

# Noninvasive Blood-Brain Barrier Opening in Live Mice

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**Abstract.** Most therapeutic agents cannot be delivered to the brain because of brain's natural defense: the Blood-Brain Barrier (BBB). It has recently been shown that Focused Ultrasound (FUS) can produce reversible and localized BBB opening in the brain when applied in the presence of ultrasound contrast agents post-craniotomy in rabbits [1]. However, a major limitation of ultrasound in the brain is the strong phase aberration and attenuation of the skull bone, and, as a result, no study of trans-cranial ultrasound-targeted drug treatment in the brain *in vivo* has been reported as of yet. In this study, the feasibility of BBB opening in the hippocampus of wildtype mice using FUS through the intact skull and skin was investigated. In order to investigate the effect of the skull, simulations of ultrasound wave propagation (1.5 MHz) through the skull using  $\mu$ CT data, and needle hydrophone measurements through an ex-vivo skull were made. The pressure field showed minimal attenuation (18% of the pressure amplitude) and a well-focused pattern through the left and right halves of the parietal bone. In experiments *in vivo*, the brains of four mice were sonicated through intact skull and skin. Ultrasound sonications (burst length: 20 ms; duty cycle: 20%; acoustic pressure range: 2.0 to 2.7 MPa) was applied 5 times for 30 s per shot with a 30 s delay between shots. Prior to sonication, ultrasound contrast agents (Optison; 10  $\mu$ L) were injected intravenously. Contrast material enhanced high resolution MR Imaging (9.4 Tesla) was able to distinguish opening of large vessels in the region of the hippocampus. These results demonstrate the feasibility of locally opening the BBB in the mouse hippocampus using focused ultrasound through intact skull and skin. Future investigations will deal with optimization and reproducibility of the technique as well as application on Alzheimer's-model mice.

**Keywords:** Blood-Brain Barrier; Focused Ultrasound; Mice; Opening .

## INTRODUCTION

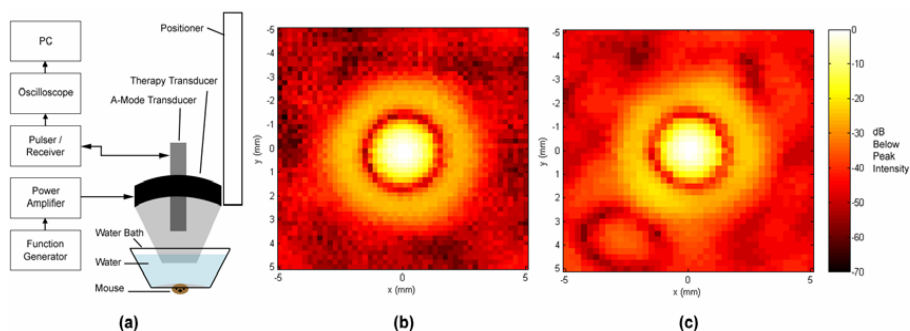
Many neurological disorders remain intractable to treatment by therapeutic agents due to the brain's natural defense: the Blood-Brain Barrier (BBB). By acting as a permeability barrier, the BBB impedes entry of virtually all molecules from blood capillaries to brain tissue, thus rendering many potent neurologically active substances and drugs ineffective simply because they cannot be delivered to where they are most needed [2]. Most of the current techniques under research do not offer transient, localized, and non-invasive targeting of a therapeutic agent [3]. The only truly transient, localized, and non-invasive opening of the BBB currently under study is with focused ultrasound (FUS). Hynynen et al. [1] showed that the BBB could be transiently opened in the presence of microbubbles and that the procedure could be monitored with MRI and MRI contrast agents. Mesiwala et al. studied the effects of

focused ultrasound on the rat's BBB at the absence of Optison [4]. Damage was seen throughout the sonicated region at every exposure tested where BBB opening was present. Certain areas within the sonicated region, however, revealed BBB opening with no visible damage. This showed the potential of opening the BBB without damaging the neurons. There is still, however, no complete understanding of the mechanism of the BBB opening and the role that the ultrasound contrast agents play. In addition to this, no study of ultrasound induced BBB opening where no neuronal damage was induced has been made.

The purpose of this paper is to lay the foundation for future studies on BBB opening in mice. Mice were chosen in particular, because many neurodegenerative disease models already exist for this animal. This paper proposes to determine whether FUS can be used to locally and transiently open the BBB in mice through intact skin and skull through the use of high resolution MRI that can provide detailed information on this phenomenon. Another purpose of this paper is to determine the feasibility of treating Alzheimer's disease with this technique by targeting the region it mainly effects, i.e., the hippocampus.

## MATERIALS & METHODS

Ultrasound waves were generated by a single-element, FUS transducer (center frequency: 1.525 MHz; focal depth: 90 mm; outer radius: 30 mm; inner radius 11.2 mm). The beam profile was obtained with a lateral full width at half maximum (FWHM) of 2 mm and an axial FWHM length of 20 mm. At the center of the FUS transducer a single-element diagnostic transducer (7.5 MHz) with a focal length of 60 mm was positioned so that the foci of the two transducers were properly aligned (Fig. 1(a)). A cone filled with degassed and distilled water was mounted on the transducer system. The water was contained in the cone by capping it with a polyurethane membrane. The FUS transducer was driven by an function generator (Agilent, Palo Alto, CA) through a 50 dB amplifier (ENI Inc., Rochester, NY) while the diagnostic transducer was driven by a pulser-receiver system (Panametrics, Waltham, MA) connected to a digitizer (Gage Applied Technologies, Inc., Lachine, QC, Canada).



**FIGURE 1.** (a) Experiment setup. Beam profile of the ultrasound beam in water at the focal region (b) at the absence of the mouse skull and (c) through the mouse skull.

The ultrasound transducers were mounted on a computer-controlled, three-dimensional positioning system (Velmex, Inc., Bloomfield, NY). Degassed distilled water in a water bath was held below the FUS transducer (Fig. 1(a)). The mouse was laid prone beneath the water bath with ultrasound gel used to eliminate the impedance mismatch between the two surfaces.

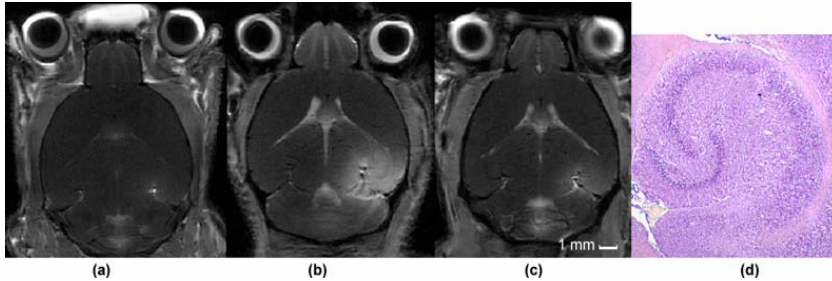
To determine whether ultrasound could be transcranially delivered using a single element focused transducer, wave propagation through the skull was simulated. A  $\mu$ CT scan (SCANCO Medical, Basserdorf, Switzerland) with a resolution of 10  $\mu$ m was performed on an excised mouse skull and provided a mapping of the porosity. From the porosity map, the maps for density, speed of sound, and the attenuation were calculated using the methods similar to those previously reported [5, 6]. In these studies, there was no significant distortion to the lateral FWHM (without skull mappings: 1.494 mm; with skull mappings: 1.551 mm), axial FWHM, beam shape, and focus location.

A two-dimensional lateral beam profile at the focus of the ultrasound beam without and with the skull were measured using a needle hydrophone (Precision Acoustics Ltd., Dorchester, Dorset, UK) (Fig. 1(b), (c)). Ultrasound through different regions of the skull were measured, however ultrasound through the parietal bones of the mouse skull on the left and right halves of the sagittal suture provided the least amount of attenuation ( $\sim$ 18% of the pressure amplitude) and minimal distortion to the beam shape and focus location when compared to other regions of the skull (i.e., the interparietal bone).

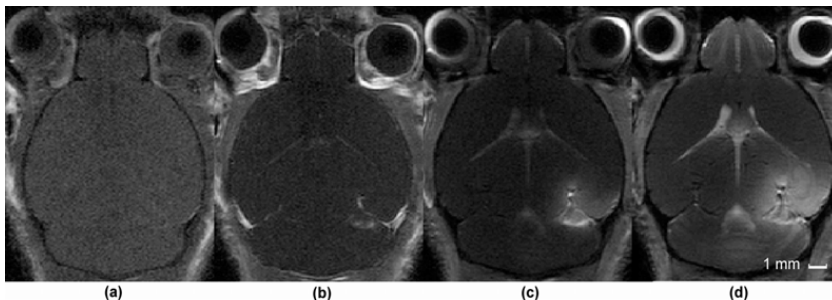
Four brown mice (Charles River Laboratories, Wilmington, MA; mass: 23 to 28 g) were anesthetized during ultrasound exposure with a mixture of ketamine (Fort Dodge Animal Health, Fort Dodge, Iowa) and xylazine (Ben Venue Laboratories, Bedford, Ohio). Before MRI scanning, the mice were switched to administration of isoflurane. During the whole scanning procedure, the mice's vital signs were monitored. All animal procedures were approved by the Columbia University Institutional Animal Care and Use Committee.

The focus of the transducer was positioned in the mouse brain using a grid positioning system. A thin metal grid was placed along the sagittal suture of the mouse skull, which could be seen through the intact skin [7]. A two-dimensional raster-scan of the grid using the diagnostic transducer was then made. The location of the hippocampus was found relative to this grid. The distance from the skull was then measured using the diagnostic transducer and the focus was placed 3 mm beneath the top of the skull, where the hippocampus was located. Using the grid positioning system and depth calculations, precise targeting of the hippocampus of the mouse brain was performed.

Pulsed wave FUS (burst rate: 10 Hz; burst length: 20 ms; duty cycle: 20%; acoustic pressures: 2.0, 2.5, and 2.7 MPa) was applied in a series of five sonications lasting 30 s each with a 30 s delay between sonications. Approximately 15 minutes prior to sonication, a bolus of 10  $\mu$ L (approximately 0.4 mL/kg ) of ultrasound contrast agent (Optison; Mallinckrodt Inc., St Louis, MO) was injected in the right femoral vein.



**FIGURE 2.** MRI and histology slides of the BBB opening. The T1 MR images were of different mouse brains after sonication and 100 min. after MRI contrast injection. Each mouse was exposed to different pressure amplitudes: (a) 2.5 MPa, and (b) 2.7 MPa, all with Optison, and (c) 2.7 MPa with no Optison. Ultrasound was focused at the hippocampus of the right side of the MR images. (c) Contrast enhancement of the hippocampus. (d) Histology image of the hippocampus at 2.7 MPa without Optison indicating no structural damage.



**FIGURE 3.** T1 MR images of a single mouse brain after sonication with a pressure amplitude of 2.7 MPa. Ultrasound was focused at the hippocampus of the right side of the MR images. The images were obtained (a) before, (b) 10 min. after, (c) 45 min. after, and (d) 100 min. after MRI contrast agent injection.

MR images were obtained with a 9.4 T system (Bruker Medical; Boston, MA). 15 minutes after sonication a T1-weighted MR Image of the mouse brain was obtained (field of view: 1.92 x 1.92 cm; matrix size: 256 x 256; slice thickness: 0.6 mm; interslice thickness: 0.70 mm). Following this, 0.5 mL of MRI contrast agents (Omniscan; Amersham Health, AS Oslo, Norway) was administered to depict BBB opening. A series of three sets of T1 and T2 images were then obtained.

## RESULTS

The MRI images were used to determine the opening of the BBB. Leakage of MRI contrast agents due to BBB disruption resulted in an increase of the pixel intensity on the T1 MR images [1]. Using this method, sonications through the mouse skull did not produce focal BBB opening near the hippocampus at pressure amplitudes below 2.0 MPa. At 2.5 MPa, there was contrast enhancement near the main blood vessel (Fig. 2(a)), and at 2.7 MPa, there was a large area of contrast enhancement indicating gadolinium permeation throughout the entire hippocampus region. Comparing

sonication with and without Optison, it was seen that Optison enhances the BBB opening (Fig. 2(b)-(c)). Initial histological studies have indicated that there was no structural damage at 2.7 MPa with no Optison (Fig. 2(d)). The slow absorption of gadolinium by the bloodstream due to its injection into the intraperitoneal cavity [8] allowed for a temporal analysis of gadolinium distribution in the brain (Fig. 3). The gadolinium is at first highly concentrated in the main vessels (Fig. 3(b)), but slowly permeates the surrounding area (Fig. 3(c)) until the entire hippocampus (Fig. 3 (d)) has been reached approximately two hours post-injection.

## CONCLUSION AND DISCUSSION

The technology and methods described here demonstrate the feasibility of transcranial, localized, non-invasive, targeted drug delivery in the brain of mice. Future studies will continue on developing safe procedures of BBB opening in mice to allow for a high survivability and long term studies. Optimization of the ultrasound parameters will be utilized in order to induce BBB opening with no or minimal (and transient) brain damage. Extensive histological studies of the potential neuronal and vascular damage will also determine the dependence of the opening on the vessel size and location. A long-term temporal analysis will be made to determine if and how long the BBB in mice stays open. Finally, the techniques described may eventually be used to test for molecular delivery to the hippocampus of Alzheimer's disease and other neurodegenerative diseases in mice.

## ACKNOWLEDGMENTS

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