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FEASIBILITY STUDY OF A CLINICAL BLOOD–BRAIN BARRIER OPENING ULTRASOUND SYSTEM

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Abstract

In this paper, we investigate the focalization properties of single-element transducers at intermediate frequencies (500 kHz) through primate and human skulls. The study addresses the transcranial targeting involved in ultrasound-induced blood brain barrier (BBB) opening with clinically relevant targets such as the hippocampus and the basal ganglia, which are typically affected by early Alzheimer's and Parkinson's disease, respectively. The targeted brain structures were extracted from three-dimensional (3D) brain atlases registered with the skulls and used to virtually position and orient the transducers. The frequency dependence is first investigated and the capability of targeting of different structures is explored. Preliminary *in vivo* feasibility is investigated in mice at this frequency. A simple, affordable and convenient system is found to be feasible for BBB opening in primates and humans capable of successfully targeting the hippocampus, putamen and substantia nigra and could thus allow for its broader impact and applications.

Keywords

Blood–brain barrier; drug delivery; microbubbles; focused ultrasound

1. Introduction

The blood–brain barrier (BBB) is a selective barrier within the neurovascular unit formed by the endothelial cells that line cerebral microvessels.^{1,2} Most molecular traffic is forced to follow a transcellular route across the BBB by the tight junctions between adjacent endothelial cells. Therefore, the BBB acts as a physical barrier impeding paracellular

transfer through the junctions. The BBB hinders the effective systemic delivery of neurological agents and biomarkers to the brain through a combination of passive, transport and metabolic barriers. Very small gaseous molecules and small molecules with high lipid solubility can diffuse freely through the lipid membranes. The main role of the BBB is to control the flow of nutrients from the lumen to the parenchyma and therefore to maintain the brain homeostasis for proper neuronal firing.³ Determining factors for the passage of molecules across the BBB are lipid solubility, charge and molecular size with a threshold range spanning between 50 Da and 400 Da.⁴ BBB remains the main limiting factor toward the development of novel treatments of neurological and neurodegenerative diseases: more than 98% of small-molecule drugs and nearly all large-molecule drugs do not cross this anatomic barrier.⁵ Therefore, potential therapeutic agents, such as growth factors^{6,7} and adenoviruses,^{8,9} do not efficiently cross the BBB when administered systemically.

Intracranial injections, mixing or attaching agents to BBB-modifying chemicals, and the chemical alteration of agents to be delivered through endogenous transport systems can be used to circumvent the BBB.¹⁰ However, these techniques are invasive, drug-specific or plagued by very poor spatial specificity. Global breaching of the BBB can be a risky process as it increases influx of all molecules and therapeutic agents in untargeted areas of the brain. An ideal method would ensure drug-independent, reversible, localized and noninvasive delivery through the BBB to minimize potential hazards. Previously, our research group has shown, along with others, that microbubble-enhanced, focused ultrasound (ME-FUS) is capable of disrupting the BBB noninvasively, transiently and selectively in small animals.^{11–13}

Clinical translation of this technique remains very challenging today. Scaling from small to large animals is a difficult task because the ultrasound beam propagation is affected by the skull thickness. Moreover, discrepancies in sound velocity and density combined with high absorption, can lead to poor focusing quality and high energy loss, especially at higher frequencies.¹⁴ High-intensity focused ultrasound (HIFU), a promising technique used in noninvasive tumor ablation, had led the way of transcranial focusing since the early 1950s.¹⁵ The introduction, in the 1990s, of piezoceramic and piezocomposite transducers capable of being driven at high voltages and the improvement of multichannel electronics¹⁶ have enabled the development of very complex methods based on prior knowledge of the skull topology that are needed in order to provide accurate focusing.^{17,18} This technique provides higher accuracy and sharper focusing compared with classic focusing techniques. An alternative is to operate at lower frequencies, but the focus can become very wide due to diffraction effects, thus decreasing the spatial resolution. This is usually used for sonothrombolysis, a technique using combined microbubble injection and FUS to dissolve clots in the brain. Besides being less sensitive to attenuation and aberration, decreasing the frequency also enhanced cavitation effects. However, working at these low frequencies can be very harmful as they may induce large secondary hemorrhages. Sonothrombolysis using unfocused ultrasound at 300 kHz can lead to patient death, as showed by a clinical trial,¹⁹ which have been suspected to be linked to unexpected enhanced cavitation in untargeted regions caused by standing waves generated within the skull.²⁰ These standing waves are more prone to occur at lower frequencies because the attenuation in the brain decreases, leading to higher reverberations. The use of focused ultrasound would have lower standing

wave risks. The standing waves are less problematic in this study case because ME-FUS uses pulsed waves at very low duty cycles (typically 0.2% to 1%) compared to HIFU or sonothrombolysis that uses continuous wave sequences, thus decreasing the risk of having enhanced cavitation effect in unintended regions of the brain. Our group has thus developed a compromise, i.e., operate at intermediate frequencies that allow transcranial propagation and sufficiently high spatial resolution with a single-element transducer. Until now, feasibility with this system has been shown in simulations and preliminary transcranial *ex vivo* validation.²¹

In this study, we investigate transcranial focusing in clinically relevant targets, especially those involved in Alzheimer's and Parkinson's disease. The hippocampus was chosen for its predominant role in early Alzheimer's disease.²² The striatum, the predominant structure of the basal ganglia, which encompass the putamen and the caudate nucleus, was chosen because of its role in the dopa-mine pathway, a pathway severely altered in Parkinson's.²³ The substantia nigra, a very small structure in the basal ganglia, is known to play an important role at the beginning of the dopamine pathway and could also be targeted depending on the drug used and the state of the disease. Most of our previous work in small animals was performed at 1.525 MHz.^{24–27} The second part of the results section will address the effect of the frequency drop in mice *in vivo* and the results will be compared with existing work at those frequencies.^{28–30} In order to pave the way for large animal experiments using this setup, the dependence of the BBB opening threshold and the bubble behavior at this particular frequency before translation to large animals. The procedure's safety at different pressure levels was also studied using histology to determine the presence of red blood cell extravasations or neuronal death and compared with previous findings at 1.5 MHz.³¹

2. Material and Methods

2.1. In vitro setup

2.1.1. Acoustic transducers—A single-element, circular focused ultrasound transducer (Riverside Institute, New York, NY, USA) with a void in its center was driven by a function generator (Agilent Technologies, Palo Alto, CA, USA) through a 50-dB power amplifier (ENI Inc., Rochester, NY, USA). The center frequency, focal depth, outer radius and inner diameter of FUS were 500 kHz, 90 mm, 40 mm and 11.2 mm, respectively. A single-element passive cavitation detector (PCD) (center frequency: 7.5 MHz, focal length: 60 mm, Olympus NDT, Waltham, MA, USA) was positioned through the center void of the FUS transducer. The two transducers were aligned so that their focal regions fully overlapped within the confocal volume. This transducer assembly was attached to a three-dimensional (3D) axis positioning system (Velmex Inc., Bloomfield, NY, USA) to be able to aim the desired target through the skull. A hydrophone (HGN-0200, Onda Corp, Sunnyvale, CA, USA, aperture 200 μ m, calibration information provided by manufacturer) is attached to a second similar 3D axis positioning system in order to scan the transcranial pressure field. The transducer was calibrated using the hydrophone readings. Its impedance was matched to 50 Ω through a matching circuit. The pressures used for BBB disruption are usually below 1 MPa and we have checked the linearity response of our transducer in that pressure range

(in water and through the skull). The electric power transmitted to the transducer remained under 3 W.

2.1.2. Skull preparation—Two human and two nonhuman primate (NHP) skulls were used for this study. For these skulls, two hemispheres were used for each location. Prior to the experiment, the skulls were degassed for one day in a sealed jar using a customized vacuum pump. In the *in vitro* experiments, the transducer was connected to the first 3D positioning system and immersed in a large water tank filled with degassed water. The human or NHP skull was also immersed in water. The hydrophone was then placed inside the skull cavity at the center plane through the virtual targeted region.

2.1.3. Targeting—Targeting was performed using a pulse-echo transducer utilizing the visible skull sutures. The 7.5-MHz pulse-echo transducer embedded through the central bore hole of the therapeutic transducer was used to map the surface of the targeted skull. The occipital protuberance (OP) that lines the inferior dorsal region and the lambda anatomical landmarks in both primates and humans (Fig. 1) was identified using time-of-flight and power spectral density measurements, whose product indicates the reflectivity of the skull. To this purpose, the pulse-echo transducer was moved using the positioning system in the lateral and ventro-dorsal directions of the skull and the time of occurrence of the peak in the power of the received RF signals was calculated in each location. The OP and lambda landmarks were then identified (Fig. 1) due to their distinct reflectivity and texture and then mapped onto a preexisting brain atlas.²¹ For each target, the orientation of sonication was chosen to be similar to the previous simulation study reported by our group.²¹ In that study, optimal orientations for the ultrasound focal spot to best match the anatomical shapes of brain structures targeted were identified. Also, as the NHP OP seemed to be hindering the ultrasonic propagation for the putamen and caudate targeting based on the simulation study, alternative orientations were selected. These alternative orientations for caudate and putamen are similar and the orientation dependence was studied *in vitro*, i.e., in the NHP case, the alternative caudate and the original putamen orientations.

2.1.4. Acoustic measurements—In order to quantify the focusing quality, pressure field measurements were performed across the geometric focus. Once the transducer was set to aim at a particular region through the skull using the procedure described in the previous section, the hydrophone was fixed at this position using the second 3D positioning system. This second positioning system was used to move the hydrophone to map the pressure field along one plane. The field of view was 2 cm in lateral length and 6 cm in the axial dimension, at a spatial step of 0.167 mm and 0.5 mm, respectively. At each point, the acoustic response was acquired on a PC workstation with an 80-MHz digital acquisition board (model 14200, Gage applied technologies Inc., Lachine, QC, Canada). For each location, the peak negative pressure was measured and represented in a two-dimensional (2D) matrix. For every case, transcranial pressure profiles, were compared with water pressure profiles, enabling quantification comparison in peak pressure amplitude, position shift and focal shape. In our previous simulation work,²¹ two other parameters were evaluated to quantify the quality of the target coverage by the ultrasound focus. For every case, we calculated the percent-of-target-overlapped, which depicted the volume fraction of

the target above the -6-dB pressure threshold, and the percent-of-beam-overlapping-target, which represents the volume fraction of the beam that falls inside the targeted region.

2.2. Bioheat equation simulation

Simulations were performed here to estimate the maximum heat dissipation increase in the skull using BBB opening acoustic sequences. Simulations were based on the discretization of the Bio-Heat equation using a 3D cubic mesh³²:

$$\rho C_p \frac{\partial T}{\partial t} = \vec{\nabla} \cdot (k \vec{\nabla} T) - \rho_b \omega_b C_b (T - T_b) + \frac{\alpha p^2}{\rho c}, \quad (1)$$

where ρ is the tissue density and C_p its calorific capacity, k is the tissue thermal conductivity and $\vec{\nabla} \cdot (k \vec{\nabla} T)$ represents the thermal diffusion. The term $\rho_b \omega_b C_b (T - T_b)$ represents the effects of perfusion, where ρ_b , ω_b , C_b and T_b are, respectively, the blood density, the perfusion rate, the blood calorific capacity and the blood temperature. The $\alpha p^2 / \rho c$ models acoustical power where α , p and c are, respectively, the tissue absorption coefficient, the pressure delivered and the speed of sound. The values used in this study for the previous parameters are summarized in Table 1. The spatial step (Δx) of this simulation was set to $\lambda/10 = 0.3$ mm in all directions and the temporal step had to be smaller than $\rho C_p \Delta x^2 / k \approx 3 = 410$ ms to respect the 3D stability criteria. The temporal step is set to 0.02 ms in order to accurately simulate the BBB opening acoustic sequence (100 cycles is equivalent to 0.2 ms, see Sec. 2.3). The values used for the simulation are described in Table 1.^{33–35}

2.3. In vivo mouse setup

In vivo experiments were conducted in mice ($N = 10$) using the same acoustic equipment and Definity® microbubbles (Lantheus Medical Imaging, MA, USA) as in Sec. 2.1. The setup and targeting procedures have been detailed elsewhere.²⁶ The pulse-echo transducer is used in that case to acquire the microbubbles acoustic signature and determine the occurrence of inertial cavitation. The BBB opening was confirmed using a vertical-bore 9.4 T MR system (Brucker Biospin, Billerica, MA, USA). Intraperitoneal (IP) injection of gadodiamide (Omniscan®, MW 573.66 Da, GE Healthcare, Princeton, NJ, USA) was performed two minutes prior to MR imaging and tracked using a spoiled gradient echo (SPGR) T1-weighted sequence, which acquired horizontal images using TR/TE = 20/4 ms, a flip angle of 25°, number of excitations (NEX) of 5, a total acquisition time of 6 min and 49 s, a matrix size of $256 \times 256 \times 16$ pixels and a field of view (FOV) of $1.92 \times 1.92 \times 0.5$ cm³, resulting in a resolution of $75 \times 75 \times 312.5$ μm³. The pulse-echo transducer was used as a passive cavitation detector (PCD) acquires acoustic emissions from microbubbles. The sonication was performed immediately after intravenous (IV) injection of 50 μL:kg⁻¹ of Definity microbubbles. The acoustic parameters are as follows: peak negative pressure ranging from 0.2 MPa to 0.6 MPa, 100 cycles, PRF 10 Hz, total sonication duration 60 s. Three hours after sonication, the mouse was sacrificed and transcardially perfused with 30 ml phosphate buffered saline and 60 ml 4% paraformaldehyde. After soaking the brain in paraformaldehyde for 24 h, the skull was removed and the brain was fixed again in 4% paraformaldehyde for six days. The post-fixation processing of the brain tissue was then

performed according to standard histological procedures. The paraffin-embedded specimen was sectioned horizontally at 6- μ m thickness section. A 1.2-mm layer from the top of the brain was first trimmed away. A total of 12 separate levels that covered the entire hippocampus were then obtained at 80 μ m intervals. At each level, six sections were acquired and the first two sections were stained with hematoxylin and eosin (H&E). A time-frequency map of the acoustic emission was generated using a customized spectrogram function²⁷ (30 cycles, i.e., 60 μ s, Chebyshev window; 98% overlap; 4096-point FFT) in MATLAB® (2010a, Mathworks, Natick, MA). Since the spectrogram provides the frequency content of the acoustic emissions from cavitating bubbles as a function of time, the duration of the cavitation activity could be assessed. The inertial cavitation dose (ICD), which is linked to the energy of the broadband signal, was also computed. All animal experiments and procedures by the Institutional Animal Care and Use Committee at Columbia University.

3. Results

3.1. Transcranial focusing quality quantification

Figures 2 and 3 show typical beam profiles of the transcranial ultrasonic pressures measured in the horizontal and transverse planes of the focus of the transducer. According to previous reports,^{27,30,31} the ratio between the pressure threshold for damage and the pressure threshold for BBB disruption remained under 2 or 6 dB. These pressure fields have been thresholded at -6 dB in order to represent the maximum extent of induced BBB opening without inducing damage. The contour of the targeted region is depicted using a blue dashed line. For each target and each skull type, these pressure profiles were acquired four times (two skulls and two hemispheres). For each acquisition, the quality of the focusing is assessed using the following parameters: attenuation compared to that of the water, lateral and axial resolution of the focus (for the lateral, small and large axis are measured to quantify the distortion of the focus), shift of the position of the focus compared to that of the water, resolution of the focus (-6 dB dimensions, roughly equivalent to half-pressure threshold) and angle tilt of the focus.

Using NHP skulls, the values of these parameters were consistent from one location to the next except for the NHP putamen targeting. The overall attenuation was found to be around -6 dB (respectively -5.66 ± 0.77 dB, -6.18 ± 0.30 dB and -5.57 ± 0.47 dB for hippocampus, caudate and vertex). The resolution of the focus was found to be acceptable compared to that of the water (lateral dimension 3.6 ± 0.1 mm, axial dimension 31.2 ± 1.2 mm). The deviation of the transverse focal spot from a circular geometry was also investigated in order to quantify the distortion of the focus. For the hippocampus, caudate and vertex, respectively, the lateral -6 dB dimensions were 3.9 ± 0.2 mm, 4.0 ± 0.1 mm and 3.7 ± 0.1 mm for the small axis and 4.2 ± 0.2 mm, 4.2 ± 0.3 mm and 4.0 ± 0.2 mm for the large axis. Corresponding axial resolutions were found to be 38.5 ± 1.7 mm, 39.2 ± 2.3 mm and 38.9 ± 1.7 mm. The displacement of the focus compared to the geometrical focus was also quantified. The shift of the focus was found to be, respectively, 0.6 ± 0.2 mm, 0.8 ± 0.1 mm and 0.5 ± 0.3 mm in the focal plane and -4.4 ± 1.3 mm, -4.1 ± 0.7 mm and -3.9 ± 1.0 mm along the geometric axis of propagation. Finally, the tilt of the focus induced by

presence of the skull was measured, which is the angle between axial dimension of the focus and the principal axis of propagation. The respective measurements were $1.21 \pm 0.11^\circ$, $1.43 \pm 0.64^\circ$ and $1.03 \pm 0.18^\circ$.

For the initial orientation calculated for targeting the putamen, the effects of the skull were stronger. The total attenuation was found to be -6.91 ± 0.88 dB. The resolution of the focus was more altered and the difference between small-axis resolution (4.3 ± 0.3 mm) and the large-axis resolution (5.2 ± 0.7 mm) was increased. The axial resolution (41.2 ± 1.8 mm) was also increased but, compared to the previous orientations, this change was less sensitive. The shift of the focus was increased by a factor of three in the lateral dimension (2.0 ± 0.7 mm) and 25% in the axial dimension (-5.0 ± 1.0 mm).

In the human skulls, the same measurements were also performed. Even though the overall effects induced by the skull were stronger, the findings were very similar. The total attenuation was measured to be -9.31 ± 0.62 dB, -9.02 ± 0.69 dB, -9.37 ± 0.65 dB and -9.05 ± 0.86 dB for the hippocampus, caudate, putamen and vertex, respectively. The lateral -6 -dB resolution was found to be 4.2 ± 0.2 mm, 4.2 ± 0.2 mm, 4.1 ± 0.2 mm and 4.0 ± 0.1 mm for the small axis in the hippocampus, caudate, putamen and vertex, respectively, and 4.5 ± 0.3 mm, 4.6 ± 0.2 mm, 4.4 ± 0.2 mm and 4.3 ± 0.1 mm for the large axis in the hippocampus, caudate, putamen and vertex, respectively. Corresponding axial resolutions were found to be 40.5 ± 1.1 mm, 42.0 ± 1.6 mm, 40.7 ± 1.3 mm and 40.3 ± 1.2 mm, respectively. The displacement of the center of the focus was found to be, respectively, 1.1 ± 0.6 mm, 1.3 ± 0.5 mm, 1.4 ± 0.3 mm and 1.2 ± 0.4 mm in the focal plane and -5.7 ± 0.6 mm, -6.5 ± 0.9 mm, -7.0 ± 0.5 mm and -6.6 ± 0.5 mm along the geometric axis of propagation. Tilt angles were measured to be $1.56^\circ \pm 0.64^\circ$, $2.13^\circ \pm 0.91^\circ$, $2.18^\circ \pm 0.80^\circ$ and $1.39^\circ \pm 0.57^\circ$. Figure 4 summarizes all the measurements with their means and standard deviations.

Target coverage estimations were calculated in each case. Table 2 summarizes the findings. For NHP skulls, the percent-of-target-overlapped was found to be $42.1\% \pm 1.4\%$, $31.0\% \pm 0.9\%$ and $21.8\% \pm 5.4\%$ for the hippocampus, caudate and putamen, respectively, while the percent-of-beam-overlapping-target was found to be $62.3\% \pm 2.4\%$, $45.1\% \pm 2.3\%$ and $27.2\% \pm 6.7\%$. For human skulls, for the hippocampus, caudate and putamen, the percent-of-target-overlapped was found to be $12.3\% \pm 2.1\%$, $13.8\% \pm 2.5\%$ and $16.6\% \pm 1.7\%$ while the percent-of-beam-overlapping-target was found to be $71.2\% \pm 1.7\%$, $54.4\% \pm 3.0\%$ and $82.1\% \pm 2.8\%$.

3.2. Skull heat dissipation simulation

The aim of this simulation was to determine whether the skull heat dissipation during BBB opening acoustic sequence can be predicted and that temperature increase is not an issue for safety. To overestimate the thermal effects, we simulated the heat dissipation in simulated homogeneous skull using 0.6 MPa (the maximum peak-rarefactional pressure applied in the experiment). Therefore, the model used provided the upper limit of the expected temperature increase during BBB opening sequence independently of the subject and skull geometry.

The temperature increase at the center of the focus is depicted in Fig. 5. After 60 s of sonication, the temperature increase is lower than 0.03°C. Irreversible changes occur above 43°C,³⁶ therefore thermal effects and potential damages are negligible when performing ME-FUS BBB opening.

This low temperature increase can be explained by the very short duty cycle used (100 cycles at 500 kHz and PRF of 10 Hz, duty cycle: 0.2%). Between each 0.2-ms pulse there is a 99.8-ms pause. In the meantime, the skull bone diffuses the heat fast enough to minimize the overall temperature increase (Fig. 5). Each individual temperature increase is almost entirely compensated during the interpulse time. Therefore, the resulting temperature increase after 60 s is negligible.

3.3. In vivo BBB opening at 500 kHz

3.3.1. BBB opening—Combined SPGR T1 MRI sequences with gadodiamide injections were used to confirm BBB opening. The contrast agent cannot penetrate the BBB therefore the deposition of the gadodiamide in the parenchyma confirmed local BBB disruption by ME-FUS.

The MR images indicated that the BBB was opened at 0.2 MPa, 0.3 MPa and 0.6 MPa (Fig. 6). The peak MR intensity enhancement at the BBB-opened region relative to the average value in the parenchyma increased by $47.3\% \pm 3.5\%$, $123.4\% \pm 14.5\%$ and $168.2\% \pm 25.6\%$ at 0.2 MPa, 0.3 MPa and 0.6 MPa, respectively. The volume of the BBB disruption was equal to 2.6 mm³, 24.6 mm³ and 67.4 mm³, respectively.

3.3.2. Cavitation detection—As a result of the deposition of gadodiamide into the brain parenchyma through the BBB opening, the MRI indicated that the BBB was opened at 0.2, 0.3 and 0.6 MPa (Fig. 6), but the spectrogram showed that the broadband response occurred at 0.30 MPa (Fig. 7). At 0.2 MPa, apart from the fundamental frequency (500 kHz), higher harmonics from the 5th to the 20th (2.5 MHz to 10 MHz) could be detected by the 7.5-MHz P/E transducer. In addition, ultra-harmonic peaks, the signature of stable cavitation, at 5.75, 6.25, 6.75, 7.25, 7.75, and 8.25 MHz were detected as indicated in Fig. 7.

The broadband response as detected by the PCD was quantified using the ICD (Fig. 7). As indicated by the ICD calculations, the ICD at 0.3 MPa and 0.6 MPa was statistically higher than at 0.2 MPa ($p < 0.05$) (Table 3), which confirmed that the threshold of inertial cavitation during the BBB opening was around 0.3 MPa.

3.3.3. Histology—The histological findings are shown in Fig. 8. In the cases of BBB opening at 0.2 MPa confirmed by MR images, no cell damage, e.g., red blood cell (RBC) extravasations or neuronal death (Baseri *et al.*, 2010), was observed after histological examination. In the cases of BBB opening at 0.3 MPa, no extra-vasations were detected in the sonicated brain regions even though a moderate broadband response was detected (Fig. 7). Brain samples sonicated at 0.6 MPa showed higher incidence of microscopic damage at multiple distinct damaged sites (Fig. 8). The exposure pressures that resulted in RBC extravasations were those associated with the highest broadband response (Fig. 7).

4. Discussion

The study presented in this paper showed that using a single spherical transducer at an intermediate frequency (500 kHz) is suitable for ME-FUS BBB opening for large animal treatment. At this frequency, the distortions induced by human and NHP skulls were acceptable. The skull interface works as an aberrating layer, therefore the foci obtained were wider than those obtained in water (approximately +12% laterally and +23% axially for NHP skulls and +21% laterally and +31% axially for human skulls). This layer also induced a displacement of the center of the focus but the lateral displacement was found to be small compared to the resolution of the transducer (less than 1 mm for NHP skulls and 1.5 mm for human skulls). Along the geometric axis of propagation, the shift of this focus is more important (around 5 mm for NHP skulls and 7 mm for human skulls). Due to its high speed of sound, the skull acts as a lens and the ultrasound waves focus ahead of the geometric focus. This change was found to be reproducible and therefore can be automatically corrected. The exact same method should be applied to correct for the energy loss across the skull. These values were found to be consistent regardless of the orientations (approximately -6 dB for NHP skulls and -9 dB for human skulls). The values of the attenuation were slightly smaller than the values found in our previous study.²¹ This may be caused by the difference in the method used for the skull preparation. Previously, the skulls were soaked in degassed water for 6 h prior to measurements. Here, the skulls were degassed in a sealed jar during 24 h. This method ensured that all small cavities in the skulls were filled with degassed water and consequently better acoustic transmission. This configuration is closer to the *in vivo* case, where cavities in the trabecular bone are filled with organic fluids. We also used the same parameters as in the previous simulation study²¹ to quantify the target coverage by the ultrasound beam. Results for human skulls were found to be very similar to *in silico* results. Focusing in the NHP skulls was found to perform better *in vitro* compared to simulations. There are two possible reasons for this. First of all, the center frequency of our transducer is inferior to the lowest frequency used in the simulations (800 kHz). Therefore, the beam shape degradation and shift of the focus are expected to be decreased. In addition, the total volume of the -6 dB focus is about three times the volume of the focus depicted in simulations and thus the target is easier to cover. For NHP skulls, the focusing quality and target coverage quantification showed that alternative orientations avoiding the occipital protuberance are more suitable than original orientations only designed to match the focus and the target shape.

Thermal increase in the skull was found to be minimal using ME-FUS sequences. The pulsed wave duty cycle applied is very low (0.2%) so that the skull between two consequent pulses has enough time to diffuse this energy. Thus, the resulting temperature increase during the whole sonication is negligible.

In the *in vivo* study, the spectrogram clearly elucidated the onset and duration of IC within a single pulse. Here, the inertial cavitation occurred at the beginning of sonication. At 0.3 and 0.6 MPa, the broadband response corresponding to the first pulse, lasted throughout the entire duration of the pulse length at all bubble sizes, which indicated that the highest pressure may fragment the microbubbles to smaller bubbles that serve as cavitation nuclei.

In the case of 0.2 MPa, only harmonics and ultra-harmonics without broadband emissions were detected. This serves as evidence that the BBB can be opened by stable cavitation only.

In our previous study, we showed that the threshold of BBB opening was at 0.3 MPa, and the inertial cavitation occurred at 0.45 MPa using 1.5-MHz FUS and Definity®.²⁷ Here, The BBB was opened at 0.2 MPa without inertial cavitation, and at 0.3 and 0.6 MPa with inertial cavitation. The mechanical index (MI) was also measured as the indicator of bubble disruption. In our previous studies, the MI value was 0.25, 0.37, and 0.49 for 0.3 MPa, 0.45 MPa and 0.6 MPa, respectively, at 1.5-MHz. In this study, the MI value was 0.28, 0.42 and 1.02 for 0.2 MPa, 0.3 MPa and 0.6 MPa, respectively, at 500 kHz. The threshold of the broadband response for both studies was close to 0.4, which is consistent with the general condition of bubble rupture threshold during BBB opening.³⁰

The histological results in this study were consistent with the existent literature that investigated the relationship between tissue damage and inertial cavitation.^{37,38} Despite the fact that the inertial cavitation occurred at 0.3 MPa, the opening was induced without any damage. In this case, inertial cavitation did not induce cell death but was sufficient to change the permeability of the endothelial cells or to transiently rupture the tight junctions. Some red-blood-cell extravasations were induced at 0.6 MPa but no extravasations could be found at 0.2 and 0.3 MPa. Even with higher ICD estimated at 0.6 MPa, the extravasations were limited to two to three sections. However, in order to investigate more specific forms of cellular damage (i.e., apoptosis), more sensitive staining protocols, such as TUNEL, will be applied in future studies.

5. Conclusion

In *in vitro* and preliminary *in vivo* mice experiments, a single-element, cavitation-guided, translational setup for ME-FUS was tested for use in NHP and humans. With a single-element driven at 500 kHz, clinically relevant targets through NHP and humans skulls were targeted *in vitro*. The focusing performance was found to be suitable without requiring MRI guidance. Thermal dissipation in the skull was found to be negligible in simulations. Preliminary *in vivo* experiments in mice showed the effect of the decrease of the pressure compared to previous work at 1.5 MHz. A safety window was proven to exist and therefore scaling to large animals and clinical translation for novel brain drug delivery applications is feasible from all aspects of safety and simplicity of the setup to be used.

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References

1. Risau W, Wolburg H. Trends Neurosci. 1990; 13:174. [PubMed: 1693235]
2. Abbott NJ, Romero IA. Mol. Med. Today. 1996; 2:106. [PubMed: 8796867]
3. Abbott NJ, Ronnback L, Hansson E. Nat. Rev. Neurosci. 2006; 7:41. [PubMed: 16371949]

4. Habgood MD, Begley DJ, Abbott NJ. *Cell Mol. Neurobiol.* 2000; 20:231. [PubMed: 10696512]
5. Pardridge WM. *NeuroRx.* 2005; 2:3. [PubMed: 15717053]
6. Nagahara AH, et al. *Nat. Med.* 2009; 15:331. [PubMed: 19198615]
7. Patel NK, Gill SS. *Acta Neurochir. Suppl.* 2007; 97:135. [PubMed: 17691299]
8. Fiske BK, Frasier MA, Sherer TB. *Exp. Neurol.* 2008; 209:28. [PubMed: 17980867]
9. LeWitt AP, et al. *Lancet Neurol.* 2011; 10:309. [PubMed: 21419704]
10. Pardridge WM. *Drug Discov. Today.* 2007; 12:54. [PubMed: 17198973]
11. Mychaskiw G, Badr AE, Tibbs R, Clower BR, Zhang JH. *Anesth. Analg.* 2000; 91:798.
12. Hynynen K, McDannold N, Vykhodtseva N, Jolesz FA. *Radiol.* 2001; 220:640.
13. Choi JJ, Pernot M, Small SA, Konofagou EE. *Ultrasound Med. Biol.* 2007; 33:95. [PubMed: 17189051]
14. Fry FJ. *Ultrasound Med. Biol.* 1977; 3:179. [PubMed: 595211]
15. Fry WJ, Mosberg WH Jr, Barnard JW, Fry FJ. *J. Neurosurg.* 1954; 11:471. [PubMed: 13201985]
16. Haar GT, Coussios C. *Int. J. Hyperthermia.* 2007; 23:85. [PubMed: 17578334]
17. Clement GT, Hynynen K. *Phys. Med. Biol.* 2002; 47:1219. [PubMed: 12030552]
18. Marquet F, et al. *Phys. Med. Biol.* 2009; 54:2597. [PubMed: 19351986]
19. Daffertshofer M, et al. *Stroke.* 2005; 36:1441. [PubMed: 15947262]
20. Baron C, Aubry JF, Tanter M, Meairs S, Fink M. *Ultrasound Med. Biol.* 2009; 35:1148. [PubMed: 19394756]
21. Deffieux T, Konofagou EE. *IEEE Trans. Ultrason. Ferroelectr. Freq. Control.* 2010; 57:2637. [PubMed: 21156360]
22. Giacobini E. *Adv. Exp. Med. Biol.* 1997; 429:235. [PubMed: 9413578]
23. Brundin P. *Movement Disord.* 2006; 21:S42.
24. Choi JJ, et al. *IEEE Trans. Biomed. Eng.* 2010; 57:145. [PubMed: 19846365]
25. Choi JJ, et al. *Ultrason. Imaging.* 2008; 30:189. [PubMed: 19149463]
26. Tung YS, Choi JJ, Baseri B, Konofagou EE. *Ultrasound Med. Biol.* 2010; 36:840. [PubMed: 20420973]
27. Tung YS, et al. *Phys. Med. Biol.* 2010; 55:6141. [PubMed: 20876972]
28. Jordao JF, et al. *PLoS One.* 2010; 5:e10549. [PubMed: 20485502]
29. Raymond SB, et al. *PLoS One.* 2008; 3:e2175. [PubMed: 18478109]
30. McDannold N, Vykhodtseva N, Hynynen K. *Ultrasound Med. Biol.* 2008; 34:834. [PubMed: 18207311]
31. Baseri B, Choi JJ, Tung YS, Konofagou EE. *Ultrasound Med. Biol.* 2010; 36:1445. [PubMed: 20800172]
32. Pennes HH. *J. Appl. Physiol.* 1948; 1:93. [PubMed: 18887578]
33. Clattenburg R, Cohen J, Conner S, Cook N. *J. Biomed. Mater. Res.* 1975; 9:169. [PubMed: 1176477]
34. Karmani S. *Curr. Orthopaed.* 2006; 20:52.
35. Weinbaum S, Jiji LM. *J. Biomech. Eng.* 1985; 107:131. [PubMed: 3999709]
36. Sapareto SA, Dewey WC. *Int. J. Radiat. Oncol. Biol. Phys.* 1984; 10:787. [PubMed: 6547421]
37. Hwang JH, Tu J, Brayman AA, Matula TJ, Crum LA. *Ultrasound Med. Biol.* 2006; 32:1611. [PubMed: 17045882]
38. Samuel S, Fowlkes JB, Miller DL. *IEEE Trans. Ultrason. Ferroelectr. Freq. Control.* 2009; 56:589. [PubMed: 19411217]

Fig. 1. Targeting images for monkey and human skulls based on combined reflectivity and time-of-flight measurements. Anatomical landmarks are clearly identified such as the occipital protuberance or lambda.

Fig. 2.

Examples of -6 dB pressure profiles obtained through a NHP skull for the four different orientations. Blue dashed lines represent the contour of the target in each case as indicated in the depicted plane. The vertex case is shown here as the optimal orientation for transcranial propagation (color online).

Fig. 3. Examples of -6 dB pressure profiles obtained through a human skull for the four different orientations. Blue dashed lines represent the contour of the target in each case as indicated in the depicted plane. The vertex case is shown here as the optimal orientation for transcranial propagation (color online).

Fig. 4.

Focusing performance assessment through human and NHP skulls. Attenuation represents the energy loss crossing the skull interface compared to that of the water. Tilt represents the angle between the axial dimension of the focus and the geometric axis of propagation. Lateral resolution and axial resolution represents the dimension of the focus (-6 dB cutoff). Lateral and axial shift represents the displacement of the center of the focus.

Fig. 5. Simulated temperature increase using a 0.6 MPa ME-FUS sequence in a homogeneous piece of skull. The graphic on the right represent a magnified view of the designated box on the left.

Fig. 6.
BBB opening at 500 KHz in the mouse hippocampus at the pressures indicated.

Fig. 7. Examples of passive cavitation detection at three different pressure levels during BBB opening in mice *in vivo*. The first row depicts the temporal backscattered signal recorded by the pulse-echo transducer. The second row represents the global frequency content of signal. The last row shows the evolution of the frequency content along time.

Fig. 8. Examples of histology results at the hippocampus obtained at three different pressure levels. At 0.2 MPa and 0.3 MPa, the microscopic slices exhibit no particular damage. Increasing the pressure to 0.6 MPa, red blood cells extravasations are clearly visible.

Table 1

Parameters used for the Bio-Heat equation.

Parameters	Values
ρ	$2200 \text{ kg} \cdot \text{m}^{-3}$
C_p	$1255 \text{ J} \cdot ^\circ\text{C} \cdot \text{kg}^{-3}$
k	$0.35 \text{ W} \cdot ^\circ\text{C} \cdot \text{m}^{-1}$
P_b	$1057 \text{ kg} \cdot \text{m}^{-3}$
C_b	$3600 \text{ J} \cdot ^\circ\text{C} \cdot \text{kg}^{-3}$
T_b	37°C
a	$34.5 \text{ Np} \cdot \text{m}^{-1} \cdot \text{MHz}^{-1}$
c	$3100 \text{ m} \cdot \text{s}^{-1}$
ω_b	0.008 s^{-1}

Table 2

Targeting coverage for the different anatomical aims through NHP and human skulls.

	%-of-target-overlapped	%-of-beam-overlapping-target
NHP hippocampus	42.1 $\pm$ 1.4	62.3 $\pm$ 2.4
NHP caudate	31.0 $\pm$ 0.9	45.1 $\pm$ 2.3
NHP putamen	21.8 $\pm$ 5.4	27.2 $\pm$ 6.7
Human hippocampus	12.3 $\pm$ 2.1	71.2 $\pm$ 1.7
Human caudate	13.8 $\pm$ 2.5	54.4 $\pm$ 3.0
Human putamen	16.6 $\pm$ 1.7	82.1 $\pm$ 2.8

Table 3

Inertial cavitation dose calculated for all the mice ($N = 10$) at three different pressure levels.

	ICD		
	0.2 MPa	0.3 MPa	0.6 MPa
Mean	0.36	13.06	75.96
STD	0.037	10.01	61.54