Original Study

Reducing Artifacts in Intracochlear Pressure Measurements to Study Sound Transmission by Bone Conduction Stimulation in Humans

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Hypothesis: Intracochlear pressure (ICP) measurements during bone conduction (BC) stimulation may be affected by motion of the pressure sensor relative to the cochlear promontory bone, demonstrating the need to cement the sensor firmly to the cochlear bone.

Background: ICP is a promising measurement tool for investigating the cochlear drive in BC transmission, but its use is not yet standardized. Previous ICP studies have reported artificially increased pressure due to motion of the sensor relative to the temporal bone. The artifact can be reduced by firmly cementing the sensor to the bone, but this is destructive for the sensor. Previous studies used a custom-made sensor; the use of commercially available sensors, however, is more generic, but also more challenging to combine with the cement. Therefore, the goals of the current study are: firstly, to evaluate a non-destructive cementing method suitable for a commercially available sensor, and secondly, to investigate ICP measurements during BC stimulation in more detail.

Methods: To study the effect of sensor cementing, three fixation conditions were investigated on six fresh-frozen temporal bones: 1) alginate, 2) alginate and dental composite, 3) alginate and dental composite, released from micromanipulators. Pressures in scala tympani and vestibuli were measured simultaneously, while velocity measurements were performed on the cochlear promontory and sensor. The ratio between sensor and promontory bone velocity was computed to quantify the relative motion.

Results: For air conduction stimulation, results were in line with those from previous ICP studies, indicating that baseline measurements were valid and could be used to interpret the results obtained with BC stimulation. Results showed that cementing the sensors and releasing them from the micromanipulators is crucial for valid ICP measurements. When the sensors were only sealed with alginate, the pressure was overestimated, especially at low and mid-frequencies. When the sensors were cemented and held in the micromanipulators, the pressure was underestimated. Compared with the scala tympani measurements, ICP measurements showed a lower scala vestibuli pressure below 1 kHz, and a higher pressure above 1 kHz.

Conclusion: Dental composite is effective as a cement to attach commercially available sensors to the cochlear promontory bone. When sensors are firmly attached, valid ICP measurements can be obtained with BC stimulation.

Key Words: Bone conduction—Bone conduction device—Bone conduction stimulation—Cochlear transmission—Fiber-optic pressure sensor—Intracochlear pressure measurements—Velocity measurements.

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Sound transmission via bone conduction (BC) is a complex process with multiple transmission pathways. Disentangling the individual pathways is challenging due to overlapping mechanisms. For example, standard stapes velocity measurements are not applicable, as they only take forward stimulation into account (i.e., via the middle ear ossicles) (1). Cochlear promontory vibrations during BC were measured by Stenfelt and Goode (2) in cadaver heads to assess the transmission properties of the skull. However, conclusions cannot be drawn from cochlear promontory velocity regarding the related cochlear mechanics of BC excitation.

To study the cochlear mechanics of BC, the pressure driving the whole cochlea and the separate cochlear ducts need detailed investigation. Differential intracochlear

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pressure (ICP), which correlates well with human hearing perception (3–5), represents the cochlear input signal of air conduction (AC) stimulation. Only a limited number of studies measured ICP during BC stimulation. Two studies confirmed the importance of the inner ear in BC perception (6,7). These preliminary studies reported artificially increased pressure during BC stimulation, induced by the motion of the pressure sensor relative to the specimen. This artifact could be reduced by mechanically immobilizing the sensor with dental composite. Both studies (6,7) used a custom-made sensor (8), but commercially available sensors would be more useful for developing a generic method to facilitate preclinical characterization of acoustic hearing implants. Commercially available sensors to study BC sound transmission were only used in a few studies, but no artificially increased pressure was reported (9–11). Currently, there is no standardized ICP method for BC stimulation, and reference data from human cadaver studies are lacking, meaning that no solid conclusions about the cochlear drive responsible for BC hearing can be made based on ICP measurements.

The goal of this study is to investigate ICP during BC stimulation using commercially available sensors. In the first step, a reversible method to attach the sensors firmly to the cochlear bone is described, and the effectiveness of this method is investigated using velocity measurements. In the second step, the effect of BC stimulation on ICP is studied in human temporal bones.

MATERIALS AND METHODS

Temporal Bone Preparation

Six fresh-frozen human TBs were used, provided by the Vesalius Institute (Anatomy & Pathology) of the University of Leuven (KU Leuven, Belgium). Ethical approval for the use of human samples was obtained by the medical ethics committee of University Hospitals Leuven (NH019 2016-06-04).

All specimens were harvested and refrigerated within 72 hours postmortem, according to the guidelines described in ASTM-F2504 (12). During the experiment, each specimen was kept moist with a saline solution. Surgical preparation consisted of a canal wall-up mastoidectomy with enlarged posterior tympanotomy and partial removal of the mastoidal portion of the facial nerve to improve access to the middle ear cavity. The cochlear wall was then thinned at the level of the scala tympani (ST) and the scala vestibuli (SV).

Stimuli and Experimental Setup

Stimulation was performed either by air, via an insert earphone (ER-3C, Etymotic Research, Illinois), or by BC, via a Baha 5 Power actuator (Cochlear Ltd., Sydney, Australia) on a BI300 implant and 6 mm abutment. A probe tube microphone (ER-7C, Etymotic Research, Illinois) was mounted in a 10 mm adaptive foam-tip to measure ear canal sound pressure near the tympanic membrane. The bone screw was implanted at 4.5 to 5 cm from the ear canal center and fixed with dental composite to simulate osseointegration (Fig. 1A). Excitation sources were driven by a stepped sine sweep between 0.1 and 10 kHz. For AC, stimulation levels of 0.1 to 0.4 V_{RMS} were used, corresponding to output levels of 102 to 114 dB SPL. The BC actuator was stimulated with 1 V_{RMS}, which corresponds to output force levels (OFLs) ranging from 105 to 135 dB (re 1 μN), as measured on a TU-1000 skull simulator (Nobelpharma Inc., Göteborg, Sweden) (13).

TBs were mounted on modeling clay to provide stability of the TB and to obtain a smooth frequency response (7,14). All measurements were done on a vibration-free table (M-VIS3048-SG2-325A, Newport Spectra-Physics, Utrecht, the Netherlands).

Quality Control

Each TB was subjected to a previous quality control check (as described in ASTM F2504-05) (12). The external ear canal and middle ear cavity were inspected with a microscope. Hereafter, the middle ear transfer function (METF); the ratio between stapes velocity and ear canal sound pressure was

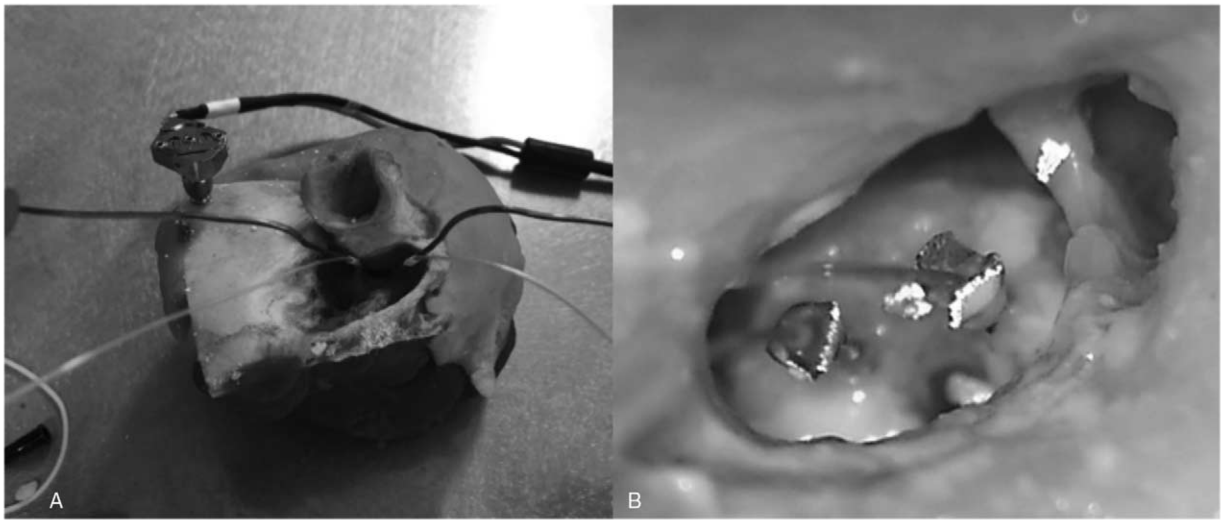

FIG. 1. Preparations used for intracochlear pressure measurements during bone conduction stimulation. *A*, View on the temporal bone mounted on modeling clay with a Baha 5 Power actuator. Pressure sensors are inserted in both scalae. *B*, Sensor insertion in scala tympani (*left*) and scala vestibuli (*right*). Reflective tapes have been placed on both pressure sensors, the promontory (center, between the scalae), and the stapes (*top right*).

compared with the defined Rosowski range (12,15) to check the middle ear functionality (Control 1) from 0.25 to 4 kHz. Stapes velocity was measured at the posterior crus with a single-point laser doppler vibrometry system, mounted onto a surgical microscope (OFV-534 Compact Sensor Head and A-HLV MM 30 Micromanipulator; OFV 5000 Vibrometer controller; Polytec GmbH, Waldbronn, Germany). During analysis, a cosine correction was applied when the incident angle between the laser beam and the stapes footplate exceeded 20 degrees.

Intracochlear Pressure Measurements

ICP was measured simultaneously in both scalae using two commercially available fiber-optic pressure sensors (FOP M-260, FISO Technologies, Canada), each with an outer diameter of approximately 310 μm, connected to a two-channel signal conditioner (VELOCE 50, FISO Technologies, Canada). Cochleostomies were drilled in both scalae using a diamond burr of 0.5 mm and a perforator of 0.35 mm while they were immersed in saline to prevent air entering the cochlea. Sensors were then inserted approximately 100 to 300 μm into the scalae, which could be visualized on the scale (per 100 μm) on the micromanipulators. Interferometric nulling of the sensors was performed after sensor insertion. Sensors were sealed using alginate (dental impression material; Alginoplast®, Heraeus Kulzer GmbH, Germany). In the fixation experiment (see below), the sensors were cemented to the cochlear promontory bone using dental composite (Dyract Seal, Dentsply Sirona, Pennsylvania). The composite was mixed with blue dye so that any leakage on the round window membrane or stapes footplate was visible, and cured with UV light for quick vulcanization. Figure 1B shows the setup of the two sensors—with reflective tape attached, for the velocity measurements—inserted in SV and ST and cemented with blue dental composite.

Dental composite has the advantage of quick vulcanization in a wet environment due to curing with UV light, and it can be removed with acetone after the experiment. The sensors were calibrated in a vibrating water column after each experiment to ensure that the sensor was not destroyed.

Velocity Measurements

A laser doppler vibrometry system was used to measure stapes velocity, as described earlier, and promontory and sensor velocity. Reflective tape ($<$1 mm^2) was attached to the optical fibers using cyanoacrylate glue, approximately 1 cm above the sensor's tip, placed so that the laser beam was in line with the sensor and avoiding additional wiggling motion. Reflective tape was also applied at the promontory, approximately 0.5 cm posterior to the round window membrane. The angle between the cochlear promontory and the laser beam was approximately 90 degrees in all specimens.

Experimental Procedure: Fixation Experiment

In the fixation experiment, a standard five-step experimental procedure was followed to test the effectiveness of the cementing method for each TB (Fig. 2). After surgery and quality control of the temporal bone (step 1), a first control measurement (Control 1) was performed (step 2) to assess middle ear functionality. After sensor insertion, alginate was applied around the sensors for the first condition, ''Alg.'' The METF and ICP were measured during AC stimulation, to check for the influence of the drilling and insertion procedure (Control 2) (step 3). Hereafter, the insert phone was removed, and

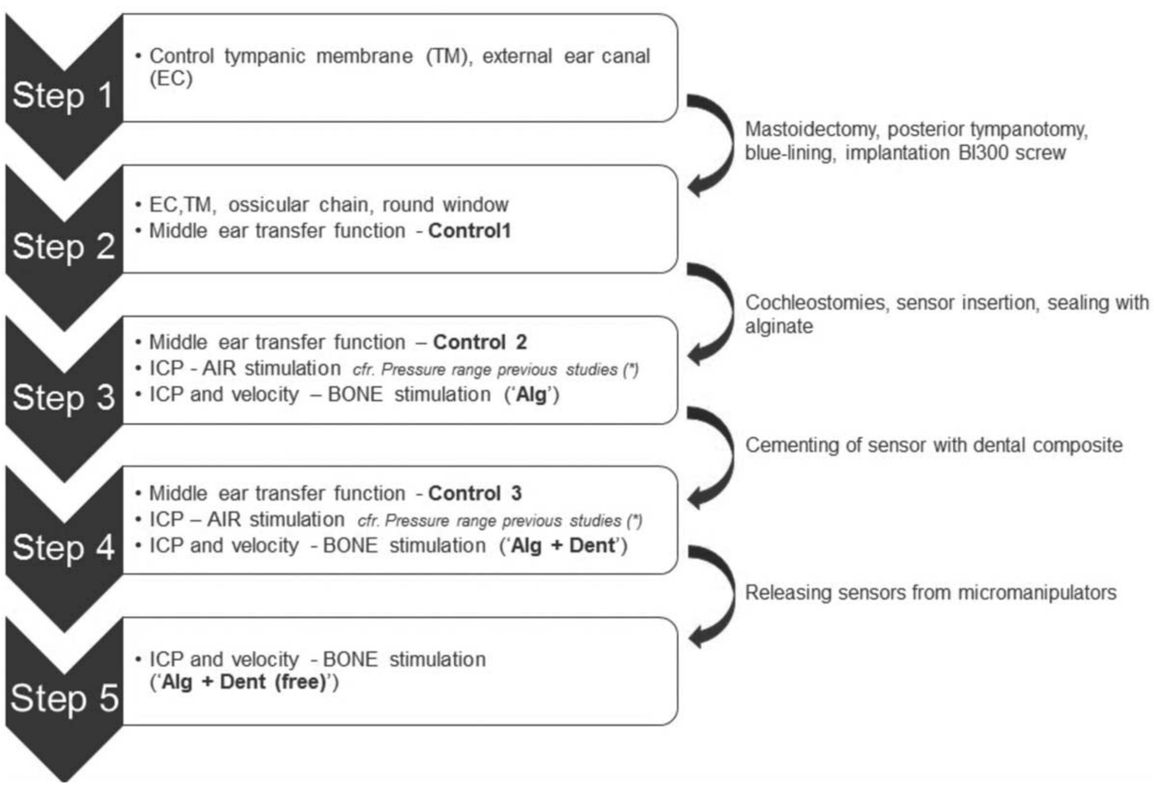

FIG. 2. Fixation experiment: overview of the experimental procedure, showing the five main steps and actions between the steps. (*) Comparison of intracochlear pressure measurements with previous studies: Nakajima et al. (3), Grössohmichen et al. (4).

promontory and sensor velocities were measured during BC stimulation. Next, the optic fibers were cemented with dental composite while still fixed in the micromanipulators, for the second condition, ''Alg + Dent.'' Throughout the application of dental cement, the round window membrane was visually inspected for leakage of composite on the membrane.

The sensors were then carefully released from the micromanipulators, while the middle ear was immersed in saline. As for the first and second conditions, ICP and velocities at the promontory and sensors were measured during BC for the third condition, ''Alg + Dent (free).'' Pressures in SV and ST, as well as METF, were measured again during AC (for Control 3) (step 4). The Control 2 and 3 measurements were compared with the Control 1 measurements (made before ''Alg'') to identify possible artifacts after cementing and after sensor release. After completions of these steps, ICP and promontory velocity during BC stimulation was measured (step 5).

Signal Generation, Acquisition, and Data Analysis

Stimulation and data recording was performed using an external soundcard (Hammerfall Multiface II, RME, Haimhausen, Germany) controlled by custom software RBA (16). A stepped sweep was generated in Matlab (Mathworks, Natick, MA) at 50 logarithmically spaced frequencies between 0.1 and 10 kHz. This sweep was presented to the specimen using a Baha 5 Power actuator (Cochlear Ltd.) or an ER●3C insert earphone powered using a LPA01 single-channel amplifier, set to a unit gain (Newtons4th Ltd., Leicester, UK).

The stimulus responses of ear canal sound pressure, velocity measurements, SV and ST signals were recorded simultaneously at a sampling rate of 96 kHz. Using a separate trigger channel, the raw signal was divided into synchronized epochs (i.e., time-windows). For each epoch, the measured signal was filtered using second-order Butterworth filters with a one-third octave bandwidth that was symmetrical around the center frequencies, before calculating the frequency-dependent response. The velocities and pressures we measured were normalized to ear canal sound pressure for AC, and to the actuator OFL measured on a skull simulator for BC.

The difference between SV and ST pressure was calculated in the time domain, and the complex differential amplitude was computed from these data. The differential amplitude was then normalized against ear canal sound pressure, which resulted in a differential pressure (ΔP). For the fixation experiment, velocity ratios were calculated by dividing sensor velocity (mm/s) with promontory velocity (mm/s) to calculate the relative motion between promontory bone and sensor.

Data analysis was performed in Matlab, graphical and statistical analyses using R (Rstudio, Boston, MA). Independent *t* tests were used to test for significant differences between the fixation conditions.

RESULTS

Vibrations of Stapes Footplate Before and After Cementing

Figure 3 illustrates the METFs of Control 1 (solid lines, before opening the cochlea), and Control 3 (dashed lines, after sensor cementing and releasing) for each TB. After drilling and sensor insertion, no change was found between Control 1 and Control 2 measurements. For each TB, the METFs of Controls 1 and 3 fell within the prescribed ASTM-standard range as adjusted by Rosowski et al. (12,15) and only minimal differences were observed in-between Control 1 and Control 3.

Air Conduction Stimulation

ICP during AC stimulation was simultaneously measured in SV and ST. Data from Nakajima et al. (3) and Grossöhmichen et al. (4) were used as a reference (shown as blue and grey shaded areas in Fig. 4), to investigate the validity and reliability of our setup. Figure 4 shows both differential pressure and phase. The recorded ΔPs of all TBs were approximately in-line with the reference data (3,4). In TB04 and TB10, magnitudes were 2 to 5 dB higher than the reference data below 1 kHz. Results were

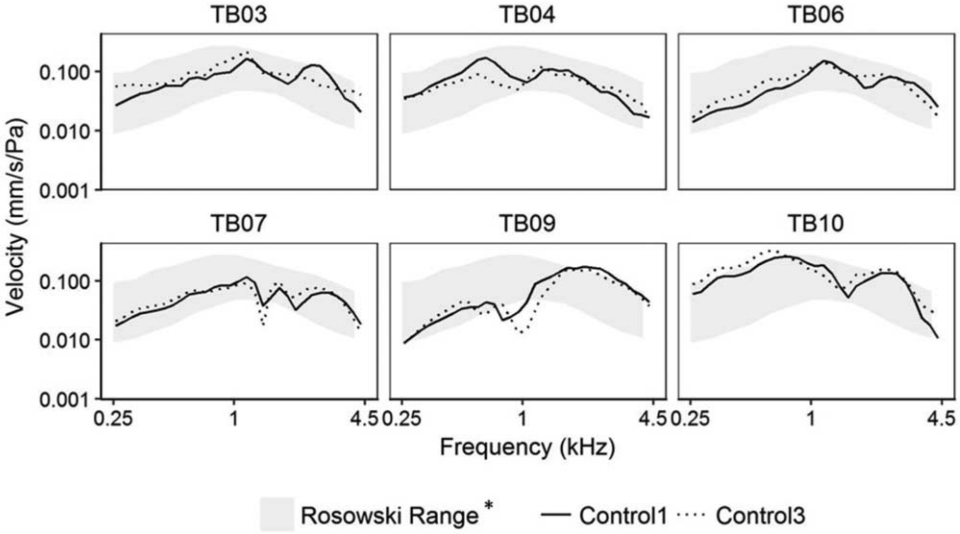

FIG. 3. Middle ear transfer functions (METFs) for each temporal bone: Control 1 (*solid lines*) represent the METFs before cochleostomy drilling and sensor insertion; Control 3 (*dotted lines*) represent the METFs after cementing and releasing. The grey shaded area represents the Rosowski range (*), which is the ASTM-range as adjusted by Rosowski et al. (12,15).

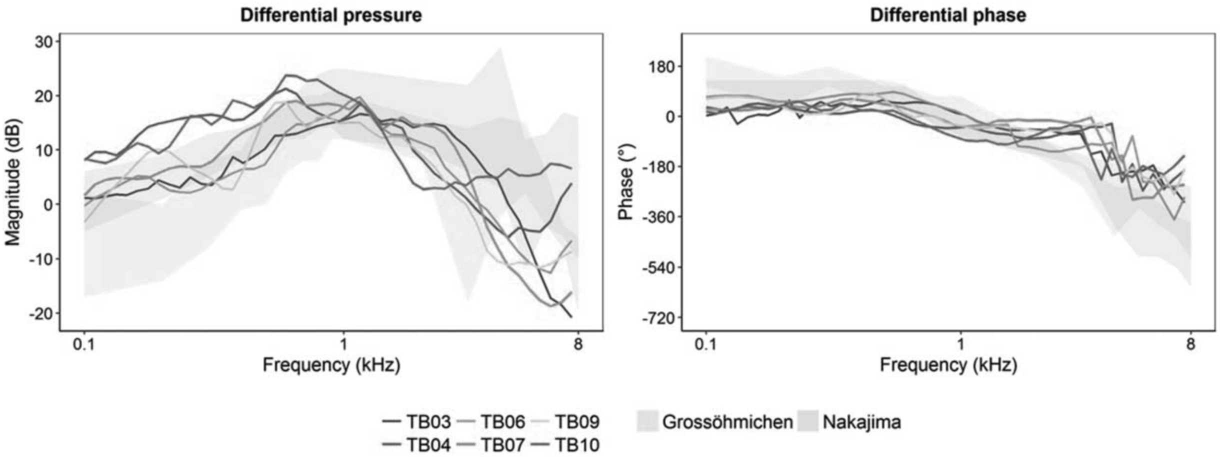

FIG. 4. Magnitude (*left*) and phase (*right*) of differential pressure during air conduction stimulation for each temporal bone. The grey shaded area displays the minimum to maximum range of Nakajima et al. (3). The blue shaded area represents the minimum to maximum range of the study of Grossöhmichen et al. (4). *See online manuscript for colored figures*.

similar to the reference data for the phase of ΔP: for all TBs, phase differences of around 90 degrees were measured below 1 kHz, which were shifted to negative phase differences above round 1 kHz. Lower normalized amplitude and phase differences than in the reference studies occurred at higher frequencies, above 4.5 kHz. This could be explained by the lower signal-to-noise-ratio in our study, due to a different output response of the loud speaker and the higher noise floor levels of the pressure sensors.

Fixation Experiment

Figure 5 shows the SV sensor motion relative to the promontory motion for each TB, as magnitude and phase, under each of the three different conditions: 1) ''Alg''; 2) ''Alg + Dent,'' and 3) ''Alg + Dent (free).'' The velocity of the ST sensor relative to the promontory is not shown, as it was similar to that of the SV sensor relative to the promontory. Large variations in both magnitude and phase were observed when the sensors were only sealed with alginate (''Alg''): the promontory motion was up to 28 dB greater and up to 20 dB smaller than that of the sensor for TB03 and TB07. In the second condition (''Alg + Dent'') and third condition (''Alg + Dent [free]''), less variation between TBs was observed. For the whole frequency range, a significant effect on the relative motion between sensor and promontory bone was found for each TB, except for TB09 at the low frequencies ($<$0.75 kHz). After sensor release (third condition, ''Alg + Dent [free]''), the deviation limits of both magnitude and phase became smaller and approached zero, indicating more similar motion between sensor and promontory bone. Results of an independent *t* test confirmed this: at low ($<$0.75 kHz) and mid (0.75–3 kHz) frequencies, a significant effect was only found in two and four of the six TBs, respectively. However, a trend ($p < 0.3$) towards an effect of fixation condition on the relative motion was found, indicating that clamping the sensor might result in artificially increased pressure. At higher frequencies ($>$3 kHz), a significant effect of fixation condition on relative motion was found in each TB. Overall, the suggestion of artificially increased pressure due to suboptimal sensor fixation is largest when the sensor is only sealed with alginate, as magnitude limits (i.e., minimum–maximum range) between –36 and 17 dB were observed. The magnitude limits become smaller in the second condition (–24 to 18 dB), when the sensor was cemented but still clamped in the micromanipulator. In the third condition, the magnitude limits approach zero and range between –3 and 14 dB. Similar results were found for the phase. Large phase differences ranging from –1000 to 500 degrees were observed in the first condition. These became smaller in the second condition and still smaller in the third condition.

The effect of the different conditions on ICP is shown in Figure 6 for each TB separately. ICP data for ''Alg'' and ''Alg + Dent'' were normalized against the pressure of ''Alg + Dent (free),'' which is considered to be the optimal condition (cfr. 0-dB-line) based on the velocity ratios (Fig. 5). An overestimated pressure of up to 10 dB at low and mid-frequencies was observed for TB03, TB06, and TB07 for ''Alg.'' An underestimated pressure in the ''Alg'' condition of up to 5 dB was found for TB09 at mid- and high frequencies. For the condition ''Alg + Dent,'' an underestimated pressure was observed for most TBs, except for TB03, which showed a higher pressure in the second condition, and TB04, where, depending on frequency, little or no pressure change was detected. Statistical comparisons to confirm the effect of fixation condition on the pressure data were performed by using an independent *t* test. Data were grouped per four frequencies, and an average pressure was calculated across all TBs. Based on graphical analysis, a pressure deviation was mainly present at low and mid-frequencies up to 2 kHz, between the ''Alg'' and ''Alg + Dent'' conditions. A significant difference ($p < 0.05$) in pressure, or a trend towards such a difference ($0.05 < p < 0.1$) was found based on statistical

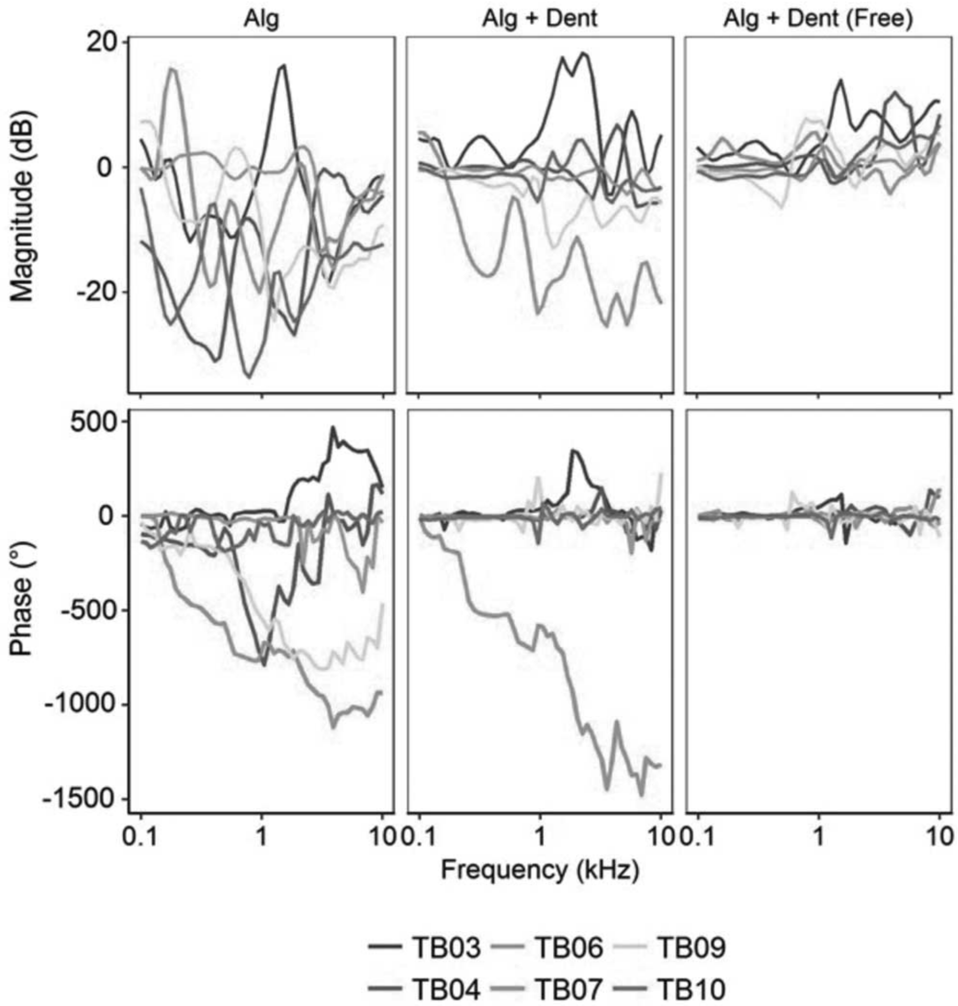

FIG. 5. Fixation experiment results: sensor motion relative to promontory motion for each temporal bone, shown as magnitude (*top*) and phase (*bottom*), under the three different conditions: alginate ("Alg"); alginate and dental composite, held in micromanipulator ("Alg + Dent") and alginate and dental composite, released from micromanipulator ("Alg + Dent [free]"). *See online manuscript for colored figures*.

testing. At higher frequencies, above 5 kHz, a significant pressure difference between both conditions was found. A trend ($p < 0.3$) was observed between the ''Alg'' and ''Alg + Dent (free)'' conditions at low and mid-frequencies, indicating that clamping the sensor can induce a pressure deviation. Above 3.5 kHz, a significant effect was also found ($p < 0.05$).

Intracochlear Pressure During Bone Conduction Stimulation

ICP magnitudes, normalized against actuator OFL and measured in the third condition ''Alg + Dent (free),'' are shown in Figure 7 for each TB. The median pressures for all six TBs for SV (red), ST (blue), and ΔP (grey) are shown in the bottom panels of Figure 7. Overall, small differences in magnitude were observed between SV and ST. The median data show a slightly higher ST pressure at low frequencies and higher SV pressure at mid-frequencies. However, the individual data of all TBs indicate that large variation existed between TBs.

The phase differences between ICP and stimulation input differed between SV and ST (see Fig. 7), indicating a pressure difference across the cochlear partition. For all TBs, a decreasing phase with increasing frequency was observed.

DISCUSSION

The goals of this study were to evaluate a non-destructive cementing method for a commercially available sensor, and to study the effect of BC stimulation on cochlear pressure. These are important steps towards understanding the underlying mechanisms of BC sound transmission.

Control Measurements Before and After Fixation

Before and after cementing, all TBs fell inside the prescribed ASTM-range as adjusted by Rosowski et al. (12,15) and the relative changes across conditions were small, indicating no effect of sensor insertion and

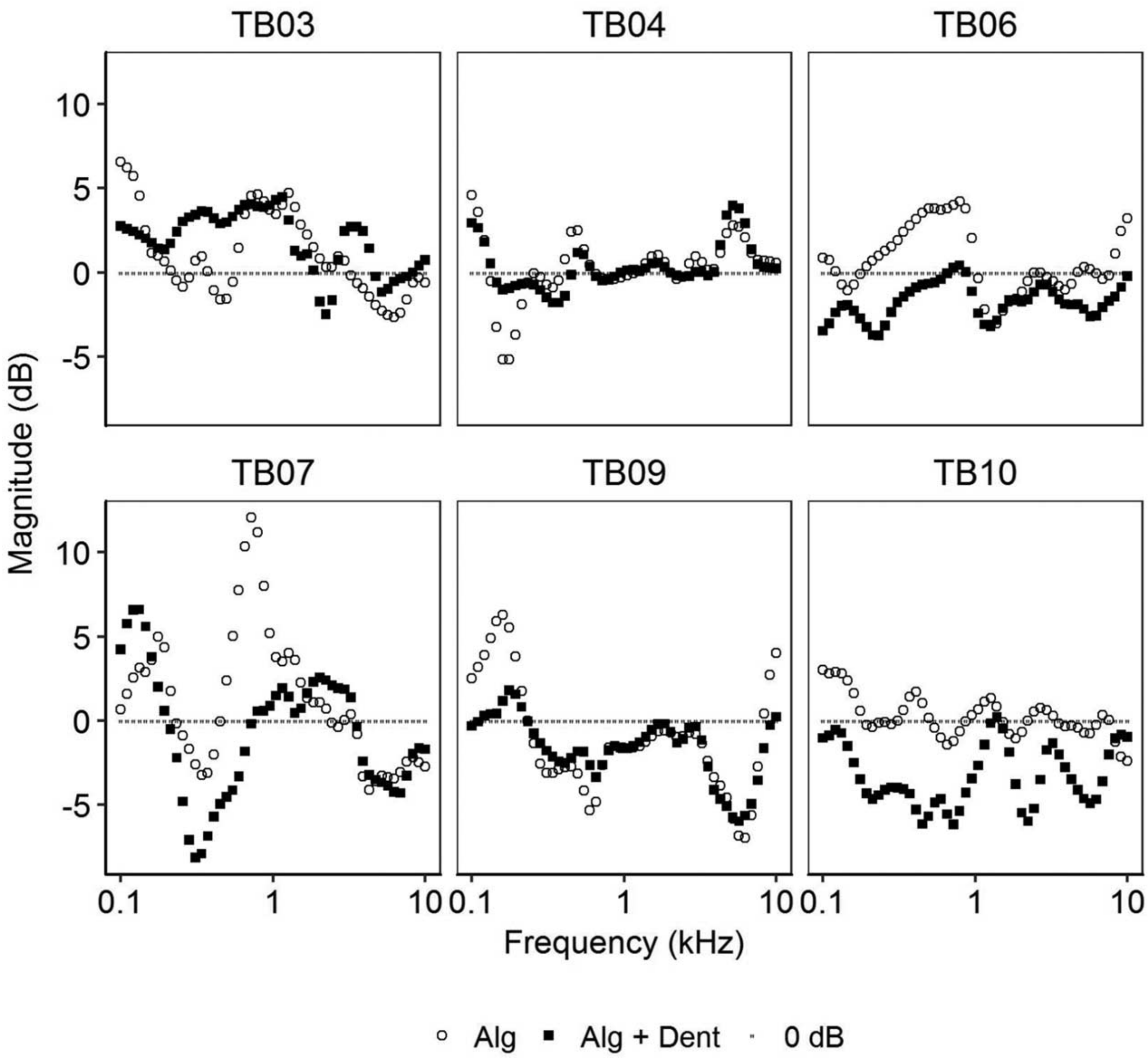

FIG. 6. Pressure ratios for the first condition (alginate, "Alg," *open circles*) and the second condition (alginate and dental composite, held in micromanipulator, "Alg + Dent," *filled circles*) relative to the third condition (0-dB-line; alginate and dental composite, released from micromanipulator, "Alg + Dent [free]").

cementing. None of the TBs showed a decrease in stapes mobilization after cementing, indicating that composite did not leak onto the footplate. The use of a dye enhances the control when applying the composite. If leakage did occur, the composite could be aspirated before the UV light was used for vulcanization.

Results from AC stimulation were consistent with previous ICP studies (3,4), indicating that our baseline measurements were valid and could be used to interpret the results we obtained with BC stimulation.

Reducing Pressure Artifacts During Bone Conduction Stimulation

Our data confirm that firmly attaching the sensors and then releasing them from the micromanipulators is crucial for valid ICP measurements during BC stimulation. The velocity data suggest that pressure deviation can occur due to motion differences between sensor and promontory bone. At all frequencies, this deviation is mainly present when the sensor is only sealed with alginate. This was confirmed by the ICP measurements, which resulted in an overestimated pressure when the sensor was sealed with alginate and held in the micromanipulators. The pressure artifact is likely to be a result of relative motion between sensor and promontory bone. Pressure was overestimated especially at lower frequencies, by up to 10 dB. When the sensor was cemented, but not released from the micromanipulator, pressure variations were also present; they disappeared when the sensor was released. In the second condition, the pressure was mostly underestimated, especially at low and mid-frequencies up to 1 kHz, since the

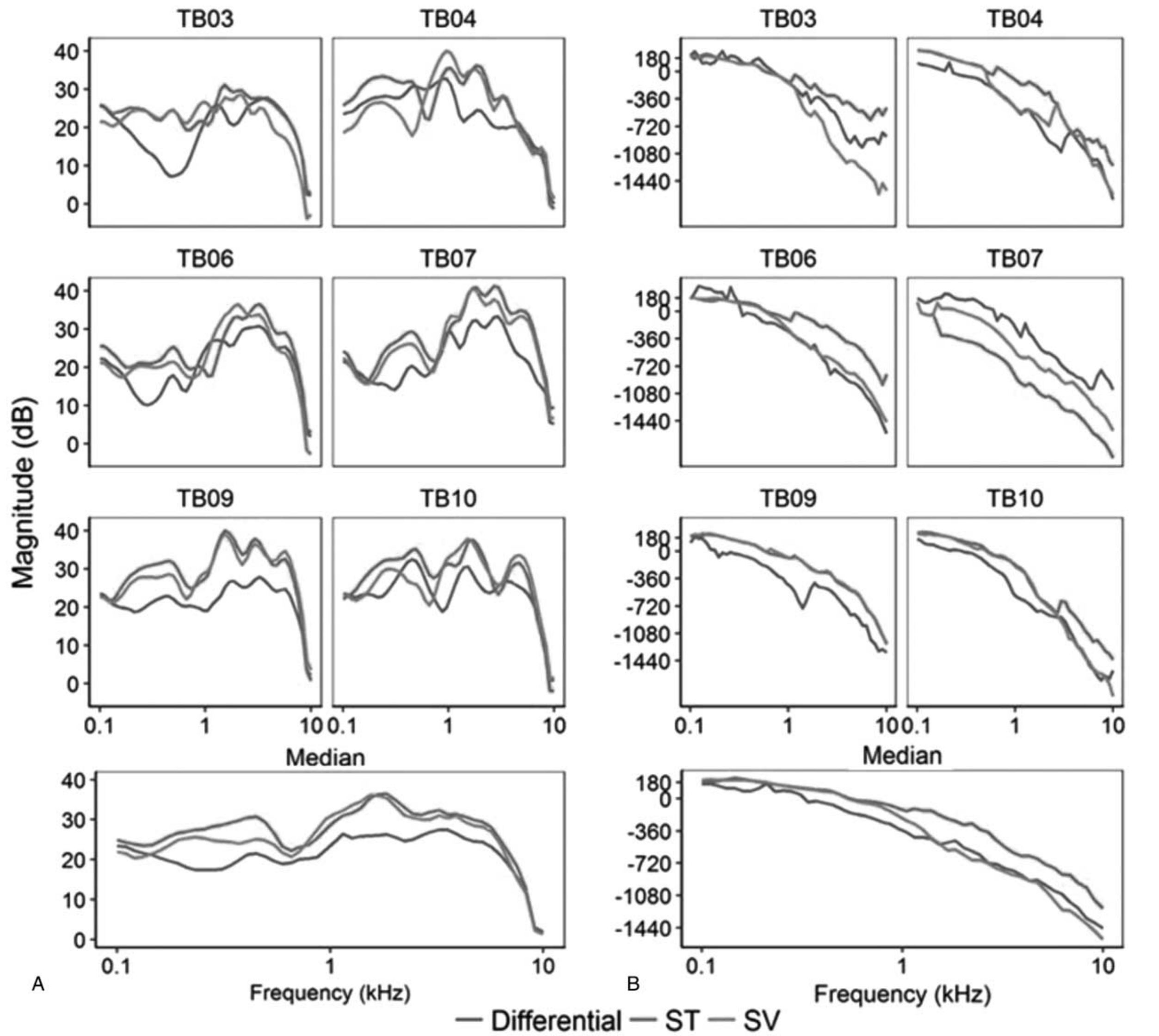

FIG. 7. Intracochlear pressure measurements for each temporal bone during bone conduction stimulation. Magnitude is presented on the *left* (*A*), phase data are on the *right* (*B*). Red lines represent scala vestibuli pressure, blue lines represent scala tympani pressure, and grey lines represent the differential pressure. The bottom panels show the median data for phase and magnitude for all six temporal bones. *See online manuscript for colored figures*.

movement of the sensor was restricted by the cement and micromanipulator.

In previous ICP studies, both custom-made (3,8,17) and commercially available pressure sensors (4,18) were used. Only a few studies have performed ICP measurements during BC stimulation with the commercially available sensors that we used, and in these the risk of artificially increased pressure with BC stimulation was not mentioned by the authors (9,10,11). Other studies used custom-made sensors to measure ICP during BC stimulation. In line with two studies, both by Olson et al. (8,16), we report that pressure artifacts were reduced when dental composite was applied around the sensor tip and the sensor was released from the micromanipulator. Olson et al. (8,16) also reported that ICP was often overestimated by up to 10 dB when the sensor was only sealed with alginate, and that in other cases ICP was underestimated due to possible leaks in the sealing. Leaks could explain the lower pressure we measured in TB09. Similar to our findings in TB04, Olson et al. (8,16) found little or no pressure difference in a few TBs, probably due to a canceling effect (i.e., a combination of leakage and artificially increased pressure), a stiff connection of the alginate sealing, or cochleostomy. In both studies by Olson et al. (8,16) a remarkable phase difference was found when the sensor was only sealed with alginate or sealed with alginate and dental composite but not released from the micromanipulator. This out-of-phase motion can artificially increase the pressure, confirming the necessity of sensor cementing. In contrast to Olson et al. (8,16), who used a custom-made sensor that was sacrificed after each experiment, we used a commercially available pressure sensor that should be reused for several experiments. A non-destructive method was therefore required. By using the most suitable solvent (acetone) and taking care in its application, we were able to reuse approximately 90% of the sensors.

Intracochlear Pressure During Bone Conduction Stimulation

Although several researchers have investigated BC transmission mechanisms using velocity and acceleration measurements or models, the exact mechanics driving the cochlear membrane remain unknown. ICP measurements can be used to investigate these driving mechanisms; however, before using these measurements to explore the cochlear drive, more in-depth investigation of the method is required. In the current study, various pressure magnitudes were found in the SV and ST across TBs during BC stimulation. In most TBs, ST pressure was greater below 1 kHz, and SV pressure was greater above 1 kHz. These results are approximately in line with those of Stenfelt et al. (19): higher volume displacements were observed below 2 kHz near the round window membrane, and above 2 kHz near the oval window.

Comparing the current study to previous ICP studies (6,7,14) in which the effect of BC stimulation was also investigated shows that small differences occurred even when the test setup was similar. In most other studies, mean SV pressure was higher than ST pressure, so that SV pressure dominated the ΔP and thus resulted in the cochlear drive in BC stimulation. In the current study, we found only small differences in pressure between both scalae, depending on sound frequency. Median SV and ST pressure were similar in each TB, which is probably due to inter-subject variation across TBs that is also prevalent in other ICP studies (3,4). Phase differences between both scalae were observed, suggesting a pressure difference along the cochlear partition and the presence of a cochlear drive. Various factors could be responsible for the differences in pressure between both scalae found in other studies. For example, Chhan et al. (6) used chinchillas, which have a lower bone density than humans and thus different wave propagation patterns. In the pilot study of Stieger et al. (7), data from only one TB were used. Variation in SV and ST pressure during BC stimulation has been reported: Stieger et al. (14) found that SV pressure was higher than ST pressure, but variations of up to 30 dB were observed between TBs and across frequencies. The implant location can influence the cochlear response in BC stimulation: Stenfelt and Goode (2) reported a transmission loss of 0.5 to 1.5 dB per centimeter away from the cochlea. In contrast to other researchers, we therefore choose to standardize implant location in all specimens, namely 4.5 to 5 cm away from the ear canal center. Further investigations are needed to explore and disentangle differences between SV and ST pressure, and the resulting pressure differences across the cochlear partition.

A mathematical modeling approach based on a three-dimensional box model has been used to describe BC transmission mechanisms. In this model, three contributors that act in combination were described: 1) fluid inertia, 2) symmetric pressure compression-expansion (depending on the impedance difference between oval and round window membrane), and 3) anti-symmetric compression-expansion (depending on the width of the basilar membrane and cochlear ducts) (20). According to this model, the variable results that we observed could be explained by asymmetric compression resulting from the geometric asymmetry between SV and ST, or by a difference in impedance loading at both scalae.

Different to Olson et al. (8,16), we used actuator OFLs, which allowed us to work with device-specific transfer functions, whereas using the output voltage in static normalization. The actuators used were also different: we used a more powerful actuator (Baha 5 Power, OFL range 105–130 dB, stimulation voltage 1 V_{rms}) than Stieger et al. (BP100 actuator, OFL range 82–104 dB, stimulation voltage 0.81 V_{rms}). Using promontory velocity is another normalization manner, as it is specimen-specific. More controlled measurements and investigation of the relations between promontory velocity and ICP measurements are needed to interpret the transfer functions. Factors such as stimulation level and non-linear effects should be taken into account.

CONCLUSION

Our study shows that, during BC stimulation, cementing and releasing the sensors from the micromanipulator is crucial for obtaining valid ICP measurements. Using our method, cochlear pressure differences can be measured reliably in both scalae. Results show that pressure magnitudes vary in both scalae across TBs, indicating that complex mechanisms are involved in BC.

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REFERENCES

1. Stieger C, Rosowski JJ, Nakajima HH. Comparison of forward (ear-canal) and reverse (round-window) sound stimulation of the cochlea. *Hear Res* 2013;301:105–14.
2. Stenfelt S, Goode RL. Transmission properties of bone conducted sound: measurements in cadaver heads. *J Acoust Soc Am* 2005;118: 2373–91.
3. Nakajima HH, Dong W, Olson ES, Merchant SN, Ravicz ME, Rosowski JJ. Differential intracochlear sound pressure measurements in normal human temporal bones. *J Assoc Res Otolaryngol* 2009;10:23–36.
4. Grossöhmichen M, Salcher R, Püschel K, Lenarz T, Maier H. Differential intracochlear sound pressure measurements in human temporal bones with an off-the-shelf sensor. *Biomed Res Int* 2016;2016:6059479.

5. Grossöhmichen M, Waldmann B, Salcher R, Prenzler N, Lenarz T, Maier H. Validation of methods for prediction of clinical output levels of active middle ear implants from measurements in human cadaveric ears. *Sci Rep* 2017;7:1–10.
6. Chhan D, Röösli C, McKinnon ML, Rosowski JJ. Evidence of inner ear contribution in bone conduction in chinchilla. *Hear Res* 2013;301:66–71.
7. Stieger C, Farahmand RB, Page BF, et al. Pressures in the human cochlea during bone conduction. *AIP Conf Proc* 2015;1703:060004.
8. Olson ES. Observing middle and inner ear mechanics with novel intracochlear pressure sensors. *J Acoust Soc Am* 1998;103:3445–63.
9. Mattingly JK, Greene NT, Jenkins HA, Tollin DJ, Easter JR, Cass SP. Effects of skin thickness on cochlear input signal using transcutaneous bone conduction implants. *Otol Neurotol* 2015;36:1403–11.
10. Banakis Hartl RM, Mattingly JK, Greene NT, Jenkins HA, Cass SP, Tollin DJ. A preliminary investigation of the air-bone gap. *Otol Neurotol* 2016;37:1291–9.
11. Farrell NF, Hartl RMB, Benichoux V, Brown AD, Cass SP, Tollin DJ. Intracochlear measurements of interaural time and level differences conveyed by bilateral bone conduction systems. *Otol Neurotol* 2017;38:1476–83.
12. ASTM. *International F2504-05: Standard Practice for Describing System Output of Implantable Middle Ear Hearing Devices*. 2005. doi:10.1520/F2504-05.
13. Håkansson B, Carlsson P. Skull simulator for direct bone conduction hearing devices. *Scand Audiol* 1989;18:91–8.
14. Stieger C, Guan X, Farahmand RB, et al. Intracochlear sound pressure measurements in normal human temporal bones during bone conduction stimulation. *J Assoc Res Otolaryngol* 2018;19: 523–39.
15. Rosowski JJ, Chien W, Ravicz ME, Merchant SN. Testing a method for quantifying the output of implantable middle ear hearing devices. *Audiol Neurotol* 2007;12:265–76.
16. Hofmann M, Wouters J. Electrically evoked auditory steady state responses in cochlear implant users. *J Assoc Res Otolaryngol* 2010;282:267–82.
17. Pfiffner F, Prochazka L, Peus D, et al. A MEMS condenser microphone-based intracochlear acoustic receiver. *IEEE Trans Biomed Eng* 2016;64:9294.
18. Greene NT, Jenkins HA, Tollin DJ, Easter JR. Stapes displacement and intracochlear pressure in response to very high level, low frequency sounds. *Hear Res* 2017;348:16–30.
19. Stenfelt S, Hato N, Goode RL, Stenfelt S, Hato N, Goode RL. Fluid volume displacement at the oval and round windows with air and bone conduction stimulation. *J Acoust Soc Am* 2004;115:797–812.
20. Xuan W, Yoon CY, Kim N. Mechanism of bone-conducted hearing: mathematical approach. *Biomech Model Mechanobiol* 2018;17: 1731–40.