10}$ values are accurate only within ~15%, based on $Q_{10}$ values of frequencies neighboring the BF and their bandwidths.

We calculated $Q_{10}$ values for animal experiments from data extracted from published post mortem (or high-level) tuning curves using “DataThief” software (https://datathief.org/).
CHAPTER 3. The human cochlear partition: Anatomical studies of osseous spiral lamina and bridge microstructure

Manuscript for this work is in preparation as: Stefan Raufer, Cornelia Idoff, Aleksandrs Zosuls, Giacomo Marino, Nathan Blanke, Irving J. Bigio, Jennifer T. O’Malley, Barbara J. Burgess, Joseph B. Nadol, John J. Guinan Jr., Hideko H. Nakajima. Microstructure of the human osseous spiral lamina and cochlear partition connecting fibers: Relevance for cochlear partition motion

SR and HHN designed research. All authors contributed to research. SR, CI, JJG, and HHN analyzed and interpreted data. SR, CI, JJG and HHN wrote the paper.
ABSTRACT

The classic view of cochlear partition (CP) motion, generalized for mammals, is derived from basal cochlear measurements in laboratory animals. Recently, we showed that the motion of the human CP at the base is substantially different from the classic view. However, interpreting some of the motion results has been impaired by a lack of knowledge regarding human CP anatomy. Previously we described a soft tissue “bridge” between the osseous spiral lamina (OSL) and basilar membrane (BM) in humans (non-existent in the classic view). Here, we show in histologically prepared human temporal-bone cross sections that the bridge is similar in radial width to the BM throughout the cochlea. Both BM and bridge widths increase linearly from ~130 μm near the base to ~500 μm near the apex. The OSL widths decrease from ~1140 μm near the base to ~360 μm near the apex. By investigating the bony microstructure of the OSL with three-dimensional reconstructions from 2 μm cross sections, we find that the OSL is very porous. The porosity is dependent on cochlear location with pore diameters up to 50 μm. Lastly, polarized light microscopy reveals that tightly-packed collagen fibers in the BM continue medially spreading out within the bridge region to connect mostly to the OSL vestibular plate. In conclusion, the human CP has a relatively wide and porous OSL and a soft-tissue bridge containing fibers connecting the BM to the OSL. These anatomical features help to explain why human CP motion is different from the classic view.
INTRODUCTION

In the classic view of the mammalian cochlea, the basilar membrane (BM) is tethered between the immobile osseous spiral lamina (OSL) and the immobile basilar crest of the spiral ligament. With this view, the only mobile part of the cochlear partition (CP) tympanic surface is the BM (Wever, 1954; Geisler, 1998; Pickles, 2013). This core assumption of an immobile OSL and basilar crest is derived from experimental measurements in the cochlear base of many laboratory animal species including guinea pigs, chinchillas, gerbils, mice, and cats (Cooper, 2000; Rhode and Recio, 2000; Lee et al., 2016; Warren et al., 2016). As a result, computational models of mammalian cochleas, including models for the human cochlea, have been developed on the premise that the OSL does not move (de Boer, 1993; Steele, de Monvel and Puria, 2009; Meaud and Grosh, 2010; Zweig, 2016). However, we have recently shown that the BM is not the only mobile structure of the CP tympanic surface in humans. In fact, in the human cochlear base, the BM accounted for only ~27-43% of the total CP volume displacement, as opposed to accounting for 100% in classic cochlear models. Furthermore, in humans there is a soft-tissue bridge between the BM and OSL. The lateral end of the bridge was reported to move almost as much as the BM in response to sound (Raufer, Guinan and Nakajima, 2019). The bridge is not present in the base of laboratory animals, nor represented in cochlear models (Raufer, Guinan and Nakajima, 2019). Figure 3.1 compares the CP anatomies of a commonly used laboratory animal (guinea pig) (Figure 3.1A) and human (Figure 3.1B). The bars below the histology indicate the lateral extent of the OSL and BM, and in humans, the bridge. The bars also illustrate the motion differences between the classic view as it would apply to the base of guinea pig (Figure 3.1C) and humans (Figure 3.1D). The illustration shows that the human OSL and bridge
move in response to sound, whereas they are stationary in the classic view and cochlear base of laboratory animals.
Figure 3.1: Histological 20 µm-thick cross sections of basal-turn cochlear partitions (CP) and illustrations of CP motion. (A) In the Guinea pig (5.5 mm from base), the CP consists of a relatively short osseous spiral lamina (OSL) attached to the basilar membrane (BM). (B) In the human (6.7 mm from the base), the CP has a wide OSL with a soft-tissue connection between the OSL and the BM (not present in the base of laboratory animals) termed the CP “bridge” (Raufer, Guinan and Nakajima, 2019). The limbus and the attachment of the tectorial membrane (TM) sit on top of the soft-tissue bridge in humans, while in laboratory animals the TM-limbus attachment sits on static OSL bone. (C-D) Motion profiles of guinea pig and human CPs. Other abbreviations are: OoC = organ of Corti; SL = spiral ligament.
Although mechanical measurements in human cochleas have shown that the OSL moves in humans (Stenfelt et al., 2003; Raufer, Guinan and Nakajima, 2019), it is unclear why a bony structure like the OSL would move. Anatomical studies in humans have identified pores in the tympanic plate of the OSL, which may explain its flexibility (Corti, 1854; Neubert, 1950; Lim, 1970; Fleischer, 1973; Küçük et al., 1991; Shepherd and Colreavy, 2004). However, the porosity of the OSL has only been investigated in small areas in the cochlear base and only from the OSL tympanic side. Knowledge of the microstructure of the OSL is minimal and the relation between mechanics and anatomy not well understood.

Anatomical details regarding the soft-tissue bridge between the OSL and BM (Figure 3.1B) and its relation to mechanics is undetermined. While the lateral end of the bridge has been shown to move as much as the BM (Raufer, Guinan and Nakajima, 2019), it is unknown which part of the bridge has functional relevance. Neubert (Neubert, 1950) described collagen fibers in what we now believe is the bridge region, but Neubert’s study with hand-drawn illustrations lacks detail.

In this study, we investigate the anatomy and microstructure of the human CP to determine the anatomical underpinnings of human CP motion. To analyze the anatomy of the human CP, we utilized three methods. First, we examined 20 μm thick histological cross-sections of human cochleas to measure dimensions of the BM, bridge, and OSL from base to apex. Second, we sectioned unconventionally thin 2-μm samples to reconstruct the three-dimensional (3D) micro-architecture of the human OSL. Third, we exploited the birefringent properties of human cochlear tissues to describe the relationship of collagen fibers within the BM and the bridge using crossed polarized light microscopy.
RESULTS

Anatomical measurements of OSL, bridge, and BM

Figure 3.2A shows an overview of the human cochlea from a mid-modiolar section of a horizontal-cut temporal bone (Merchant and Nadol, 2010). Detailed views of three locations from base to apex are shown in Figure 3.2B-D. Widths of the OSL, CP bridge and BM versus cochlear longitudinal location are plotted in Figure 3.2E (details in Table 3.1). The OSL width decreased from base to apex (black), but the BM width (red) increased from base to apex. Our BM width measurements agree with previous BM measurements (dotted red line in Figure 3.2E (Wever, 1938)). Notably, the soft tissue area between the BM and OSL—the CP bridge (blue line in Figure 3.2E)—had about the same width as the BM and increased from base to apex at a similar rate as the BM. It is remarkable that the bridge and BM widths are almost equal throughout the length of the cochlea and increase at the same rate from base to apex. Furthermore, the widths of the bridge, BM, and OSL add up to an overall CP width of about 1.3 mm, which was similar from base to apex (purple line in Figure 3.2E).

In the base of laboratory animals, the limbus and its attachment to the tectorial membrane sit on top of a static bony OSL (Figure 3.1A), but Figures 3.1A and 3.2 show that this is not universal across mammals. In humans, the limbus rests on both the CP bridge and OSL in the base, and exclusively on the CP bridge in the apex (Figure 3.2). In all cochlear turns, the attachment of the tectorial membrane to the limbus in humans lies above the soft-tissue bridge, that is, above a mobile structure, not above stationary bone as assumed in the classical view of cochlear mechanics.
Another anatomical aspect is the difference in width between the OSL tympanic plate versus the OSL vestibular plate (see Figure 3.2D). For measurements, we defined the width of the bridge as the distance between the ends of the OSL vestibular plate and the BM. However, the OSL vestibular plate was sometimes shorter than the tympanic plate. In ~54% of the cases (across specimens and locations), the vestibular plate was longer than the tympanic plate (difference of 72.6 μm ± 35.7μm SD), in ~33% of the cases the vestibular and tympanic plates had the same length, and in ~13% of the cases, the tympanic plate was slightly longer than the vestibular plate (by 58.5 μm ± 32.8). There was no systematic change from base to apex in these differences. Lastly, the thickness of the OSL, defined as the distance between the vestibular and tympanic plates, was ~50 μm at the lateral end and ~150 μm near the modiolus (c.f. Figure 3.2D). For all three longitudinal measurement points in Figure 3.2, the OSL thickness did not change significantly from base to apex.
Figure 3.2: Width measurements of the basilar membrane (BM), bridge, and osseous spiral lamina (OSL). (A) An example mid-modiolar cross section of a human cochlea prepared using a horizontal cut. (B-D) Detailed views of the cochlear partition (CP) approximately 28 mm, 19 mm, and 12 mm from the base. Panel C shows measurements of BM, bridge, and OSL widths. Panel D shows thickness measurements of the OSL at three radial locations. The measurements in C and D were done in each location in each bone. The image in panel D was flipped horizontally. (E) Measurements of BM (red), bridge (blue), and OSL (black) widths as a function of cochlear location. The data were collected from mid-modiolar horizontal (n=15), vertical (n=6), and Poeschel (n=1) sections of normal human temporal bones. The solid lines represent linear fits to the data, error bars indicate plus/minus one standard deviation (data provided in Table 3.1). The purple data are the sums of BM, bridge, and OSL measurements and show nearly constant CP width of 1.3 mm from base to apex. Reference data of BM width from Wever (1938) shown as a red dotted line. Other abbreviations are: TM=tectorial membrane; OoC=organ of Corti; RM=Reissner's membrane.
Table 3.1 Mean and standard deviations of the widths of the basilar membrane (BM), bridge, and osseous spiral lamina (OSL) from 21 different bones at different distances from the base in the cochlea. The number of bones (n) measured at each location are given in parenthesis in the standard deviation (SD) columns. The number of specimens varied slightly because in some sections landmarks (like the organ of Corti, or modiolus) were not obvious.

<table>
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<th>Bridge width (µm)</th>
<th>OSL width (µm)</th>
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<td>35 (n=15)</td>
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</tr>
<tr>
<td>30</td>
<td>471</td>
<td>30 (n=6)</td>
<td>519</td>
</tr>
<tr>
<td>31.6</td>
<td>573</td>
<td>63 (n=14)</td>
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</tbody>
</table>
**OSL anatomy**

The two plates of the OSL are comprised of thin ossified connective tissue between which auditory-nerve fibers travel to the organ of Corti. In histological sections with a standard thickness of 20 µm, the OSL plates appear solid and uniform (e.g. Figures 3.1 and 3.2). However, it has been reported that there are micro perforations of the OSL (Corti, 1854; Neubert, 1950; Lim, 1970; Fleischer, 1973; Küçük et al., 1991; Shepherd and Colreavy, 2004). To investigate OSL micro perforations, we made ~100 serial sections of 2 µm thickness for three different locations in one cochlea of a 63 year old male (1 mm, 9 mm, and 12 mm from the base). From these we reconstructed a large area of OSL bone at each location (see Methods). These 3D reconstructions are shown in a side view in Figure 3.3 and in a top-down view in Figure 3.4. The inserts in Figure 3.3 show examples of single 2 µm sections where the OSL perforations are readily visible, indicated with arrows.

The 3D reconstructions show that there are small bony pillars connecting the vestibular (green) and tympanic (purple) plates, but these bony connections are sparse. Another characteristic is that the lateral tip of the vestibular plate is slightly tilted downwards (towards scala tympani) (see inserts in Figure 3.3A-C). The area tilted downward corresponds to the area where the fibers coursing through the bridge anchor to the OSL (described in more detail below). The vestibular plate shows a thicker medial and lateral third and notably thinner middle part.

The porosity of the OSL plates is most apparent in the top-down view (Figure 3.4), which shows systematic differences in the perforations between the tympanic (purple) and vestibular (green) plates as well as across longitudinal locations. The tympanic plate was generally more porous than the vestibular plate, and more apical locations had more perforations than basal locations. At 12.6 mm from the base, the porosity was 20% of the tympanic plate versus 10% of
the vestibular plate; at 9 mm from the base, 8% of tympanic plate and 8% for the vestibular plate; and at 1 mm from the base, 4% of the tympanic plate versus 3% of the vestibular plate. Pore sizes reached up to 50 µm. The pores of the vestibular plate were mainly around its center (Figure 3.4A-C, green). For the vestibular plate, the medial one-third and lateral one-third were mostly solid bone with little porosity, while the middle third was porous. The differences in OSL porosity across the OSL width are most obvious in the most apical location (green in Figure 3.4A).
Figure 3.3 Side views of 3D reconstructions of the osseous spiral lamina (OSL). (A-C) OSL tympanic plate (purple) and vestibular plate (green), 12 mm, 9 mm, and 1 mm from the base (longitudinal location estimated based on the relationship of Figure 3.2E). Inserts in A-C show examples of single two-micron sections from the same locations. Arrows in the inserts indicate OSL perforations. Scale bar applies to main panels, not inserts.
Figure 3.4 Top view of 3D reconstructions of the osseous spiral lamina (OSL). (A-C) OSL tympanic plate (purple) and vestibular plate (green), 12 mm, 9 mm, and 1 mm from the base. Perforations of different shapes and sizes are visible in all reconstructions. The tympanic plate in the most apical reconstruction was particularly porous. The overall porosity of the tympanic and vestibular plates was similar for more basal regions. The arrow in panel C indicates a possible crack through the most basal tympanic plate.
**Bridge anatomy**

Figure 3.5 shows histologic sections of the human CP with regular light microscopy (left column with “1” next to panel letter, panels A1-D1) and the same sections with crossed-polarized light microscopy (left column, panels A2-D2). In crossed-polarized light, the birefringent collagen fibers of the BM became visible as bright bands (see Methods for details). Enlarged polarized-light images, focused on the bridge region, are shown in right column panels A3-D3.

The birefringent fibers (from lateral to medial) appear to connect to the medial part of the spiral ligament via fanned out filaments, to course through the BM, and to continue into, and to traverse through the bridge, finally connecting to the OSL. The fibers within the bridge appear to be a continuation of the BM fibers. The fibers fan out as they run medially to attach to the OSL. The fibers clearly attach to the vestibular plate of the OSL; additionally, connections to the tympanic plate are visible (Figure 3.5A3). Overall, the appearance of the bridge fibers and BM fibers are very similar.

Towards the apex of the cochlea, the signal of the polarized light gradually weakened, and the fibers of the BM as well as within the bridge were generally less obvious. This may be because the thickness and amount of collagen fibers in the BM decreases from base to apex by approximately five-fold (Liu et al., 2015). Thus, the polarized light illuminates less birefringent material in the apex. In our most apical measurement location, the fibers of the BM as well as the fibers within the bridge were hardly visible (Figure 3.5D).
Figure 3.5 Crossed polarized light microscopy showing connecting fibers in human histological sections. (A1-D1) show H&E stained 20 μm histological sections with regular light microscopy at 10X magnification and four longitudinal locations (1 mm, 6.7 mm, 12.3 mm, and 24.1 mm) from the base. (A2-D2) show the same sections as in A1-D1, but with crossed polarized light microscopy, which makes connecting fibers between the basilar membrane (BM) and the osseous spiral lamina (OSL) visible as bright bands. (A3-D3) show the same as in A2-D2, but with a magnification of 20X, detailing the fibers of the bridge and BM. The signal of the BM and connecting fibers decreases in apical parts of the cochlea, likely because the thickness of the BM decreases. Scalebars in panel A are 100 μm and apply to all panels.
DISCUSSION

In this study we highlight that the human cochlear partition does not conform to the generalized classic view of the mammalian cochlea. In particular, the human cochlea has a CP bridge that is not considered in cochlear models and not present in the base of laboratory animals (mouse, gerbil, guinea pig, chinchilla, cat, as shown in Raufer, Guinan and Nakajima (2019) Figure S1). The bridge is similar in width to the BM and both increase in width from base to apex but remain equal in width at each longitudinal location. In contrast, the human OSL occupies a large percentage of the CP in the base but its width decreases from base to apex. The changing widths balance so that the overall width of the CP stays approximately the same. Using 3D reconstructions of the OSL bony structure, we found that the OSL tympanic and vestibular plates have regions of high porosity. Birefringent fibers course through both the BM and the bridge, providing a structural element that appears to tie both regions together. Below we consider whether the roles of the OSL porosity and the existence of fibers within the bridge may explain the motion of the human CP measured Raufer et al. (Raufer, Guinan and Nakajima, 2019).

Comparison to other studies of OSL microstructure

The microanatomy of the OSL has previously been investigated in a number of studies, but these studies did not quantify the porosity of the OSL bone, were limited to a small area, and/or were done in animals (Corti, 1854; Neubert, 1950; Lim, 1970; Fleischer, 1973; Küçük et al., 1991; Shepherd and Colreavy, 2004; Rask-Andersen et al., 2012).

Shepherd and Colreavy (2004) quantified the pore sizes of the human OSL at different longitudinal locations using electron microscopy, but only at the modiolar attachment of the
tympanic plate and in a small ~40x40 µm range. They reported the perforation diameters to be small (5-7 µm on average) but these may not be representative of more lateral OSL regions where we found pore sizes up to 50 µm in diameter (Figure 3.4A). Kucuk et al. (1991) analyzed the bony structure of the modiolus and its OSL attachment; they reported the tympanic plate to have a high porosity but that the vestibular plate had no porosity. We estimate that the measurement location in figure 3 of Kucuk et al. (1991) was near the end of the basal turn where we found the vestibular plate to be highly porous (cf. Figure 3.4A). In addition, in our specimen the porosity of both the vestibular and tympanic plates increased at more apical locations. Finally, Fleischer (1973) described the microanatomy of the OSL in an elephant and Lim (1970) studied the OSL in guinea pigs, but neither study quantified the porosity of the OSL.

Numerous bony pillars between the vestibular and tympanic plates of the human OSL were reported by Fleischer (1973) and Rask-Andersen et al. (2012). We found only a few bony connections between the two plates (Figure 3.3), and it is unclear whether these are the same as those described by Fleischer and Rask-Andersen et al. In our 2 µm histological sections, structures connecting the tympanic and vestibular plates were clearly visible (inserts in Figure 3.3A-C). The bony connections between the tympanic and vestibular plates are very thin (Fleischer 1973, Rask-Andersen et al. 2012) and it could be that our 2 µm thick sections cannot resolve the bony pillars between the two OSL plates. The connections we observed did not stain with Toluidine blue, even though this stained the bone of the vestibular and tympanic plates, suggesting the possibility that these connections are not all bone.

An elaborate organization of the OSL bone was described by Neubert (1950) (Neubert’s sketches look remarkably similar to Corti’s (1854) drawing). Our finding that the vestibular plate has three distinct radial regions agrees with Neubert’s description of the OSL bone organization.
However, we cannot confirm Neubert’s description of mesh-like bone close to the modiolus, circular bone in the middle, and radial bone close to the bridge-region. Our reconstructions, based on a side view of the osseous spiral lamina with a thickness of 2 µm (see Methods) may not be able to capture the highly organized bone growth suggested by Neubert and Corti.

Our study was based on a laborious reconstruction from only one human temporal bone, but over a ~200 µm longitudinal length at three longitudinal locations and for the entire radial width of the OSL (~1000 µm). Further, with our 3D surface reconstructions it was very easy to quantify the porosity of the OSL over a large area. Future studies will likely provide further details about OSL anatomy.

**Comparison to other studies of bridge fibers**

The fibers within the bridge appear continuous with the collagen fibers of the BM fibers and have a similar appearance in our polarized light images (Figure 3.5). However, with our setup it is not possible to quantify the material of the bridge fibers and determine whether they contain (the same kind of) collagen as the BM (Iurato, 1962; Dreiling, Henson and Henson, 2002). Agrawal et al. found elastin expressed in the bridge region and Liu et al. collagen 2 markers in the bridge region, suggesting that the bridge fibers likely contain collagen (Liu et al., 2015; Agrawal et al., 2018). A study that discussed bridge fibers more explicitly was Neubert (1950). Neubert’s “extension fibers”, which he claims are collagen fibers, could be the same as our bridge fibers. Whether the bridge fibers consist of collagen (and which kind) needs future investigation. It is likely that the bridge fibers contain collagen, as the intensity of the polarized light through the bridge fibers (and hence the birefringence) appears very similar to that of the BM collagen fibers in Figure 3.5. This issue could be resolved with an immunohistochemical or
a more sophisticated optical approach, as in (Kalwani et al., 2015; Liu et al., 2015; Agrawal et al., 2018).

We visualized the birefringent bridge fibers in only one orientation (see Methods), and within the plane of the BM fibers. Kalwani et al. (2013) used birefringence imaging to generate fiber orientation maps and reported a bifurcation for the BM fibers at the base of the limbus. Such bifurcations can be appreciated in regular light microscopy in human specimens, but to resolve whether a bifurcation of the fibers occurs in humans, a sophisticated immunohistochemical or optical approach is necessary. For example, generating quantitative birefringence maps, similar to Kalwani et al. (2013), would enable (i) investigation of the course of the BM and bridge fibers at all orientations in a plane and (ii) determination of the material properties of the bridge fibers.

Finally, an open question is whether the bridge fibers are anchored in the tympanic or vestibular plate of the OSL, and in which manner. Some anatomists claimed that the human BM is exclusively anchored at the tympanic plate of the OSL, while others claimed that BM fibers attach to both the tympanic plate and vestibular plate (Neubert, 1950; Liu et al., 2015; Agrawal et al., 2018). It is furthermore assumed that the BM attaches only to the lateral tip of the OSL. We found that the bridge fibers connected mostly to the vestibular plate of the OSL, but also found remnants connecting to the tympanic plate (Figure 3.5). The connections to the vestibular plate were more numerous. The bent shape of the OSL vestibular plate towards scala tympani at its lateral end (Figure 3.2, 3.3) constitutes an intriguing anatomical modification that allows the fibers of the bridge to attach over a relatively large radial width on the OSL vestibular plate without changing direction (Figure 3.5). This supports our observations that most bridge fibers attach to the vestibular plate of the OSL. In the apical locations, the weak signal strength (likely
due to less collagen fibers in the apex (Liu et al., 2015)) prevents us from drawing conclusions concerning more apical locations.

**Implications of the OSL and bridge anatomy for cochlear partition motion**

It is generally assumed that cochlear mechanics are similar across mammals. However, the anatomical architecture surrounding the BM and organ of Corti vary considerably between laboratory animals and humans (Raufer, Guinan and Nakajima, 2019). In the base of laboratory animals, motion measurements of the cochlear partition tympanic surface show that the BM is the major moving structure with a simple beam motion (Figure 3.1A). However, Raufer, Guinan, and Nakajima (2019) recently showed that the human cochlear partition has a very different motion compared to the classic view derived from laboratory animals (Figure 3.1B).

Here, we quantified pores in the tympanic and vestibular plates of the OSL. Although in the most basal cochlear location the OSL did not show a high porosity in our specimen (only 3-4% of the OSL were porous, Figure 3.4C) the fact that the OSL is very thin and very wide (~1200 μm in the base) may explain why the OSL was observed to move in the base (Raufer, Guinan and Nakajima, 2019). The wide OSL deflects and the motion increases from the medial attachment near the modiolus to more lateral places. For more apical locations where the OSL width decreases, the increased porosity may allow for the OSL to move in more apical locations, such as observed around the 2 kHz characteristic frequency location (12 mm from the base) by Stenfelt et al. (2003). The OSL appeared stronger (less porous) in the base than in more apical locations, in agreement with the need for a stronger support for the stiffer BM in the base (Fleischer, 1973).
The fibers within the bridge have similar birefringent properties as the BM, which may explain why the bridge has similar motion compared to the BM (Raufer, Guinan and Nakajima, 2019). The bridge fibers are likely the functionally relevant parts within the bridge that provide stiffness. In human, a striking relationship preserved from base to apex is that the BM and bridge width are about the same (Figure 3.2). Previous motion measurements showed that the maximum motion of the CP near the base of the cochlea was near the boundary between the bridge and BM (Raufer, Guinan and Nakajima, 2019). It is likely that the maximum CP motion at different longitudinal locations is also near the BM-bridge boundary because the width relationship between the bridge and BM are preserved longitudinally. The organ of Corti sits only above the BM, and the maximum motion being near the BM-bridge boundary affects the rocking motion of the organ of Corti. The motion of the organ of Corti in humans will differ from the classic view, where the maximum motion is near the center of the BM (see discussion in Raufer, Guinan and Nakajima (2019)). Thus, it is likely that the input to the sensory mechanism at the organ of Corti, specifically the shearing motion between the tectorial membrane and hair cell stereocilia, will differ from the classic view in humans (Raufer, Guinan and Nakajima, 2019). However, until more detailed measurements are available, we can only speculate about the consequences of the human CP anatomy on the micromechanical motions within the organ of Corti.

A less speculative theory we consider for the role of an exceptionally wide and mobile OSL and the existence and motion of the bridge is the following: The motion of the OSL and bridge will increase the volume compliance of the CP. As a result, the more compliant CP will operate at a lower frequency range. Certainly, low-frequency hearing and limited high-frequency hearing are unique features of large land-living mammals like humans (Manoussaki et al., 2008; Manley, 2012), which could be achieved by anatomical modifications described in this report.
METHODS

Measurements of cochlear partition dimensions. Mid-modiolar histological sections of normal cochleae of 13 females and 8 males aged between 11-99 years (mean 70.2 years) were used to measure anatomical structures of the cochlear partition. Horizontal bone cuts (n=15), vertical bone cuts (n=6), and bone cuts in the Poeschl plane (n=1, contralateral side of one specimen used for vertical cut) were used to obtain measurements from up to 11 locations in the cochlea. Specimens were chosen from the temporal bone collection at Massachusetts Eye and Ear and had already been prepared before the start of this study by the techniques described in (Merchant and Nadol, 2010). The thickness of the sections was 20 µm; the temporal bone specimens were decalcified, embedded in celloidin and stained with H&E (hematoxylin and eosin). The specimens were photographed using a Nikon E400 microscope and a Nikon DS-Ri2 color camera with a resolution of 4080×3072 pixels.

Five different measurements were performed in each of the cochlear sections (c.f. Figure 3.2). The structures measured were (i) the width of the basilar membrane, (ii) the width of the CP bridge, (iii) the width of the OSL, (iv) the height of the OSL including the nerve fibers, and (v) the width difference between the tympanic and vestibular plates of the OSL. The width of the BM was measured following the approach of Bhatt et al. (2001) and Liu et al. (2015). The width of the CP bridge was defined as the width between the BM and the lateral end of the OSL vestibular plate. The width of the OSL was defined as the width between the lateral end of the vestibular plate of the OSL and the modiolus. The height of the OSL was defined as the transverse distance between the two OSL plates. The measurements were made using the software Image J, analyzed in MATLAB (version R2013b). Figure panels were arranged in Adobe Illustrator CS6.
Creating a three-dimensional model of the OSL microstructure. For reconstructing the microstructure of the OSL, we cut 2 µm thick sections of the CP from a specimen embedded in plastic 2.5 hours post-mortem by standard methods at the Otopatology Laboratory at Mass Eye and Ear. The cochlea was from a 63 year old male and we investigated three different longitudinal locations of that cochlea (1 mm, 9 mm, and 12 mm from the base). For every location, we cut 100-120 2 µm serial sections. The sections were cut in a cross-sectional view using an ultramicrotome (LKB Bromma, 2128 Ultramicrotome). Every section was put in a separate drop of deionized water on a glass slide. The water evaporated after placing the slide on a hot plate at ~110-120°C for ~20-30 seconds. When dry, the sections were stained with Toluidine blue on the hot plate for 7-8 seconds, after which the stain was washed off with regular water. The remaining water evaporated by drying the stained sections on the hot plate before and a cover slip was mounted on the slide.

Each of the 2 µm sections was photographed with the Nikon camera system described above. The images were pre-processed in Adobe Photoshop CS6, where one color channel was extracted to reduce the image size. Depending on which channel showed the best contrast, the blue or green channel of the RGB image was chosen. Images of damaged sections were removed and replaced with duplicates of images before or after the damaged section—this was the case in 2-3% of the cases. After pre-processing of the images, the stack of images was imported into Amira 6.4.0, where the 3D reconstructions were obtained. In Amira, the stack of images was aligned semi-automatically by using the Amira alignment tool. The OSL bone was selected by using the ”Magic Wand” tool in Amira. The stained bone of the OSL could be clearly distinguished from the surrounding soft tissue and structures. The tympanic and vestibular plates of the OSL were selected separately to reconstruct the 3D models of both OSL plates using the
Amira “Generate Surface” and “Surface View” functions. The 3D model could be rotated to obtain a face-on view of the two plates of the OSL as presented in Figure 3.4. To quantify the porosity of the OSL, we used the software Image J 1.8.0.

**Visualizing bridge fibers with birefringence microscopy.** Birefringence refers to the property of anisotropic media by which light propagating in specific directions experiences different indices of refraction (hence, different propagation velocities) for different orientations of the optical polarization. It is a consequence of the inherent difference in the optical polarizability of an anisotropic medium along different axes. A relative phase delay, termed the optical retardance, accumulates between the two polarization components of the light. The effect on polarized light propagating through the sample is to mix its polarization states.

The simplest form of polarized light microscopy entails linear polarizers in the illumination and detection arms that have transmission axes at 90° to each other. Without a sample in the object plane, no light would reach the camera through the crossed polarizers; but, when a specimen is placed between the polarizers, its structurally anisotropic features (such as areas of spatially organized collagen fibers) will generate a bright image against the dark background. In short, the sample induces relative retardance between the components of the incident linear polarization, leading to a mixed polarization state, providing enhanced contrast for anisotropic structures (see, for example, (Hecht, 2002)).

In our studies, to visualize fibers within the CP bridge, a differential interference contrast (DIC) microscope (Nikon E400) was used, with one of the Nomarski prisms removed. The specimens were illuminated with polarized light, where the white light from the lamp source was filtered by a linear polarizer before the specimen, and a crossed linear polarizer (oriented at 90°
with respect to the illumination polarizer) after the specimen (and objective lens). In the images shown in Figure 3.5, however, the background intensity is not fully dark. This is due to the fact that only one of the Nomarski prisms was removed from the DIC setup. While this results in less-than-optimum contrast for birefringent structures (e.g., the bridge fibers), the advantage is that non-birefringent, stained structures can still be seen at the same time. To obtain maximum contrast of the bridge fibers, the specimen was oriented with the long axis of the fibers at ~45° with respect to the crossed polarizers (Murphy, 2001). The highly anisotropic structure of collagen fibers renders them strongly birefringent, so they are still contrast-enhanced with this compromised setup. We studied the collagen fibers within the bridge region in a large number of specimens and chose representatives for Figure 3.5.

We tried different slice thicknesses (2 µm, 6 µm, and 20 µm) and stains (Pentachrome, Gomori Trichrome, Mallory Aniline) and concluded that the regular 20 µm, H&E stained sections (same specimens as used for cochlear partition anatomy measurements) were most appropriate for this study and accurately represented the anatomy of the bridge fibers. The images shown in Figure 3.5 are representatives of a large pool of specimens analyzed.

The images from the four different locations in Figure 3.5 are from four different specimens. Because of aging of the sample, speckles appeared in the images taken with crossed polarized light (see Figure 3.6). The speckles were more prominent in specimens that were mounted a long time ago (10 years and longer). The speckles in the empty background were removed in Adobe Photoshop. Importantly, only the empty background was edited in Adobe Photoshop, speckles close to or within tissue were not removed.
Figure 3.6 Image of the cochlear partition visualized with polarized light microscopy. Here, the speckles created by the aging sample were not removed digitally and are visible as white dots in the whole image. In Figure 3.5, the speckles in the empty space were removed in Adobe Photoshop to increase readability of the figure. No modifications were done in areas occupied by cochlear structures.
CHAPTER 4. Low-frequency hearing in humans: Characterizing sound flow in ears affected by a mechanical inner ear pathology


SR, and HHN designed research. SR, SFM, and HHN conducted research. SR analyzed data. SR, SFM, and HHN wrote manuscript.
ABSTRACT

The transmission of infrasound within the human ear is not well understood. To investigate infrasound propagation through the middle and inner ear, stapes and round window membrane velocities were measured to very low frequencies (down to 0.9 Hz from 2000 Hz) in fresh cadaveric human specimens. Results from ear-canal sound stimulation responses show that below 200 Hz, the middle ear impedance is dominated by its stiffness term, limiting sound transmission to the inner ear. During air-conduction, normal ears have approximately equal volume velocities at the oval (stapes) and round windows, known as a two-window system. However, perturbing the impedance of the inner ear with a superior semicircular canal dehiscence (SCD), a pathological opening of the bone surrounding the semicircular canal, breaks down this simple two-window system. SCD changes the volume velocity flow in the inner ear, particularly at low frequencies. The experimental findings and model predictions in this study demonstrate that low-frequency auditory and vestibular sound transmission can be affected by a change in the inner-ear impedance due to an SCD.
INTRODUCTION

It is widely believed that human hearing is insensitive to infrasound, defined as frequencies below 20 Hz. However, there is evidence that infrasound can alter the processing of sound in the cochlea. For example, animal studies show that exposure to infrasound can modulate the endocochlear potential, leading to a change in the electrochemical voltage that drives the receptor current through the transduction channels of the auditory hair cells (Salt and DeMott, 1999; Salt et al., 2013). Moreover, high intensity, low frequency bias tones can alter distortion product otoacoustic emissions and shift the frequency and level of spontaneous otoacoustic emissions, indicating that cochlear processing is affected by infrasound in humans (Hensel et al., 2007; Marquardt et al., 2007; Kugler et al., 2014). The long-term consequences of exposure to infrasound are not clear, but subjective reports claim that exposure to infrasound affects sleep habits, disrupts work performance, and compromises the well-being of the population (e.g. review Baliatsas et al. 2016). Given these observations, it is worthwhile to objectively investigate how infrasound propagates through the human middle and inner ear, which we address here.

In addition to studies of hearing thresholds, it has been shown that the vestibular system, equipped with specialized low frequency sensing organs, is also sensitive to acoustic stimulation (Young, Fernández and Goldberg, 1977; Møller and Pedersen, 2004). The experiments in the mentioned studies rely on infrasound entering the inner ear via the middle ear. However, the mechanical constraints that the middle ear and inner ear impose on the transmission of infrasound are unknown.

To understand how infrasound is transmitted, this study describes low-frequency middle ear and inner ear transfer functions in fresh cadaveric normal ears. This study also determines
how these transfer functions change due to perturbation of inner-ear mechanics. We will specifically focus on semicircular canal dehiscence (SCD), a disorder characterized by an abnormal opening of the bone surrounding the superior semicircular canal, which was shown to change the transmission of sound especially at low frequencies (Chien et al., 2007; Pisano et al., 2012; Niesten et al., 2015). With a prevalence of 0.7% to 1.9% in the US population, SCD is being recognized as a relatively common otologic pathology (Minor et al., 1998; Carey, Minor and Nager, 2000). Vestibular and auditory symptoms, many of which are induced by low-frequency sounds, including vertigo and autophony, are debilitating for the patients (Minor, 2005).

In the normal ear, air-conducted sound is transmitted through a “two-window” system, consisting of the oval window and the round window membrane (RWM). In the two-window system, the volume velocity of the oval window is equal to the volume velocity of the round window—no appreciable volume velocity is lost to another sound-conducting path in the inner ear (Stenfelt, Hato and Goode, 2004)\(^1\). In patients with SCD, an additional sound-conducting path, known as a “third window,” is added within the inner ear, changing the distribution of volume velocities. Figure 4.1 shows a circuit diagram of a normal ear (panel a) and an ear with SCD (panel b). In the normal ear, the volume velocity \( U \) at each node is identical \( (U_{\text{Normal}}^{\text{Normal}} = U_{\text{Dif}}^{\text{Normal}} = U_{\text{RWM}}^{\text{Normal}}) \). However, in the SCD-affected ear, the volume velocity is divided in the vestibule and can flow through 1) the series impedances of the cochlear partition \( (Z_{\text{Diff}}) \) and RWM \( (Z_{\text{RWM}}) \); or 2) the SCD impedance \( (Z_{\text{SCD}}) \). \( Z_{\text{Diff}} \), the “differential impedance” across the

\(^1\) Micro openings, such as the cochlear and vestibular aqueducts, can be neglected in AC stimulation as their impedance magnitude is much higher than the impedance of the oval window and round window.
cochlear partition is defined as the impedance at the base of the cochlea (near the oval and round windows), and is also influenced by the helicotrema (Nakajima et al. 2009). \[ Z_{\text{Diff}} = \frac{P_{\text{Diff}}}{U_{\text{Diff}}}, \]

where the differential pressure across the partition \( P_{\text{Diff}} \) is defined as \[ P_{\text{Diff}} = P_{SV} - P_{ST} \]

\( P_{SV} \) is the pressure in scala vestibuli and \( P_{ST} \) the pressure in scala tympani near the oval and round windows) (Nakajima et al., 2009). Experiments on fresh cadaveric human specimens show that SCD causes decreases in RWM velocities, \( P_{SV}, P_{ST}, \) and \( P_{\text{Diff}} \) at frequencies below 1000 Hz, resulting in decreased low-frequency air-conducted sound transmission across the partition, consistent with low-frequency hearing loss in SCD patients (Pisano et al. 2012; Niesten et al. 2015; Minor 2005). Additionally, SCD results in shunting of volume velocity from the oval window to the SCD, resulting in sound-induced stimulation of the ampulla of the vestibular system and vertigo known as Tullio phenomenon (Minor et al., 1998).
Figure 4.1: Schematic and impedances of the inner ear. a) The circuit of the normal inner ear consists of the differential impedance of the cochlear partition ($Z_{\text{Diff}}$), and the impedance of the round window membrane ($Z_{RWM}$). $Z_{\text{Diff}}$ is not a local impedance of the basilar membrane, but defined as the differential pressure across the partition at the base, over the volume velocity of the stapes ($U_{\text{Stapes}}$), i.e. $Z_{\text{Diff}} = (P_{SV} - P_{ST})/U_{\text{Stapes}}$, where $P_{SV}$ and $P_{ST}$ are pressures in scala vestibuli and scala tympani at the base. $Z_{\text{Diff}}$ is also influenced by the helicotrema. The volume velocities of the stapes ($U_{\text{Stapes}}$), across the partition ($U_{\text{Diff}}$), and the round window membrane ($U_{RWM}$) are shown with arrows and are equivalent during air conduction in normal ears. b) Circuit model for the SCD case includes an impedance and volume velocity for the semicircular canal ($Z_{SCD}$, $U_{SCD}$). Figure adapted from Stieger et al. (2013).
An SCD functions as an acoustic volume velocity leak between the inner ear vestibule and the middle cranial fossa. Because of the low-pass characteristics of a small opening, an SCD predominantly influences auditory and vestibular thresholds at low frequencies (Gopen et al. 1997 have shown these low-pass characteristics for the cochlea aqueduct). These changes have been observed in clinical studies and temporal bone experiments, but because previous measurements were made only for frequencies above 100 Hz, the effects of an SCD on intracochlear pressures, RWM velocities, audiometric thresholds, and vestibular thresholds were small (0-15 dB) (Chien et al., 2007; Pisano et al., 2012; McEvoy et al., 2013; Milojcic et al., 2013; Niesten et al., 2015). Without lower frequency information, we lack an understanding of the acoustic effects of SCD.

In this present study, we characterize stapes and RWM velocities for the first time down to very low frequencies of 0.9 Hz. We then determine the effects of a mechanical perturbation to the inner ear (in form of an SCD) on stapes and RWM velocities at low frequencies and infrasound. To characterize and understand the mechano-acoustic mechanisms behind SCD, we apply a lumped element network model.

MATERIALS AND METHODS
Temporal bones. The study was approved by the Institutional Review Board of the Massachusetts Eye and Ear. Five fresh human temporal bones (without the use of fixative) were used for this study. Upon death of the donor, the temporal bones were removed from the skull base using a Schuknecht plug cutter (J. Nadol, 1996) at Massachusetts General Hospital. Minutes after removal, the specimens were stored in 250 ml of 0.9% saline with two drops of betadine.
solution and stored in a refrigerator. The saline solution was renewed every 24 hours between the bone extraction and the experiment. Three of the temporal bones were kept in the refrigerator before the experiment (1-6 days) and two temporal bones were frozen shortly after extraction and then defrosted for experiments. To defrost the frozen temporal bone specimens, we placed it in room-temperature saline for 30 minutes. Experiments were conducted at room temperature. The age range of the donors was between 28 and 85 years.

To enable access to the middle-ear ossicles and the round window membrane (RWM), we exposed the middle ear cavity and epitympanic space by drilling out the bone surrounding the posterior-lateral aspect of the temporal bone. We severed the stapedial tendon to access the area close to the stapes and to have an unobstructed view of the posterior crus of the stapes. Initial measurements of stapes and RWM velocities were performed to confirm that no air entered the cochlea during the preparation process. A phase difference of one-half cycle between stapes and RWM velocities at frequencies between 50-300 Hz was a good indicator that no air was enclosed in the inner ear (Nakajima et al. 2009). After baseline measurements for the normal ear, a dehiscence was introduced at the lateral aspect of the semicircular canal near the arcuate eminence with varying size between 0.21 mm$^2$ and 0.7 mm$^2$ across specimens. We used a micro-measurement device that allowed us to measure the SCD under the operating microscope to within a certainty of 50-100 microns. It is rather difficult to control the SCD size during an experiment, which is why the sizes differed, but the sizes of the SCDs we introduced were comparable to other studies and within the range of what is observed in patients (Pisano et al., 2012; Niesten et al., 2015). The lateral aspect of the canal was covered by a fluid column of about 0.5 mm to prevent air from entering the inner ear (see Pisano et al. 2012; Niesten et al. 2015 for details). The time between baseline measurements and SCD measurements were as
much as 30 minutes. Similarly, patching the SCD to restore the baseline measurements took up to 30 minutes. Thus, the time elapsed between initial and final measurements could have been up to 1h. The middle ear was kept moist during that time to prevent it from drying.

We used normal specimens without known middle and inner-ear pathologies. In this series of experiments, we included five out of eight experiments (three experiments were abandoned early). The five experiments that were completed had baseline stapes velocities that were within or near the range of Rosowski et al. (2007) standards and had a signal-to-noise ratio >10 dB. We did include an experiment with a stapes velocity resonant frequency near 350 Hz (this low resonance might be due to a compliant middle-ear system). Because this study focuses on low frequencies, we included this experimental result (below 200 Hz, the data was within the standard, we plotted the data with a dashed line, distinguishing it from the other experiments that are plotted with solid lines). Fresh human cadaveric temporal bone specimens including those previously frozen have been shown to have very similar middle and inner ear macro-mechanical properties to that of the living human, which is why a temporal bone model is appropriate for this work (Rosowski et al., 1990; Chien et al., 2009). Because of the high consistency of our data, agreement with previous studies (Chien et al. 2007; Niesten et al. 2015; Pisano et al. 2012 to 100 Hz), and our ability to reverse the effects of our manipulation (a strong experimental control), five temporal bones seemed sufficient for this technically challenging study.

**Sound Stimulation.** Acoustic pure tones between 0.9 Hz and 2 kHz (3 points per octave in the frequency range 0.9 – 6 Hz; 5 per octave between 5-100 Hz; and 10 points per octave between 50-2000 Hz) were generated by a Beyerdynamics DT 770M headphone driver and delivered to the ear canal via a flexible polyethylene tube. The time to measure all frequencies for each
condition was 3-4 minutes. The sound levels employed were between 75-110 dB SPL, i.e. in the linear operating range of the middle ear (Greene et al., 2017). Details regarding the generation of high-intensity, low-frequency pure tones with commercial headphone drivers can be found in the appendix, and have also been presented elsewhere (Hensel et al. 2007). All data acquisition in response to sound (from microphone and vibrometry) used a sampling frequency of 500kS/s.

**Microphone.** The sound pressure level in the sealed ear canal was monitored with a Brüel & Kjær (BK) infrasound microphone (Type 4964 pre-polarized cartridge, Type 2671-W-001 preamplifier, and 1704-A-001 signal conditioner). A polyethylene tube with an inner radius of 1.5 mm and 2 inches in length was used to connect the microphone to the ear canal. Mounting putty was used to seal the microphone tube as well as the speaker tube to the ear canal. The infrasound microphone was calibrated in a standard manner (see Appendix for details).

**Velocity measurements.** A commercial laser Doppler vibrometry system (Polytech CLV 700) was used to measure velocities of the posterior crus of the stapes and the RWM. Three to six auto reflective, metal coated beads (50 μm in diameter and approximately 0.07 μg each) were placed on the posterior crus of the stapes and the center of the RWM to enhance the signal-to-noise ratio for stapes and RWM measurements. Keeping the stapes footplate dry for measurement is difficult because the SCD region needs to be under fluid. We find that the posterior crus and footplate center have similar measurements at low frequencies (<2 kHz) if the laser is as close to perpendicular to the plane of the footplate and if the laser direction is parallel to the plane defined by the posterior and anterior crus of the stapes (to prevent measuring superior-inferior rocking of the stapes). Stapes measurements were made at an angle of
approximately 30 degrees with respect to the axis of the piston-type motion; a cosine-correction was not taken into account because it would change the magnitude only by about 1 dB. The measurement location and laser-beam angle before and after SCD did not change. For the RWM measurements, we tried to be consistent across specimens with the placement of the beads near the center of the RWM. The RWM is not a perfectly circular membrane, but has been described as a hyperbolic paraboloid with almost a flat area near the center. The noise floor of the laser measurement was determined by measuring the velocity of the cochlear promontory during acoustic stimulation.

To allow for low-frequency velocity measurements, the noise floor of the system needed to be reduced considerably. Four interventions enabled measuring stapes and RWM responses down to 0.9 Hz. First, the temporal bone holder was placed on an air table inside a double-walled booth with an elevated floor to isolate it from vibratory building noise. Second, the microscope, where the laser system was attached to, was mounted on the same air-floating table, reducing the differential modes between specimen and laser system. Third, the position of the laser was enforced by supporting the headpiece of the microscope with rigid rods and clamps to further minimize relative motion between the specimen and laser system. Fourth, relatively high sound pressure levels (between 90-110 dB SPL at low frequencies, checked for linear operation detailed below) were used to record stapes and RWM velocities.

**Harmonic distortions and noise sources.** Figure 4.2a shows an example of the sound pressure in the ear canal (continuous solid line) and the spectrum during the presentation of a 6 Hz pure tone, indicated as $f_0$. The odd-order harmonic distortion products are indicated. The nonlinearities, produced by the loudspeaker, were 30-40 dB (~1%) below the level of the probe
frequency for all stimulus frequencies used. The acoustic noise floor in our sound-attenuating chamber is considerably higher at low frequencies—the slope of the noise floor below 200 Hz is approximately -40 dB/decade. Figure 4.2b shows an example of a raw stapes velocity measurement (thick solid line) next to the velocity of the cochlear bone in response to the same acoustic input at the ear canal. At around 3 Hz and 12 Hz, the laser Doppler vibrometer (LDV) picked up considerable vibration of the specimen, possibly due to vibrational building noise in the environment. For our measurements in normal ears, a signal-to-noise ratio of >10 dB was maintained at all frequencies.
Figure 4.2: Distortion and noise of the acoustic stimuli: a) Sound pressure measured in the ear canal (continuous line) with a typical noise floor (dotted line) during the presentation of a 6 Hz pure tone. Harmonic distortions of the loudspeaker were 30-40 dB below the primary level for all frequencies. b) Stapes velocity measurements compared to the velocity of the cochlear promontory during sound stimulation (raw measurements, not referenced to ear canal pressure).
Details regarding sound generation and microphone calibration. The loudspeaker (driver of a Beyerdynamics DT 770M) was removed from the headphone housing and mounted on a flat wooden board to limit the volume in front of the loudspeaker membrane to about 2 cm$^3$ (see Figure 4.3). At the center of the wooden board, a hole was drilled to tightly attach a flexible polyethylene tube to deliver sound to the ear canal. The inner diameter of the polyethylene tube was 4 mm with a length of 2 inches. In the experiment, the polyethylene tube that is attached to the loudspeaker on one end was sealed to the ear canal on the other end with mounting putty.

During microphone calibration (Figure 4.3), we simulated the ear canal volume with a body of a plastic syringe. The total volume of the acoustic field consisted of the ear canal volume, the volume in front of the loudspeaker membrane, the volume of the polyethylene tube connecting the loudspeaker to the ear canal, and the volume of another polyethylene tube used to connect the microphone to the ear canal.

The microphone was calibrated in a standard manner, using a 2 cm$^3$ uniform tube (the body of a 6 ml Monoject plastic syringe) that simulated the ear canal (see Figure 4.3). The polyethylene tubing coming from the loudspeaker was attached to one end of the syringe body and held in place with a perforated foam ear plug. The $\frac{1}{4}$" BK microphone was placed at the other end of the syringe body (reference location) where the tympanic membrane would be, leaving a total volume of 2 cm$^3$ between the microphone cartridge and the end of the polyethylene loudspeaker tubing. We perforated the wall of the syringe body near the microphone to attach another polyethylene tube with an inner radius of 1.5 mm and 2 inches in length (used in the actual experiment) at which end the probe microphone was located. We exchanged the position of the BK microphone from the reference location to the probe location but had another microphone cartridge at the other location to keep the acoustic field unchanged.
Not surprising for the long wavelengths, we observed small differences (within 1 dB) between the two measurement locations for frequencies between 0.9 Hz and 2 kHz, which we accounted for by correcting the response of the probe-tube microphone by the calibration file.
Figure 4.3: Schematic of the set-up to calibrate the probe tube microphone. In order to generate high-intensity low-frequency sound pressures with a conventional loudspeaker, the acoustic field has to be closed and the volume minimized. The total volume of the acoustic field consist of the volume in front of the loudspeaker membrane, the polyethylene (PE) tube connecting the loudspeaker to the ear-canal, the ear canal volume (simulated by the body of a syringe), and the PE tube with adapter for the probe tube microphone.
RESULTS

Stapes and Round window membrane velocities in normal ears

Figure 4.4a shows the magnitude and phase response of stapes velocity transfer functions (SVTFs; stapes velocity referenced to ear-canal pressure) for five fresh human specimens. We show reference data (95% confidence interval across 13 studies) from Rosowski et al. (2007) as gray shaded area.

For individual specimen (Figure 4.4a colored lines), the magnitudes of the SVTFs between 0.9 Hz and 300 Hz are increasing proportionally by about 20 dB/decade (dashed black reference line indicates an increase of 20 dB/decade). The frequency corresponding to the peak magnitude varies from 350 Hz to 1 kHz across specimens, and the absolute values of the maximum vary from $1 \times 10^{-4}$ to $2 \times 10^{-4}$ m/s Pa$^{-1}$. For higher frequencies, SVTFs generally decrease with increasing frequency. However, this magnitude decrease was not necessarily monotonic and the phase varied with frequency. Complex motions of the stapes can lead to distinct anti-resonances at frequencies above 2 kHz (Rosowski et al., 2007). Such complex behavior is not observed at frequencies below 300 Hz, where the magnitudes of the SVTFs increase monotonically with frequency. Below 300 Hz, the velocity of the stapes leads the ear canal pressure by a quarter-cycle, as can be observed in the phase response. The monotonically increasing magnitude and quarter-cycle phase relative to the ear canal pressure imply a stiffness-dominated middle ear and is consistent with previous studies (Rosowski et al., 2007; Greene et al., 2017).

An interesting new observation is that below 10 Hz, the phase of the SVTF starts deviating from 0.25 cycles towards 0 cycles, consistent with the SVTF becoming more resistive.
toward lower frequencies. In the same frequency range, the increase in magnitude is less than 20 dB/octave. Below 10 Hz, the middle ear may have additional resistive components (frictional losses) that are not accounted for in current middle ear models, which predict a perfectly horizontal phase at low frequencies (Kringlebotn, 1988; Rosowski and Merchant, 1995).

Figure 4.4b shows the velocity of the RWM referenced to the sound pressure in the ear canal. Similarly to the stapes, the velocity of the RWM increases by about 20 dB/decade for frequencies between 1-200 Hz. RWM velocity magnitudes, measured at the center of the RWM, are generally higher than the velocity of the posterior crus of the stapes (as a reference, the horizontal grid lines in each graph represent the same magnitudes). This observation is consistent with previous measurements of RWM motion, where it was shown that the center of the RWM moves considerably more than the stapes footplate (Stenfelt, Hato and Goode, 2004). The phase of the RWM is lagging the ear canal pressure by a quarter-cycle at frequencies below 200 Hz, leading to a phase difference between stapes and RWM of 0.5 cycles (the RWM bulges out when the stapes pushes in).
Figure 4.4: Normal data for five ears: a) Individual stapes velocities ($v_{Stapes}$) referenced to ear canal pressure ($P_{EC}$). Gray-shaded area is normative data from Rosowski et al. (2007). One specimen had a low resonance (at 350 Hz, blue dashed line) with similar response to other ears at lower frequencies. b) Individual round window membrane velocities ($v_{RWM}$) from the center of the RWM referenced to $P_{EC}$. Black dashed lines in panels (a) and (b) indicate an increase by 20 dB/decade. The artifact was determined by measuring the vibration of the cochlear promontory during sound stimulation.
The effect of semicircular canal dehiscence (SCD) on stapes and round window membrane velocities

Figure 4.5 follows the same format as Figure 4.4, but the measurements were recorded after a dehiscence was introduced at the lateral aspect of the semicircular canal near the arcuate eminence. The size of the dehiscence varied between 0.21 mm$^2$ and 0.7 mm$^2$ across bones.

After SCD, stapes vibration (Figure 4.5a) are similar to measurements in normal ears (before SCD, Figure 4.4a). For frequencies between 0.9 Hz and 200 Hz, the magnitude of SVTFs increase by 20 dB/decade and the velocity of the stapes are leading the ear canal pressure by 0.25 cycles. Thus, in SCD-affected ears, the volume velocity entering the inner ear is not notably different from the normal ear for low frequencies.

The response of the RWM (Figure 4.5b), unlike the stapes, is greatly affected by the dehiscence in the otic capsule. Compared to the reference measurements in Figure 4.4b, the slope of the magnitude is more variable and almost twice as large, i.e. 35-40 dB/decade with SCD (a black dashed line of 40 dB/decade is plotted in Figure 4.4b for reference). At frequencies below 300 Hz, the phase response of the SCD-affected ears is more positive compared to the normal ears in Figure 4.4b.
Figure 4.5: Superior canal dehiscence (SCD) data for five ears: a) Individual stapes velocities ($v_{Stapes}$) referenced to ear canal pressure ($P_{EC}$) with SCD. b) Individual round window membrane velocities ($v_{RWM}$) referenced to $P_{EC}$. The black dashed line in panel (a) indicates an increase by 20 dB/decade; the black dashed line in panel (b) by 40 dB/decade. The data represented by the purple line is in the noise below 10 Hz and is not reliable in this frequency range.
Another way to quantify the effects of SCD on stapes and RWM velocities is by plotting the ratio (difference in log-domain) between the SCD and normal condition. In Figure 4.6a, the SCD effect on stapes velocities is plotted for the individual tested specimens. Four out of the five tested specimens show a slight increase in stapes velocity after an SCD was introduced. The increase in stapes velocity is \( \leq 5 \) dB and similar across frequencies. One of the tested specimens shows a slight decrease in stapes velocity after the dehiscence was created, but the effect is <3 dB across frequencies. On average, stapes velocities increased by 1.89 dB. The phase is unchanged for frequencies below 300 Hz. Above 300 Hz, we observe slight differences of 0.05-0.1 cycles in the individual experiments, but no notable changes on average.

Figure 4.6b shows that with SCD, RWM velocities exhibit a profound decrease at low frequencies compared to the reference measurements: the changes in RWM velocity magnitude due to SCD show decreases of 10-35 dB per decade from high to low frequencies (100 Hz to 1 Hz). This is accompanied by a change in phase by +0.2 to +0.25 cycles (Figure 4.6b). The frequency range at which the SCD causes a systematic decrease in RWM velocity magnitude with decreasing frequency varies greatly across ears, leading to a much larger variance after SCD as compared to our reference measurements. In one specimen the RWM exhibits a decrease in velocity for frequencies below 1000 Hz (green line), whereas another specimen exhibits a decrease in RWM velocity only below 200 Hz (red line). Similar observations were made in Chien et al. (2007) though their low frequency limit was 100 Hz (gray shaded areas in Figure 4.6) and thus, the effect of an SCD they observed was much smaller.

The decrease in RWM velocity and change in stapes velocity was reversible in all specimens when the SCD was patched, as indicated by thin black lines in Figure 4.6.
Figure 4.6: Effect of SCD: a) Changes in magnitude and phase of stapes velocity ($v_{\text{Stapes}}$) due to superior semicircular canal dehiscence (SCD). On average (bold black line), the stapes velocity increased by 1.89 dB after SCD. The SCD effect was reversible (plotted with thin black lines) within 1 dB in four specimens and to within 2 dB in one specimen. Gray-shaded area represents 95% confidence interval from Chien et al. (2007) b) Change in round window membrane velocity ($v_{\text{RWM}}$) due to SCD.
Round window membrane velocities referenced to stapes velocities

A useful way by which stapes and RWM velocities can be analyzed is by determining the ratio between RWM velocity and stapes velocity. This provides insights into losses occurring between the oval window and round window.

Figure 4.7a plots RWM velocities referenced to stapes velocities for the normal ear. The positive values of the magnitude imply that the velocity of the RWM center is higher than that of the stapes. Although it is known that the volume velocities of the oval window and round window are generally equal in air conduction, it is not surprising that the center of the RWM moves more than the posterior crus of the stapes (Stenfelt, Hato and Goode, 2004). While the stapes is rigid and the excursion of the posterior crus is similar to the piston-type excursion of the whole oval window at these low frequencies, the RWM is very compliant and the excursion near the center of the membrane (our measurement location) is largest compared to other locations on the RWM (Voss, Rosowski and Peake, 1996; Stenfelt, Hato and Goode, 2004). In our experiments, the velocity of the center of the RWM is approximately 5-15 dB larger than the velocity of the posterior crus of the stapes. For frequencies below 300 Hz, the difference between stapes and RWM velocity is independent of frequency.

In the normal ear (Figure 4.7a), the phase response of the stapes and RWM velocities are generally 0.5 cycles out of phase for frequencies between 10 and 200 Hz, which indicates that the cochlear impedance is normal and the fluid in the cochlea is incompressible and free of air bubbles, and that there are no unusual fluid leaks or dehiscences of the otic capsule. In two out of five specimens, a perfect half-cycle relationship is observed for frequencies as low as 0.9 Hz (green and blue lines); four out of five bones show a perfect half-cycle difference down to 10 Hz (all but the purple line). The purple line deviates from the half-cycle below 10 Hz, which could
be explained by: experimental error (too much time elapsed during measurement of stapes and RWM velocities resulting in the accumulation of fluid on the RWM from residual saline in the surrounding middle ear space), small amounts of air in the cochlea, or an unusually low-impedance leak (for example through the aqueducts).

In Figure 4.7b, RWM velocities referenced to stapes velocities are shown for the SCD-affected specimens. Compared to the flat magnitude ratio results of normal ears in panel 6a, the SCD ratio magnitudes increase by about 20 dB/octave with increasing frequencies. The phase relationship of one-half cycle between stapes and RWM velocity is completely diminished at frequencies below 300 Hz, where it is converging to -0.3 to -0.25 cycles. The measurements in Figure 4.7b imply that low-frequency volume velocity is directed away from the RWM, towards the dehiscence.
Figure 4.7: Round window membrane velocities ($v_{RWM}$) referenced to stapes velocities ($v_{Stapes}$):

a) Individual normal data and b) after superior semicircular canal dehiscence (SCD). The black dashed line in panel b indicates a change of 20 dB/decade.
A circuit model for the inner ear with SCD

*Estimating the SCD Effect on $P_{\text{Diff}}$ with RWM velocity measurements:* To better understand and quantify the mechano-acoustic mechanisms involved, we use the lumped-element circuit model shown in Figure 4.1. The impedances, volume velocities, and pressures are complex numbers (magnitude and phase). The circuit in Figure 4.1 can be used to establish a relationship between the pressures in the inner ear and the RWM velocities. The circuit of the *normal* inner ear consists of a volume velocity source ($U_{\text{stapes}}^{\text{Normal}}$, at the stapes footplate), an impedance of the cochlear partition ($Z_{\text{Diff}}$, which includes the helicotrema), and the impedance of the round window membrane ($Z_{\text{RWM}}$). For the pressures in the inner ear, the cochlea input drive is defined as the differential pressure $P_{\text{Diff}}$ across the partition, i.e. $P_{\text{Diff}} = P_{SV} - P_{ST}$, where $P_{SV}$ and $P_{ST}$ are the pressures in scala vestibuli and scala tympani, measured in the cochlear base far from the partition. Note that the cochlear impedance $Z_C$ (as used in other studies) is not equal to $Z_{\text{Diff}}$, but defined as $Z_C = P_{SV}^{\text{Normal}}/U_{\text{stapes}}^{\text{Normal}} = Z_{\text{Diff}} + Z_{\text{RWM}}$ (Puria and Allen, 1991; Shera, 2007; Nakajima et al., 2009).

$P_{\text{Diff}}$ is an important measure, as it estimates the input drive to the cochlea and has frequency responses near identical to neurophysiological measures, such as cochlear microphonic (assuming that the neurosensory mechanism is intact, Dancer & Franke 1980; Nakajima et al. 2009). As shown in Figure 4.1, the differential pressure across the partition can be written as:

$$
P_{\text{Diff}}^{\text{Normal}} = U_{\text{RWM}}^{\text{Normal}} Z_{\text{Diff}}
$$

(1)

$$
P_{\text{Diff}}^{\text{SCD}} = U_{\text{RWM}}^{\text{SCD}} Z_{\text{Diff}}.
$$

(2)
By combining Eq. 1 and 2, the effect of SCD on the differential pressures, $\Delta P_{Diff}$, can be expressed by means of the change in RWM velocities, i.e.:

$$\Delta P_{Diff} = \frac{P_{Diff}^{SCD}}{P_{Diff}^{Normal}} = \frac{U_{RWM}^{SCD}}{U_{RWM}^{Normal}} \approx \frac{v_{RWM}^{SCD}}{v_{RWM}^{Normal}}.$$  \hspace{1cm} (3)

Equation 3 states that measuring a change in RWM velocity due to SCD is an estimate of a change in $P_{Diff}$, $\Delta P_{Diff}$. Because $\Delta P_{Diff}$ closely resembles a change in hearing sensitivity due to a macromechanical (not sensorineural) change, the change in RWM velocity as seen in Figure 4.6b can also be used to predict SCD-related hearing loss. Following the results in Figure 4.6b, we predict a low frequency hearing loss due to SCD, which is consistent with clinical findings (Minor et al., 1998). Furthermore, because volume velocity is shunted away from the cochlea towards the dehiscence, SCD patients can experience sound-induced dizziness (Tullio phenomenon) due to the shunted volume velocity stimulating the vestibular system (Minor et al., 1998).

**Determining the acoustic properties of an SCD:** We can use our experimental results in conjunction to a computational model to determine the acoustic properties of an SCD. In Figure 4.8 a & b, the values for $Z_{Diff}$ and $Z_{RWM}$ are similar to what has been used previously (Nakajima et al., 2009; Frear, Guan, Stieger and Nakajima, 2018). Notably, the most updated estimate of $Z_{Diff}$ consists of an RL element in parallel (instead of a resistor alone as used in older models), with $R=3.04\times10^{10}$ Nsm$^{-5}$ and $L=6.46\times10^{7}$ Ns$^2$m$^{-5}$ (Figure 4.8b). The round window impedance $Z_{RWM}$ is an RLC element in series with $R=1.47\times10^{10}$ Nsm$^{-5}$, $L=7.76\times10^{5}$ Ns$^2$m$^{-5}$, and $C=3.59\times10^{-14}$ N$^{-1}$m$^5$. The elements used for the impedances are presented in Figure 4.8b.
Figure 4.8: Modeling results: a) Impedance model employed with b) the respective lumped elements. c) Measured (black): experimental results from this study (same as average in Figure 4.6b). Model (green): model predictions from Eq. 7 using the parameter elements in panel (b). Measured $\Delta P_{Diff}$ (gray): actual measured change in $P_{Diff}$ from Pisano et al. (2012); Niesten et al. (2015) (n=5). $\Delta P_{Diff} = \frac{P_{Diff}^{SCD}}{P_{Diff}^{Normal}}$. 

\[
\begin{align*}
R_{Diff} &= 3.04 \times 10^{16} \text{ Ns}^{-1} \\
L_{Diff} &= 6.46 \times 10^{6} \text{ Ns}^{-2} \\
R_{RW} &= 1.47 \times 10^{16} \text{ Ns}^{-1} \\
L_{RW} &= 7.76 \times 10^{6} \text{ Ns}^{-2} \\
C_{RW} &= 3.59 \times 10^{-14} \text{ N}^{-2} \text{ m}^{-3}
\end{align*}
\]
Equation 3 states that the change in $P_{\text{Diff}}$ after an SCD ($\Delta P_{\text{Diff}}$) can be estimated by the change in RWM velocity $v^\text{SCD}_{\text{RWM}} / v^\text{Normal}_{\text{RWM}}$ (plotted in Figure 4.6b). Furthermore, using the model in Figure 4.1a and 4.1b, we can derive equations:

$$U^\text{Normal}_{\text{RWM}} (Z_{\text{Diff}} + Z_{\text{RWM}}) = U^\text{Normal}_{\text{Stapes}} (Z_{\text{Diff}} + Z_{\text{RWM}})$$

(4)

$$U^\text{SCD}_{\text{RWM}} (Z_{\text{Diff}} + Z_{\text{RWM}}) = U^\text{SCD}_{\text{Stapes}} (Z_{\text{Diff}} + Z_{\text{RWM}}) || (Z_{\text{SCD}}),$$

(5)

where the symbol “$||$” indicates the element is in parallel, as shown in Figure 4.1b. By taking the ratio of Eq. 5 and Eq. 4, we obtain the ratio of the RWM velocities before and after SCD:

$$\Delta P_{\text{Diff}} \approx \frac{v^\text{SCD}_{\text{RWM}}}{v^\text{Normal}_{\text{RWM}}} \approx U^\text{SCD}_{\text{RWM}} \left( \frac{U^\text{SCD}_{\text{Stapes}} (Z_{\text{Diff}} + Z_{\text{RWM}}) || (Z_{\text{SCD}})}{U^\text{Normal}_{\text{Stapes}} (Z_{\text{Diff}} + Z_{\text{RWM}})} \right).$$

(6)

Including our observation that stapes velocities increase by 1.89 dB (24%) after SCD (Figure 4.6a), Eq. 6 simplifies to:

$$\Delta P_{\text{Diff}} \approx 1.24 \left( 1 + \frac{Z_{\text{RWM}} + Z_{\text{Diff}}}{Z_{\text{SCD}}} \right)^{-1}$$

(7)

The SCD impedance $Z_{\text{SCD}}$ is found by fitting Eq. 7 with the known $Z_{\text{RWM}}$ and $Z_{\text{Diff}}$ to our experimentally measured changes in RWM velocities, enabling us to model $\Delta P_{\text{Diff}}$ (Figure 4.8c). The above derived relationships assume that 1) the measurement location on the stapes and RWM are the same for the normal and SCD ears, 2) the vibration modes of the stapes and RWM are unchanged by an SCD, and 3) the proportionality of the volume velocity to the measured...
point velocity of the stapes and RWM is the same for the normal and SCD ears, so that volume velocities are estimated by point velocities.

In Figure 4.8c, we plot the average of our experimental data \( \frac{v_{RWM}^{SCD}}{v_{RWM}^{Normal}} \), with black lines), the model calculations using Eq. 7 (green lines), and the average \( \Delta P_{Diff} \) obtained from experimental intracochlear pressure measurements (from Pisano et al. 2012 and Niesten et al. 2015 with gray lines). The values for the SCD impedance \( Z_{SCD} \) that result in the model fit in Figure 4.8c are: \( R_{SCD} = 2.5 \times 10^{10} \text{Nsm}^{-5} \) and \( L_{SCD} = 1.0 \times 10^{7} \text{Ns}^2 \text{m}^{-5} \). The computational model results closely follow our experimental data from velocity measurements. Furthermore, the model and our experimental data closely follow the experimental intracochlear pressure data as predicted by Eq. 3. Thus, we can refer to the change in RWM velocities as a change in the cochlear input drive, or \( \Delta P_{Diff} \).

In Figure 4.8c, we observe that the SCD affects \( \Delta P_{Diff} \) predominantly at low frequencies. At frequencies above 1 kHz, the SCD has only minor effects on the input drive. This is observed in the individual experimental results (Figure 4.6b colored lines) as well as in the model fit (Figure 4.8c green line), where the curves converge to magnitude changes of almost 0 dB for frequencies greater than 1 kHz. At lower frequencies, the SCD leads to a substantial loss in the cochlear input drive (large negative \( \Delta P_{Diff} \)). The magnitude decreases systematically as the frequency decreases, and the phase is converging to 0.25 cycles at low frequencies. For example, at 1 Hz the loss is as large as 40 dB. The SCD is acting like a first-order low-pass shunt, allowing low frequency volume velocities to pass more easily through the SCD impedance. As a result, the cochlear input drive is decreasing with decreasing frequency. Because the cochlear input drive is decreasing at low frequencies and the volume velocity passes through the semicircular
canal, the vestibular input drive (across the ampulla of the semicircular canal) increases at low frequencies with SCD.

**Sensitivity of model parameters**

To investigate whether the variability observed in Figure 4.8c is due to the variability of the SCD impedance or other elements in the circuit, we investigate the sensitivity of the parameters in our model. In Figure 4.9a and 4.9b, the SCD impedance $Z_{SCD}$ and the resistive component ($R_{SCD}$) of $Z_{SCD}$ are multiplied with scalars. Changing the SCD impedance in this manner changes $\Delta P_{Diff}$ (the extent to which the cochlear input drive is affected by SCD). Differences in the SCD impedance may explain the variation across ears observed in Figure 5b. The resistive component ($R_{SCD}$) of the SCD impedance alone can explain most of the low-frequency variation in SCD effect (Figure 4.9b), suggesting that the acoustic mass of the SCD plays a minor role at low frequencies compared to its resistive losses.

Figures 4.9c and 4.9d explore the consequences of altering the RWM impedance (and compliance, $C_{RW}$) on SCD’s effect on cochlear input drive, $\Delta P_{Diff}$. For our model, we use an average value for the round window membrane impedance ($Z_{RWM}$) across many experiments, however, $Z_{RWM}$ varies by at least an order of magnitude across specimens (Nakajima et al., 2009; Frear, Guan, Stieger and Nakajima, 2018). Changing the impedance or compliance of the RWM has a similar effect on $\Delta P_{Diff}$ as varying the SCD impedance, and can account for the variance we observe in the experimental measurements. The less compliant the RWM, the greater impact an SCD has on the cochlear and vestibular input drives. In Pisano et al. (2012) and Niesten et al. (2015), we showed that despite keeping the SCD size and location the same, $\Delta P_{Diff}$ differed across ears. These observations are consistent with our current modeling results in Figure 4.9c.
and d, where we show that the compliance of the RWM plays a critical role in how severely an SCD changes the pressures within the inner ear. The almost identical appearance of Figures 4.9c and 4.9d suggests that variations in the RWM compliance, as opposed to variations in the acoustic mass or resistive losses, play a more direct role in how $\Delta P_{Diff}$ is affected.
Figure 4.9: Perturbation of model parameters: The gray line in each panel represent the average experimental data (Figure 4.6), and the black continuous lines represent the best model fit to the experimental data. a) The SCD impedance and b) SCD resistance are multiplied with scalars ranging from 0.3 to 3. c) The round window membrane impedance and d) compliance are multiplied by scalars from 0.3 to 3. $\Delta P_{\text{Diff}} = \frac{P_{\text{SCD}}^{\text{Diff}}}{P_{\text{Norm}}^{\text{Diff}}}$. 
DISCUSSION

Ossicular motion at low frequencies and third-window effects

In Figure 4.10, we present a summary of our main findings. In normal ears (continuous lines), both stapes and RWM velocities are monotonically increasing with a slope of 20 dB/decade for frequencies between 0.9 Hz and 200 Hz. The phase at these low frequencies is close to ±0.25 cycles, although we observe a slight decrease in phase below 10 Hz, possibly due to resistive losses causing the phase to become more real (or closer to 0 cycles). The velocity of the center of the compliant RWM is about 10 dB higher than the velocity of the posterior crus of the stapes. Although the area ratio between the stapes footplate and RWM (2.39/3.85) would only predict a difference of 4 dB (if we assume that the volume velocities of stapes and RWM are equal, see Stenfelt et al. 2004), this assumption rests on a piston-like motion of both the stapes and RWM. Whereas the piston-like motion is a reasonable assumption for the stapes at low frequencies, the RWM behaves like a drum, clamped at its edges. As a result, the velocity of the center of the RWM is higher compared to other locations on the membrane and approximately 10 dB above the velocity of the stapes crus. Below 300 Hz, the half-cycle relationship between stapes and RWM is consistent with a simple relative motion between the two measurement locations (the RWM bulges out when the stapes pushes in).

Surprisingly, in normal ears (Figure 4.10), RWM motion appears to be more consistent (smaller standard deviation) across ears than the stapes motion at frequencies below 300 Hz. In the tested frequency range, SVTFs are barely affected by an SCD (dashed lines in Figure 4.10a). On the other hand, velocities of the RWM are changed by an SCD in a predictable manner.
(dashed lines in 4.10b), making it a promising tool to investigate impedance changes in the inner ear, such as SCD.

An important finding from this study is that the SCD induced a decrease in RWM velocities that is frequency dependent. In some bones, an SCD affects RWM velocities at frequencies below 2 kHz, whereas other specimens are affected only at frequencies lower than 200 Hz. Thus, inner-ear dehiscences can be left unnoticed when the frequency range of the measurements is not appropriately chosen. By extending the frequency range to lower frequencies, inner ear dehiscences can be reliably detected by measuring RWM velocities (Figure 4.10b).
Figure 4.10: Summary of normal and SCD data: a) Average stapes velocity ($v_{\text{Stapes}}$) referenced to ear canal pressure ($P_{\text{EC}}$). b) Average round window membrane velocity ($v_{\text{RWM}}$) referenced to $P_{\text{EC}}$. Shaded areas indicate ±1 standard deviation.
The effect of the size of the semicircular canal dehiscence

We investigated the effect of SCD size on stapes and RWM velocities in one of the five specimens (blue line in Figures 4.4-4.7). Figure 10a shows stapes velocities in the normal case (black continuous line) and its response under varying SCD sizes (colored lines). As was concluded in Figure 4.6, stapes velocities are generally unaffected by an SCD. The size of the SCD does not change this conclusion.

Interestingly for RWM velocities (Figure 4.11b), we observe than an increase in SCD size from the size of a pinhole (approximately 50 µm diameter) to a 0.2x0.2 mm dehiscence, increases the effect on RWM velocities. However, further increasing the size of the dehiscence by another millimeter or so did not produce much effect on RWM velocity.

Our current observations with RWM velocities are consistent with our previous findings from intracochlear pressure measurements (Pisano et al., 2012; Niesten et al., 2015) – an increase in SCD size decreases the sound transmission across the cochlear partition at low frequencies. However, once the SCD size exceeds certain dimensions, SCD effects on $P_{Diff}$ and RWM velocities are independent of the size of the SCD. It was also shown that the saturation size varied considerably across ears (Pisano et al., 2012; Niesten et al., 2015). Predictions were made by Songer and Rosowski and Kim et al., that the SCD size effect saturated when the area of the SCD equals the cross-sectional area of the canal (Songer and Rosowski, 2006; Kim, Steele and Puria, 2013). However, our experimental results from Pisano et al. and Niesten et al. contradicted this prediction (Pisano et al., 2012; Niesten et al., 2015). The cross-sectional diameter of the canal is approximately 1 mm. We found that the low-frequency decrease in sound transmission continued up to a length of 2 mm in some specimens (Pisano et al., 2012; Niesten et al., 2015). In the present study, our SCD size (between 0.21 and 0.7 mm$^2$) were
similar or below the cross-sectional area of the canal (~0.7 mm$^2$). We used these sizes because it is technically challenging to repair larger SCD sizes to completely reverse the effects of SCD. Our model is able to explain the consequences of SCD on the cochlear input drive below this saturation region. Figure 4.9b suggests that resistive losses (likely along the walls of the semicircular canal, as well as from the opening of the dehiscence itself) rather than an increase of the acoustic mass could be responsible for the size-specific decrease in the cochlear input drive at low frequencies.

In this present study (as well as in our earlier intracochlear pressure studies) where SCD size was similar across ears, the variance of our results in Figure 4.5b is likely attributed to anatomical variations across bones. Our present modeling results in Figure 4.9 show that changes in RWM impedance and compliance, which can vary by an order of magnitude across ears (Nakajima et al., 2009; Frear, Guan, Stieger and Nakajima, 2018), can change how an SCD affects the cochlear input drive, $\Delta P_{\text{Diff}}$. An increase in $Z_{\text{RWM}}$ (through decreasing the compliance of the RWM) results in a larger decrease of the cochlear input drive due to SCD. Therefore, our study may have implications for round window reinforcement surgery, where tissue and/or glue is used to stiffen the RWM (decrease its compliance) in hopes of reducing symptoms associated with SCD (Silverstein et al., 2014). Our present findings are inconsistent with this surgical notion. In fact, round window reinforcement may possibly worsen the auditory and/or vestibular symptoms associated with SCD at low frequencies.
Figure 4.11: Effect of semicircular canal dehiscence (SCD) size on a) stapes velocity ($v_{\text{Stapes}}$) and b) round window membrane velocity ($v_{\text{RWM}}$) referenced to ear canal pressure ($P_{\text{EC}}$). The data is presented from one specimen with different SCD sizes.
Effective dimensions of the semicircular canal with dehiscence

The anatomical dimensions of the superior semicircular canal can be used to calculate the acoustic mass and resistance within the canal associated with an SCD, based on equations for narrow tubes (Beranek, 1986). The acoustic impedance of a narrow tube is $Z = R + j\omega L_A$, where $R = 8\eta l/(\pi a^4)$ and $L_A = 4\omega l/(3\pi a^2)$ (see footnote 2 for details).

We extracted average values for $l$ and $a$ from Muren et al. (1986). The length $l$ refers to the distance between the vestibule and the opening of the canal near the arcuate eminence (about one third of the full revolution of the superior semicircular canal) and the radius $a$ refers to the inner radius of the bony part of the semicircular canal interfacing the inner-ear fluid. Using $l=1.44\text{ mm}\times120^\circ/360^\circ = 0.48\text{ mm}$ and $a = 1.06\text{ mm}/2 = 0.53\text{ mm}$, we obtain values of $R_A = 1.4\times10^7$ Ohm and $L_A = 7.3\times10^5$ Ohm (our modeling results in Figure 4.8c yielded: $R_{SCD} = 2.5\times10^{10}$ Ns$^2$m$^{-5}$ and $L_{SCD} = 1.0\times10^7$ Ns$^2$m$^{-5}$). Compared to the experimental results, the values of the analytical solution are 2-3 orders of magnitude lower. We can obtain similar results to our model predictions when we reduce the diameter of the semicircular canal to an effective diameter of 20% of the anatomical measurements, yielding $R_A = 8.6\times10^9$ Ohm and $L_A = 1.8\times10^7$ Ohm.

Much of the area of the semicircular canal is taken up by the membranous labyrinth (especially near the crista), which we hypothesize will change the effective resistive losses and mass of the perilymph considerably.

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2 $\eta$ = viscosity of water = $8.90 \times 10^{-4}$ Pa; $l$ is the length of the narrow tube, $a$ the radius of the narrow tube, and $\rho$ the density of water = 1000kg/m3.
CONCLUSIONS

At low frequencies and infrasound, the middle ear considerably limits the sound energy transmitted to the inner ear. In normal ears, stapes velocity transfer functions and RWM velocities increase by 20 dB/decade for frequencies between 1 Hz and 200 Hz. At low frequencies, stapes and RWM velocities are remarkably similar across specimens (within 10 dB). When a dehiscence is introduced in the superior semicircular canal, stapes velocity is relatively unaffected, whereas RWM velocity decreases in a predictable, frequency-dependent manner, similar to the effect of SCD on $P_{Diff}$. With SCD, volume velocity is shunted away from the cochlea, and towards the vestibular system to the opening of the semicircular canal, when stimulated with sound at the ear canal. Thus, our results are consistent with classic SCD symptoms such as low frequency hearing loss and/or sound-induced dizziness, also known as Tullio phenomenon. The effect of an SCD on RWM velocity is larger for lower frequencies, indicating that an opening in the bony labyrinth affects auditory and vestibular thresholds especially at infrasonic frequencies.

Our findings show that changing impedances in the inner ear can have a considerable effect on (infra)sound transmission through the auditory and vestibular system. Pathological mechanical changes can result in impedance changes that greatly affect sound transmission. These findings suggest that perhaps some individuals may have mechanical pathologies resulting in unusual susceptibility to infrasound sound transmission.
CHAPTER 5. Low-frequency hearing in humans: Experimental and clinical evaluation of a mechanical inner ear pathology

This work is under review as: Y. Song Cheng*, Stefan Raufer*, Xiying Guan, Christopher F. Halpin, Daniel J. Lee, Hideko H. Nakajima. Superior canal dehiscence similarly affects cochlear pressures in temporal bones and audiograms in patients.

*YSC and SR equally contributed as first authors. YSC, SR, XG, CFH, DJL, and HHN designed research. YSC, SR, XG, and HHN conducted research. YSC and SR analyzed data. YSC, SR, XG, CFH, DJL, and HHN wrote manuscript.
ABSTRACT

The diagnosis of superior canal dehiscence (SCD) is challenging and audiograms play an important role in raising clinical suspicion of SCD. The typical audiometric finding in SCD is the combination of increased air-conduction (AC) thresholds and decreased bone conduction thresholds at low frequencies. However, this pattern is not always apparent in audiograms of patients with SCD, and some have hearing thresholds that are within the normal reference range despite subjective reports of hearing impairment. In this study, we used a human temporal bone model to measure the differential pressure across the cochlear partition ($P_{\text{Diff}}$) before and after introduction of an SCD. $P_{\text{Diff}}$ estimates the cochlear input drive and provides a mechanical audiogram of the temporal bone. We measured $P_{\text{Diff}}$ across a wider frequency range than in previous studies and investigated whether the changes in $P_{\text{Diff}}$ in the temporal bone model and changes of audiometric thresholds in patients with SCD were similar, as both are thought to reflect the same physical phenomenon.

We measured $P_{\text{Diff}}$ across the cochlear partition in fresh human cadaveric temporal bones before and after creating an SCD. Measurements were made for a wide frequency range (20 Hz to 10 kHz), which extends down to lower frequencies than in previous studies and audiograms. $P_{\text{Diff}} = P_{SV} - P_{ST}$ is calculated from pressures measured simultaneously at the base of the cochlea in scala vestibuli ($P_{SV}$) and scala tympani ($P_{ST}$) during sound stimulation. The change in $P_{\text{Diff}}$ after an SCD is created quantifies the effect of SCD on hearing. We further included an important experimental control – by patching the SCD, to confirm that $P_{\text{Diff}}$ was reversed back to the initial state.

To provide a comparison of temporal bone data to clinical data, we analyzed AC audiograms of patients with symptomatic unilateral SCD (radiographically confirmed). To
achieve this, we used the unaffected ear to estimate the baseline hearing function for each patient, and determined the influence of SCD by referencing AC hearing thresholds of the SCD-affected ear with the unaffected contralateral ear.

\( P_{\text{Diff}} \) measured in temporal bones (n=6) and AC thresholds in patients (n=53) exhibited a similar pattern of SCD-related change. With decreasing frequency, SCD caused a progressive decrease in \( P_{\text{Diff}} \) at low frequencies for all temporal bones and a progressive increase in AC thresholds at low frequencies. SCD decreases the cochlear input drive by approximately 6 dB per octave at frequencies below \( \sim 1 \text{ kHz} \) for both \( P_{\text{Diff}} \) and AC thresholds. Individual data varied in frequency and magnitude of this SCD effect, where some temporal-bone ears had noticeable effects only below 250 Hz.

We found that with decrease in frequency the progressive decrease in low-frequency \( P_{\text{Diff}} \) in our temporal bone experiments mirrors the progressive elevation in AC hearing thresholds observed in patients. Our findings suggest that testing AC thresholds at frequencies below 250 Hz would detect a bigger change in AC thresholds in SCD affected ears, improving diagnosis.
INTRODUCTION

Superior canal dehiscence (SCD) describes the anatomical defect of the superior semicircular canal (SSC), most commonly found along the floor of the middle fossa near the arcuate eminence (Minor et al., 1998). This defect of the bony labyrinth creates an abnormal connection or ‘third window’ between the inner-ear fluid and the fluid and tissue of the cranial vault. As a result, the transmission of sound in the inner ear is altered, which then manifests as auditory and/or vestibular symptoms (Minor et al., 2001; Rosowski et al., 2004).

The challenge faced in diagnosing SCD is that presenting symptoms of aural fullness, distortion in hearing and dizziness are non-specific and common to the patient population seeking care in the otolaryngologist’s clinic. Unfortunately, these challenges delay recognition of SCD in some patients, and in others who are wrongly diagnosed, some may undergo various inappropriate treatments. Carey et al. analyzed 1000 temporal bones and found dehiscences of the superior canal in 0.5% of specimens. Within this population with dehiscences, it is unclear how many were symptomatic, but raises the possibility that SCD is not as uncommon as previously thought (Carey, Minor and Nager, 2000).

Audiometric testing in patients with SCD typically reveals increased air conduction (AC) thresholds and decreased bone conduction (BC) thresholds in frequencies <2kHz, resulting in a characteristic low frequency air-bone gap (ABG) (McEvoy et al., 2013; Mehta et al., 2014). These audiometric findings are believed to correspond respectively with impaired hearing of AC sounds and the paradoxical hypersensitivity to vibrations (BC stimulus) transmitted through the body (e.g., autophony and hearing one’s footsteps, heart beat and eye movements in the affected ear) (Mikulec et al., 2004; Merchant and Rosowski, 2008).
The decrease in AC hearing with SCD has been investigated in several studies using animal models (Carey et al., 2004; Songer and Rosowski, 2010), human temporal bones (Pisano et al. 2012; Niesten et al. 2015; Raufer et al. 2018), and theoretical models (Mikulec et al., 2004; Songer and Rosowski, 2007; Kim, Steele and Puria, 2013). These studies have provided evidence that during AC stimulation an SCD acts as a shunt, leading to an alternate path for the flow of volume velocity towards the labyrinth, and decreasing the input to the cochlea.

By directly measuring changes in inner ear pressures in human temporal bones using micro-fiberoptic sensors, we have demonstrated that an SCD decreases the pressure difference across the cochlear partition (Pisano et al., 2012; Niesten et al., 2015). The pressure difference across the cochlear partition, expressed as the cochlear input drive ($P_{\text{Diff}}$), has been shown to be a good estimate of hearing (assuming the neurosensory components are normal). Animal experiments have demonstrated that $P_{\text{Diff}}$ have the same magnitude and phase frequency response characteristics as cochlear microphonic, a neurophysiological recording of hearing (Dancer and Franke, 1980; Nedzelnitsky, 1980). In earlier temporal bone experiments, we simulated SCD conditions by creating a defect in the SSC and observed a decrease in $P_{\text{Diff}}$ below 1 kHz, which is consistent with the pattern of low-frequency AC hearing loss seen in SCD patients (Pisano et al., 2012; McEvoy et al., 2013).

In this study, we measured the effects of SCD on $P_{\text{Diff}}$ over a wider frequency range (20 Hz to 10 kHz) than in earlier temporal bone studies to examine whether $P_{\text{Diff}}$ decreases in a predictable manner at low frequencies. In addition, to explore the potential value of testing AC hearing thresholds at frequencies lower than 250 Hz for diagnosing SCD, we directly compared our experimental findings in temporal bones with clinical audiometric data collected from patients diagnosed with unilateral SCD. To compare audiometric with experimental data, we
selected patients with unilateral SCD and referenced AC hearing thresholds of SCD-affected ear with thresholds of unaffected contralateral ear. Assuming that hearing is symmetric at baseline, and that SCD does not affect sensorineural hearing, the difference in hearing thresholds between the SCD-affected ear and the unaffected ear for each patient is analogous to comparing $P_{Diff}$ measured before and after creating a SCD in each temporal bone experiment.

MATERIALS AND METHODS

**Temporal bone preparation.** We used fresh and previously frozen temporal bones that were removed (J. B. J. Nadol, 1996) within 24 hours post-mortem. Detailed descriptions of our experimental setup and methods may be found in (Nakajima et al., 2009; Pisano et al., 2012). In brief, the temporal bones were dissected and the middle ear cavity was accessed via the facial recess. The bone overlying the positions for the scala vestibuli (SV) and scala tympani (ST) sensors, and the lateral aspect of the SSC was carefully thinned with a diamond burr. Micro-fiberoptic pressure sensors developed by Olson (1998) were positioned in the SV and ST through small cochleostomies created under fluid (to prevent air entering the inner ear) and sealed in position using dental impression material (Jeltrate, L.D. Caulk Co.). Once the sensors were in position and hermetically sealed, fluid in the middle ear space was removed and the dental impression material was reinforced with a layer of dental cement. Reflective beads that increase the signal strength of velocity measurements using laser Doppler vibrometry (Polytec CLV, Waldbronn, Germany) were positioned on the posterior crus of the stapes, the center of the round window (RW), and the cochlear promontory. The sound stimulus was delivered using a dynamic
speaker (Beyerdynamic DT 48, Heilbronn, Germany), which was sealed to the external ear canal together with a probe-tube microphone (Etymotic Research ER 7).

**Temporal bone experiments.** $P_{SV}$, $P_{ST}$, ear canal pressure ($P_{EC}$), stapes velocity ($V_{Stapes}$), and RW membrane velocity ($V_{RW}$) were measured in response to AC pure tones between 20 and 10,000 Hz at levels between 70 - 120 dB SPL. Responses were averaged in the time domain (10 averages of 100 ms long intervals per frequency) and analyzed in the frequency domain (details in (Nakajima *et al.*, 2009; Stieger, Rosowski and Nakajima, 2013)). After sensors were sealed in place, measurements were made to ensure normal condition. Stable magnitudes of $V_{Stapes}$ and $V_{RW}$ before and after sensor insertions and a half cycle phase difference between $V_{Stapes}$ and $V_{RW}$ for frequencies below 500 Hz, confirmed that the inner ear was free of fluid leaks and air bubbles that could affect measurements. Baseline measurements were first performed on the intact temporal bone (normal condition) and $V_{Stapes}$, $V_{RW}$, as well as $P_{SV}$ and $P_{ST}$ were checked to determine they were within normal ranges by comparing to prior reports (Chien *et al.*, 2007; Nakajima *et al.*, 2009; Greene *et al.*, 2017; Frear, Guan, Stieger, Rosowski, *et al.*, 2018).

Approximately 1.25 mm length SCD was then created while at least 2 mm fluid covered the SSC using a diamond burr along the lateral aspect of the SSC near the arcuate eminence. Pressure and velocity measurements were repeated after the SCD was created. To confirm that the measured changes could be attributed to the SCD, the defect was then repaired by placing a small piece of paper over the defect (to prevent occlusion of the canal lumen), followed by Jeltrate over the paper, which sets to a rubbery consistency to form a hermetic seal. Repeated measurements of intracochlear pressures were performed after SCD repairs to verify that the SCD effect was reversible by restoring the baseline condition. We experimented on 6 temporal bone specimens
in total. Ages of the donors ranged from 23 to 78 years old with a mean age of 58.0 years old. There were 3 males and 3 females, and 4 left and 2 right ears.

**Data analysis in temporal bones.** In the temporal bone experiments, cochlear input drive ($P_{\text{Diff}} = P_{SV} - P_{ST}$) arises from vector subtraction of pressures measured simultaneously at the base of the cochlea in scala vestibuli ($P_{SV}$) and scala tympani ($P_{ST}$), in response to sound stimulation at the ear canal. These quantities are represented with italics as they are complex entities having both magnitude and phase. The effect of SCD on hearing, estimated by $P_{\text{Diff}}$, can be quantified by calculating the change in $P_{\text{Diff}}$ when an SCD is created ($[\text{effect of SCD on } P_{\text{Diff}}] = P_{\text{Diff}}^{[SCD]} - P_{\text{Diff}}^{[\text{Normal}]}$).

**Audiometric findings in SCD patients.** To determine the effect of SCD on hearing in patients, we selected subjects with symptomatic unilateral SCD and compared AC hearing thresholds between the SCD-affected ear and the contralateral “normal” or baseline ear (as an estimate of baseline hearing for each subject).

Audiometric thresholds of subjects were tested at 250, 500, 1000, 2000, 4000 and 8000 Hz on SCD patients seen at the Massachusetts Eye and Ear as a routine part of their clinical evaluation. Both AC and BC thresholds were measured, but only AC thresholds are reported here. Diagnosis of SCD syndrome was based on clinical presentation, audiometric testing, cervical vestibular-evoked myogenic potential (cVEMP), and high-resolution temporal bone cone beam CT imaging. This study only included definitive SCD as interpreted by our radiologists in our institution, and the average length was 4.0 mm ± 1.17 mm (standard deviation). A dehiscence was defined as appearance on CT of a lack of bone overlying the SSC.
in 2 consecutive image on one view as per Lookabaugh et al. 2014 (Lookabaugh et al., 2014). We excluded subjects with asymptomatic SCD (incidental radiological findings), history of other otologic diseases (e.g. Eustachian tube dysfunction, Ménière’s disease, chronic otitis media or sudden sensorineural hearing loss), prior ear surgery (e.g. ossiculoplasty, tympanoplasty, stapedectomy), contralateral defects (i.e. bilateral SCD) or “near-dehiscences” (thin bone over SSC) on CT. The audiograms of 53 patients who met our inclusion criteria for unilateral SCD were analyzed. This consisted of 32 females and 21 males, with an average age of 49.3 ±10.8 (standard deviation) years old at diagnosis. Of these, 30 patients had SCDs on the left and 23 on the right. The use of the audiometric data for this study was approved by the Human Studies Committee of the Massachusetts Eye and Ear.

**Data analysis of audiometric data.** Normal distribution of the audiometric data was tested with a Shapiro-Wilk test. Because the clinical data were not normally distributed, we used the Wilcoxon signed-rank test to compare thresholds of the SCD-affected versus the baseline ear. For multiple comparison of the six audiometric frequencies tested, a Bonferroni correction was applied and statistical significance was defined as a *p*-value smaller than \( \alpha = 0.05/6 = 0.0083 \). All analysis was performed in Matlab (MathWorks, MA, USA).
RESULTS

Temporal bone experiments – $P_{\text{Diff}}$ decreases progressively with decreasing frequency at low frequencies with SCD

Creating an SCD resulted in progressive drop in $P_{SV}$, $P_{ST}$, as well as in the cochlear input drive ($P_{\text{Diff}}$) in all six specimens at low frequency, which is consistent with earlier studies (Pisano et al., 2012; Niesten et al., 2015). Figure 5.1A demonstrates the drop in $P_{\text{Diff}}$ in the SCD condition (red solid line) from initial control (black solid line). Figure 5.1A also shows an important experimental control: that the SCD effect is reversible by resurfacing the defect with dental impression material (dashed grey line). Repair of the SCD was considered successful if post-reversal pressure measurements were within 2 dB of the measurements made before creating the SCD, and is essential to confirm that the measured changes after SCD are not due to other causes during an experiment. Figure 5.1B shows the effect of SCD on $P_{\text{Diff}}$ expressed in change in $P_{\text{Diff}}$ is progressively larger as the frequency decreases.

Figure 5.2 plots the effect of SCD on $P_{\text{Diff}}$ for each of the six specimens, along with ±95% CI of the mean of the six specimens (gray shaded area). In all specimens, the drop in $P_{\text{Diff}}$ caused by an SCD becomes progressively larger as the frequency decreases. This drop in $P_{\text{Diff}}$ generally begins at around 500 Hz and the effect increases by about 6 dB per octave with decreasing frequency, resulting in a 12-22 dB decrease in $P_{\text{Diff}}$ at 20 Hz. Note that while the effect of SCD on $P_{\text{Diff}}$ in all specimens follows a predictable trend (progressive pressure loss as frequency decreases), the frequency where SCD starts affecting $P_{\text{Diff}}$ varies among specimens (as reported in (Pisano et al., 2012; Niesten et al., 2015)). In some specimens, the influence of SCD on $P_{\text{Diff}}$ starts around 1 kHz, while in others the decrease in $P_{\text{Diff}}$ is only apparent below 250 Hz.
Figure 5.1: Effect of SCD on $P_{\text{Diff}}$ in a representative specimen. **A**) $P_{\text{Diff}}$ in normal (black line) and SCD condition (red line). The dashed grey line represents $P_{\text{Diff}}$ after repair of SCD, which reverses the effect of SCD. **B**) Change in $P_{\text{Diff}}$ due to SCD across frequency.
Figure 5.2: Change in $P_{\text{Diff}}$ in temporal bones due to SCD. Each colored line represents the effect of SCD on a temporal bone specimen. The grey shaded area represents the 95% CI above and below the mean of all experiments. The effect of SCD is more severe as the frequency decreases, but there is inter-ear variability in the effect of SCD.
**Audiometric thresholds — SCD results in low-frequency air-conduction hearing loss**

By referencing AC thresholds of the SCD-affected side to the contralateral unaffected side we found that in frequencies above 2 kHz, the thresholds of the SCD-affected ear and contralateral baseline ear are similar (Figure 5.3). At frequencies below 1 kHz, the SCD-affected ear shows elevated thresholds compared to the unaffected ear. Note that the SCD-affected ears have larger inter-quartile ranges than baseline ears at low frequencies, suggesting that the effect of SCD on hearing threshold is variable across ears. In Figure 5.3B, we can see that on average, SCD caused an increased AC thresholds below 2 kHz. This effect is progressively larger as the frequency decreases. We found statistically significant higher hearing thresholds for the SCD ears at 250 Hz ($p=1.2\times10^{-6}$, $\alpha = 0.0083$), 500 Hz ($p=1.1\times10^{-4}$, $\alpha = 0.0083$) and 1 kHz ($p=2.8\times10^{-4}$, $\alpha = 0.0083$). At 2 kHz, 4 kHz, and 8 kHz no statistical significance between the baseline and SCD-affected ear was found ($p=0.02$, 0.039, and 0.38, respectively, $\alpha = 0.0083$).
Figure 5.3: Air conducted (AC) hearing thresholds of subjects with unilateral SCD. 

**A)** Averaged AC hearing thresholds of unilateral SCD-affected ears (red solid line) and contralateral unaffected ears (black solid line). Circles within the box represent the median and the edges of the box represent the 25th and 75th percentiles, respectively. Whiskers cover approximately ±2.7 of the standard deviation and circles indicate outliers outside that range. 

**B)** The individual differences in hearing threshold between the SCD-affected ear and normal-hearing across patients. Hearing thresholds of the affected and unaffected ears are statistically different at 250, 500 and 1000 Hz, indicated by *. Arrows indicate outlier outside the plotted range (one outlier per arrow).
DISCUSSION

The concept of a pathological “third window” is often used to explain the effects of SCD on inner ear mechanics. During AC hearing, the SCD decreases the temporal bone inner ear pressures and shunts volume velocity away from the cochlea, resulting in a decrease in the cochlear input drive. Theoretical models have predicted that AC hearing loss with SCD (or decrease in $P_{Diff}$) are more pronounced as the frequency decreases (Songer and Rosowski, 2006; Kim, Steele and Puria, 2013; Raufer, Masud and Nakajima, 2018). Both our audiometric findings from patients with unilateral SCD and temporal bone studies supports this prediction, reaffirming current understanding of SCD as a pathological “third window” of the cochlea.

Superior canal dehiscence predominantly affects low-frequency hearing because the flow of sound (volume velocity) depends on the relative acoustic impedances between RW and SCD which changes at low frequency. Acoustic impedance of SCD would be the mass of the fluid between the oval window and the SCD, which is a function of frequency, i.e. impedance of mass ($Z \propto \omega M$, where $\omega$ is the radian frequency and $M$ the acoustic mass, thus $Z$ increases with frequency). At low frequencies, this SCD impedance is small thus sound is shunted through the SCD. At high frequencies, the high impedance of mass prevents sound from being shunted through the SCD (Figure 5.5, described below).

Bone conduction hearing, in contrast to AC hearing, is known to be slightly enhanced in some patients with SCD in the lower frequencies, contributing to a low frequency ABG (Carey et al. 2000). While the influence of SCD on AC hearing is well understood, the pathological mechanism behind the effect of SCD on BC hearing is not fully understood. Proposed theories include: transmission of sound waves via non-osseous routes through the SCD from movement of the dura or cerebral spinal fluid, changes to the effect of middle- and inner-ear inertance
effect, and enhancement of bone compression (Brantberg, Bergenius and Tribukait, 1999; Stenfelt and Goode, 2005; Stenfelt, 2015). But these hypotheses have not been proven to account for the changes seen with SCD (Brantberg, Bergenius and Tribukait, 1999; Merchant and Rosowski, 2008). Changes in AC and BC thresholds are both important clues in audiometric diagnosis of SCD, but in some patients, the change in AC hearing thresholds at 250 Hz may be too small to detect because normal reference ranges are wide (-10 to 20 dB HL).

Comparing audiometric data and temporal bone data

The change in $P_{Diff}$ due to SCD determined from our temporal bone experiments mirrors the change seen with AC hearing thresholds from clinical audiograms. Figure 5.4 directly compares the effect of SCD on $P_{Diff}$ ($\pm$95% CI around the average) seen in Figure 5.2 to the effect on clinical AC hearing thresholds shown in Figure 5.3. At low frequencies (500 Hz - 1 kHz), SCD is associated with a decrease with decreasing frequency by approximately 6 dB per octave in both $P_{Diff}$ and AC hearing. Above 2 kHz, minimal differences to the baseline measurements are observed. Both the temporal bone and clinical data followed similar patterns, but in the audiometric data, SCD started affecting AC thresholds at higher frequencies than in our temporal bone experiments.
Figure 5.4: Comparison of effects due to superior canal dehiscence (SCD) on $P_{\text{Diff}}$ in temporal bone specimens and on air-conducted (AC) hearing thresholds in patients. The grey shaded region (95% CI around the average) plots the SCD-induced change in $P_{\text{Diff}}$. The red data show the difference between AC hearing thresholds of SCD affected ear and contralateral unaffected ear in patients with unilateral SCD.
The reason for this difference is unclear, but clinical SCD were generally larger (4.0 mm) than the SCD created in our temporal bone experiment (1.25 mm). Niesten et al. (2015) explored the influence of SCD size and location, and found that in each ear, size but not location, appeared to influence the amount by which SCD affects $P_{\text{Diff}}$. We are unable to “correct” for the effect of size in the present temporal bone experiments using findings from Niesten et al. 2015 because of the low number of earlier experiments that succeeded in enabling the study of effect of size (up to and beyond 4 mm). Regardless, even for a given size of the SCD, the measured SCD effect varied across ears (Pisano et al., 2012; Niesten et al., 2015), and the effect of size varied across ears as well (Niesten et al., 2015). It is possible that this wide variability may reflect anatomical difference between temporal bone or subjects.

To reference the SCD-affected ear and the contralateral “normal” or baseline ear, we made the assumption that hearing is symmetric at baseline. The Epidemiology of Hearing Loss study looked at almost 4000 adult participants and reported that the inter-ear differences were small with an averaged difference of 1.4 dB with a range of -0.8 to 4 dB based on the published data. We also made the assumption that SCD does not affect sensorineural hearing. Multiple experiments and models of SCD have affirmed that SCD mainly affects the mechanical transmission of sound in the inner ear (Minor et al., 2003; Rosowski et al., 2004; Attias et al., 2011; Kim, Steele and Puria, 2013). McEvoy et al. performed a comparison between hearing in SCD affected ears with both age matched controls, and with contralateral unaffected ears in patients with unilateral SCD, and concluded that hearing loss in SCD is low frequency and conductive (McEvoy et al., 2013). Interestingly, McEvoy et al. raised the possibility that SCD may predispose patients to sensorineural hearing loss, which may explain the difference between our temporal bone and audiometric data. However, similar to McEvoy et al, we excluded patients
with known profound SNHL, and therefore cannot draw conclusions about the prevalence of
SNHL in SCD from our dataset.

**Effects of SCD vary across temporal bone specimens**

Our temporal bone measurements, consistent with previous experiments, revealed a
considerable variability across specimens (Pisano *et al.*, 2012; Niesten *et al.*, 2014). In some
specimens, the influence of SCD on $P_{\text{Diff}}$ is apparent at frequencies below 1 kHz, while in others
the drop in $P_{\text{Diff}}$ only begins below 250 Hz (Figure 5.2). The variability in the change in hearing
thresholds was seen in our patient population as well. Together, this suggests that in some
patients, SCD-related hearing loss may be missed by conventional audiograms, and that testing
hearing thresholds below 250 Hz may overcome this variability because the effect of SCD is
progressively larger as the frequency decreases.

We have studied parameters that can influence the effect of SCD by computational
modelling. Figure 5A illustrates a computational model used by Raufer *et al.* to investigate SCD
effects on $P_{\text{Diff}}$ (Raufer, Masud and Nakajima, 2018). Figure 5.5B demonstrates that changing the
parameters of SCD impedance (change in mass and resistance) can change the model function
such that it changes the frequency where $P_{\text{Diff}}$ starts to decrease. Beside the differences in size
that would affect the mass and resistance of the SCD in our model, it is possible that other
influences could affect inner ear sound transmission due to SCD, such as changing the
impedances in the inner ear (Raufer, Masud and Nakajima, 2018). Thus, the amount by which
SCD affects $P_{\text{Diff}}$ and hearing is a complex interaction of many variables, and what variables are
responsible for the variations in SCD effects across ears is presently unknown.
Figure 5.5: Model for SCD: A) The model includes the pressure in scala vestibuli ($P_{SV}$) and scala tympani ($P_{ST}$) and the effect of the SCD (change in impedance). B) A relatively large SCD could affect hearing thresholds more and at higher frequencies (red), whereas a small SCD may affect hearing thresholds less and outside audiometric test frequencies (blue). (Details of the model in (Raufer, Masud and Nakajima, 2018) with parameters $Z_{Diff}$: $R_{Diff} = 3.04 \times 10^{10}\ \text{Nsm}^{-5}$, $L_{Diff} = 6.46 \times 10^{7}\ \text{Ns}^2\text{m}^{-5}$; $Z_{RW}$: $R_{RW} = 1.47 \times 10^{10}\ \text{Nsm}^{-5}$, $L_{RW} = 7.76 \times 10^{5}\ \text{Ns}^2\text{m}^{-5}$, $C_{RW} = 3.59 \times 10^{-14}\ \text{N}^{-1}\text{m}^5$. Yellow: $R_{SCD} = 1.7 \times 10^{10}\ \text{Nsm}^{-5}$, $L_{SCD} = 2.0 \times 10^{7}\ \text{Ns}^2\text{m}^{-5}$. Blue: $R_{SCD} = 4.5 \times 10^{10}\ \text{Nsm}^{-5}$, $L_{SCD} = 4.0 \times 10^{7}\ \text{Ns}^2\text{m}^{-5}$. Red: $R_{SCD} = 0.6 \times 10^{10}\ \text{Nsm}^{-5}$, $L_{SCD} = 0.8 \times 10^{7}\ \text{Ns}^2\text{m}^{-5}$.)
Limitations

The temporal bone experimental model does not fully represent clinical factors that may influence intracochlear pressure and auditory symptoms in SCD patients. Creating an SCD reliably causes a drop in low-frequency $P_{\text{Diff}}$, but some patients have no auditory or vestibular symptoms, despite having a large SCD as revealed by CT imaging. This may be due to anatomical relations between SCD and adjacent dura and brain (Luers et al., 2014), which may seal or plug the dehiscence in situ.

Clinical utility of measuring hearing thresholds below 250 Hz

At present, the clinical utility of audiograms as a screening tool for SCD is limited by its poor sensitivity. The impact of SCD on AC hearing thresholds between 250 Hz – 1000 Hz is small (Figure 5.3). Threshold at 250 Hz is about 20 dB, which would generally be considered a small or clinically insignificant loss in hearing. This study demonstrates that SCD causes a consistent progressive decrease in cochlear input drive $P_{\text{Diff}}$ as the frequency decreases (Figure 5.2). Audiometric testing of AC thresholds below 250 Hz could therefore improve the sensitivity of audiograms in detecting SCD because the effect on hearing thresholds are larger as frequency decreases. Assuming the effect of an SCD on AC hearing thresholds is comparable to the effect of an SCD on $P_{\text{Diff}}$ (Figure 5.4), we estimate that audiometric testing at 20 Hz will reveal an average AC hearing threshold of 35 to 40 dB in SCD patients. An estimate is shown in Figure 5.6 where extrapolation was made at low frequencies with a linear fit (on the log-axis) to the average hearing thresholds between 250 Hz and 1 kHz. There are other causes of low-frequency hearing loss, such as otosclerosis, but acoustic reflex and wideband acoustic immittance can
generally help differentiate ossicular fixation from SCD (Nakajima et al., 2012, 2013; Merchant et al., 2014).

Current audiometric testing equipment can already probe AC hearing thresholds at 125 Hz, and standard headphones (e.g., Beyerdynamics DT48, Sennheiser HD280) and in-ear headphones (Etymotic Research ER2) have the capacity to test frequencies down to 20 Hz, especially with an in-the-canal ear tip to occlude the ear canal. Importantly, hearing thresholds for normal-hearing subjects are already established between 20 Hz and 12.5 kHz (cf. ISO 226:2003), providing a basis for interpreting hearing levels at frequencies below 250 Hz. The clinical evaluation of a cohort of SCD patients is the next step in testing this hypothesis. Testing additional frequencies during a routine audiogram may slightly increase testing time, but likely will not have a big effect in most clinics. As such, we envision lower frequency audiometry being applied based on either presenting symptoms suspicious for third window pathology or detection of low frequency ABG with intact acoustic reflexes.
Figure 5.6: Predicted air-conduction hearing levels from pure-tone audiometric testing at frequencies below 250 Hz (dashed lines for prediction). The data for the normal and SCD condition were extrapolated with a straight line (on a log-axis) for low frequencies from average hearing thresholds between 250 Hz and 1 kHz.
CONCLUSIONS

The effect of SCD on inner-ear sound transmission is similar for fresh cadaveric temporal bones and SCD patients. Every temporal bone measured in this study exhibited an SCD-induced low-frequency decrease in sound transmission, and on average with approximately 6 dB reduction per octave decrease in frequency. Direct comparison between temporal bone and patient data shows that measuring AC audiograms below 250 Hz may improve the sensitivity of audiometry as a screening tool for SCD, because SCD-related AC hearing loss is often below the conventional frequencies tested and the effect is more prominent at lower frequencies.
CHAPTER 6. Conclusions and future studies
The thesis presents studies relevant to passive cochlear mechanics in the cochlear base and studies regarding low-frequency hearing in humans. Both research areas have promising future directions. For example, for a follow-up to Chapter 5, we started testing low-frequency hearing in SCD patients to test our prediction that audiometric air-conducted low-frequency threshold shifts could be used as a diagnostic tool for identifying SCD patients. Regarding future studies based on Chapters 1-3 (human cochlear mechanics), we present preliminary findings below, which will likely steer future research in that direction. We will pursue three interrelated efforts to gain a better understanding of the mechanics of the human cochlea: (i) measuring the anatomy and motion of the human CP and its components, especially near the hair-cell stereocilia, using optical coherence tomography (OCT), (ii) establishing how the mechanics of this motion relate to the anatomy of the cochlea, and (iii) developing and applying finite element (FE) models based on these measurements. Below, we present preliminary findings of OCT measurements in fresh cadaveric specimens to quantify both the anatomy and motion of the human CP, and preliminary findings on FE modeling of a human cochlear cross-section.

**Preliminary findings: In-situ imaging and motion measurements of human CP structures using optical coherence tomography**

*Introduction:* The motion of the cochlear partition (CP) determines the mechanical input to the hair cells, which determine auditory nerve responses. In Chapter 3 we described that in the base of laboratory animals (typically rodents), the CP consists of the basilar membrane (BM) attached to the bony osseous spiral lamina (OSL). This BM motion is similar to that of a simply supported beam and is the primary contributor to the surface motion of the CP compared to the
relatively negligible motion of the OSL. However, we noted that the CP anatomy of the cochlear base in the human shows major differences from the base of smaller laboratory animals. In the base, the OSL occupies the majority of the width of the CP in human. Furthermore, there exists a soft-tissue ‘bridge’ between the OSL and the BM in the human, which has about the same width as the BM and which is absent in the cochlear base of laboratory animals.

*Methods:* We used a ThorLabs Ganymede-III-905 nm center wavelength, spectral-domain OCT system combined with custom measurement system software VibOCT and SyncAv (Ravicz *et al.*, 2018 AIP) to measure the transverse motion of the basal CP in fresh human temporal bones at multiple radial locations, including along the OSL, the soft-tissue bridge, and the BM. Measurements were made through the intact round window membrane as well as after removing it. Ear-canal sound-driven CP motion was measured along the CP with a spatial resolution of 10 μm and at each point from 0.1 to 10 kHz in 1/4th-octave steps below the best frequency of about 15 kHz. Axial resolution of the OCT system is 1.83 μm in air. Lateral resolution depends on the lens used, and was 8 μm for the Thorlabs LSM03-BB objective lens and 4 μm for the Mitutoyo NIR 10X objective lens.
Preliminary findings: Figure 6.1 (top) shows an OCT image taken through the round window (RW) from a temporal bone harvested 16 hours postmortem from the left of a 76-year-old male. The OCT measurements began 17 hours postmortem and were completed within 24 hours postmortem. At the bottom of Figure 6.1 is a histological view of a different specimen for anatomical reference. In the OCT image, we can identify the OSL, bridge (B), BM, spiral ligament (SL), limbus (L), tectorial membrane (TM), and OoC (including the reticular lamina (RL), tunnel of Corti between the inner and outer pillar cells, and the space of Nuel between the outer pillar cell and outer hair cells). With OCT, we are able to scan to locations of structures to within about 1 μm accuracy radially and 2 μm axially to make vibration measurements at the CP surface and deeper into structures of the OoC, using methods similar to those of other groups (Gao et al., 2014; Lee et al., 2015, 2016; Ramamoorthy et al., 2016; Ren, He and Kemp, 2016; Warren et al., 2016; Cooper, Vavakou and Heijden, 2018).

Figure 6.2a plots the transverse motion of the scala-tympani surface of the CP against radial positions measured with VibOCT. The x-axis radial location has the origin x=0 at the OSL-bridge junction. The y-axis is normalized to the maximum velocity, which occurs near the bridge-BM connection. These OCT-based measurements of the OSL, bridge, and BM displacements are consistent with earlier measurements made in a different fresh specimen using LDV, which are plotted in Figure 6.2b for comparison.
Figure 6.1: (Top) OCT image obtained through the round window and scala tympani of a fresh human temporal bone. (Bottom) Histology of a representative cochlea (different cochlea from OCT) near the base for reference. L=Limbus, B=Bridge, SL=Spiral ligament.

Figure 6.2: CP Velocity re max versus CP location for (a) OCT and (b) LDV for two different ears. Colored lines for frequencies.
We have also demonstrated that we can perform high-resolution vibrometry of various CP elements. For example, in Figure 6.3 we show that in this specimen, RL and BM motion measured across many points were similar across a wide frequency range with <5 dB magnitude. However, there may be systematic differences between the motion of the BM and RL (Figure 6.3). At the center of the BM (Location 4 and 5 in Figure 6.3), the RL showed larger transverse motion than the BM, whereas at more medial and more lateral locations the motion of the BM and RL were almost identical (Locations 2, 3, 6, and 7). At the most medial location (Location 1), the BM moved more than the RL in this specimen. The observed differences were small and within 5 dB. However, for OoC micromechanics the differential motion of the BM and RL could have important implications.

We investigated differences in transverse motion at different radial locations. Figure 6.4 plots magnitude ratios to compare three points across the partition: near the medial end of the BM (Location 1), near the center of the BM (Location 4) and near the lateral end of the BM (Location 7). The blue line in Figure 6.4 shows that BM motion close to the inner hair cells (Location 1) was larger than near the BM center (Location 4). This is in agreement with findings from Chapter 2, where we report the largest BM displacement close to the inner pillar and hair cells instead of the center of the BM near the outer pillar cells. The yellow line in Figure 6.4 shows that a similar trend is seen for the RL. However, the magnitude difference between Locations 1 and 4 for the RL is not as large as for the BM. The red line in Figure 6.4 shows that the BM is moving less near its lateral end compared to its center, in agreement with findings from Chapter 2. The purple line contrasts the magnitude of RL motion at Locations 4 and 7 and shows that RL is reduced at the lateral end, but more so than BM motion. These differences
between BM and RL motion across the CP may bear important implications for OoC micromechanics and have to be investigated in a systematic manner in future studies.

Figure 6.3: Relative motion of the basilar membrane (BM, downward-pointing arrows) and reticular lamina (RL, upward-pointing arrows).

Figure 6.4: Magnitude ratios of the basilar membrane (BM) and reticular lamina (RL) at different radial locations.
Lastly, Figure 6.5 shows measurements from a 59-year-old male left ear extracted 14 hours postmortem with high magnification providing 4-µm lateral resolution. The higher resolution allows clear definition of the RL and TM. The labels near the surface of the RL furthermore identify the brightness due to the IHC and OHC cuticular plates and the head of the pillar cells (P). Furthermore, we see the outer tunnel lateral to the OHCs. Our initial OCT measurements in human show excellent images and displacement measurements, demonstrating our capability to pursue this research in the future.

**Limitations:** The brightness scans of the OCT provide limited quality to register some of the anatomical landmarks of the CP to within a few micrometers. Individual variation in anatomy across bones furthermore limits the use of anatomical measurements from histologically prepared specimens for comparison. For example, the exact location of the BM/bridge boundary in Figures 6.3 and 6.5 are difficult to determine. Some landmarks like the lateral end of the limbus or the tunnel of Corti are prominent and robust features and can be determined to within a few micrometers. The contrast of the brightness scan can be adjusted during an experiment (in real-time), aiding in defining additional landmarks. In the future we plan to prepare histological sections of the contralateral side of an OCT measured cochlea, providing a more reliable anatomical guide for the measured CP motion. Better anatomical registration of the OCT will make our motion measurements more reliable, but it is unlikely that the main conclusion of our work will change as the main landmarks of the CP are already clearly distinguishable with the current setup.
Preliminary findings: Finite element modeling

We will build FE models rooted in measured anatomy and material properties of human, testing and constraining them with motion measurements. Because it is not possible to measure in vivo sound-transduction details of hair-bundle motions in humans, computational modeling remains critical for predicting and gaining a detailed quantitative understanding of cochlear mechanics. One key advantage of FE models is that they can be constrained by anatomically realistic dimensions and material properties when calculating functional behavior. Improvements in multi-physics FE-simulation software and the availability of powerful multi-core computers make the proposed approach well suited for the future. We will further our understanding of human hearing mechanics through the application of a series of increasingly sophisticated human FE models, built with methods similar to those that have been used for existing animal models.

Figure 6.6 shows a first modeling approach with a simplified anatomy of the cochlear partition. The important features for this model are: (i) the bony part of the CP (dark green in Figure 6.6), (ii) the soft tissue including the bridge and nerve fibers (green in Figure 6.6), and
(iii) the BM (red in Figure 6.6). Details of the OoC were neglected in the model, but simplified outline of the OoC was included in Figure 6.6 for illustrative purposes. The details of the bridge fibers were also not taken into account, but all soft tissue (including nerve fibers and bridge) had the same Young’s modulus. No fluid interactions were considered for this model. Parameters for the FE model are as follows: $E_{BM} = 10$ MPa (Young’s modulus); $E_{softTissue} = 1$ MPa; $E_{Bone} = 1$ GPa; $\rho_{SoftTissue} = 1100$ kg/m$^3$ (density); $\nu_{SoftTissue} = 0.45$ (Poisson’s ratio); Damping = 0.1; $\rho_{Bone} = 2000$ kg/m$^3$; $\nu_{Bone} = 0.3$; $P_0 = 1$ Pa (pressure).

Figure 6.6: Illustration of the human cochlear partition consisting of the bony osseous spiral lamina, soft tissue (including nerve fibers and bridge), and the BM. Abbreviations: BM = basilar membrane; BC = basilar crest; SL = spiral ligament.
**Preliminary findings:** Figure 6.7 shows the meshed FEM model. The three data graphs show sensitivity analyses of different parameters: The Young’s modulus of the BM, the soft tissue, and bone of the OSL. The graphs are normalized to the respective maximum of the graph. The results for the baseline parameters are plotted as black lines in each of the graphs. With the chosen parameters, the BM is moving more than the OSL. The displacement of the BM is influenced by surrounding structures in that the maximum of the displacement is not at the center of the BM.

In the first sensitivity analysis, the effect of the Young’s modulus of the BM was investigated. The results are shown in the upper right panel in Figure 6.7 where the Young’s modulus of the BM was reduced from 10 MPa (base parameter) to 1 MPa. Reducing the Young’s modulus of the BM is motivated by the fact that the BM is composed of layers and that the effective Young’s modulus might not be that of collagen alone (one could also argue that the layers of cells might increase the stiffness of the BM). A relatively small elastic modulus of the BM leads to a pronounced response of the BM and less involvement of the OSL. The location of the maximum does slightly change as a function of the Young’s modulus of the BM. As the stiffness decreases, the location of the maximum shifts towards the center of the BM.

Changing the Young’s modulus of the soft tissue (lower left graph in Figure 6.7) led to the following observations: A more elastic material of the soft tissue led to a larger displacement of the bridge area between the OSL and the BM. Furthermore, the relative displacement of the OSL is considerably larger when the Young’s modulus of the soft tissue is decreased. Interestingly, the maximum displacement of the CP shifted medial towards the bridge area. These characteristics are consistent with our experimental findings from Chapter 2.
Lastly, a sensitivity analysis of the Young’s modulus of the bony OSL was performed. We showed in Chapter 5 that the OSL is not completely ossified, which could change the Young’s modulus of the OSL considerably. When the Young’s modulus of the bone is decreased, we observe a much larger displacement of the OSL and a shift of the maximum displacement towards the bridge between the OSL and BM. The shift of the peak towards the bridge is consistent with the experimental data from Chapter 2 and can be achieved by the correct combination of BM, soft tissue, and OSL Young’s modulus. Summarizing the sensitivity analyses of the different parameters, we showed that the model is able to capture important characteristics observed in the experiment. A future FE model will take into account experimental data from Chapter 2, detailed anatomical information from Chapter 3, and the micromechanics of the organ of Corti.
Figure 6.7: Meshed finite element model and sensitivity analyses of different parameters. Similar motion profiles as reported in Chapter 2 are possible by changing the Young’s modulus of the BM, soft tissue, or OSL by one to two orders of magnitude. Base parameters (black lines) are:

\[ E_{BM} = 10\text{MPa}; \quad E_{\text{softTissue}} = 1\text{MPa}; \quad E_{\text{Bone}} = 1\text{GPa}. \]
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doi: 10.1073/PNAS.1810766115.


