Impedance Measurements of the Human Basilar Membrane

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Abstract
The cochlea is a mechanical frequency analyzer, owing its characteristics to the impedance of the basilar membrane (BM). In humans, the acoustic impedance of the BM has never been measured and the stiffness of the human BM has not been revised since von Bekesy’s experiments. We measured intracochlear pressures in scala vestibuli and velocities of the BM 1.2 mm from the base of the cochlea. By taking the ratio of the pressure and velocity measurements, the specific acoustic impedance (Z) is calculated. At low frequencies, where the BM impedance is stiffness-dominated, the stiffness is extracted by multiplying the imaginary part of Z by the angular frequency. Our results show that the specific acoustic impedance of the BM is decreasing by 6 dB per octave at frequencies between 100 – 10,000 Hz, with a phase close to -90 degrees. The real part of the impedance is positive and slightly increasing at low frequencies. The imaginary part is negative and dominating Z at low frequencies. The specific acoustic stiffness at the measurement location amounts to 0.85 GPa/m ±0.3 GPa/m, which is about one order of magnitude higher than von Bekesy’s estimates of the static stiffness.

Introduction
The cochlea is a mechanical frequency analyzer and as such depends on the passive mechanics of the inner ear. From early hearing theories, mechanical measurements of the basilar membrane (BM), and psychoacoustic experiments, it has been established that the ear works as an acoustic prism, analyzing the frequency components of a sound mixture mostly independent from each other.

In order to develop realistic physical models of a biological system, its mechanical parameters, such as the stiffness, have to be known. The acoustic impedance (and stiffness) of the BM is a key parameter to understanding the function of the cochlea, as it governs the traveling wave. The impedance and/or stiffness of the BM have been measured in guinea pig and gerbil utilizing a variety of methods (for an overview see [1]). However, in humans, the acoustic impedance and stiffness have not been determined experimentally; current estimates of the basal stiffness of the human BM vary by two orders of magnitude [2, 3]. Because the anatomy (and most likely the physiology) of the human inner ear differs markedly from that of other species, impedance and stiffness measurements from small laboratory animals cannot be used to infer the impedance and/or stiffness of the human BM.

The goal of this study is to establish reference measurements of the acoustic impedance (Z₄) for the human BM, against which cochlear models can be tested. This is achieved by simultaneously measuring the pressure in scala vestibuli (P₃V) and the velocity of the BM (v₃M) from scala tympani in fresh human cadaveric temporal bones, i.e. Z₂ = P₃V/v₃M. We analyze and discuss the magnitude and phase, and real and imaginary part of the impedance of six specimens.

Materials and Methods

Temporal Bones
We report results for six specimens. Fresh human cadaveric human temporal bones were removed from the skull base using an intracranial approach. Minutes after removal, the specimens are stored in 250 ml of 0.9% saline with two drops of betadine solution and refrigerated. The first step in preparing a temporal bone specimen for an experiment is to remove soft tissue on the outside of the bone (muscle tissue, fat, and vessels). We then use a facial recess approach to get access to the middle ear cavity (for details see [4]). When possible, we leave the stapedial tendon intact. However, in order to access the area close to the stapes for placement of the pressure sensor (below), this is not always possible. The cochleostomy for the pressure sensor (~200µm) is hand-drilled shortly before the sensor is inserted, but the otic capsule near the oval window is prepared in advanced and thinned to make hand-drilling more effective. Initial measurements of stapes and round window membrane (RWM) velocities were performed to check whether air entered the cochlea. A phase difference of one-half cycle between stapes and RWM velocities (at frequencies between 50 Hz and 300 Hz) is a reliable indicator that no air is enclosed in the inner ear. All experiments were concluded within 48 h post mortem. We used left ears only, the donors were between 53 to 78 years old (mean = 60.2 years).

Figure 1a shows an image of a temporal bone specimen taken during an experiment. Arrows point to the stapes, pressure sensor, round window membrane (RWM) area, and BM. The RWM was removed to enable velocity recordings from the BM. Figure 1b shows a mid-modiolar cross section of a human temporal bone to highlight where the pressure sensor was placed with respect to the cochlear partition.

Sound Stimulation
Acoustic pure tones between 100 Hz and 24 kHz (10 per octave) were generated by a Radio Shack driver (400-1377, Fort Worth, TX) and delivered to the ear canal through flexible tubing. The sound pressure level in the ear canal was monitored with a broadband microphone (Knowles EK-23103-000, Itasca, IL). A metal sleeve, inserted through the bony part of the ear canal, was used to place the microphone 1 mm from the umbo. Sound pressure levels did not vary as a function of frequency (isoinput) but varied across experiments (from 94-110 dB SPL) depending on the noise floor of the velocity measurements.
Pressure Measurements

Fiber-optic pressure sensors were used to record pressures in the vestibule [4]. We calibrated our pressure sensors in a column of water using a shaker (Bruel and Kjaer 4290, Naerum, Denmark) with an external accelerometer. After an initial calibration in the shaker, we inserted the pressure sensor into the vestibule. A cochleostomy with a diameter of approximately 200 µm was hand-drilled near the oval window. The opening was kept under saline to prevent air from entering the cochlea. The sensor was then hermetically sealed to the bone with dental impression material before the middle ear space was cleared of saline. After the initial intracochlear measurement, the sensor was extracted from the cochlea and calibrated again in the shaker. If the sensor sensitivity remained within 2 dB of the initial calibration, intracochlear pressure measurements were considered accurate.

The insertion of the pressure sensor is done with the RWM intact. The half-cycle phase difference between stapes and RWM velocity measurements (and their respective magnitudes) is used to determine whether the insertion of the pressure sensor was successful. Stapes and RWM membrane velocities are unchanged after a successful insertion of the sensor.

Velocity Measurements

A laser Doppler vibrometer (Polytec CLV 700, Irvine, CA) was used to measure velocities of the posterior crus of the stapes, RWM, and BM. 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 velocity recordings from the BM. The laser beam was perpendicular to the BM. Stapes measurements were made at an angle of approximately 30 degrees with respect to the footplate, but a cosine correction was not applied. The acoustic coupling of the sound in the ear canal to the temporal bone specimen was determined by measuring the velocity of the cochlear promontory; the noise floor of the laser system was determined by measuring responses without acoustic stimulation.

Before the BM recordings, we carefully removed the opaque RWM with the sharp blade of a hypodermic needle (25Gx5/8”). The removal of the RWM is necessary to allow for an unobscured view of the cochlear partition. To prevent fluid from exiting the cochlea and air entering through the open round window space, the temporal bone was oriented with the RWM parallel to the table.

Calculation of the Specific Acoustic Impedance and Stiffness

We use a technique based on intracochlear pressure measurements to determine the acoustic impedance and stiffness of the human BM in intact cochleae [1]. Dong and Olson showed that the impedance of the BM can be reliably estimated about one-half octave below the characteristic frequency by simultaneously recording the pressure in scala vestibuli \(P_{SV}\) and the velocity of the BM from scala tympani \(v_{BM}\). This approach uses acoustic stimulation in the ear canal (in our case 100 Hz – 24 kHz) and avoids direct contact with the BM [1].

The specific acoustic impedance \(Z_A^S\) is obtained by dividing \(P_{SV}\) by \(v_{BM}\), i.e.

\[ Z_A^S = P_{SV}/v_{BM}. \]

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

\[ Z_A^S = P_{SV}/v_{BM} \propto R + j(M\omega - S/\omega), \]  

(1)

where \(\omega\) is the radian frequency, \(R\) the resistance (or damping), \(M\) the mass, and \(S\) the stiffness of the system. Although the impedance of the BM, as determined by our measurements, is not that of a simple harmonic oscillator (the real part is frequency dependent and the phase exceeds 90° at high frequencies), Eq. 2 holds for the long-wave regime, which is about one-half octave below the characteristic frequency (CF) of the BM [1].

Note that \(Z_A^S\) is a complex entity. The magnitude of \(Z_A^S\) is calculated by dividing the magnitude of \(P_{SV}\) by the magnitude of \(v_{BM}\), i.e.

\[ |Z_A^S| = |P_{SV}|/|v_{BM}| \quad [\text{Pa}\cdot\text{s}/\text{m}] \]

The phase of \(Z_A^S\) is calculated by subtracting the phase angle of \(v_{BM}\) from the phase angle of \(P_{SV}\), i.e.

\[ \angle Z_A^S = \angle P_{SV} - \angle v_{BM} \quad [\text{radians}] \]

At low frequencies, where \(Z_A^S\) is stiffness-dominated, the acoustic stiffness \(K_A^S\) is extracted by multiplying the imaginary part of \(Z_A^S\) by the angular frequency, i.e.

\[ K_A^S = \Im(Z_A^S) \cdot \omega \quad [\text{Pa}/\text{m}] \]
Results

Characteristics of the specific acoustic impedance and stiffness of the human BM

The specific acoustic impedance is obtained by dividing the pressure $p$ by the velocity $v$ (see Eq. 1). In Fig. 2a, the magnitude and phase of $Z_A^S$ of the BM are shown for six specimens. From 400 Hz to 10 kHz, $Z_A^S$ decreases by approximately 6 dB per octave. At 1 kHz, $Z_A^S$ amounts to approximately 0.14 GPa s/m and at 5 kHz to around 0.02 GPa s/m. The phase below 10 kHz is close to negative 0.25 cycles (-90º), ascribed to a reactance in the form of a stiffness element. The frequency range for which the BM is stiffness-dominated is between 400 Hz and 10 kHz.

In Fig. 2b, the real and imaginary parts of $Z_A^S$ are plotted. The real part is positive and slightly increasing at low frequencies, indicative of viscous losses. The imaginary part is negative and decreasing at low frequencies. Overall, the imaginary part is dominating the impedance at low frequencies, leading to the observed quarter-cycle phase shift between pressure and velocity. The acoustic impedance measurements from gerbil show a negative real part, and are not in agreement with our observations [1].

The specific acoustic stiffness ($K_A^S$), plotted in Fig. 2c, is calculated by multiplying the imaginary part of $Z_A^S$ by the radian frequency. This is only possible because the phase of $Z_A^S$ is close to -0.25 cycles, implying that the impedance is dominated by the reactive (imaginary) part. The average $K_A^S$ of the BM shown in Fig. 2c amounts to 0.85 GPa/m ±0.3 GPa/m for the measurement location 1.2 mm from the base of the cochlear.

Conclusions

In this study, we measured the acoustic impedance and stiffness of the BM 1.2 mm from the base in human cochleae. The impedance of the BM is decreasing by 6 dB/octave at low frequencies, implying a stiffness-dominated system. The real part of the impedance is positive and increasing at low frequencies; the imaginary part is decreasing at low frequencies and dominating the impedance. The specific acoustic stiffness of the BM, estimated by multiplying the imaginary part of the impedance with the radian frequency, amounts to 0.85 GPa/m 1.2 mm from the base.

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