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Research article

Influence of inner ear impedance on middle ear sound transfer functions

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A R T I C L E I N F O

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Round window stiffness
Cochleostomy

A B S T R A C T

Introduction: For experimental studies on sound transfer in the middle ear, it may be advantageous to perform the measurements without the inner ear. In this case, it is important to know the influence of inner ear impedance on the middle ear transfer function (METF). Previous studies provide contradictory results in this regard. With the current study, we investigate the influence of inner ear impedance in more detail and find possible reasons for deviations in the previous studies.

Methods: 11 fresh frozen temporal bones were prepared in our study. The factors related to inner ear impedance, including round window membrane stiffness, cochleostomy, cochlea fluid and cochlea destruction were involved in the experimental design. After measuring in the intact specimen as a reference (step 1), the round window membrane was punctured (step 2), then completely removed (step 3). The cochleostomy was performed (step 4) before the cochlear fluid was carefully suctioned through scala tympani (step 5) and scala vestibuli (step 6). Finally, cochlea was destroyed by drilling (step 7). Translational and rotational movement of the stapes footplate were measured and calculated at each step. The results of the steps were compared to quantify the effect of inner ear impedance changing related to the process of cochlear drainage.

Results: As the inner ear impedance decreases from step 1 to 7, the amplitudes of the METF curves at each frequency gradually increase in general. From step 6 on, the measured METF are significantly different with respect to the intact group at high frequencies above 3 kHz. The differences are frequency dependent. However, the significant decrement of rotational motion appears at the frequencies above 4.5 kHz from the step 5.

Conclusion: This study confirms the influence of inner ear impedance on METF only at higher frequencies ($\geq$3 kHz). The rotational motions are more sensitive to the drainage of fluid at the higher frequency. Study results that found no influence of cochlea impedance may be due to incomplete drainage of the cochlea.

1. Introduction

Experimental studies on cadaveric temporal bone (TB) specimen are widely used to explore middle ear mechanics as well as to develop surgical procedures or devices for hearing rehabilitation. Measurements of the middle ear transfer function (METF) are a very important tool in such experiments [1]. The METF characterizes the sound transfer from the tympanic membrane (TM) through the

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ossicular chain into the inner ear, taking sound pressure at the TM as input and sound pressure in the cochlea as output. Experimentally, the sound pressure in the inner ear is often replaced by the sound induced vibration of the stapes footplate measured with Laser Doppler Vibrometry (LDV) in practice.

For some investigations on middle ear reconstruction, it can be advantageous, in terms of measurement approach, to remove the inner ear. Actually, the drainage process results in a change in acoustic-mechanical impedance. The inner ear input impedance is largely a result of the liquid medium inside the cochlea, so it can be expected that the presence or absence of cochlear fluid strongly affects the METF [2]. However, previous studies provide contradictory results concerning the effects of draining the cochlea on stapes displacement. Gyo et al. find that the displacement of the stapes, after cochlear destruction by severing, increases by about 10 dB at frequencies above 0.6 kHz [3]. The analog circuit models of Goode et al. seem to support this [4]. Lord et al. report almost opposite results [5]. They found no changes at higher frequencies and a slight decrement in stapes motion at low frequency. In their study the internal auditory meatus was drilled in 5 specimens till the medial stapes footplate (SFP) was exposed. Another study of 10 TBs found that draining of the cochlear fluid changes the motion patterns of the stapes at higher frequencies and results in an increase in METF throughout the measured frequency range, with 5 dB at 300 Hz, about 10 dB from 800 Hz on up to about 20 dB at 6 kHz [6]. Gan et al. found considerably lower changes. Aspirating the cochlear fluid from the scala tympani (ST) through the round window (RW) by use of a syringe with a silastic tube has been shown to result in a maximum increase of 3.4 dB at 4 kHz in 6 TBs which is also confirmed by their finite element method (FEM) prediction [7,8].

Part of the different results may be attributed to measurement methods and sometimes very small numbers of experiments, e.g. Ref. [3]. The observed minor changes between the different preparations might also be explained by the function of the cochlear aqueduct (CA), a small canal connecting the cochlea's perilymphatic fluid and cerebrospinal fluid (CSF) surrounding the brain, plays a role in regulating cochlear pressure and fluid homeostasis [9,10]. It allows more or less fluid to flow between the cochlea and the CSF, which could result in differences in the pressure dynamics of the perilymph [11]. The size of the CA and its course vary in humans [12]. Such individual anatomical variations in the CA and in the preparations (position of the cutting plane of the CA), may increase variability in studies on human TBs. In some cases, air bubbles can form relatively quickly in the cochlea without this already being visible on the round window (RW). A metrological check for air bubbles in the cochlea is therefore particularly important (phase difference of the vibrations at stapes and round window).

Another apparent reason may be the different methods of drainage used in these studies, e. g. cochlear destruction totally by severing, drilling along the internal auditory to expose the medial surface of footplate, or just suction of cochlear fluid without any structural damage to the anatomy of the cochlea. These various preparation methods likely cause varying changes of impedance, which in turn would have different effects on the METF. However, the specific impacts are unknown yet. Therefore, a more comprehensive study under a standardized experimental setup is necessary to examine the effect of inner ear impedance reduction on METF quantitatively. Changes in inner ear impedance may already occur when opening the cochlear via round window or cochleostomy. As the stiffness of the normal round window membrane (RWM) is rather low compared to the stiffness of the stapedial annular ligament, the influence of the RWM on METF is usually neglectable[2]. However, RWM stiffness may vary individually also due to non-obvious pathological changes [13]. The second and main influencing factor is the cochlear fluid. As fluid removal might have been incomplete in previous studies, a defined stepwise procedure to remove the fluid will be used in this study.

Stapes footplate motion is composed of different modes of vibration, with piston-like motion being dominant at $f \leq 2$ kHz and higher modes (such as rocking motion) becoming more important at higher frequencies [6,14]. However, the majority of previous

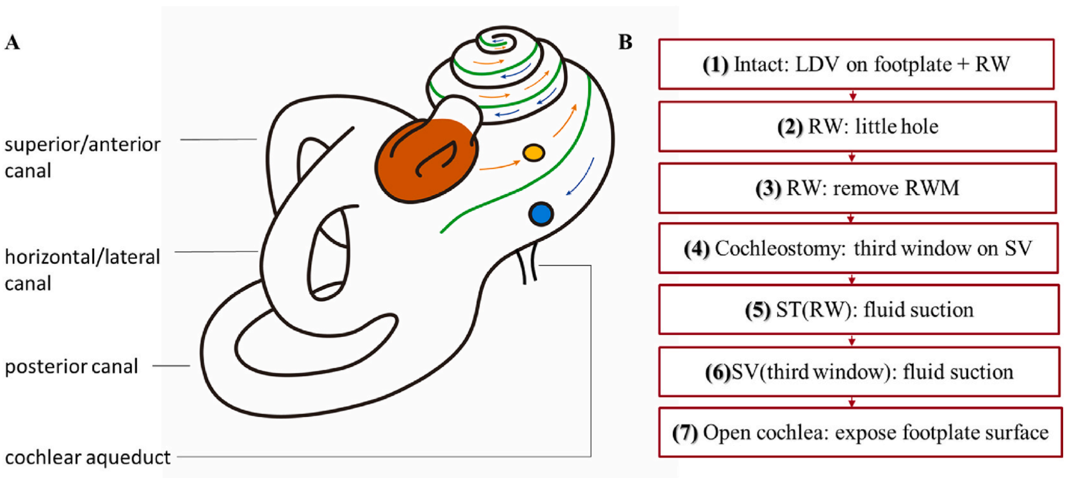

Fig. 1. A: Schematic structure of the inner ear: blue disc refers to round window membrane (RMW), red disc refers to oval window (OW), yellow disc refers to the third window made by cochleostomy. Blue arrow represents the fluid running in the scala tympani (ST), red arrow represents the fluid running in the scala vestibular (SV),. B: Experimental step 1–7. The vibrations at three points on the footplate were measured with Laser Doppler Vibrometer (LDV) at each step. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

studies used single point LDV measurements, where results may vary with the exact measurement position. In this study we therefore used three point measurements to obtain the main translation and rotation of the footplate and their change with cochlear drainage.

2. Methods and materials

2.1. Preparation

Eleven freshly frozen human cadaveric TBs were prepared for this study. Before preparation, they were evaluated for normal middle ear and TM using microscopy. After defrosting they were thawed in water at 4 °C for 24h and then warmed to room temperature before the measurement.

Mastoid approach and posterior tympanotomy were used to reach the middle ear cavity. The facial recess was opened to expose the stapes footplate. All middle and inner ear structures were kept intact during preparation. If necessary, the RWM was fully exposed by soft tissue removal and/or drilling of a bony coverage.

2.2. Experimental protocol

Measurements at the specimen were performed with intact and successively drained cochlea in 7 steps (see also Fig. 1).

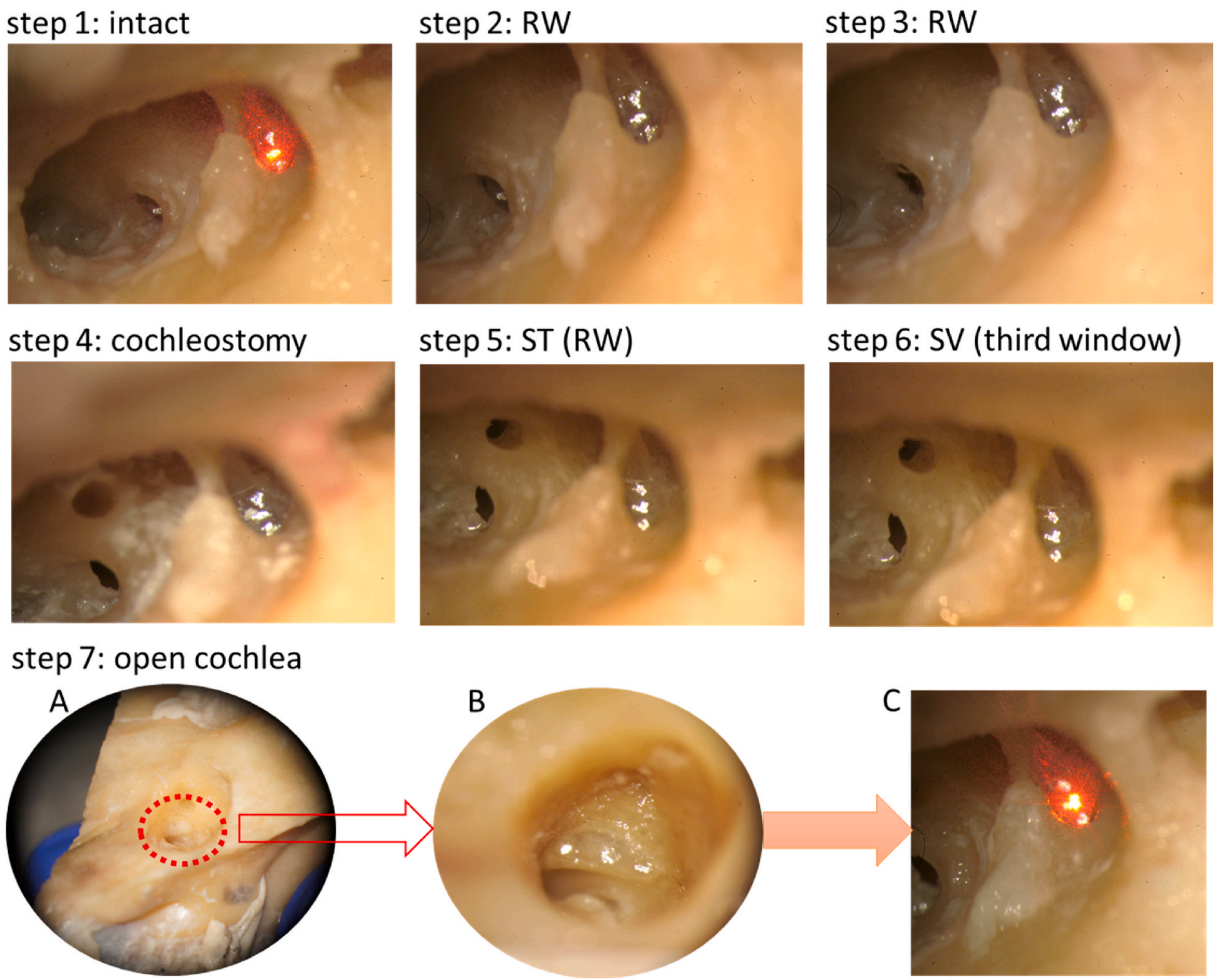

Fig. 2. Sequential photos of steps 1–7 in experiment, with laser measurement focus points marked by reflective foils, from up to down in order of the focus at crus anterior, center point, and crus posterior. Step 7 is detailed in images A–C: A shows how the cochlea is destroyed and the location of drilling, B displays the vision of footplate medial side inside cochlea afterwards, and C depicts the visual field employed for LDV measurement.

1. Initially, the vibration of footplate was measured as the reference in the intact ear. In order to check for air bubbles in the inner ear, vibration of the round window membrane was also measured. The phase difference between RWM and footplate must be around 180° for an intact, completely fluid filled cochlear [15,16].
2. In order to investigate in detail the effect of varying degrees of RWM stiffness reduction, the RWM was first punctured with a hole (∅ 1 mm) with a syringe.
3. Then the RWM was removed completely.
4. A cochleostomy was drilled 3 mm above the RW into the scala vestibuli (SV) using a ∅ 0.8 mm diamond burr. For prevention of perilymph leakage, the underwater technique, namely middle ear filled with water, was utilized during cochleostomy. This was a preparatory step so that the liquid could be removed in the later steps. This step can also show whether cochleostomy has any more influence on METF than the round window.
5. Next, the cochlear was drained by careful suction of the fluid through the RW.
6. To conduct a complete cochlear drainage, possible remaining fluid was removed by suction through the cochleostomy. In both steps drainage was confirmed by visual inspection with the microscope only. Complete removal of the fluid cannot be guaranteed.
7. Finally, the medial part of cochlea was destroyed by drilling near the internal auditory meatus, in an angle perpendicular to the footplate, to open the cochlear cavity such that the footplate undersurface was exposed. An intact annular ligament of stapes and partial cochlear bony structures such as cochlear basal turn, round window and cochleostomy window, were retained afterwards. Fig. 2 illustrates the specifics of this step.

The vibrations of three points on the footplate (midpoint, crus anterior and crus posterior) were measured with the LDV for each experimental step. Steps 1–3 took approximately 5 min each, steps 4–6 took approximately 15 min each, totaling around 1 h. Step 7, involving cochlear drilling with water, took about 45 min. The results of these steps were compared with baseline measurements to quantify the effect of drainage process on the translational and rotational movement of the stapes footplate.

2.3. LDV measurement

A hole (∅ 1 mm) was drilled in the lateral wall of the external ear canal, just above the umbo. A probe microphone (ER7c, Ethymotic Research, Inc., Elk Grove Village, Illinois, U.S.A.), measuring the input sound pressure to the TM, was placed 3 mm in front of the umbo. An earphone (EARtone 3A) was inserted into the ear canal to provide sound excitation. Multisine excitation signal from 0.05 to 6 kHz was generated at a sound pressure level of 100 dB SPL (256 spectral lines). Three reflective foils with a size of approx. 0.05 mm^2 were placed at the footplate center, crus anterior and crus posterior separately, along the long axis of SFP, to improve the signal quality of the laser. One reflective foil was placed at the center of the RWM. The vibration velocity of these points was measured by a laser Doppler vibrometer LDV (laser head CLV 700, controller CLV 1000, Polytec PI, Waldbronn, Germany). The laser head was mounted to an operating microscope (OPMI 111, Carl Zeiss, Oberkochen, Germany) and the laser beam (spot size 0.1 mm2) was positioned by use of a micromanipulator (self-construction). Velocity was mathematically integrated to the displacement. METF was determined as the stapes footplate displacement with respect to the excitation sound pressure in front of the TM. Round Window Transfer Function (RWTF) was determined as the RWM displacement with respect to the excitation sound pressure in front of the TM. METF and RWTF were measured as frequency response functions with 20 averages.

LDV measurements were carried out at an angle of about 30° out of the stapes axes (footplate to head). As a result, the measured METF magnitude is approx. 1.2 dB lower than the actual magnitude. No cosine correction was carried out, as the METF of the different

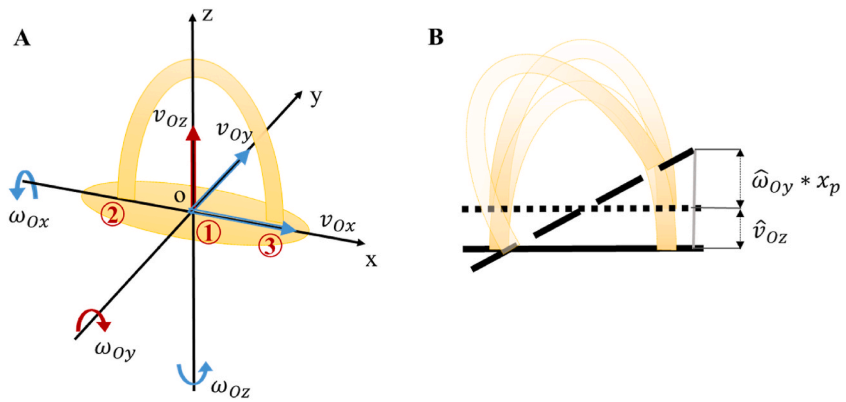

Fig. 3. A: The 3D motion of the stapes footplate includes translational velocity components and the angular velocity components. In our study, the translational velocity v_{Oz} and the rotational component ω_{Oy} (red arrows) are calculated from the measuring data v_{1z}, v_{2z} and v_{3z} at targets 1, 2, and 3. B: The solid line is the original position of the footplate. Dotted line is the position of the footplate for (piston-like) translation motion only. Long dashed line is the real position of the footplate for combined translation and rotation. The motion at the edge of the footplate can be composed from the translation part v_{Oz} and the rotation part $\widehat{\omega}_{oy} * x_p$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

states (step 1–6) within a preparation are always compared with each other and the measurement angle remains the same during this process (see also below).

The METF measured at step 1 were compared with standard ranges in the literature to exclude functionally abnormal specimen, i.e. such specimen that exhibit pathological sound transmission of the intact middle ear [1]. Also in step 1 the phase difference between METF and RWTF was determined. Values around 180° were indicative of a cochlea without air bubbles. Subsequently, the steps 1–7 were performed on the specimen as per experimental protocol. In order to ensure the accuracy and comparability of the measurements, initially a microscopic view field to simultaneously observe the footplate and RW was found, after the specimen was fixed on the experimental table. Then the specimen was not moved, only minor angle adjustments of microscope as necessary, for laser measurement from step 1–6. Repositioning accuracy of the LDV to the three measurement positions was about 0.1 mm (This results from the very small foils). The specimen was also returned back to the same measuring position after cochlea destroy (step 7), with a repositioning accuracy of the angle of the LDV measurement of about 5° (i.e. $<$0.5 dB variability in METF magnitude). The vibrations of 3 points on the footplate were measured with the LDV at each step.

2.4. Calculation of SFP rotational motion

Here the method stated by Hato et al. was taken to calculate the rotational motion of the SFP[6].

The motion of the stapes as a rigid body can be described by three translations and three rotations of the footplate center point (see Fig. 3A). These are the translations in the long and short axis of the footplate v_{Ox}, v_{Oy} , the translation perpendicular to the footplate surface v_{Oz} and the three rotations around these axes $\omega_{Ox}, \omega_{Oy}, \omega_{Oz}$. From these motions only $v_{Oz}, \omega_{Ox}, \omega_{Oy}$ are relevant for sound transfer to the cochlear, as they induce a volume velocity of the inner ear fluid. We focus on v_{Oz}, ω_{Oy} in our study, as ω_{Ox} is of minor importance and rather difficult to determine exactly.

With LDV we are able to measure motion in z-direction at various points of the footplate. The three measured points are located near the middle of the footplate (point 1), near crus anterior (2) and near crus posterior (3). They were chosen along the long axis of SFP to calculate the rotational motion across the short axis.

The relationship for the motion at any measurement point at the SFP is

$$v_{pz}\left(x_p\right) = v_{Oz} - \omega_{Oy} * x_p$$

Where x_p is the distance of the measurement point to the footplate center point. The distances x_p were obtained from photos of the stapes footplate with the LDV spots. A mean footplate length of 2.83 mm was used for all specimen [17]. The distances were calculated with ratio equations.

With our three measurement points we get an over determined system of equations for the translation v_{Oz} and rotation ω_{Oy}:

$$\begin{bmatrix} v_{1z} \\ v_{2z} \\ v_{3z} \end{bmatrix} = \begin{bmatrix} 1 & -x_1 \\ 1 & -x_2 \\ 1 & -x_3 \end{bmatrix} \begin{bmatrix} v_{Oz} \\ \omega_{Oy} \end{bmatrix} \text{ or } \begin{bmatrix} v_{1z} \\ v_{2z} \\ v_{3z} \end{bmatrix} = X \begin{bmatrix} v_{Oz} \\ \omega_{Oy} \end{bmatrix}$$

This system can be solved with a minimum least square approach:

$$\begin{bmatrix} v_{Oz} \\ \omega_{Oy} \end{bmatrix} = \left(X^T X\right)^{-1} X^T \begin{bmatrix} v_{1z} \\ v_{2z} \\ v_{3z} \end{bmatrix}$$

In order to compare translation and rotation components, the rotational component ω_{Oy} was multiplied by the half length of the footplate long axis (1.415 mm) to get the translation (due to rotation) at the edge of the footplate (see Fig. 3B). The ratio of translation due to footplate rotation is given by:

$$ratio = \frac{\widehat{\omega}_{Oy} * 1.415\ mm}{\widehat{v}_{Oz}}$$

2.5. Comparison of single point and three points method

In most studies the METF is determined from single point measurements of stapes motion at a point near but never exact at the center of the footplate. In our study this corresponds to the measurement at point 1. The translation v_{Oz} of the footplate, however, would be a better measure for the METF as it corresponds to footplate volume velocity (assuming a symmetric footplate) and thus to induced inner ear fluid pressure. We therefore also compare v_{1z} and v_{Oz} for every experimental step.

2.6. Statistical analysis

All data were log-transformed to a dB-scala as previous METF measurements indicated a log-normal distribution[1]. All statistical analyses were done on this transformed data. Data evaluations were performed at frequencies of 0.25 kHz, 0.5 kHz and further in steps of 0.5 kHz up to 5 kHz.

The distributions of the results over the eleven specimens were tested for normality with the Kolmogorov-Smirnov-test. The null hypothesis couldn't be rejected for frequencies of 1 kHz and above for all experimental steps. At lower frequencies there are some deviations from the normal-distribution assumption, but the Q-Q-plot shows that these deviations are rather small. As the *t*-test behaves quite robust against limited divergences from the normality assumption, 2-sided t-tests were used for comparative statistics [18]. A threshold value of $p < 0.05$ was used for statistical significance. A correction of the p-level is not needed here, which would be necessary only if the aim of the study would be to draw generalized conclusions across all groups together. Each *t*-test in this study is independent, assessing different hypotheses with independent variables like RWM stiffness, cochleostomy, cochlear fluid, and cochlear destruction.

3. Results

All specimens used for the experiment were validated and tested positive against literature data, which implies that every specimen used in this study has a free movement of the stapes despite individual anatomical variations existing [1]. Coherence was used as a criterion for measurement quality. Only measurement data with a coherence better than 0.6 were included in the evaluation. The 95% confidence interval of the METF magnitude is then + -2 dB [19]. The frequency range for the analysis was therefore restricted to 0.2–5 kHz. This frequency range still covers 94% of the weighted range relevant for speech intelligibility [20].

3.1. *The influence of drainage process on METFs*

The results of mean METF at each step obtained from eleven temporal bones are plotted in Fig. 4. Small changes in METF are visible for frequencies above 800 Hz. Changes seem to increase with every experimental step. Fig. 5 and Table 1 show these changes in more detail. The variation of individual measurements increases with every experimental step (Fig. 5). Changes become significant only for steps 6 and 7 and for frequencies≥3 kHz (red values in Table 1).

3.1.1. *Cochlear opening*

The METF does not change after RWM puncturing (Fig. 5A). There is also very little variation in the results from the individual specimen. The same holds true for complete removal of the RWM. Although results from single specimen show some change for this step. The variation in the results from the individual specimen already increases slightly in this step.

The variation in results increases even more when additionally a cochleostomy is performed in step 4 (Fig. 5C). These variations are more pronounced at higher frequencies (≥800 Hz), where the systems mass dominates the transfer function. This could be a sign of small fluid loss in the cochlea. There is also no significant change in METF for this step, compared to the intact cochlea.

3.1.2. *Cochlear fluid*

Removing the cochlear fluid significantly changes the METF at higher frequencies (≥3 kHz) but only at step 6 with additional suction via opening of the cochleostomy. After cochlear fluid drainage from both ST and SV the METF increases by about 7 dB for $f \geq 3$ kHz. Fluid suction just via the round window already shows the same trend of changes in METF but without statistical significance. Variations in the results are now up to 15 dB at higher frequencies (≥3 kHz) and around 5 dB and even less below 800 Hz. The results can be summarised as follows: the more completely the liquid is drained, the more the METF increases. The increasing variation in results across specimen indicates possible incomplete fluid suction.

3.1.3. *Cochlear destroy*

Comparison measured between step 7 with step 1 finds statistically significant difference at high frequency above 3 kHz in eleven

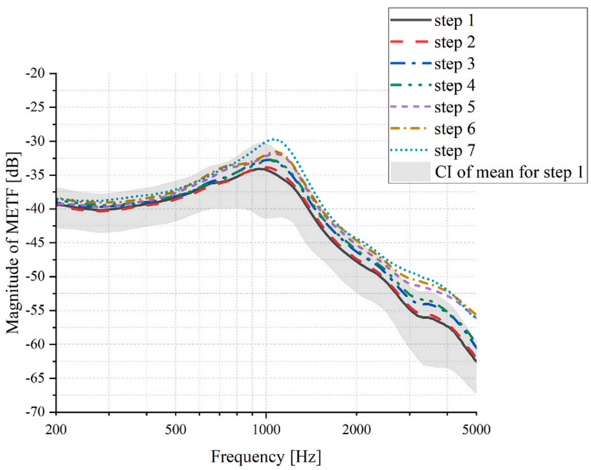

Fig. 4. Mean METF (n = 11) for every experimental step from intact (step 1) to cochlear drilling (step 7).

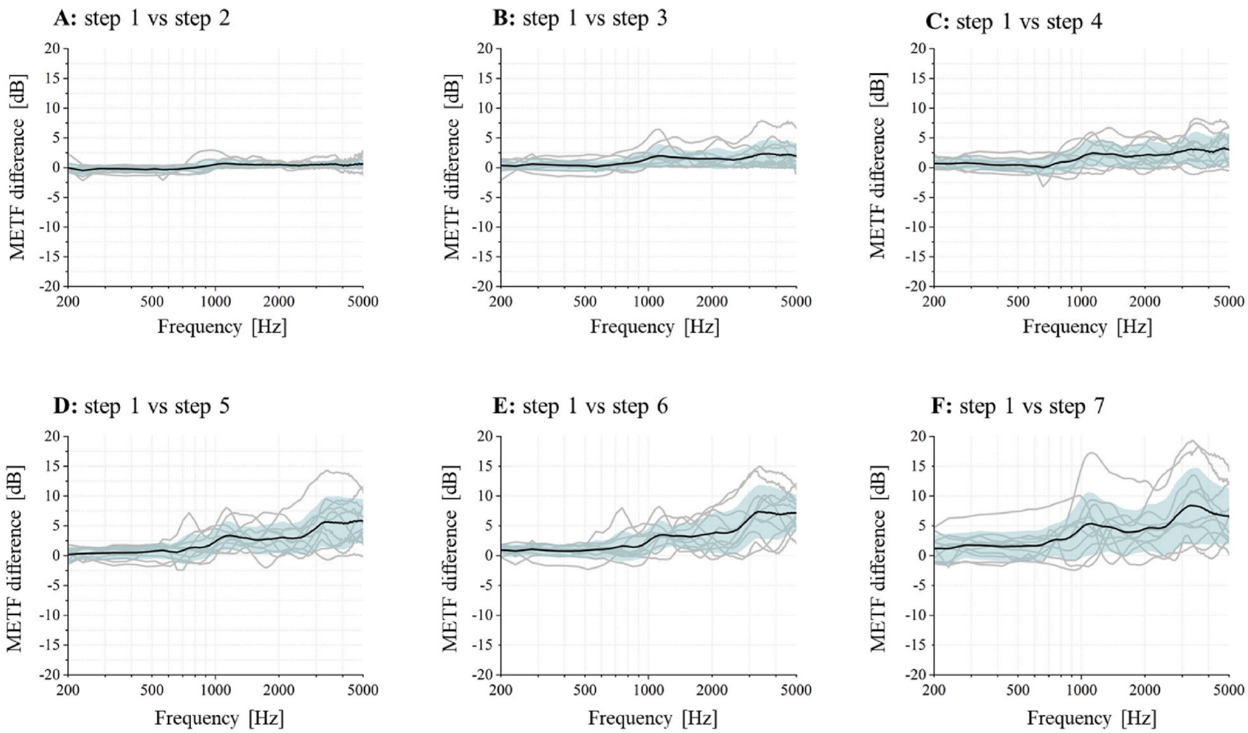

Fig. 5. METF difference resulting from stepwise destruction and draining of the cochlea compared to intact reference (n = 11). A–F: cochlea drained and destroyed from step 2 (punctured round window membrane) to step 7 (cochlea destroyed). Gray curves are individual specimen results, black curves are mean and the blue area presents the confidential interval of the mean. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
The statistical analysis for the difference of METFs between every step with intact group.

	Frequency (kHz)	0.25	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5
step 1 vs 2	difference	-0,471	-0,205	0,48	0,505	0,519	0,342	0,523	0,602	0,398	0,463	0,545
	p value	0,821	0,928	0,867	0,861	0,86	0,914	0,878	0,877	0,913	0,897	0,875
step 1 vs 3	difference	0,266	0,319	1,464	1,615	1,541	1,324	1,774	2,329	2,218	2,197	1,858
	p value	0,894	0,885	0,615	0,578	0,611	0,681	0,607	0,555	0,55	0,552	0,606
step 1 vs 4	difference	0,645	0,437	1,674	2,087	2,166	2,156	2,745	3,059	2,78	3,051	2,957
	p value	0,75	0,842	0,552	0,45	0,444	0,473	0,408	0,429	0,43	0,372	0,384
Step 1 vs 5	difference	0,357	0,767	2,206	2,72	3,014	3,002	4,655	5,573	5,508	5,759	5,797
	p value	0,862	0,732	0,445	0,329	0,287	0,317	0,161	0,14	0,131	0,125	0,135
Step 1 vs 6	difference	0,765	0,892	2,264	3,265	3,782	3,989	**6,293**	**7,302**	**7,065**	**7,026**	**7,116**
	p value	0,708	0,698	0,444	0,231	0,169	0,147	**0,033***	**0,032***	**0,028***	**0,039***	**0,047***
Step 1 vs 7	difference	1,165	1,583	4,458	4,322	4,551	4,823	**7,605**	**8,308**	**7,503**	**6,806**	6,472
	p value	0,554	0,447	0,136	0,116	0,087	0,065	**0,009***	**0,015***	**0,017***	**0,040***	0,061

temporal bones. After cochlear destroyed, we measured larger increases and variations of results in METF magnitude than previous steps, a mean increment less than 1.6 dB for $f < 1$ kHz, a mean increment of about 4.5 dB for 1 kHz $\leq f < 3$ kHz, and a mean increment of 7.6 dB for 3 kHz $\leq f < 5$ kHz. However, the increment is slighter at 4.5 kHz than that between step 6 with step 1, and becomes insignificant at 5 kHz.

3.2. The role of rotation in the change of footplate motion with drainage process

Fig. 6 illustrates the change of rotational motion at every step. When the relative motion level is close to 0 dB, the ratio of translational displacement at x-z edge caused by rotation to the translational movement at the center point is nearly 1. This implies that the percentage of rotation taken up in the overall footplate motion reaches to maximum. For intact measurements, the mean ratio magnitude is approximately −17 dB at 0.2 Hz, reaching the minimum around −20dB values below 1 kHz. The rotational component tends to a stable increment with frequency from 1 kHz, with a ratio of about −8 dB around 5 kHz. Some curves in Fig. 6 rise at low frequencies, which is probably negligible measurement errors.

Fig. 7 and Table 2 demonstrate the difference of rotational ratio between every step with the intact reference. The comparisons between step 2, 3, 4 with step 1 find the factors of RWM's stiffness and cochleostomy do not result in any statistically significant change. Reducing RW stiffness causes only a mean decrement of about 1 dB to rotational motion of SPF, cochleostomy causes a mean decrement of 2 dB. The differences between step 5, 6, 7 with step 1 are statistically significant. After cochlear fluid drained only from ST, the mean decrement of 7.4–8 dB occurs in rotational component for 4–5 kHz. After drainage from both SV and ST, the mean decrement of 5.6–7.6 dB for the range of 3–5 kHz. After cochlea drilling, the mean decrement of 5.8–8.85 dB for 3–5 kHz, where it is deserved to notice that the decrease appears slighter above 4 kHz rather than a stable decrement with frequency when inner ear impedance absent. The relative rotational ratio of step 7 has similar values with step 6, suggesting that cochlear destruction and merely complete cochlear drainage perform the similar effect on rotational motion of SPF.

3.3. *Comparison of single point and three points method*

As shown in Fig. 8, the statistical validation shows that the different value between v_{1z} and v_{Oz} at every step are very small close to 0 dB in the entire measurement frequencies. It suggests that almost no difference exists between the results from single point method and three points method.

4. Discussion

4.1. *The influence of cochlear drainage on METFs*

4.1.1. *Intact versus completely drained cochlear*

The complete draining of the cochlea (steps 6, 7) does result in significant increase of more than 6 dB across the frequency range of 3–5 kHz. Here the group of step7 can be used as a reference to confirm whether the drainage by suction method was complete. In theory steps 6 and 7 should induce similar effects (both are fully drained state). However, actually step 7 shows larger increase and variability during 0.8–1.5 kHz. The variability may be due to the more invasive drilling work. Such stronger intervention could cause damage to the delicate structures of the middle ear (especially annular ligament), leading to increased variability in METFs. The influence of complete drainage on the METF appears to be frequency dependent, with a stronger effect and statistically significant mean change at higher frequencies ($f \geq 3$ kHz), which is consistent with Merchant et al. that the resistance of cochlear fluid dominates the inner ear impedance above 2 kHz [2]. The fact that the increase in METF is significant above 3 kHz but becomes insignificant at 5 kHz may be due to the complex motion of the stapes. The translational component of the overall motion of the stapes footplate, as measured by METF, is reduced significantly at higher frequencies, with the increasing prominence of the rotational component dominated by cochlear fluid [21]. Consequently, the increase in METF is not as noticeable as it is at lower frequencies.

4.1.2. *Steps of cochlear drainage and influence on results*

A detailed analysis of cochlear drainage methods in previous literature revealed varying drainage levels, which is the probable

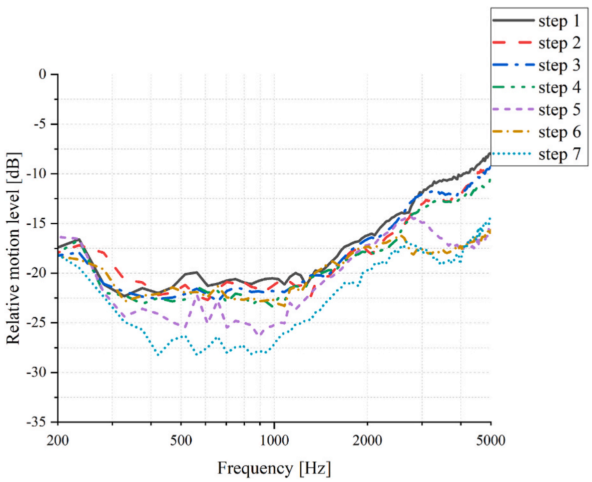

Fig. 6. Mean ratio of relative magnitudes of x-z edge velocities due to rotation with respect to the footplate center velocity in the z direction (n = 11).

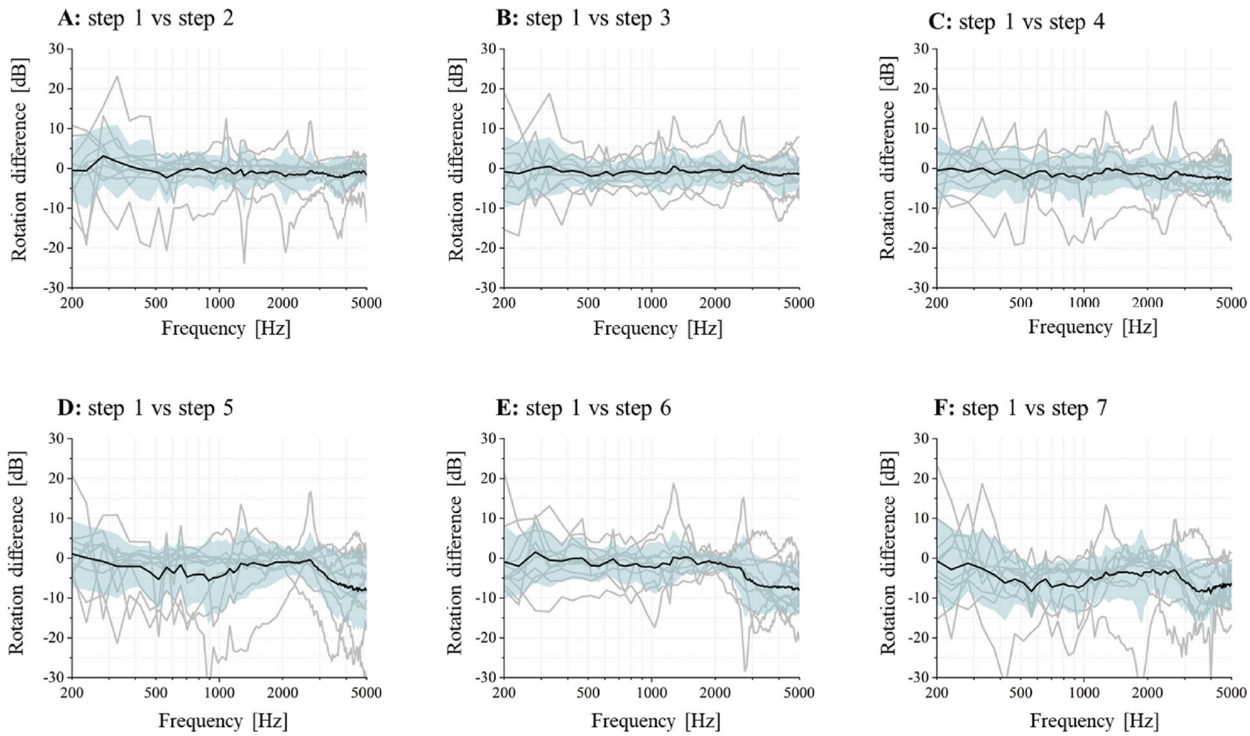

Fig. 7. Ratio of rotational to translational motion of the stapes footplate compared with intact cochlea reference (n = 11). A–F: cochlea drained and destroyed from step 2 (punctured round window membrane) to step 7 (cochlea destroyed). The blue area in the figure refers the confidential interval of the mean. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
The statistical analysis for difference of rotational ratio between every step respect with intact reference.

	Frequency (k Hz)	0.25	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5
step 1 vs 2	difference	-0,586	-1,084	-0,777	-1,221	-1,726	-1,519	-1,088	-2,05	-1,709	-1,098	-1,648
	p value	0,82	0,745	0,811	0,683	0,529	0,502	0,635	0,515	0,545	0,677	0,55
step 1 vs 3	difference	-1,315	-1,994	-1,263	-1,025	-0,373	-0,686	-0,091	-1,353	-1,783	-1,4	-1,22
	p value	0,611	0,57	0,664	0,705	0,862	0,756	0,968	0,637	0,517	0,572	0,615
step 1 vs 4	difference	-0,025	-2,538	-2,825	-0,569	-1,646	-2,518	-1,408	-2,158	-2,208	-2,28	-2,568
	p value	0,992	0,549	0,485	0,842	0,536	0,409	0,588	0,449	0,336	0,286	0,23
Step 1 vs 5	difference	0,064	-5,33	-4,797	-1,363	-1,003	-0,848	-2,978	-6,066	-6,775	**-7,403**	**-8,038**
	p value	0,983	0,269	0,316	0,662	0,671	0,707	0,285	0,091	0,051	**0,035***	**0,024***
Step 1 vs 6	difference	-2,015	-1,838	-2,101	0,127	-1,192	-2,301	**-5,646**	**-7,107**	**-7,02**	**-7,354**	**-7,602**
	p value	0,409	0,583	0,551	0,962	0,563	0,276	**0,042***	**0,016***	**0,008***	**0,007***	**0,006***
Step 1 vs 7	difference	-2,864	-6,199	-6,913	-3,589	-3,518	-3,909	**-5,813**	**-8,389**	**-8,849**	**-7,5**	**-6,289**
	p value	0,338	0,104	0,092	0,259	0,222	0,155	**0,026***	**0,006***	**0,007***	**0,005***	**0,009***

cause for the conflicting study results [3–7]. To address these conflicts, we devised a seven-step investigation protocol to effectively clarify the influence of different drainage levels on outcomes. By pre-steps of RWM removal and cochleostomy, we were able to drain the cochlear fluid from the ST and SV through the RW and an artificial third window on the cochlea stepwise. After the fluid was completely drained from both windows, we destroyed the cochlea entirely by drilling.

We were not able to find a statistically significant mean change to sound response caused by cochlear drainage only from ST. Only when perilymphatic fluid was drained from both SV and ST, the mean stapes displacement exhibits a statistically significant increase. The complete draining of the cochlea (steps 6, 7) does result in significant increase, although partial draining (step 5) does not. This fact offers an explanation to solve the contradictory results in this regard. Previous study results, which find no influence of cochlea impedance, may be due to incomplete drainage of the cochlea. As well as in the comparison between Gan et al., our results show a

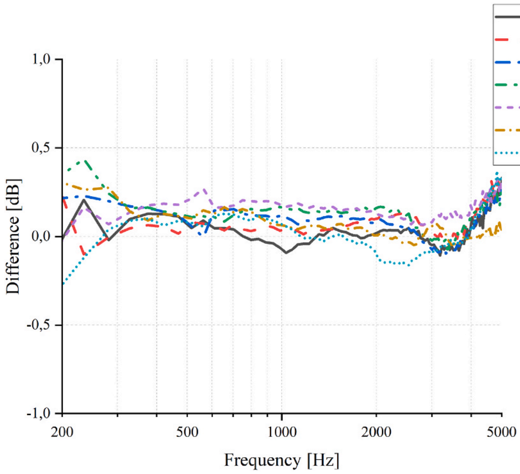

Fig. 8. Difference between the displacement magnitude measured at a central point of the footplate and the calculated translation magnitude (from the three points measured at the footplate). Mean over all specimen (n = 11) for each experimental step.

larger value of increment at high frequencies 3–5 kHz [7]. This is probably also due to our more complete drainage of cochlea from both SV and ST after cochleostomy, where they only drained from SV.

4.1.3. Comparison with literature data

We compare our findings with previous studies that claimed that they drained the TB cochlea, and separate the literature simply into two paragraphs based on different draining methods used: fluid suction only or cochlea destroyed.

In a single TB, Voss reports the magnitude of the stapes movement increases 1–2 dB below 1 kHz and 5–7 dB above 1 kHz due to perilymphatic fluid leak after RW puncture [22]. They do not introduce the exact drainage procedure, but confirm the draining of the cochlea drained by showing their data of the impedance reduction to be consistent with the measurement in the cat [23]. But the difference found by Voss is with huge individual characteristics [22]. By manipulating the cochlear fluid to vary the inner ear pressure from 0 to 400 mm H_2O on 10 fresh TBs, Myers et al. report a mean maximal change in stapes movement varied from 1.7 to 8.4 dB in the range of 0.4–5 kHz [24]. However, the major effect occurs below 1.0 kHz, opposite with our results, the reason probably is that the distention of the annular ligament produced by the artificial high inner ear pressure, which affects the inner ear impedance at low frequency. Gan et al. aspirate the cochlear fluid by syringe with silastic tube only from ST through RW[7]. Their results from six TBs demonstrate that the cochlear impedance changes if the perilymphatic fluid is drained. After cochlear fluid drainage from RW, the METF increase is 1 dB below 1 kHz, and reaches 3.4 dB maximum at 4 kHz after gradual rise from low frequency to high frequency. Their results are also demonstrated by their finite element modeling prediction[8]. We notice that our results is similar with Gan et al., but the maximum values differ [7].

Although it must be noted that Gyo et al. only included one specimen and there are some notable differences in the methods (measurement point at stapes head instead of SFP), the METF change resulting from complete cochlear destruction is consistent with findings of Hato et al. and ours [3,6]. The cochlea destruction increases stapes motion at the entire frequency range but mainly affects higher frequencies. However, the maximum increment is at the resonant frequency around 1 kHz. Although we also have individual TBs reaching a similar maximum incremental value at 1 kHz, after statistical calculation, we demonstrate that the significant mean increment occurs merely above 3 kHz, with the mean maximum of 8 dB at 3.5 kHz. This is in contradiction to the results of Lord et al., which a slight decrement in stapes motion below 1 kHz, and no statistically significant difference in 5 TBs above 1 kHz [5]. Nevertheless, the prediction of the electrical analog model from the same publication shows an increase at all frequencies, which is in agreement with our findings. Since the experimental setups are similar, one possible reason could be that Lord et al. did not check if there was air entering the inner ear in the process of the freezing and thawing of specimen before intact measurement [5]. Such air bubbles in the cochlea might cause a reduction of the inner ear impedance even when the specimen is intact and produce misleading results [25]. In order to exclude these specimens with perilymphatic air bubbles from our experiment, this study tested and ensured a 180-degree phase difference between the RW and footplate at start.

4.1.4. Rotational movements with intact versus drained cochlea

The ossicular chain has a complex motion pattern during middle ear sound transmission. Non-symmetric motions of TM stimulate footplate rocking, while the shape of the malleus handle and the deformable joint shape both contribute[26]. Excitation of the stapes head parallel to the footplate finally results in rotational motions [21]. For this reason, it seems sensible to take a closer look at how the change of rocking motion contributes to the final change caused by cochlear drainage process. Our results indicate a close correlation between the alteration in rotational motion (Table 1) and the change of METF (Table 2) during draining process.

For step 1, Our results of intact group are similar to Heiland et al., and they show a similar trend of increase towards higher frequencies but are generally lower than the ratios reported by Hato et al. [6,27]. As shown in Fig. 9, the mean ratios reported by

Heiland et al. and Hato et al. have minimum of −20 dB at 0.5 kHz, and reach 5 dB SPL at 5 kHz, Sim et al. report minimum of −12 dB at 0.5 kHz, maximum of −3 dB SPL around 5 kHz [6,14,27].

A noteworthy finding is that with partial drainage (step 5), a statistically significant decrease in rotational motion was observed, while significant change in METF occurred only at complete drainage (step 6). This shows that the rotational component is more sensitive to cochlear drainage than the overall motion at high frequencies, which is in accordance with Hato et al. [6].

For steps 6 and 7, we can see that the rotational motion component keeps decreasing with frequency for the entire range of 0.2–5 kHz. This indicates that when the cochlea is drained, the motion becomes more piston-like again at higher frequencies. As a result, the difference in Table 2 is less at low frequencies, where the higher modes are less dominant even in the intact perilymphatic state. The curve at step 7 is consistently about 5 dB SPL lower than the results reported by Hato et al. across the range of 0.4–4 kHz (see Fig. 9) [6]. Our data suggests that rotation plays lager role in overall SFP motion above 4 kHz. An abnormal upward trend observed in the curve below 0.4 kHz may be attributed to measurement error caused by unstable low-frequency coherence, where coherence below 0.6 indicates that the presence of noise or other sources of distortion can interfere with the measurement.

4.2. Influencing factors

To achieve partial and complete drainage, the steps of RWM removal and cochleostomy were included in our experimental protocol. These factors, closely related to inner ear impedance as well as biomechanical environment of middle ear, can potentially affect METF and stapes motion [28]. Their statistical insignificance further confirms the exclusion of their interference in the evaluation of drainage procedure. Additionally, we considered the evaluation of calculation tools, which could also impact the results. All these influencing factors are discussed collectively here.

4.2.1. RW stiffness

In our study, though individual TBs did exhibit stronger effects especially when the RWM was removed completely (step 3), we find no statistically significant change in the mean (see Fig. 5 and Table 1). That can be attributed to the fact that the RWM has a good compliance to sound pressure (since that is its function), so one would expect the effect of decreasing stiffness to be small [15].

4.2.2. Cochleostomy

Cochleostomy is a very common operation during cochlea implant surgery. In our study cochleostomy has a slight incremental effect (up to 3 dB) on METF, however, based on the available data the change is not statistically significant. Despite cochleostomy without subsequent connective tissue sealing, we still find a similar result in the mean with Pazen et al. [29]. Our findings about rotational motion of SFP is also comparable with them. Pazen et al. find that after cochleostomy is drilled and closed again with connective tissue, the changes of METF and the volume velocity of oval window (rotational ratio) in six TBs are statistical insignificant, suggesting that cochleostomy does not significantly alter cochlear input impedance [29].

4.2.3. Single point versus 3-point measurements

We compare the results of center point of SFP, v_{1z} and v_{Oz}, obtained from single point method and three points method at each step. The statistical validation shows no significant difference between the two methods at each step, suggesting that actually the center point measured by single point method has almost same location with that calculated by three points method. Thus, the single point method is reliable enough to represent the movement of footplate relative to three points method.

4.3. Experiment's limitations

4.3.1. TB freshness

TB freshness always raises the concerns about its potential unpredictable impact on experiment results. In our study, step 1–6 cumulatively took about an hour before step 7, which involved cochlear drilling with water. This duration in air exposure would lead to gradual drying and diminished freshness of the TBs, which may affect METF measurements. Therefore, following previous experience, we applied the standard procedure in our laboratory for METF measurements on TBs, which includes the maintenance of the TBs wetness during the experiment. Moreover, for further examination this issue, we did a pre-experiment on 2 TBs with cochlea destroyed, it revealed only no more than 1 dB shift of METFs in 1-h fully air exposure. But sample size of pre-experiment is small, marking one limitation of the study.

4.3.2. Increasing cochlear impedance

From a clinical perspective, the cochlear mechanics of round window reinforcement and resulting increased cochlear impedance (for instance in CI surgery) are of high interest. We did not include round window reinforcement in our experimental design, due to difficulties in quantifying the effect of reinforcement and experimental time control related to TB freshness. However, there is extensive literature on this subject. Most publications show a drop around 2 dB at the lower frequencies below 1 kHz on stapes movement [30].

5. Conclusion

This study confirms the influence of the reduced inner ear impedance on the METF, induced by complete drainage of cochlea, is

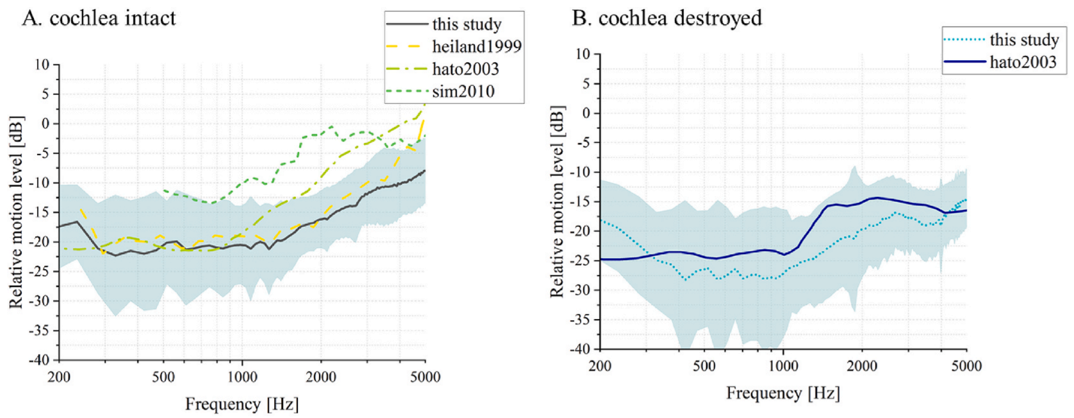

Fig. 9. Mean ratio of rotational to translational stapes footplate motion compared with literature data. A: rotation of SPF with intact cochlea, B: rotation of SPF with cochlea destroyed. The blue area in the figure refers the confidential interval of mean from this study. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

mainly important at higher frequencies ($\geq$ 3 kHz). Even when drainage is not complete, significant decrease of rotational motions appears above 4.5 kHz. The results suggest that various preparation methods, which have different degrees of cochlear drainage, will have different influence on inner ear impedance. Its influence on the METF results should be considered in the calculation and analysis of the results. This study summarizes the effects of cochlear drainage process related to inner ear impedance on the stapes motion, providing reference values for relevant studies. Among them, rotation motion plays an important role and deserves further study with more precise methods.

Data availability statement

Data associated with the study has not been deposited into a publicly available repository and data will be made available on request.

Ethics approval and consent to participate

All the experiments were performed under the approval from the Ethics Commission at the Technische Universität Dresden (BO-EK-422092022).

CRediT authorship contribution statement

Sijia Zhai: Data curation, Formal analysis, Funding acquisition, Investigation, Visualization, Writing – original draft. **Matthias Bornitz:** Methodology, Resources, Supervision, Writing – review & editing, Software, Validation. **Till Moritz Eßinger:** Visualization, Writing – review & editing. **Zhaoyu Chen:** Writing – review & editing. **Marcus Neudert:** Conceptualization, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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