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Ultrasound insertion loss of rat parietal bone appears to be proportional to animal mass at sub-megahertz frequencies

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Abstract

Transcranial ultrasound therapy is an increasing area of research for non-invasive treatments in the brain including targeted drug delivery. Measurements of ultrasound transmission through rat parietal bone at five frequencies (0.268 MHz, 0.841 MHz, 1.409 MHz, 1.972 MHz and 2.53 MHz) were performed at 88 locations in 22 *ex vivo* rat skullcaps (Wistar) using a fiber-optic hydrophone system. At submegahertz frequencies the skull insertion loss was found to be proportional to animal mass, while at higher frequencies this trend was lost. Maps of the transverse pressure profile of the transducer before and after skull insertion showed increased distortion effects at higher frequencies. Parietal bone thickness was measured and was found to increase with increasing body mass. Additional measurements were made through mouse and rabbit skulls at 2.53 MHz. At this frequency, aberration effects through mouse skull were negligible, while large distortions were observed through rat and rabbit skull. Preclinical transcranial ultrasound studies in rats may be improved by scaling applied powers according to body mass in order to produce more consistent *in situ* pressures.

Keywords

Transcranial ultrasound; Focused ultrasound; Insertion loss

Introduction

There is an increasing interest in the development of ultrasound interventions for brain disorders. Focused ultrasound provides a non-invasive alternative to traditional surgical interventions. Its potential has been demonstrated in preclinical work for procedures such as blood-brain barrier disruption (BBBD) [Hynynen et al., 2001; McDannold et al. 2006; Choi et al., 2007] and clinically in tissue ablation procedures for the treatment of chronic pain [Martin et al., 2009] and brain tumors [McDannold et al., 2010]. It was previously believed that successful ultrasound brain therapy required a portion of the bone from the skull to be removed to create viable acoustic window. While ultrasound attenuates strongly through skull bone at high frequencies [Fry and Barger, 1978], frequencies in the sub-megahertz range have potential for use in transcranial therapy. However, in preclinical animal studies, where the skull bone is thin, a wider range of frequencies can be used while still achieving a sufficiently high pressure at the focus. Although most preclinical work is conducted at frequencies below 2MHz, frequencies as high as 8 MHz have been used [Bing et al., 2009]. Knowledge of mean skull insertion loss values at different frequencies is important for

estimating *in situ* pressures during treatments. This is especially important in procedures such as BBBD where an under-estimate of delivered pressures could result in undesired tissue damage. Further, in order to minimize error in pre-clinical experiments, a better understanding of the ultrasound losses as they apply to individual animals is desirable.

In a previous study on BBBD, interanimal variability in post treatment T1w contrast enhancement was observed, even though a modified pulse was used to remove standing waves, and thus reduce the error on *in situ* pressure estimates [O'Reilly et al., 2011]. Skull thickness variability was suggested as one possible cause of the observed differences. We hypothesize that younger, lighter animals will have thinner skull bones and that these differences in skull bone thickness will have an impact on the ultrasound losses through the skull.

This study attempts to characterize the ultrasound losses through the skull bone as a function of animal mass in a rat model.. This would offer a relatively simple way to estimate *in situ* acoustic pressures during therapy.

Material and methods

The acoustic field was generated using an in-house constructed spherically focused transducer (5 cm aperture, FN=1) with a fundamental frequency of 0.268 MHz. The transducer could be driven at its fundamental frequency and 3rd, 5th, 7th and 9th harmonics (0.841 MHz, 1.409 MHz, 1.972 MHz, 2.53 MHz) using a set of external matching circuits.

The transducer was mounted in a large, rubber lined tank filled with de-ionized, degassed water (fig.1). A planar fiber-optic hydrophone with an active tip diameter of 10 μ m (Precision Acoustics Ltd., Dorset, UK) was mounted on a three axis scanning system in front of the transducer. A detailed description of the hydrophone system can be found in Morris et al., 2009. The fiber-optic hydrophone was calibrated by comparison with a factory calibrated fiber-optic hydrophone calibrated over the range 1 – 30 MHz, and with a scanning laser vibrometer [Harland et al., 2003]. The sensitivity of the hydrophone ranged from approximately 0.17–0.26V/MPa over the range of frequencies examined. The transducer was driven using a function generator (AFG3102, Tektronix, TX, USA) and bursts of approximately 100–200 μ s in length. Long bursts were used to imitate the conditions most commonly used in *in vivo* transcranial ultrasound experiments. The transducer was driven sufficiently low power as to avoid non-linear propagation and non-linear hydrophone response. Input voltages at each frequency were selected in order to ensure adequate signal-to-noise ratio. While the higher harmonics had poorer efficiencies, these were offset by sharper foci and higher focal gains. As a result, large changes in input voltage between frequencies were not necessary. The signal acquired from the fiber-optic hydrophone was transferred from oscilloscope (Tektronix AFG3021B Digital Phosphor Oscilloscope) to computer via a GPIB interface using LABVIEW software (National Instruments, TX, USA). Digitization rates of 12.5 – 125 MHz were used depending on the ultrasound frequency. The focus of the transducer was found by performing raster scans of the acoustic field in the transverse plane and then performing line scans along the acoustic axis.

Measurements were taken through formalin-fixed *ex vivo* skullcaps from 22 Wistar rats (255–539 g). Formalin-fixation of fresh skulls has been shown to produce a minimal change in skull bone properties [Fry and Barger, 1978]. All skull samples were obtained from prior animal studies which were conducted with the approval of the institution's Animal Care Committee. Skull thickness was measured at four locations through the parietal bone in each skull using calipers. Measurements were taken away from the sutures where the bone might be thicker. The skullcaps were degassed in a vacuum jar for a minimum of one hour prior to

performing measurements in order to remove any trapped air. The skullcaps were mounted on a three-axis stage in front of the transducer with the transducer focus at a depth of 5 mm from the skullcap to imitate the most common *in vivo* sonication depth. For each of the five frequencies, measurements were taken at four locations through the parietal bone of each skull cap and compared with the original field scans. The ultrasound transmission through the skull was defined by

$$Transmission = \frac{P_{skull}}{P_{water}} \quad (1)$$

where P_{skull} is the pressure at the transducer focus measured through the skullcap, and P_{water} is the pressure at the transducer focus measured directly in water. Since pressure is directly proportional to the hydrophone output, transmission was calculated using the hydrophone output voltage before and after skull insertion. To account for possible focal shifts, the peak pressure near the focus after skull insertion was used to calculate the transmission value, even if this location did not correspond exactly to the location of the focus in water. To determine the reproducibility of the measurements, setup and measurements were repeated ten times through one skull (animal mass 290 g) at the fundamental frequency and 9th harmonic. Analysis was performed in MATLAB (Mathworks, MA, USA).

Additionally, the ultrasound field distortions through one mouse skull (wild type, approx. 30 g) and one rabbit skull (New Zealand White, approx. 3 kg) were measured at 2.53 MHz and compared with measurements from the rat skulls. The mouse and rabbit skulls were formalin fixed and were prepared in the same manner as the rat skulls, degassing under vacuum pressure prior to the measurements. The rabbit skull was degassed for 3 hours due to its larger size.

Micro CT images (Enhanced Vision Systems MicroCT) were obtained of one skull from each species (mouse, rat, rabbit) and reconstructed at 27 μ m resolution.

Results

The average skull thickness measured in the 22 rat skulls was 0.71 ± 0.03 mm. Figure 2 shows coronal micro CT images of one rat skull compared with mouse and rabbit skullcaps, and with the wavelengths of ultrasound used in this study. The average parietal bone thickness in each animal is shown in Figure 3 as a function of mass. The thickness increased proportionally with mass. Figure 4 shows the mean transmission through each skull versus rat mass. A line of best fit and its equation are displayed on each plot. The average transmission was 91.2 ± 1.7 % at 0.268 MHz, 66.6 ± 1.7 % at 0.841 MHz, 60.5 ± 2.0 % at 1.409 MHz, 63.8 ± 2.7 % at 1.972 MHz and 56.7 ± 2.8 % at 2.530 MHz. At the lower frequencies a linear trend is visible with transmission decreasing as the body mass increases. However, beginning at the fifth harmonic, the data begins to spread and the trend is fully lost at the seventh and ninth harmonics. The results of the reproducibility analysis are detailed in Table 1. The measurements were found to be much more reproducible at the fundamental frequency than at the 9th harmonic. This is consistent with the greater variability in results within each skull at the higher frequencies, seen in fig.5 as much larger error bars at the 7th and 9th harmonics. Figure 5 shows intensity plots of the normalized pressure distributions at the five frequencies before and after insertion of a skull. At the lower frequencies the profiles before and after insertion are comparable. As the frequency increases the focus begins to distort through the skull, with the most obvious differences visible at the ninth harmonic. Corresponding plots of phase difference are also shown in fig.

5. The phase were normalized to the location of highest pressure intensity so that this location represents a phase change of 0. Like the pressure distributions, the greatest distortions in phase are visible at the 9th harmonic. Large focal deformations were seen in some skulls at 2.53 MHz. This was found to be highly location dependent within the skulls. Some locations caused high deformations while others in the same skull resulted in minimal distortion. This was additionally found to be independent of animal mass. Different animals of comparable mass showed very different amounts of beam distortion at the ninth harmonic. Figure 6 shows pressure profiles and phase difference images through skulls from two animals of similar mass, one where no significant focal distortions are visible and one which has large focal distortions. The acoustic field distortions through mouse, rat and rabbit skull at 2.530 MHz are illustrated in figure 7. Almost no field distortion was observed through the mouse skull, while the focus was highly distorted in the rabbit skull.

Discussion

At sub-megahertz frequencies through-skull transmission in rat skull is proportional to animal mass. This is likely due to the fact that larger animals are also older animals with thicker skull bone. The most likely explanation for the linear ultrasound transmission to mass relationship is that the wavelength of the ultrasound at lower frequencies is large relative to the skull bone thickness and therefore variations in the thickness result in small phase distortions. Losses in the sub-megahertz regime are more likely attributable to other attenuation mechanisms such as reflection and absorption. However, as the frequency increases and the wavelength approaches the thickness of the bone, wave distortion begins to have an effect on the pressure distribution producing focusing or defocusing effects dependent on geometry. Assuming a speed of sound of approximately 2900 m/s in bone [Fry and Barger, 1978], the wavelengths used in this study ranged from approximately 1.1–10.8 mm (fig.2). Rat skull bone ranged in thickness from approximately 0.5–1 mm, while the mouse skull was closer to 0.2 mm and the rabbit skull was thicker than 1.5 mm. Greater phase changes were observed at the higher frequencies and in the thicker animal skulls. The transverse pressure profiles through skull (fig.5) show limited distortion at low frequencies. However, at higher frequencies the distortion was occasionally severe. In some skulls, the skull bone was seen to have a focusing effect at higher frequencies, resulting in a higher than expected *in situ* pressure. As the frequency is increased, the variations in transmission within each skull also increased highlighting the geometry dependence. This is seen in the standard deviations in figure 4, which spread as the frequency increases. At the 5th, 7th and 9th harmonics resonance may also be a confusing factor as some of the skull thicknesses measured correspond to the half-wavelength resonance in bone at these frequencies [Aarnio et al., 2005].

Refraction and diffraction were considered to be contributing mechanisms to the observed aberrations. Their individual contributions to the aberration effects were not measured in this study. The purpose of this study was not to develop a model to investigate the different mechanisms affecting ultrasound propagation in the skull but rather to find a simple method to provide a more accurate estimate of *in situ* pressures. *In vivo* these estimates will be affected by standing waves in the skull cavity when the long pulses are employed [O'Reilly et al., 2010], as are used in most blood-brain barrier studies and as are required in thermal therapy or ablation studies. However, they will provide a better starting estimate for prescribing treatment parameters.

At the lowest frequency measured (0.268 MHz) an increase in pressure was seen through some skulls (fig. 4). Standing waves were not detected from the axial scans performed behind the skullcap. At the lowest frequency the focus is not sharp and signal noise may contribute to this apparent increase in focal pressure. The forward and reflected electrical

power from the transducer was not measured during the experiments. However it is possible that standing wave formation occurred between the transducer and the skullcap.

Ultrasound behavior through the mouse and rabbit skulls was as expected, with very little observed distortion through the mouse skull and large aberrations through the rabbit skull. Few measurements were taken through the mouse and rabbit skulls and only a single skull from each animal was used so firm conclusions cannot be made without further investigation. However, it may be that for preclinical studies investigating the effects of higher frequency transcranial sonications, mouse models may be the most appropriate. This could include studies where a very tight focal spot is desired, such as for clot lysis or displacement in stroke research. Further characterization of the ultrasound propagation in different animal models may help in selecting the most appropriate animal model and ultrasound parameters for preclinical studies.

The results of this study suggest that at submegahertz frequencies, animal mass should be considered when estimating *in situ* pressures in order to minimize inter-animal variability. Further, while sufficiently high pressures can be generated through rat skull at higher frequencies, the variability of the ultrasound transmission must be considered and experiments may be subject to a higher level of uncertainty. It may be that in order to achieve repeatable results at higher frequencies in rats a greater control of the acoustic field is required. This could possibly be achieved using a phased array [Hynynen and Jolesz, 1998] instead of a single element transducer and applying corrected focusing based on MRI or CT images of the skull [Hynynen and Sun, 1999; Clement and Hynynen, 2002; Aubry et al., 2003].

This study was performed with one transducer geometry in primarily a single animal model. It is possible that results could be affected by the aperture and focus of the transducer. Additionally the depth of the focus within the skull will impact results. It is expected that phase aberration effects will increase with greater distance from the skull. However, since the rat skull cavity is only approximately 10 mm in depth, conditions where the focus is very far from the skull are not relevant to *in vivo* conditions. The transducer and acoustic parameters were selected to as closely match *in vivo* experimental conditions as possible but may be less suitable for evaluating transmission during other types of transcranial ultrasound, such as imaging. As described above the use of a skullcap rather than a full skull permitted evaluation of the lateral pressure profile but did not model the standing wave conditions previously seen in *ex vivo* rat skull studies [O'Reilly et al., 2010]. The study also did not consider sonications through the midline or sutures, or through lateral portions of the skullcap where the skull curvature and the angle of incidence of the ultrasound might play a greater role. While the changes in insertion loss with mass are likely due to the increasing thickness of the parietal bone, bone density and microstructure may also be contributing factors, although these were not considered in this study. Finally, the animals studied in this experiment were young, healthy animals and weight gain was assumed to relate to overall animal growth. These relationships may not apply when considering animals which have lost weight due to illness, as might occur in tumor models. Studies in different animal models such as mouse, rabbit and pig would be useful to determine the extent to which these relationships are applicable.

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Figure 1.
Experimental Setup

Figure 2. Coronal micro CT images of a mouse skullcap (top), rat skullcap (middle) and rabbit skullcap (bottom). Scale bars show the approximate wavelengths of the ultrasound in bone from 0.268–2.53 MHz.

Figure 3. Average skull thickness in 22 rat skull caps as a function of animal mass. A line of best fit and its equation are shown. Error bars indicate one standard deviation on the mean.

Figure 4.

Ultrasound transmission through skull versus rat mass for 0.268 MHz(a), 0.841 MHz(b), 1.409 MHz (c), 1.972 MHz (d) and 2.530 MHz(e). A line of best fit and its equation are shown for each frequency. Error bars indicate one standard deviation on the mean.

Figure 5.

Contour plot showing the normalized pressure profile of transducer focus in the transverse plane at five frequencies in water (a–e) and in water through a rat skullcap (f–j). Contours at intervals of 0.1 are shown. (k–o) Corresponding absolute phase difference maps from 0 to π with contours at intervals of 10%.

Figure 6.

Normalized pressure map of transducer focus in the transverse plane at 2.53 MHz in water (a, b) and in water through a rat skullcap (c, d) through two different skull caps from animals of similar mass. Contours at intervals of 0.1 are shown. Large distortions are visible in (c) but not (d). (e, f) Corresponding absolute phase difference maps from 0 to π with contours at intervals of 10%.

Figure 7.

Normalized pressure map of transducer focus in the transverse plane at 2.53 MHz in water (a–c) and in water through a mouse skullcap(d), a rat skullcap (e) and a rabbit skullcap(f).Contours at intervals of 0.1 are shown.(g–f) Absolute phase difference maps for the three species normalized.(g–i) Corresponding absolute phase difference maps from 0 to π with contours at intervals of 10%.

Mean (mean $\pm$ S.D.), maximum and minimum values for 10 measurements in one skull. Values at four different measurement locations for f_0 and $9 f_0$ are presented.

Table 1

Location	0.268 MHz		2.53 MHz	
	Mean	Max.	Min.	Max.
1	0.83 $\pm$ 0.02	0.86	0.80	0.62
2	0.79 $\pm$ 0.02	0.83	0.76	0.72
3	0.82 $\pm$ 0.02	0.85	0.80	0.85
4	0.79 $\pm$ 0.01	0.81	0.76	0.80