

# Feasibility of Transcranial, Localized Drug-delivery in the Brain of Alzheimer's-model Mice Using Focused Ultrasound

James J. Choi<sup>1</sup>, Mathieu Pernot<sup>1</sup>, Scott A. Small<sup>3</sup>, and Elisa E. Konofagou<sup>1,2</sup>

<sup>1</sup>Department of Biomedical Engineering, <sup>2</sup>Department of Radiology, <sup>3</sup>Department of Neurology  
Columbia University, New York, NY, U.S.A., 10027

**Abstract**— Therapeutic agents are difficult to deliver to the brain because of brain's natural defense: the Blood-Brain Barrier (BBB). It has been shown that Focused Ultrasound can produce reversible and localized BBB opening in the brain when applied in the presence of ultrasound contrast agents [1]. However, a major limitation of ultrasound in the brain is the strong phase aberration and attenuation of the skull bone. Thus, despite the high potential of non-invasive adaptive focusing techniques, no study of trans-cranial ultrasound-targeted drug treatment in the brain has been reported as of yet. In this study, the feasibility of BBB opening in the hippocampus of Alzheimer's-model mice using focused ultrasound through the intact skull and skin was investigated. A high power focused transducer (1.5 MHz central frequency) was mounted on a 3D positioning system and a 7.5 MHz single element diagnostic transducer was placed through the center of the focused transducer and aligned with the high power beam in order to achieve high precision targeting. In order to investigate the effect of the skull, simulations of ultrasound wave propagation 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 was attenuated) and a well-focused pattern through the left and right halves of the parietal bone. In in-vivo experiments, the brains of four mice were sonicated through intact skull and skin. Ultrasound sonications were set to a burst length of 20 ms at a 20% duty cycle and was applied 5 times for 30 s per shot with a 30 s delay between shots. The acoustic pressure ranged from 2.0 to 2.7 MPa. Prior to sonication, ultrasonographic contrast agents (Optison; 0.05 mL) were injected in the mice intravenously. Contrast-enhanced high resolution T1 and T2-weighted MR Imaging (9.4 Tesla) with an in-plane resolution of 75  $\mu$ m was able to distinguish opening of large vessels in the region of the hippocampus. These results demonstrate the feasibility of potentially, locally opening the BBB in the mouse hippocampus using focused ultrasound through the intact skull and skin. Future investigations will deal with optimization and repeatability of the technique as well as feasibility on Alzheimer's-affected mice.

**Keywords:** Ultrasound; Focused Ultrasound; HIFU; Blood-Brain Barrier.

## I. 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]. As a result, traversing the BBB is the rate-limiting factor in brain drug delivery development [3].

The BBB is a specialized vascular system consisting of endothelial cells connected together by tight junctions [2]. The luminal and abluminal membranes line the inner wall of the vessel and act as the permeability barrier. The combination of tight junctions and the two membranes characterizes the BBB with low permeability to lipophilic, large, and ionic substances. Certain molecules such as glucose and amino acids, however, act as exceptions by being actively transported.

A successful drug delivery system requires transient, localized, and non-invasive targeting of a therapeutic agent. Many of the current techniques currently under research do not offer all these attributes simultaneously. Several pharmaceutical companies use the technique known as "lipidization," which is the addition of lipid groups to the polar ends of molecules to increase the permeability of the agent [3]. The main drawback with this technique is that it increases the permeability of the drug not only in the targeted region but also the entire brain and body. As a result, there is a limit to how much a person can intake before the side effects become rampant. A second technique under study is neurosurgical-based drug delivery methods, which involve the invasive plantation of drugs into a region by a needle [3]. Through the use of diffusion, the drug can be localized to the targeted region. Diffusion, however, reduces by 90% in only 0.5 mm from the initial location. In addition to this, invasive procedures require traversing unharmed brain tissue causing potentially further damage. Other techniques include the use of drugs mixed with a solvent and drugs with adjuvants attached to disrupt the BBB [3]. The disruption with these techniques is through dilation, contraction, and other methods. The main concern, however, is that disruption of the BBB is non-localized in the brain and that the solvent and adjuvants are potentially toxic. There is also the delivery of drugs through endogenous transporters, which may involve intense medical chemistry to modify the drugs as well as complete understanding of the structure and functionality of these endogenous transporters [3]. This technique may provide a delivery technique specific to the brain, which is non-toxic, but requires special attention to each individual molecule and is still not localized to a targeted region. The only truly transient, local, and non-invasive opening of the BBB under study right now is with focused ultrasound (FUS).

The opening of the BBB using ultrasound was first observed by Vykhodtseva et al. while investigating the effects of FUS on the rabbit brain [4]. Hynynen et al., further studied this phenomenon and showed that the BBB could be transiently opened in the presence of microbubbles and that the procedure could be monitored with MRI and MR contrast agents [1]. Mesiwala et al. studied the effects of focused ultrasound on the

rat BBB in the absence of Optison [5]. 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. Hynynen et al., further investigated this phenomenon with and without Optison to search for a threshold of BBB opening and neuronal damage and understand the mechanism of opening [6-9]. These mechanisms included transcytosis, transendothelial openings, widening of interendothelial clefts and opening of the tight junctions, and the free passage through the injured endothelial lining. There have, however, been no study of ultrasound induced BBB opening where no neuronal damage was induced. In addition to this, there is still no complete understanding of the mechanism of the BBB opening.

The purpose of this paper is to determine whether FUS can be used to locally open the BBB in mice through intact skin and skull and that high resolution MRI (9.4 Tesla) can provide detailed information on this phenomenon.

## II. MATERIAL & METHODS

### A. Animals

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; 75 mg per kg of body mass) and xylazine (Ben Venue Laboratories, Bedford, Ohio; 3.75 mg per kg of body mass). Before MRI scanning, the mice were switched to administration of isoflurane. During the whole scanning procedure, the mouse's vital signs were monitored. All animal procedures were approved by the Columbia University Institutional Animal Care and Use Committee.

### B. Ultrasound Equipment

Ultrasound was generated by a single-element focused 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 half intensity peak diameter of 2 mm and a beam length of 20 mm. Through the a small hole in the FUS transducer was placed an A-mode 7.5 MHz transducer with a focal length of 60 mm. The face of the A-mode transducer was extended 30 mm away from the surface of the therapeutic transducer without significantly distorting the FUS beam (Fig. 1.a). A removable cone was mounted on the transducer system and was filled with distilled water that was degassed with a vacuum-based system. The water was contained in the cone by capping it with a polyurethane membrane. The FUS transducer was driven by an Agilent function generator through a 50 dB amplifier (ENI Inc., Rochester, NY) while the A-mode transducer was driven by a pulser-receiver system (Panametrics, Waltham, MA) connected to a digitizer (Gage Applied Technologies, Inc., Lachine, QC, Canada).

The ultrasound system was mounted on a 3-Dimensional Velmex positioning system (Velmex, Inc., Bloomfield, NY) controlled via a PC. Distilled degassed water in a water bath was held below the FUS transducer (Fig. 1(a)). The mouse was

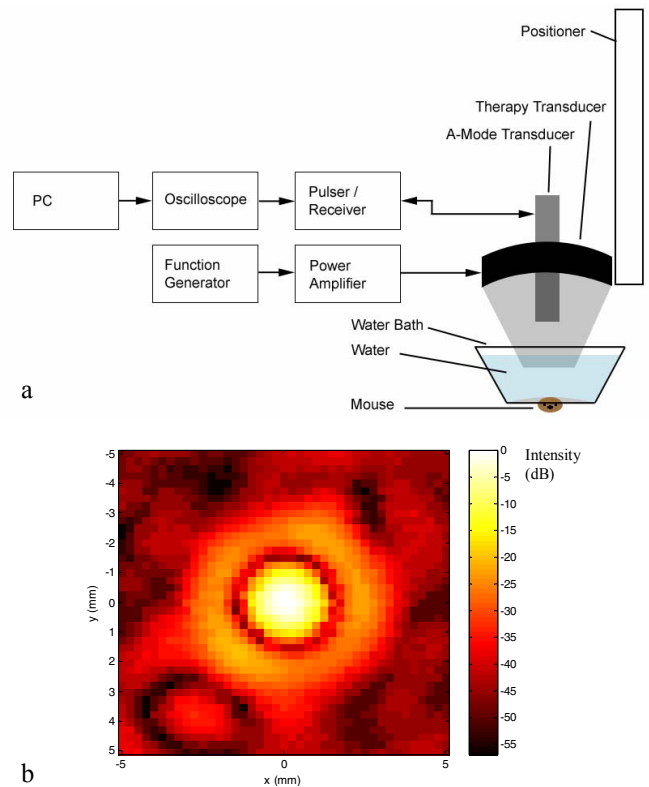


Figure 1. (a) Experimental Setup. (b) Beam profile of the ultrasound beam through the left parietal bone. The values shown are in decibels below the peak intensity.

laid prone beneath the water bath with ultrasound gel used to provide an interface between the two surfaces.

### C. Trans-Cranial Ultrasound

To determine whether ultrasound could be transcranially delivered, wave propagation through the skull was first simulated. A  $\mu$ CT scan with a resolution of 10  $\mu$ m was performed on an excised mouse skull and provided a mapping of the porosity, density, speed of sound, and attenuation. The methods of producing these maps were similar to those previously reported [11, 12]. In these studies, no significant distortion to the half-peak beam width (without skull mappings: 1.43 mm; with skull mappings: 1.48 mm), length, or shape were seen.

Hydrophone measurements of the ultrasound beam through the skull were made (Fig. 1(b)) revealing a well-formed beam. Ultrasound through the parietal bones of the mouse on the left and right halves of the sagittal sutures provided the least amount of attenuation when compared to other regions of the skull. An attenuation of 18% of the pressure field was measured with minimal distortion to the beam shape.

### D. Sonications

The focus of the transducer was positioned in the mouse brain using a grid positioning system. In this method, it was noted that the sutures of the mouse skull were seen through the skin of the mouse and could be used as anatomical landmarks

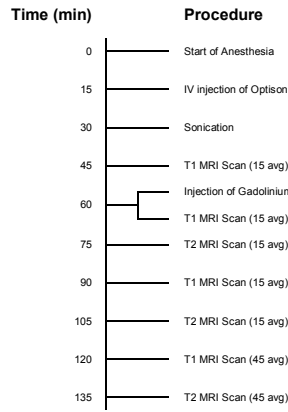


Figure 2. Timeline of the experiment.

for targeting purposes. A thin metal grid was then placed in alignment with these landmarks and a 2-dimensional scan using the A-mode transducer was made. The location of the hippocampus could then be found relative to this grid. The distance from the skull was then measured using the A-mode transducer and the focus was placed 3 mm beneath the top of the skull. Using the grid positioning method and depth calculations, precise targeting of the hippocampus of the mouse brain was performed.

Pulsed wave FUS (burst rate: 10 Hz; burst count: 30400; duty cycle: 20%) was applied in a series of five sonications lasting 30 s each with a 30 s delay between sonications. The tested acoustic pressure amplitudes were 2.0, 2.5, and 2.7 MPa.

Approximately 15 minutes prior to the start of each sonication, 10  $\mu$ L (approximately 0.4 mL/kg ) of ultrasound contrast agents (Optison; Mallinckrodt Inc., St Louis, MO) that contained microbubbles (mean diameter: 3.0-4.5  $\mu$ m; concentration: 5.0-8.0  $\times 10^8$  bubbles per mL) was injected in the right femoral vein (Fig. 2).

### E. Magnetic Resonance Imaging

MR Images were obtained with a 9.4 T system (Bruker Medical; Boston, MA). The mouse was placed prone on a plastic tub, which was inserted into a 3.8 cm diameter birdcage coil. 15 minutes after sonication, but before gadolinium injection, a T1-weighted MR Image 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). Once the scan was finished 0.5 mL of MR contrast agents (Omniscan; Amersham Health, AS Oslo, Norway) was administered to depict BBB opening. After injection of this contrast agent, a series of six alternating T1-weighted and T2-weighted images were then obtained (Fig. 2).

### F. MR Image Analysis

The area of the contrast enhanced region was calculated by applying a threshold relative to the non-sonicated (unenhanced) region. This provided a clear differentiation between unaffected and contrast enhanced regions. The approximate area of the contrast enhanced region was then calculated.

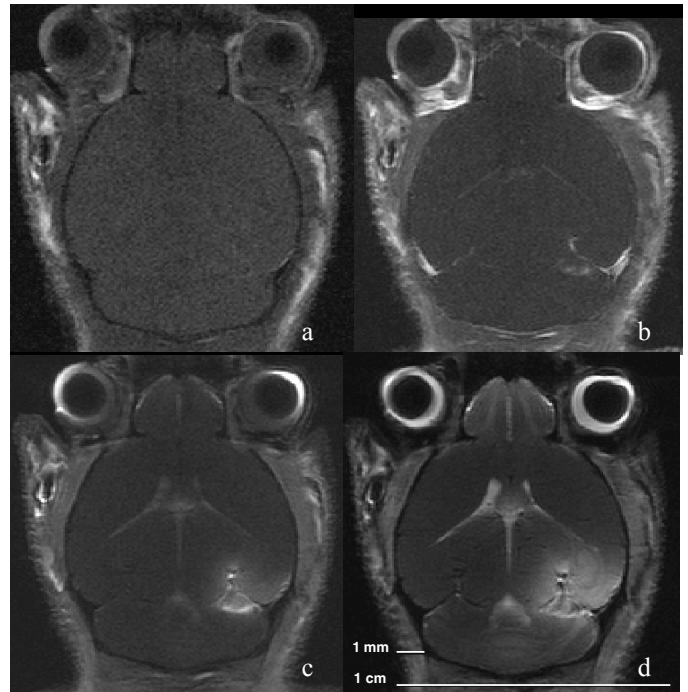


Figure 3. T1 MR images of the mouse brain after sonication with a pressure amplitude of 2.7 MPa. Ultrasound was focused at the hippocampus of the right side of the MR images. The left side of the MR images were not targeted and acted as a control. (a) Before gadolinium injection, there was no visible difference between the left and right side of the MR images. (b) Immediately after injection, however, gadolinium began to leak through the main arteries near the hippocampus. (c) 30 minutes after injection, permeation throughout the region increased until (d) 1 hour after where gadolinium permeated the entire hippocampus region.

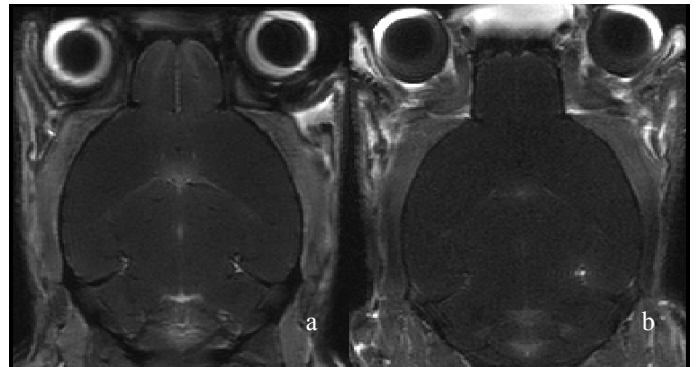


Figure 4. T1 MR images (45 averages) of the mouse brain 1 hour after sonication and MR contrast injection with a pressure amplitude of (a) 2.0 MPa and (b) 2.5 MPa. Ultrasound was focused at the hippocampus of the right side of the MR images. The left side of the MR images were not targeted and acted as a control. (a) No contrast enhancement was visible on the MR image at 2.0 MPa. (b) The contrast enhancement at 2.5 MPa, however, was high.

## III. RESULTS

MRI was used to determine the opening of the BBB. Leakage of MR 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 produced focal BBB opening near the hippocampus at pressure

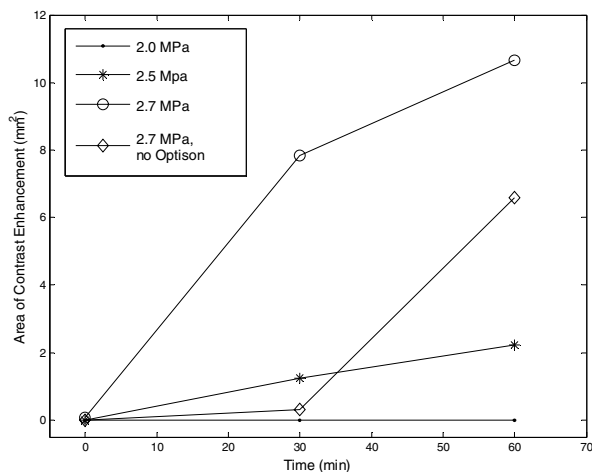


Figure 5. Area of contrast enhancement versus time for different pressure amplitudes.

amplitudes greater than 2.0 MPa (Fig. 3 and 4). The temporal nature of BBB opening around the hippocampal region can clearly be seen in Fig. 3. The gadolinium is at first highly concentrated in the main arteries but slowly permeates the entire hippocampus. At a lower intensity of 2.5 MPa, there is a smaller amount of contrast enhancement, and at 2.0 MPa, there is no contrast enhancement, possibly indicating no BBB opening (Fig. 4). The area of contrast enhancement decreased with decreasing ultrasound intensity while increasing with time at the highest pressure amplitudes (Fig. 5).

#### IV. DISCUSSION

This study demonstrates for the first time, to our knowledge, opening of the BBB through the intact skull and skin in mice without any phase aberration corrections at the transducer level. There was minimal attenuation and distortion of the beam resulting in a predictable and consistent ultrasound beam through the parietal bone of the skull. Precise targeting of the focused ultrasound was accomplished with an A-mode transducer and a grid positioning method that utilized the sutures of the skull as anatomical landmarks. Mice were chosen for our study mainly because there are many neurological models already available for it. This method of trans-cranial ultrasound provides an easy method for delivering ultrasound through the skull for future studies on the effects of opening the BBB with ultrasound as well as other ultrasound studies on the brain. This paper dealt with, but was not limited to, Alzheimer's-model mice and achieved its goal by opening the BBB in the hippocampus.

In addition to the previous findings, this paper wished to investigate the BBB opening using high-resolution MR imaging (9.4 T) and to determine whether detailed information on the BBB opening could be studied. Figure 3(c) provides an example of the detail possible with high-resolution MR imaging. With this magnet, more detailed analysis of the area enhanced by the MRI could be made including measurement of the area being affected by the BBB opening (Fig. 5). Also, the vessel density and size seem to play a significant role in the way drugs are spread through the BBB.

#### V. CONCLUSION

The technology and methods described here show the feasibility of trans-cranial, localized, non-invasive, targeted drug delivery in the brain of mice. Future studies will be concerned with optimizing the ultrasound parameters to induce BBB opening with no or minimal damage. Histological studies of the possible neuronal damage will also be assessed as well as the affects of the importance of 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-affected mice.

#### ACKNOWLEDGMENT

This study was supported by a Special Development Award from the Whitaker Foundation and the Defense Advanced Research Projects Agency. The wave propagation simulation software was provided by Dr. Michael Tanter of the Laboratoire Ondes et Acoustique, Paris, France.

#### REFERENCES

- [1] K. Hynynen, N. McDannold, N. Vykhodtseva, F. A. Jolesz, "Noninvasive MR imaging-guided focal opening of the blood-brain barrier in rabbits," *Radiology*, vol. 220, pp. 640-646, 2001.
- [2] L. L. Rubin, J. M. Staddon, "The cell biology of the blood-brain barrier," *Annu Rev Neurosci*, vol. 22, pp. 11-28, 1999.
- [3] W. M. Pardridge, "The blood-brain barrier: bottleneck in brain drug development," *NeuroRx*, vol. 2(1), pp. 3-14, 2005.
- [4] N. I. Vykhodtseva, K. Hynynen, C. Damianou, "Histologic effects of high intensity pulsed ultrasound exposure with subharmonic emission in rabbit brain in vivo," *Ultrasound Med Biol*, vol. 21, pp. 969-979, 1995.
- [5] A. H. Mesiwala, P. D. Mourad, "Monitoring of biologic effects of focused ultrasound beams on the brain," *Radiology*, vol. 224(1), pp. 294-297, author reply pp. 296-297, 2002.
- [6] A. H. Mesiwala, L. Farrell, H. J. Wenzel, et al, "High-intensity focused ultrasound selectively disrupts the blood-brain barrier in vivo," *Ultrasound Med Biol*, vol. 28, pp. 389-400, 2002.
- [7] K. Hynynen, N. McDannold, H. Martin, F. Jolesz, N. Vykhodtseva, "The threshold for brain damage in rabbits induced by bursts of ultrasound in the presence of an ultrasound contrast agent (Optison)," *Ultrasound Med Biol*, vol. 29, pp. 473-481, 2003.
- [8] N. McDannold, N. Vykhodtseva, F. A. Jolesz, K. Hynynen, "MRI investigation of the threshold for thermally induced blood-brain barrier disruption and brain tissue damage in the rabbit brain," *Magn Reson Med*, vol. 51(5), pp. 913-923, 2004.
- [9] N. Sheikov, N. McDannold, N. Vykhodtseva, F. Jolesz, K. Hynynen, "Cellular mechanisms of the blood-brain barrier opening induced by ultrasound in presence of microbubbles," *Ultrasound Med Biol*, vol. 30(7), pp. 979-989, 2004.
- [10] K. Hynynen, N. McDannold, N. A. Sheikov, F. A. Jolesz, N. Vykhodtseva, "Local and reversible blood-brain barrier disruption by noninvasive focused ultrasound at frequencies suitable for trans-skull sonications," *Neuroimage*, vol. 24(1), pp. 12-20, 2005.
- [11] C. Connor, K. Hynynen, "Patterns of Thermal Deposition in the Skull During Transcranial Focused Ultrasound Surgery," *IEEE Trans Biomed Eng*, vol. 51(10), pp. 1693-1706, 2004.
- [12] G.T. Clement, K. Hynynen, "A non-invasive method for focusing ultrasound through the human skull," *Phys Med Bio*, vol. 47(8), pp. 1219-1236, 2002.