Difference of auditory brainstem responses by stimulating to round and oval window in animal experiments

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ABSTRACT
To ensure the safety and efficacy of implantable hearing aids, animal experiments are an essential developmental procedure, in particular, auditory brainstem responses (ABRs) can be used to verify the objective effectiveness of implantable hearing aids. This study measured and compared the ABRs generated when applying the same vibration stimuli to an oval window and round window. The ABRs were measured using a TDT system 3 (TDT, USA), while the vibration stimuli were applied to a round window and oval window in 4 guinea pigs using a piezo-electric transducer with a proper contact tip. A paired t-test was used to determine any differences between the ABR amplitudes when applying the stimulation to an oval window and round window. The paired t-test revealed a significant difference between the ABR amplitudes generated by the round and oval window stimulation (t = 10.079, α < .0001). Therefore, the results confirmed that the biological response to round window stimulation was not the same as that to oval window stimulation.

KEYWORDS
animal experiment; auditory brainstem response; implantable hearing aids; oval window stimulation; round window stimulation

Introduction
Recently, several types of implantable hearing aids are under development. To ensure the safety and efficacy of implantable hearing aids, animal experiments are an essential developmental procedure, in particular, auditory brainstem responses (ABRs) can be used to verify the objective effectiveness of implantable hearing aids.

In the case of implant middle ear hearing aids (IMEHDs), which have been developed and commercialized since the 1980s, the input acoustic energy is applied to an oval window. However, recent research has been focusing on the use of a round window. There are 2 opening windows into the inner ear. The one is oval window and the other is round window. The sound is changed to the vibration by the tympanic membrane. The vibration travel through the 3 ossicles and into the inner ear. The oval window is the intersection of the middle ear with the inner ear and is directly contacted by the stapes. The vibrations have been amplified over 20 times when they contacted the tympanic membrane by the 3 ossicles. The round window is situated below and little behind the oval window. As the stapes footplate moves into the oval window, the round window membrane moves out, and this allows movement of the fluid within the cochlea.

The performance and effectiveness of oval window stimulus-type IMEHDs have already been verified based on the stapes footplate response according to ASTM F2504-05. Plus, the same method has been used to confirm the performance and effectiveness of round window stimulus-type IMEHDs, as it was assumed that the biological response to round window stimulation would be the same as that to oval window stimulation.

New study results were also recently published on this topic in the Journal of Hearing Research. Accordingly, to confirm the abovementioned results, this study measured and compared the ABRs generated when applying the same vibration stimuli to an oval window and round window. The ABRs were
measured using a TDT system 3 (Tucker-Davis Technologies, USA), while the vibration stimuli were applied to a round window and oval window in 4 guinea pigs using a piezo-electric transducer (PZT) with a proper contact tip. A paired t-test was used to determine any differences between the ABR amplitudes when applying the stimulation to an oval window and round window.

**Measurement of ABRs**

The ABR is an auditory evoked potential signal that is extracted from electrical activity in the brain and recorded via electrodes placed on the scalp. Generally, sound stimuli applied to the tympanum are used to generate ABRs. However, vibrations were applied to an oval window and round window by a PZT, SK4748-2 (Morgan Electro Ceramics, United Kingdom), and used to generate ABRs in this study. The specifications of the PZT used here provided in Table 1.

The in-vivo animal experiments for ABR measurement were performed with the approval of Kyungpook National University Industry Foundation (approval number, KNU 2014-0123). A schematic diagram of the animal experiment is shown in Fig. 1.

The electric pulse used to generate the vibration on a PZT was created by the TDT System3 hardware. The PZT was positioned near the round window and oval window of the target animal using a micro manipulator as shown in Fig. 1.

Needle electrodes were used in the ABR measurement. The needle electrode was unipolar electrode, EL450 (BIOPAC system, USA) that was Teflon-coated stainless steel needles. Dimensions of electrode was 37 mm(long) and 300 um(diameter). The active electrode was placed on the vertex and the reference electrode was placed on the mastoid. The generated ABR signal from the vibration of the PZT was amplified in the pre-amplifier, a 4-channel MEDUSA preamp (Tucker-Davis Technologies, USA) and analyzed by a real-time processor in the TDT system3. The TDT system 3 recorded the ABRs in the animals. The recording was started with stimulation and lasted for 10 ms. Parameters of the BioSigRP that is recording software for TDT system3, were set up as follows: the bandpass filter was a 300–30,000 Hz filter, 60 Hz notch was used, gain was 20 times, the sampling frequency was 24.4 kHz and the artifact rejection feature on. The average of 500 waveforms was displayed on a PC monitor during the experiments using operating software (BioSigRP, TDT).

The target animals were 4 Hartley guinea pigs without hearing problems, with weights ranging from 130 g to 170 g. The guinea pigs were fully anesthetized during the experiments. The anesthetic agent was mixtures of tiletaminezolazepam (1.8 mg/100 g) and xylazine hydrochloride (0.7 mg/100 g) intramuscularly. Surgical preparation of the animal was

| Specification               | Value                                                                 |
|-----------------------------|----------------------------------------------------------------------|
| Dimensions [mm]             | 1.0 × 1.0 × 1.8                                                       |
| Material                    | PZT506(PXE55)                                                         |
| Dielectric thickness [um]   | 20                                                                   |
| Number of layers            | 90                                                                   |
| Average capacitance [nF]    | 27.5 (@ 120 Hz, 1.0 V, ±10 %)                                        |

**Table 1.** The piezoelectric transducer (PZT) specifications.
performed by the qualified medical doctor from the Otorhinolaryngology-Head and Neck Surgery. The mastoidectomy was performed to open the middle ear cavity. The soft tissue of the external auditory canal was removed for insert the vibration transducer. The part of promontory was removed for facilitate access to the round window. The body temperature of guinea pigs were monitored and maintained at 38°C during the animal experiment.

The electric pulse used to generate the vibration stimulus was a tone-burst signal and the amplitude of the applied signal was decreased from 9 V to 0.005 V (5 dB step). The maximum amplitude of the signal applied to the PZT was set to 9 V to avoid saturation of the output signal, because the maximum amplitude of the output signal from the TDT system is 10 V. The frequencies of the applied signals were 2 kHz, 4 kHz, 8 kHz, and 16 kHz. ABR measurement was performed when the vibration stimuli was applied to a round window and oval window of the target animals.

**Statistical analysis of animal experiment results**

From the animal experiments, tone-burst ABR (TBABR) signals were acquired when the vibration stimuli were applied to a round window and oval window. In this study, the targets of statistical analysis were the ABR amplitudes and hearing threshold, the latter of which can be easily obtained by the ABR test. The TBABR consisted of 5 waves, and the peak value of wave III was set to the amplitude of the ABR, because wave III arises from second-order neuron activity and its peak value appears to be the largest in most cases.

![Figure 3. The experiment setup for ABR measurement.](image)

![Figure 4. Some of the experimental results when stimulation was applied to (A) the oval window and (B) round window.](image)
In this study, 2 null hypotheses were established to verify that the biological response to round window stimulation would be the same as that of oval window stimulation. The first null hypothesis was that the amplitude of ARB to round window stimulation was the same as that of ABR to oval window stimulation with the same vibration. The other null hypothesis was that the threshold of ABR to round window stimulation was the same as that of ABR to oval window stimulation.

To reject these null hypotheses, a paired T-test was performed using SPSS Statistics software, version 21 (IBM, USA). The paired T-test can be used when there is one measurement variable and 2 nominal variables, and also when one of the nominal variables has only 2 values. In the paired T-test for the first null hypothesis, the measurement variable was the amplitude of ARB and the nominal variables were the positions to apply the vibration (the round window and oval window) and intensities of vibration. The number of vibration intensity was 14 levels. In this study, the intensity of the vibration was replaced with the amplitude of the applied electrical signal to the PZT, because only one PZT was used throughout the study. The amplitudes of electrical signals applied to the PZT were 9.000, 5.061, 2.846, 1.600, 0.900, 0.506, 0.285, 0.160, 0.090, 0.051, 0.028, 0.016, 0.009, and 0.005 V.

In the paired T-test for the other null hypothesis, the measurement variable was the threshold of ABR and the nominal variables were the positions to apply the vibration and the amplitudes of the electrical signals applied to the PZT.

**Measurement of vibration intensity**

The output vibrations of the PZT were measured using a laser vibrometer system, the OFV5500 (main

![Figure 5](image-url).

*Figure 5.* The experimental results of (A) guinea pig 1, (B) guinea pig 2, (C) guinea pig 3, and (D) guinea pig 4.
controller) and OFV551 (laser probe) (Polytec GmbH, Germany). The measured output vibrations of the PZT within the applied input signals are shown in Fig. 2.

The displacement of vibration on the PZT can cover the range of an ossicle which was prescribed from ASTM F2504 at 94 dB SPL sound input, within the suggested amplitudes of the input electrical signal.

**Measurement of ABR**

ABR measurements were performed in normal hearing guinea pigs to ensure normal distribution of measured data. Guinea pigs were selected by a sound pressure ABR test before surgery and a diagnosis after surgery. The experiment setup to acquire the ABRs that were generated when applying the vibration stimuli to an oval window and round window is shown in Fig. 3.

Experiments were performed on anti-vibration table. Some of the experimental results are shown in Fig. 4.

The measured ABRs were generated using 4 stimulation frequencies, 14 stimulation intensities, and 2 stimulation locations. The amplitudes of measured ABRs are shown in Fig. 5.

All guinea pigs had normal hearing, and the ABR amplitudes were obtained from animal experiments. The hearing thresholds of guinea pigs are provided in Table 2. The hearing thresholds show the different values according to the location of the stimulus point.

**Results of statistical analysis**

The number of amplitudes was obtained 112 from each guinea pig. The paired-T test was performed using amplitude data that was generated by stimuli to the oval window and round window. The correlation of paired samples was 0.779 (p < .0001). In the results of paired samples testing, the t value was 10.079, the degree of freedom was 221, and the significance (2-tailed) was p < .0001.

The number of thresholds was obtained 8 from each guinea pig. The paired samples correlation of hearing thresholds was 0.521 (p < .039). In the results of paired samples testing, the t value was −2.252, the degree of freedom was 15 and the significance (2-tailed) was p = .040.

**Discussion and conclusion**

This study measured and compared the ABRs generated when applying the same vibration stimuli to an oval window and round window. The ABRs were measured using TDT system 3 hardware, while the vibration stimuli were applied to a round window and oval window in 4 guinea pigs using a piezo-electric transducer with a proper contact tip. A paired t-test was used to determine any differences between the ABR when applying the stimulation to an oval window and round window. The target data of paired T test were the amplitudes of ABRs and hearing thresholds.

The paired t-test revealed a significant difference between the ABR amplitudes generated by the round and oval window stimulation (t = 10.08, α < .0001). Therefore, the results confirmed that the biological response to round window stimulation was not the same as that to oval window stimulation.

But, there is no significant difference between the hearing thresholds generated by the round and oval window stimulation (t = −2.252, α = .040). In this case, the threshold results are not reliable, because the degree of freedom was only 15. It meant that the number of samples for an accurate analysis was few. If the number of samples is extended to obtain the hearing threshold, the analysis results for the hearing threshold may support a significant difference between oval window stimulation and round window stimulation.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

### Table 2. The hearing thresholds of guinea pigs.

| Guinea pig | Hearing threshold (from stimulus to the oval window) [v] | Hearing threshold (from stimulus to the round window) [v] |
|------------|----------------------------------------------------------|----------------------------------------------------------|
|            | 2 kHz | 4 kHz | 8 kHz | 16 kHz | 2 kHz | 4 kHz | 8 kHz | 16 kHz |
| 1          | 0.051 | 0.009 | 0.005 | 0.051 | 0.051 | 0.051 | 0.016 | 1.6 |
| 2          | 0.028 | 0.005 | 0.016 | 0.285 | 0.051 | 0.051 | 0.028 | 0.9 |
| 3          | 0.09  | 0.09  | 0.009 | 0.051 | 0.09  | 0.09  | 0.051 | 0.285 |
| 4          | 0.09  | 0.09  | 0.09  | 0.16  | 0.506 | 0.16  | 0.09  | 1.6 |
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