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● *Original Contribution*

ACOUSTIC TRANSMISSION FACTOR THROUGH THE RAT SKULL AS A FUNCTION OF BODY MASS, FREQUENCY AND POSITION

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Abstract—In many transcranial ultrasound studies on rats, the transmission factor is assumed to be independent of animal weight and losses resulting from non-normal incidence angles of the beam are not accounted for. In this study, we measured acoustic transmission factors through 13 excised skulls of male Sprague-Dawley rats weighing between 90 and 520 g, at different positions on each skull and at 1, 1.25, 1.5, 1.75 and 2 MHz. Our results revealed that insertion loss through rat skull increases linearly with both body mass and frequency and strongly depends on the position, decreasing from the front to the back and from the midline to the lateral sides. Skull thickness also scales linearly with body mass. Reflection explains the main part of the insertion loss compared with attenuation and aberration. These data are helpful in predicting the acoustic pressure at the focus in the brain. (E-mail: benoit.larrat@cea.fr) **© 2018 World Federation for Ultrasound in Medicine & Biology. All rights reserved.**

Key Words: **Acoustic transmission, Insertion loss, Rat skull, Focused ultrasound.**

INTRODUCTION

In recent years, transcranial focused ultrasound (FUS) in the brain has emerged as a very promising therapeutic tool to replace or complement state-of-the-art therapies. For example, spectacular improvements have been observed in the tremor conditions of drug-resistant patients by thermally ablating tiny areas deep in the brain in a remote way (Elias et al. 2016). For all *in vivo* transcranial applications, precise knowledge of the peak negative pressure (PNP) at the focus is key to ensuring both the efficiency and safety of the procedures. Indeed, the acoustic pressure is directly related to the energy deposition in the tissues. This energy deposition must be sufficient to trigger the desired biological effects while avoiding undesired effects at the focus or out-of-focus lesions.

As an example, in high-intensity focused ultrasound for thermal ablation, the temperature rise has to be high enough to ablate cancerous cells (Dervishi et al. 2013; Pauly et al. 2006) or regions of the thalamus for essential tremor (Elias et al. 2016), yet the energy deposition must be controlled to prevent burning of the surrounding tissue (Shea et al. 2017).

Another promising application of FUS in the brain is the temporary blood–brain barrier opening (Hynynen et al. 2001). Combined with microbubbles injected in the blood flow, low-intensity FUS can permeate the vessel walls and allow drugs to enter the brain (Aryal et al. 2014; Marty et al. 2012). In this case, the PNP must be high enough to ensure significant oscillations of the circulating microbubbles to exert mechanical stress on vascular endothelium (McDannold et al. 2008) while staying low enough to strictly avoid implosion of microbubbles and permanent damage to the endothelial cells of the blood vessels (McDannold et al. 2012). Transcranial neurostimulation also strongly depends on the use of adequate intensity and control of the pressure distribution behind the skull (Deffieux et al. 2013). Reversibly, new transcranial imaging modalities that are developed at the pre-clinical stage thus far, such as functional ultrasound imaging (Tiran et al. 2017), photoacoustic imaging (Lavaud et al. 2017) and passive cavitation detection (Arvanitis et al. 2016), benefit from a good knowledge of skull attenuation and heterogeneity, thereby allowing for a gauge of the sensitivity required for the design of acoustic detectors.

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To estimate PNP *in situ*, the first step is to calibrate the ultrasound transducer in a water tank. A second and influential step is correction for ultrasound insertion loss through the skull. The insertion loss results from the addition of several effects contributing to a decrease in the PNP at focus: aberration, reflection, absorption and scattering. The global skull insertion loss strongly depends on the animal species (Porto et al. 2013) because skull thickness, skull shape and bone structure are unique to each species. Even among a given species, skull insertion loss varies between individuals, especially in large animals (Asahara 2013) and from one skull location to another.

Most new FUS developments are first performed in rodents. Because it is larger compared with the mouse, the rat is a widely used animal model (Magnin et al. 2015; Mead et al. 2017; Yuan et al. 2016). Unfortunately, only one previous study has reported on the attenuation of rat skulls (O'Reilly et al. 2012). That study used skulls from Wistar rats weighing from 250 to 550 g. The authors reported a linear dependency of acoustic insertion loss with body mass at submegahertz frequencies, but no dependency at higher frequencies.

In the study described here, we investigated a larger range of body mass (90−520 g) and a different rat strain, Sprague-Dawley. We focus our measurements in the 1- to 2-MHz range.

METHODS

Acoustic setup

The transducer used for this study was a mono-element concave MR-compatible (diameter = 25 mm, focal depth = 20 mm; Imasonic, Voray-sur-l'Ognon, France) transducer with a central frequency of 1.5 MHz. The transducer was previously calibrated in a de-gassed water tank, and the focal spot was mapped at different frequencies ranging from 1 to 2 MHz. The transducer was mounted on a fixed holder in the tank filled with deionized water. A calibrated hydrophone (HGL-0200, preamplifier AH-2020, Onda, Sunnyvale, CA, USA) was used to measure acoustic pressures. Its active surface at the tip is a 200-μm-diameter disk. It was mounted on a micrometric three-axis positioning stage and placed in front of the transducer. The transducer was driven by a portable generator and amplifier (Image Guided Therapy, Pessac, France).

For all measurements, the pulses were 10 periods long with a 0.1-s pause between pulses. Two periods at the beginning and at the end of each pulse were excluded to ensure a purely monochromatic measurement. The electrical power was set to obtain an approximately 0.8-MPa PNP at focus in free water (at 1.5 MHz). The signal acquired by the hydrophone was directed to an oscilloscope (WaveRunner 44Xi, LeCroy, Chestnut Ridge, NY, USA), and the signal was an average of 50 measurements. The peak-to-peak voltage was measured on screen and converted into acoustic pressure thanks to the calibration data provided by the hydrophone manufacturer.

Skulls

Thirteen skulls were excised from Sprague-Dawley male rats ranging from 90 to 520 g in body mass. The skulls were obtained from previous studies approved by our local ethics committee (Project Authorization No. 12-058, Site Authorization No. B-91-272-01). After removal of as much tissue as possible, the skulls were boiled in a solution of water and sodium bicarbonate and then preserved in phosphate-buffered saline with azide. Skulls were never dry stored. The skulls, mounted on a micrometric three-axis positioning stage, were placed in the water tank. The water de-gassed for 15 min prior to any measurement. The de-gassing system was provided by Image Guided Therapy. The skulls were placed so that the focal spot of the ultrasound beam was approximately 5 mm under the skull, to mimic a realistic *in vivo* experiment with this transducer. The skulls were visually oriented with a normal incidence. The whole cone of the ultrasound beam intersected the skull for all measurements. The distance between the transducer center and skull surface was kept roughly constant for all measurements (16 ± 1 mm), which made the beam cross the skull over a circular surface 6 ± 1 mm in diameter.

Transmission measurements

For all acoustic measurements through skulls, the hydrophone was moved on the three axes to find the maximum pressure. It is to be noted that this location was never found to be farther than 0.1 mm from its location without the skull. The transmission factor was then defined as the ratio

$$\tau = P_{\text{skull}}/P_{\text{free}} \quad (1)$$

where P_{skull} is the acoustic pressure at focus through the skull, and P_{free} the acoustic pressure at focus in free water. The voltage in output of the hydrophone was proportional to the acoustic pressure, and the transmission factor was directly calculated by obtaining the ratio of the voltages.

In the first experiment, three transmission measurements were done on 10 skulls at three different positions along the interhemispheric line—front, middle and back—as illustrated in Figure 1. The front position corresponds to the striatum, often used in diffusion experiments after ultrasound induced blood−brain barrier opening (Magnin et al. 2015) or to implant tumors for ultrasound treatments (Sun et al. 2017). The middle