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Preclinical evaluation of a low-frequency transcranial MRI-guided focused ultrasound system in a primate model

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Abstract

This study investigated thermal ablation and skull-induced heating with a 230 kHz transcranial MRI-guided focused ultrasound (TcMRgFUS) system in nonhuman primates. We evaluated real-time acoustic feedback and aimed to understand whether cavitation contributed to the heating and the lesion formation. In four macaques, we sonicated thalamic targets at acoustic powers of 34–560 W (896–7590 J). Tissue effects evaluated with MRI and histology were compared to MRI-based temperature and thermal dose measurements, acoustic emissions recorded during the experiments, and acoustic and thermal simulations. Peak temperatures ranged from 46–57°C, and lesions were produced in 5/8 sonicated targets. A linear relationship was observed between the applied acoustic energy and both the focal and brain surface heating. Thermal dose thresholds were 15–50 cumulative equivalent minutes at 43°C, similar to prior studies at higher frequencies. Histology was also consistent with earlier studies of thermal effects in the brain. The system successfully controlled the power level and maintained a low level of cavitation activity. Increased acoustic emissions observed in 3/4 animals occurred when the focal temperature rise exceeded approximately 16°C. Thresholds for thermally-significant subharmonic and wideband emissions were 129 and 140W, respectively, corresponding to estimated pressure amplitudes of 2.1 and 2.2 MPa. Simulated focal heating was consistent with the measurements for sonications without thermally-significant acoustic emissions; otherwise it was consistently lower than the measurements. Overall, these results suggest that the lesions were produced by thermal mechanisms. The detected acoustic emissions, however, and their association with heating suggest that cavitation might have contributed to the focal heating. Compared to earlier work with a 670 kHz TcMRgFUS system, the brain surface heating was substantially reduced and the focal heating was higher with this 230 kHz system, suggesting that a reduced frequency can increase the treatment envelope for TcMRgFUS and potentially reduce the risk of skull heating.

Introduction

Thermal ablation using transcranial MRI-guided focused ultrasound (TcMRgFUS) is a rapidly advancing less-invasive alternative to surgical resection and radiosurgery that is in clinical trials for the treatment of brain tumors (1,2) and for functional neurosurgery applications (3–7). The currently-available TcMRgFUS device focuses a high-intensity 650-670 kHz ultrasound wave through the intact skull into the brain using a large (30 cm diameter) hemispherical, 1024-element phased array transducer that is integrated with a 1.5T or 3T MRI scanner. The large transducer is used to distribute the acoustic energy over a large surface area to avoid overheating the skull (8,9), which strongly absorbs ultrasound energy and thus rapidly heats. The phased array can electronically steer the focal point away from its geometric focus and is used to correct for skull-induced aberrations (10–12). These phase and amplitude corrections are calculated using information gathered from CT scans (13,14). MRI and MR temperature imaging (MRTI) (15) are used to plan, monitor, and evaluate the procedure.

Since acoustic absorption increases with ultrasonic frequency, a relatively low frequency is used to allow for effective transmission through the skull. Simulations and experiments over a range of 500 kHz to 1.5 MHz suggested that a frequency of approximately 700 kHz is optimal for focusing through the skull while minimizing energy deposition in the bone (8,9). With these factors, along with cooling of the scalp throughout the procedure, heating sufficient to create small lesions within the brain is possible. However, at this frequency the treatment envelope of the device – the regions that can be safely targeted – is relatively small and restricted to central brain areas. This restriction is largely due to skull heating. As the focal region moves away from centrally-located targets, the angles between many of the transducer elements and the bone become oblique, which leads to decreased acoustic transmission in those areas (16). In such cases, the effective area of the skull where the energy is distributed is reduced. Furthermore, clinical experience has shown that sufficient focal temperatures cannot always be achieved even in thalamic targets (17), locations that are nearly ideal for this device.

Skull heating can be further reduced using an even lower frequency. A lower frequency also markedly reduces skull-induced aberrations (18) and enables a larger range where the focal region can be effectively steered compared to a similar higher-frequency transducer. However, lowering the acoustic frequency also reduces absorption (and thus heating) at the focus. In addition, since the threshold for acoustical cavitation, the “nucleation” of microbubbles and their subsequent interaction with the ultrasound field, decreases when the ultrasound frequency is reduced, the risk of uncontrolled cavitation activity needs to be considered. When microbubbles are present, their interaction with the acoustic field results in nonlinear scattering of the field and increased heating (19).

While some microbubble activity may be desirable to improve the focal heating (19), it should be kept to a low level. Over time, as the acoustic wave reflects and scatters from the bubbles in the focal region, additional bubbles are nucleated along the beam path. Boiling can also rapidly occur. These effects can lead the focal heating to shift towards the transducer in an unpredictable manner (20). Furthermore, the microbubbles can increase in

size and violently collapse, a process termed inertial cavitation, and result in undesired mechanical tissue damage and microhemorrhage (21). Cavitation is further complicated since it has a threshold that varies in different tissues and an onset that is unpredictable and rapid. These complications are exacerbated with transcranial sonication by the challenges of predicting the acoustic pressure at the focal point. Recording the acoustic emissions using a passive detector (hydrophone) can detect the presence of cavitation activity through the production of large acoustic signals at fractions ($1/2$, $3/2$, etc.) and harmonics ($2\times$, $3\times$, etc.) of the acoustic frequency and with broadband signals that occur when the microbubbles collapse (22). By monitoring these emissions, one may be able to rapidly control the output of the TcMRgFUS device to minimize or optimize the cavitation activity produced during sonication in real time (23,24).

A low frequency clinical prototype TcMRgFUS device operating at 220–230 kHz with such control has been developed. The device has been evaluated with simulations (25–27), experiments in tissue-mimicking phantoms and ex vivo human skulls (27,28), and tests *in vivo* in pigs after removing the outer skull bone (29). While such studies are invaluable, they are inadequate for determining the safety and effectiveness of the device in a large brain with an intact skull. These studies have also not tested the closed-loop cavitation control that has been integrated into the device.

To begin to answer these questions, we evaluated thermal ablation with a 230 kHz TcMRgFUS system in a large animal model. Thalamic targets were sonicated in rhesus macaques at different exposure levels while obtaining MRTI and recording the acoustic emissions. Temperature and acoustic data obtained during the sonications, post-FUS MRI, and histological data were examined to determine whether the induced tissue effects were consistent with thermal effects, or if cavitation played a significant role. The pressure field and heating in the brain was simulated and compared to the experiments. Real-time control based on passive acoustic emission monitoring was evaluated to ensure that excessive cavitation activity was prevented, even when very high exposure levels were used. Finally, the thermal data acquired here was compared to a prior study in macaques (30) with the 670 kHz version of this FUS system that is currently being evaluated in clinical trials for brain tumors (1,2) and functional neurosurgery applications (3–7) and that was recently approved by the United States Food and Drug Administration for the treatment of essential tremor. We anticipate that if the focal heating is improved and the skull-induced heating is reduced compared to the previous primate study, it would suggest that this device can indeed increase the treatment envelope for TcMRgFUS thermal ablation. Such an improvement could increase the number of patients that can be treated with this technology and enable treatments at brain regions that are currently not targetable with TcMRgFUS.

Methods

Animals

All experiments were performed in accordance with procedures approved by the Harvard Medical School Institutional Animal Care and Use Committee. Tests were done in four adult rhesus macaques. Each animal was anesthetized with ketamine (15 mg/kg/h i.m.) and xylazine (0.5 mg/kg/h i.m.) or with 4 mg/kg/hr ketamine and Dexmedetomidine (0.01–0.02

mg/kg/h) and intubated. The head was shaved, and a catheter was placed in a leg vein. During the procedure the heart rate, blood oxygenation levels, and rectal temperature were monitored. Body temperature was maintained with a heated water blanket.

A thalamic target in each hemisphere was sonicated as described below. In Monkey 1 (male, 13.7 kg) and Monkey 3 (female, 13 kg), the two targets were sonicated over two sessions a week apart. In Monkey 2 (female, 9.5 kg), both targets were sonicated in a single session. Monkey 4 (male, 14 kg) was sonicated in three weekly sessions, with a location in one hemisphere sonicated twice. Monkey 1 was not euthanized. Monkeys 2, 3 and 4 were sacrificed 2, 12, and 48 hours after the last session, respectively. They were anesthetized with ketamine (15mg/kg i.m.) and then euthanized with an overdose of pentothal (100mg/kg). They were then perfused transcardially with 1 L 0.9% NaCl, followed by 2 L 10% buffered formalin phosphate. The brains were removed and placed in 10% buffered formalin phosphate. The brains were photographed and cut into blocks. Blocks containing the thalamus and areas on the brain surface were paraffinized and sectioned at 5 μ m. They were stained with Hematoxylin and Eosin (H&E) or H&E and Luxol Fast Blue (H&E-LFB). The thalamic targets and brain surface were examined to determine whether the tissue effects were consistent with thermal damage, or if there was evidence of cavitation-induced hemorrhage.

Device

The device tested was the ExAblate 4000 low-frequency TcMRgFUS system (InSightec). It consists of a 30 cm diameter hemispherical 1024-element phased array transducer operating at 230 kHz coupled with a 1024-channel driving system, a treatment planning workstation, and a water system that cools, circulates, and constantly degasses the water inside the transducer. The water temperature was maintained at 14–15°C, and the dissolved oxygen concentration was less than 1 ppm. The driving system allows for individual control of the phase and amplitude for each phased array element in order to steer the focal point to different targets.

The focal half-intensity width and length produced by the transducer in water were provided by the manufacturer and were 3.0 and 5.8 mm, respectively. Previous calibration of the system with a calibrated hydrophone and an ex vivo macaque skull (31) estimated that 1 W of acoustic power corresponds to a peak negative pressure amplitude of 223 kPa. The system was integrated with a clinical 3T MRI unit (GE Healthcare). Imaging was performed with a 14 cm diameter receive-only surface coil (constructed in-house). For clinical use, the hemisphere transducer is mounted on its side and coupled to a patient's head via a water-tight membrane. To remove the need for this membrane and to simplify the experiments, the transducer was rotated 90° so that it could be simply filled with water like a bowl. The animal was placed supine on the table with its head tilted backwards so that the top of the head was submerged in the water. Figure 1 shows the experimental setup.

The TcMRgFUS device corrects skull-induced aberrations using acoustic modeling and geometric and density information gathered from CT scans. CT scans (Ceretom, Danvers, MA, USA) were obtained of each animal several days before the experiments. The images were reconstructed with a voxel volume of $0.49 \times 0.49 \times 0.65$ mm using a bone kernel.

Before the sonications, the CT were registered to the treatment planning MRI using the ExAblate software. The position of the transducer within the MRI frame of reference was automatically obtained using MRI tracking coils integrated into the transducer housing.

MRI

A fast spoiled gradient echo sequence was used for MRTI (15) in a single plane (repetition time (TR): 29 ms; echo time (TE): 13 ms; flip angle (FA): 30°, bandwidth (BW) ± 5.7 kHz; matrix: 256×128; voxel dimensions: 1.1 × 2.2 × 3.0 mm). After sonication, images were obtained with a T2-weighted fast spin echo sequence (TR/TE: 4500/85 ms; echo train length (ETL): 8; BW: ± 15.6 kHz; averages: 2; matrix: 256×256; FOV: 12 cm; voxel dimension: 0.5×0.5×3 mm), a T1-weighted fast spin echo sequence (TR/TE: 500/13 ms; ETL: 4; BW: ± 15.6 kHz; averages: 4; matrix: 256×256; FOV: 12 cm; voxel dimension: 0.5×0.5×3 mm), and a three-dimensional T2*-weighted spoiled gradient echo sequence (TR/TE: 33/19 ms; FA: 15°; BW: ± 15.6 kHz; averages: 1; matrix: 256×256×36; FOV: 12 cm; voxel dimension: 0.5×0.5×1 mm). Axial T1-weighted FSE images were acquired before and after an injection of Gd-DTPA (Magnevist, Berlex Laboratories) at a concentration of 0.2 mmol/kg to detect blood-brain barrier breakdown. The brain from Monkey 4 was imaged 24h *post mortem* using a 7T MRI (Biospec, Bruker) and an 86 mm volume coil. T2-weighted RARE images (TR/TE: 3500/51.8 ms; ETL: 14; BW ± 50 kHz; averages: 6; matrix: 256×300; FOV: 8 cm; voxel dimension: 0.3×0.3×2mm) were acquired.

Sonications

After treatment planning imaging and registration of the MRI and CT scans, a target in the thalamus was selected. To localize the focal point, short (10–15s), continuous-wave sonications were applied at acoustic powers of 40–50W while obtaining MRTI. These test sonications were repeated with different imaging orientations to ensure that the focal point was correctly targeted in the brain. Then, longer (40–86 s) sonications were applied with automated closed-loop feedback. This feedback was based on the acoustic emissions, which were recorded with passive cavitation detectors (PCD's) that were integrated in the TcMRgFUS transducer. Pre-determined levels of spectral energy at half the frequency of the TcMRgFUS device (i.e., the subharmonic) were used as inputs to this controller. The frequency bands and control levels were suggested by the manufacturer. During sonication, the acoustic power was slowly ramped up to the maximum value specified by the user while maintaining a set level of cavitation activity. Acoustic emissions at the subharmonic is indicative of microbubble activity, and a marked increase in activity, along with broadband emissions, is indicative of inertial cavitation (22).

The acoustic emissions recordings were analyzed offline. The device stored the acoustic spectra obtained during each sonication. The average signal over frequency ranges of 115 ± 5 and 73.6 ± 30 kHz was tabulated for each spectrum. The signals at the latter frequency range were assumed to come from wideband emission. For each sonication we found the mean of the strongest 5% of the signals. Artifacts were present in some of the spectra. They were removed by excluding recordings where large signals were present in a frequency band that was normally silent.

We also recorded acoustic emissions using in-house assembled PCD's for offline analysis. They were placed in the acoustic beam path 6–11 cm away from the geometric focus of the TcMRgFUS device. One PCD was a 0.7×4 cm element that was slightly focused (radius of curvature: 15 cm). This PCD was air-backed and had a resonant frequency of 610 ± 10 kHz. A digital band-reject filter (Model 3944, Krohn-Hite Corp) was used to reduce the signal at 230 kHz and to amplify the received signal. The second PCD was a flat circular element (radius: 1 cm) that was also air-backed and had a resonant frequency of 120 ± 10 kHz. This PCD was filtered using a passive band reject filter (Allen Avionics) to remove signal at 230 kHz. The acoustic emissions were then recorded using a high-speed digitizer (NI PXI-5124, National Instruments) and analyzed using software written in Matlab (Version R2013, MathWorks).

Simulations

The sonications were simulated using a 3-D Pseudo-Spectral Finite Difference Time Domain method (k-Wave, MATLAB toolbox) (32) that solved the discretized wave equations for elastic wave propagation on a finite difference grid (33). With this approach the stress tensor and the velocity field is transformed to the spectral domain at each time step; derivatives are calculated and inverse transformed to the spatial domain. The simulator was modified to split the shear wave and compressional wave components of the elastic wave (34). In soft tissue, absorbed power density was calculated using the average of last ten cycles (steady state) of the pressure (stress) field:

$$Q_f = \frac{2\alpha_c}{\rho c_c} \frac{1}{10T} \int_{t_{end}-10T}^{t_{end}} p(t)^2 dt$$

where $p(t)$ is the pressure field, α_c (1/m) is the compressional wave absorption coefficient, ρ (kg/m^3) is the density of the medium, c_c (m/s) is the compressional wave velocity, T (s) is one period of the ultrasound wave. Inside the skull, absorbed power density was calculated using the compressional and shear wave velocity components:

$$Q_f = 2\alpha_c \rho c_c \frac{1}{10T} \int_{t_{end}-10T}^{t_{end}} u_c(t)^2 dt + 2\alpha_s \rho c_s \frac{1}{10T} \int_{t_{end}-10T}^{t_{end}} u_s(t)^2 dt$$

where $u_c(t)$ is the compressional wave particle velocity, $u_s(t)$ is the shear wave particle velocity, α_s (1/m) is the shear wave absorption coefficient, c_s (m/s) is the shear wave velocity.

The simulation model was formed using MRI and CT scans of the animal and the TcMRgFUS transducer. CT/MRI registration was performed using 3-D Slicer ver.4.4.0 (35) using the Expert Automated Registration module. After registration, the MRI and CT data of the head were resampled at 1-mm resolution on the same volume. The material parameters used in the simulations were extracted from the CT images. The brain tissue was assumed to be homogeneous. The skull was extracted manually using a threshold for the intensity value. The image data was converted to Hounsfield units to determine the porosity of the skull (14). Then the skull density was calculated from this data assuming a linear relationship between

skull porosity and a maximum density of 2200 kg/m^3 (14). Compressional speed of sound ($c_{skull,c}$) and absorption of the skull ($\alpha_{skull,c}$) were calculated by interpolating the tabulated values for 270 kHz frequency in Pichardo et al. (36). Compressional absorption at 230 kHz was obtained by multiplying the interpolated values by 230/270. The shear wave absorption ($\alpha_{skull,s}$) was taken as $(90/85)\alpha_{skull,c}$ and the shear wave speed $c_{skull,s}$ was taken as $(1400/2700) c_{skull,c}$ (37). The model was discretized in 1 mm ($\sim \lambda/7$) steps in each direction. A 230-kHz sinusoidal waveform was applied to the array elements for a total simulation duration of 300 μs , which correspond to 45 cm propagation distance. Additional details on the simulations can be found in Top et al. (26). The temperature rise was simulated using a finite-difference time-domain formulation of Pennes's bioheat equation (38). The temporal sonication power profile data from the experimental study was used to calculate the absorbed power density input as a function of time. The spatial grid was the same as in the elastic wave simulations, and the temporal time step was 10 ms. Parameters used for the acoustic and thermal simulation are described in Table 1 and Figure 2.

Data Analysis

In MRTI, phase changes were converted to temperature changes using the temperature sensitivity of the water proton resonant frequency of $-0.01 \text{ ppm}/^\circ\text{C}$ (44). The maximum temperature rise achieved at each focal hotspot was calculated and compared to the heating on the dorsal brain surface induced by ultrasound absorption in the skull. Temperature changes measured in MRTI along with the rectal temperature were used to estimate the thermal dose achieved at the focus (45). The thermal dose uses the temperature/time trajectory to estimate the cumulative equivalent minutes at 43°C (CEM 43°C). Thermal damage at the focus was compared to isodose contours drawn at 240 and 18 CEM 43°C . A thermal dose of 240 CEM 43°C is often used as a conservative threshold for thermal ablation (46). The lower value is the threshold for damage found in the brain using MRTI (47).

Temperature measurements within the skull were not possible because a lack of MR signal in bone. Instead, we evaluated the temperature changes on the brain surface induced by skull heating. The entire brain was first manually segmented in an average of all of the FSPGR magnitude images used for MRTI. Then the dorsal brain surface within the transducer path was identified. The average temperature rise was found in a one voxel wide strip on the brain surface. We also used second, more conservative (worse-case) metric that attempted to take into account non-uniform heating. The mean temperature rise of the hottest 5% of the voxels within 6-7 voxels (approximately 7 mm) of the dorsal brain surface was identified. These criteria were determined ad-hoc in a previous study (1) with an aim of including only the hottest voxels but with a sufficient number to average out noise or other temperature measurement artifacts, which were assumed to be randomly distributed around zero. The temperature measurements at the brain surface from one sonication in Monkey 4 were excluded due to head motion. The same analysis was performed on the simulated temperature maps.

Phase instabilities in the MRTI were removed in post-treatment analysis by correcting for phase changes in brain areas that were not expected to be heated using a method described previously (1). To define these areas, the segmentation of the brain surface was eroded at the

edges by 6-7 voxels (approximately 7 mm) and a circular 1 cm region of interest (ROI) surrounding the focal target was excluded. The phase-difference distribution in the remaining, presumably non-heated voxels was then fit to a non-specific smooth surface using a regularized, piecewise linear (low order spline) surface model. The regularization is based on the Laplacian of the surface, and is used to penalize deviations from smoothness in the fitted surface. This surface fit was then extrapolated into the heated regions at the focal point and outward to the brain surface and subtracted off. All analysis was performed using Matlab.

Heating at the focal point and on the brain surface were plotted as a function of the acoustic energy and the acoustic energy density on the outer skull surface, respectively. Least squares linear regression and correlation coefficients were evaluated. For heating on the brain surface, weighted least squares regression was used with weights determined by the standard deviations of the measurements.

The magnitude of the acoustic emissions was compared to the focal temperature rise and to the applied acoustic power and energy. To estimate thresholds for thermally-significant cavitation activity, we determined whether the cavitation signal was above a threshold level defined *ad hoc* to be three times the average cavitation signal of all the sonications where the temperature rise was less than 10°C. Probit regression was used to estimate the probability for enhanced cavitation activity as a function of the temperature rise and the acoustic power along with 95% confidence intervals. Reported thresholds are values where the probability of enhanced activity was 50%. The 95% confidence intervals are reported in square brackets.

Results

MRI findings

Heating at the focal point was evident at each target in every session. The peak temperature at the eight targets ranged from 46-57°C, with corresponding accumulated thermal dose estimates between 2.8 and greater than 240 CEM43°C. The temperature rise at the focal point increased linearly with the applied acoustic energy (Figure 3A). This relationship was similar for the targets in Monkeys 1-3. Heating in Monkey 4 was less efficient and was considered separately. In Monkeys 1-3, the temperature rise increased at a rate of $2.6 \pm 0.3^\circ\text{C}$ per kJ (R^2 : 0.81). In Monkey 4 it increased at a rate of $1.3 \pm 0.3^\circ\text{C}$ per kJ (R^2 : 0.59). At the focus, the temperature measurements obtained in both sagittal and coronal MRTI were consistent with this linear increase. On the brain surface, however, higher temperatures were observed with sagittal MRTI (Figure 3B). The hottest regions observed on the brain surface measured in sagittal MRTI increased linearly with the applied acoustic energy at a rate of 99.4 ± 11.4 and $72.0 \pm 20.9^\circ\text{C}$ per kJ/cm^2 , for Monkeys 1-3 (R^2 : 0.88) and Monkey 4 (R^2 : 0.71), respectively. When considering a one voxel-wide strip covering the dorsal brain surface, the temperature rise increased at a rate of $47.4 \pm 8.3^\circ\text{C}$ per kJ/cm^2 (Monkeys 1-3; R^2 : 0.78) and $37.7 \pm 10.3^\circ\text{C}$ per kJ/cm^2 (Monkey 4; R^2 : 0.66). The brain surface measurements were normalized to the area of the outer skull surface, which was 54, 48, 47, and 55 cm^2 for Monkeys 1-4, respectively. These data are summarized in Table 2.

Figure 4 and Figure 5 show example MRTI and post-sonication imaging for two animals. Immediately after sonication, tissue damage was evident in five of the eight sonicated locations. Four of the lesions were evident in both T2- and contrast-enhanced T1-weighted MRI at this time. In these cases, the accumulated thermal dose at the focus was greater than 240 CEM43°C, and the size of these lesions were consistent with 240 CEM43°C isodose contours. In Monkey 4, the lesion was only evident as a tiny spot of BBB disruption in contrast-enhanced MRI shortly after sonication. In this case, the peak accumulated thermal dose was less than 240 CEM43°C. In the three locations without MRI-evident damage, the thermal dose at the focus was 15 CEM43°C or less. Hypointense areas were not evident in any location immediately after sonication in T2*-weighted imaging.

Two locations were imaged at later times. In Monkey 1, a thalamic target in each hemisphere was sonicated in two sessions one week apart. After one week, the lesion increased in size (Figure 4). This animal was not euthanized and is in good health more than one year after the sonications. MRI acquired 19 months after FUS showed only small hyperintense regions at the lesion sites. Monkey 4 was euthanized 48 h after sonication, and the formalin-fixed brain was imaged at 7T the next day (Figure 5). Immediately after sonication, the lesion was only evident as a tiny enhancing region (Figure 5B) and not detected on T2-weighted imaging. At 48h, the lesion was clearly observed in the *post mortem* T2-weighted imaging (Figure 5C–D). In both targets imaged a second time, the size of the lesion at the later time point was consistent with size of the MRI-based thermal dose contours drawn at 18 CEM43°C (the threshold found earlier in rabbits (47)).

A small area of BBB disruption was evident next to the inner skull surface in or adjacent to the cortex after the second session in Monkey 1 (Figure 6). Despite this damage, the animal recovered without issue. More extensive cortical damage was evident in the other animals. The location of the damage did not correspond to the imaging plane used for MRTI, so we could not correlate brain surface damage to the thermal dose. The maximum dose for the hottest 5% of voxels on the brain surface in the imaging plane was 0.5, 5.7, 27, and 12 CEM43°C for Monkey 1–4, respectively. No damage was observed on the brain surface in the area that included the MRTI plane; however in Monkey 2 the space between the brain surface and skull (probably CSF or meninges), including that in this area, was enhancing after Gd-DTPA administration.

Passive cavitation monitoring

The sonications were controlled by a closed-loop feedback system based on subharmonic acoustic emissions recorded from passive cavitation detectors embedded in the transducer of the TcMRgFUS system. The level of cavitation activity was generally constant during the sonications and in most cases, the emissions magnitude was not sufficient to trigger any change in the exposure level. High power sonications were tested with Monkey 2. The exposures were controlled as expected (Figure 7A) during these sonications, and when the cavitation activity was too high, the sonication was halted (Figure 7B). In the first two sessions in Monkey 4, a high noise level was present in the recordings, leading to unnecessary exposure control. In the last session in that animal, one sonication was halted due to evident cavitation activity. Investigation of the MRTI showed that the monkey's head

shifted during the sonication, which may have perturbed bubbles near the water surface and resulted in cavitation activity.

Figure 8 plots the magnitude of the subharmonic and wideband emission as a function of the focal temperature rise, the acoustic power, and the applied energy. There was a clear relationship between the magnitude of both subharmonic and wideband emissions and the temperature rise at the focus, and this relationship was similar in all the monkeys. The emissions also increased as a function of power. However, the results in Monkey 4 did not follow the same trend as the other cases, and strong emissions were not observed at higher power levels. The temperature rise and power levels at which enhanced acoustic emissions were observed are listed in Table 3. Thermally-significant emissions were defined as signals where the magnitude was more three times higher than the average emissions of all the sonications where the peak temperature rise was less than 10°C. This threshold is indicated by horizontal dotted lines in Figure 8. Table 3 lists values where the probability for enhanced emission was 50% as determined by probit regression. These regressions, along with 95% confidence intervals, are shown as the shaded areas in Figure 8. No clear relationship was observed between the applied acoustic energy and emissions strength.

The cavitation activity was also recorded using custom detectors placed closer to the head. These recordings were evaluated offline and compared to the recordings made with the detectors integrated into the transducer. In the first three sessions (Monkey 1–2), detectors with center frequencies at approximately 105 and 610 kHz were used. In these cases, the subharmonic emissions were consistent with those measured by the integrated detectors. In addition to emissions at the subharmonic, harmonic and ultraharmonic emissions were also observed. Otherwise, there was nothing evident that was not detected with the limited bandwidth of the manufacturer's detectors. Some of the later tests used detectors with a center frequency of 1 MHz, which also showed emissions consistent with the integrated detectors. In one case, one of the detectors was coupled to the eye with sterile lubricant and recordings were made transorbitally. These recordings through the thin bone in the orbital cavity were similar to those recorded through the thicker outer skull bone.

Simulations

An example map of the maximum temperature achieved during a sonication in Monkey 4 along with the corresponding simulation are shown in Figure 9. In this case, the temperature rise at the focus (12.4°C) was similar to the predicted value (13.1°C). The average heating of the hottest 5% of the voxels on the brain surface was also similar (6.8°C, 6.2°C for measurement, simulation), but the location of the hottest measurements was different than the simulations (arrows in Figure 9). The comparison between peak measurements and simulation for all sonications is shown in Figure 10. A good correlation (R^2 : 0.67) was observed for the peak temperature rise at the focus (Figure 10A). When the measured focal temperature rise was greater than approximately 14°C, the simulations consistently under-predicted the experimental findings (Figure 10B). The measurements and simulations were similar on average on the brain surface over all of the sonications (Figure 10C–D), but there was more variation (R^2 : 0.49).

The simulated peak pressure amplitudes at the focus were tabulated for each sonication. At 1W, the simulations predicted a peak negative pressure amplitude of 184–189, 168–185, 184–192, and 171–184 kPa in Monkeys 1–4, respectively, at the thalamic targets. Using these values instead of the applied acoustic power, the thresholds for thermally-significant subharmonic and wideband emissions were estimated to be 2.1 [1.8–2.8] MPa and 2.2 [1.9–3.6] MPa, respectively, for Monkeys 1–3. These thresholds were 12% lower than would be expected from the acoustic power thresholds estimated in Table 3 and extrapolating from our estimates of the pressure amplitude obtained using an ex vivo macaque skull (223 kPa at 1W).

Histology

Shortly after sonication, a lesion with a well-defined core (diameter: 2–3 mm) was observed in the sonicated target in Monkey 2 (Figure 11A). The lesion was characterized by high-temperature coagulative necrosis, where the central core appeared to be “heat-fixed”. The core was a pale-stained area with the cracked appearance, which might have resulted from the sectioning and staining process of the thermally coagulated tissues. Few tissue elements could be recognized in the most central area. At the periphery of the central core, the blood vessels were congested; the necrotic (dead) neurons had dark, shrunken nuclei and homogeneous cytoplasm, and the myelinated fibers were fragmented. The central core was separated from the surrounding tissue by a thin (~30 µm) rim of vacuolated tissue. Beyond this rim, there was a region (width: 235–500 µm) where most cells were found dead or appeared to be dying (yellow contour in Figure 11A). Some of these cells had dark, shrunken nuclei and homogeneous cytoplasm, while others were darkly stained. The nerve fibers were disorganized and matrix was vacuolated. This surrounding region represented an inner boundary zone of the lesion. The size of the central core was similar to the dimensions of the area that reached 240 CEM43°C (red circle in Figure 11A). The size of the inner boundary zone was consistent with the dimensions of the 18 CEM43°C contour (orange circle in Figure 11A). The difference between the lesion boundaries and these contours was less than the size of a voxel in the MRTI. There was also an outer boundary zone, where most of the tissue elements were likely viable. We could not compare the shape of the isodose contours to that of the lesion in histology since the histological sections in this animal were cut in the axial plane while the thermometry was obtained in sagittal images.

A sagittal section of the lesion examined two days after sonication in Monkey 4 is shown in Figure 11B. The lesion was pale-stained and lacked a normal structure. Disorganized nerve fibers were observed throughout the lesion, most of the capillary blood vessels were congested, and the matrix was vacuolated. The tissue in some areas surrounding larger blood vessels appeared mostly intact (* in Figure 11B). The size and shape of the lesion was consistent with the 18 CEM43°C isodose contour; their dimensions agreed within the dimensions of a voxel in MRTI. The extent of the lesion followed the pattern of the white matter, and a gap was present in the middle of the lesion (also evident in the MRI shown in Figure 5D) that corresponded to a region with few fibers. The head moved during one of the sonications in this animal, which may have resulted in error in the thermal dose estimation and might also explain the gap in the lesion.

Damage on the brain surface was evident in Monkeys 2–4. A heat-fixed area approximately 2.5 mm thick was observed in the cortex of Monkey 2. There were a few subdural hemorrhages and a small blood vessel with damaged walls in this area. No evidence of bleeding was observed. A thermal lesion approximately 1.25 mm thick was found in a cortical area of Monkey 3. No hemorrhages were present in this animal. Tiny subdural damage was found in Monkey 4.

Discussion

We can compare these results with previous work in nonhuman primates with a similar TcMRgFUS system that operated at a higher frequency. With that system, which operated at 670 kHz and had 512 active elements, the average normalized peak temperature rise on the brain surface was 130°C per kJ/cm² for 20s sonications (30), more than twice the value measured here at 230 kHz (Table 2). Heating at the focus was not detected at the exposure levels used in that study, which were up to 6 kJ, while in the current study they were clearly detectable and high enough to create thermal lesions. While having fewer elements (512 vs. 1024) may have resulted in imperfect aberration correction and contributed some to the lower focal temperature in the earlier study, we do not expect that it would be a major effect with the thin monkey skull. Given the large difference in the findings, these results thus suggest that reducing the frequency will increase the “treatment envelope” where TcMRgFUS can be safely applied in the brain. This improvement might enable the use of this technology for clinical applications in brain regions that are currently out of reach for the approved TcMRgFUS device, such as many brain tumors and functional neurosurgery targets.

The experimental findings suggest that the lesions were produced by predominately thermal mechanisms. Evidence for this is three-fold. First, the temperature rise increased linearly as a function of the exposure level. If significant microbubble activity were present, we might expect an abrupt jump in temperature rise as the power level increased past a certain threshold (48). Such a jump was not observed. Second, the MRI-based thermal dose measurements and contours predicted the onset and extent of the lesions. Previously, we measured a thermal dose threshold between 12–40 CEM43°C in rabbit brain, with 18 CEM43°C the value representing a 50% probability for thermal damage (47). Here, the threshold was between 15–45 CEM43°C, consistent with that threshold. Shortly after sonication, the size of the lesions was consistent with isodose contours drawn at 240 CEM43°C, a conservative threshold used to predict high-temperature thermal ablation (49). In the two animals imaged at later times, the lesion dimensions were consistent with the 18 CEM43°C threshold. Finally, the histological appearance of the lesions was consistent with thermal lesions observed previously in the brain in this temperature range (47,50,51). If significant inertial cavitation were involved in the lesion formation, we would have expected to have seen hemorrhagic lesions (29), or at least some petechiae. However, that was not the case. Additionally, in the lesion in Monkey 4, areas within the lesion near large blood vessels appeared to be relatively intact, which can occur in thermal lesions when the blood vessels act as a heat sink.

On the other hand, these results also suggest that cavitation may have been present at the focus and could have played a role in enhancing the heating there. Both cavitation monitoring systems detected activity consistent with both subharmonic and broadband emissions, and the onset of an increase in these emissions was correlated with an increase in the temperature rise at the focus. While we could not confirm the cavitation activity originated from the focus, these results suggest that a low-level of cavitation activity may enhance the focal heating without producing hemorrhages and result in thermal ablation of the tissue. As discussed below, the simulations were consistent with such enhanced heating in the temperature range where cavitation activity was detected. If present, this enhancement could have come from increased absorption at the focus due to microbubble generation (19,48). It is also possible that the enhanced heating was the result of reduced blood perfusion due to vasospasm, which has been observed with sonication above the inertial cavitation threshold (52) and in studies with injected microbubble agents (53). It is interesting that in Monkey 4, where no thermally-significant cavitation was observed, preserved tissue was evident around some of the blood vessels (Figure 11B). Perhaps if microbubbles were present, FUS-induced vasospasm could have resulted in greater heating in that monkey.

The thresholds for thermally-significant subharmonic and wideband emissions in Monkeys 1-3 were 2.1 [1.8-2.8] MPa and 2.2 [1.9-3.6] MPa, respectively. We are unaware of prior work measuring cavitation thresholds in the brain at such a low frequency. These thresholds are higher than the threshold for thermally-significant cavitation found *in vivo* in muscle (1.8 MPa at 230 kHz) (48). The mechanical index (MI), defined as the peak negative pressure divided by the square root of the acoustic frequency, is a metric that is used to relate mechanical acoustic effects at different frequencies. This metric was developed based on theoretical formulation of inertial cavitation thresholds in water and blood (54). The MI threshold for inertial cavitation found here (4.6 [4.0–7.5]) is similar to the value of 4.7 estimated from a study by Lele et al. in the cat brain at 2.7 MHz (22) but lower than the values of 7.4–8.6 estimated from thresholds found by Vykhodtseva et al. at 0.94 and 1.72 MHz in the rabbit brain (55).

The simulations were in good agreement with the measured temperature rise at the focus. However, we should note that the peak temperature rise depends strongly on the acoustic and thermal properties, and published values vary considerably. For example, we used a literature value for brain tissue attenuation (0.114 dB/cm at 230 kHz) from Goss et al., who tabulated measurements from a number of other studies over a range of frequencies (42). Other attenuation measurements are substantially different when extrapolating to 230 kHz (40). This uncertainty in attenuation and in other parameters could have led to systematic errors in our estimates of the pressure amplitude and focal temperature rise. What was evident, however, is that the difference between the measured and simulated focal temperature was consistently higher when the measured temperature rise was in the range when thermally-significant cavitation activity was observed. Since the simulations did not include the effects of cavitation, these results are thus consistent with microbubble activity at the focus increasing the heating.

The heating efficiency was substantially lower in Monkey 4 than in the other animals, and thermally-significant cavitation activity was not observed, even though similar exposure levels were employed. The reason for this difference is not clear and may have been due to differences in skull properties or other unknown factors. This monkey was a mature male, while Monkey 1 was a pre-pubescent male, and Monkeys 2 and 3 were female. The simulations suggested that the pressure amplitudes were lower in the targets in this animal than in most of the other cases, but this difference does not appear to be sufficient to explain the results.

The heating and damage that was produced on the brain surface does not reflect what will occur in a human. The human skull has an outer surface area 5–6 times larger than a macaque monkey, so the acoustic energy will be distributed over a much larger area. It is not straightforward to extrapolate our experimental findings to a human since the human skull is thicker than that of a macaque. Higher exposure levels will be needed to achieve ablation-level temperatures at the focal point, and the dynamics of the heating of the brain surface by the skull might be different. However, as noted above, the comparison between this study and the earlier work at 670 kHz (30) suggest that reducing the frequency to 230 kHz should reduce skull-induced heating on the brain surface.

This study had several limitations. The number of animals used in the experiments was small, and due to skull heating we were able to examine sonications only in the centrally-located thalamus. Experiments in a larger sample might reveal cavitation-related adverse effects that were not observed here, and results may differ in other structures where the acoustic absorption, perfusion, cavitation thresholds, and other factors may be different. Sonication in brain tumors or in patients who received radiation or chemotherapy should be carefully considered since the vasculature in such cases may be compromised and potentially pose a higher risk for cavitation-induced side effects. Passive acoustic mapping (56–58) or other techniques that could localize the cavitation activity are needed to confirm that the cavitation activity was occurring only at the focus.

Conclusions

Skull-induced heating limits the portions of the brain that can be targeted by TcMRgFUS with the currently-available device operating at 650–670 kHz. The results of the present study suggest that this lower-frequency TcMRgFUS system can increase the targetable areas in the brain that can be thermally ablated. The temperature and thermal dose measurements, along with the MRI-evident tissue effects, were all consistent with thermal ablation being the dominant mechanism in the production of these lesions. Cavitation activity was present, but it was maintained at a low level by the closed-loop feedback system. This activity suggests that microbubbles in the focus may have accompanied or enhanced the heating. The effects on tissue, as observed in histological evaluation, are comparable to thermal effects seen in earlier studies at higher frequencies and no destructive mechanical effects or hemorrhages were observed. The MRTI measurements found an increase in cavitation activity when the temperature rise exceeded 16.6°C. Simulations of the heating agreed on average with the measurements, but were consistently lower for the sonications where thermally-significant cavitation activity was observed. Thermally-significant acoustic

emissions occurred in three of the monkeys with a threshold of 221 W and an estimated pressure amplitude of 2.2 MPa. The cavitation control system was successful in quickly halting the sonication when larger activity was detected.

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Figure 1.

Coronal T2-weighted image of a macaque monkey superimposed on a diagram of the experimental setup. The animal (Monkey 2) was placed on its back with its head tilted back so that it was partially submerged in temperature-controlled degassed water. The position of the geometric focus is indicated by the green “x”. The FUS beam was steered to the thalamic target using the phased array transducer. Acoustic emissions were recorded during sonication using two PCDs mounted in the beam path as well as by detectors integrated into the hemisphere transducer (not shown). The inset shows an axial contrast-enhanced T1-weighted image after creating a lesion in the thalamus.

Figure 2. Speed of sound (–) and absorption (– –) values used for the compressional wave propagation in the skull (36) as a function of density.

Figure 3.

Temperature rise at the focal point (**A**) and on the brain surface (**B**) as a function of the applied acoustic energy and energy density, respectively. The relationship between the temperature rise and the exposure levels was similar for Monkeys 1-3. Less heating was observed for Monkey 4, so it was examined separately. A linear relationship was observed for focal heating, and this relationship was consistent for MRTI obtained in both coronal (open symbols) and sagittal planes (filled symbols). Such a relationship was only evident for sagittal MRTI for brain surface heating, and coronal measurements are not shown in (**B**). For these measurements, the brain surface was segmented, and the hottest 5% voxels were identified (mean $\pm$ SD shown).

Figure 4.

MRTI (top) and post-FUS imaging (bottom) obtained in two sessions in Monkey 1. Thermal dose contours at 18 (orange) and 240 (red) CEM43°C were calculated from the MRTI. The plot shows the peak temperature rise as a function of time at the focus for these two sonications (solid line: hottest voxel; dotted line: mean of 3×3 voxel region). Immediately after each session, a small lesion was observed in contrast-enhanced T1-weighted MRI (CE-T1WI). The dimensions of these areas were consistent with the 240 CEM43 contours at this time. At week 2, the lesion produced in the first session was largely non-enhancing in CE-T1WI, and it appeared in T2-weighted imaging (T2WI) with an increased size that was consistent with the dimensions of the 18 CEM43°C contour. Nineteen months later, only small regions hyperintense in T2-weighted imaging remained. Bar: 1 cm

Figure 5.

MRTI (A) and post-FUS imaging (B–D) obtained in Monkey 4. A thermal dose contour at 18 CEM43°C was calculated from the MRTI and is shown in orange. Immediately after the session, a tiny spot of BBB disruption was observed in contrast-enhanced T1-weighted MRI. A lesion was not observed in T2-weighted imaging at that time. This animal was euthanized three days after FUS, and the formalin-fixed brain was imaged post mortem at 7T. The lesion was clearly visible at that time in T2-weighted MRI. Superposition of the 18 CEM43°C thermal dose contour on this lesion [inset in (D)] was consistent with the size of the MRI-evident lesion. Bar: 1 cm.

Figure 6. Post-FUS contrast-enhanced MRI showing a small region with damage next to the skull after the sonications in the second session with Monkey 1. Left: Axial; Right: Coronal. Bar: 1 cm

Figure 7.

Power control via acoustic emissions recordings. The signal around the subharmonic (115 kHz) was used to modulate the power level in real time. The system aimed to keep the emissions at a safe level and halted the sonication if the total emissions exceeded a setpoint. In the example shown in (A), the emissions did not exceed this setpoint. In the example shown in (B), the sonication was aborted due to excessive cavitation. (C): Emission spectra from the second example obtained using a PCD integrated into the transducer. This detector had a resonant frequency near the subharmonic; higher frequencies were filtered out. The gray bands indicate the frequency ranges analyzed here for subharmonic and wideband emissions. The black spectrum was obtained at the start of the sonication. The red shows the spectrum with the greatest emission. (D) Emission spectra at these two times obtained using a custom PCD. Here the gray band shows the resonant frequency of the detector, which was 610 ± 10 kHz. Note the presence of higher harmonics and ultraharmonics.

Figure 8.

Acoustic emissions magnitude as a function of the peak temperature at the focus, the acoustic power, and the acoustic energy. A clear relationship between focal heating and both subharmonic and wideband emissions was observed, and this relationship was evident for all animals. There was also a relationship between emissions and the acoustic power, except for in Monkey 4 where enhanced signals were never observed. The different symbol shapes represent measurements obtained during the different sessions. The sigmoid curves represents the probability for having thermally-significant emissions, which was defined here as having signal higher than three times the average signal from all the sonications when the temperature rise was less than 10°C. This threshold is indicated by a dashed line. This probability was estimated using probit regression; the shaded areas are 95% confidence intervals of this fit. The point at which the probability was 50% is indicated by “+” symbols.

Figure 9.

Measured and simulated sagittal temperature maps. **Left:** Maximum temperature map obtained with MRTI for a sonication in Monkey 4. Erroneous signals were observed near the skull base (**) due to low signal and on the dorsal brain surface from a blood vessel (*).

Right: Corresponding simulation. The measured temperature rise at the focus was similar to the simulation prediction. The heating pattern on the brain surface was also similar, but the location of the greatest heating was near the back of the head in the experiment, but near the front in the simulation (arrows). Bar: 1 cm.

Figure 10.

Simulated vs. measured temperature rise at the focus and on the brain surface. **(A):** A good correlation (R^2 : 0.67) between measurements and simulations was observed for the focal heating. **(B):** The simulations and measurements at the focus agreed on average for low temperatures. The measured temperatures were consistently higher than the simulations predicted when the focal temperature rise was above approximately 14°C. **(C–D):** On average, the simulated and measured temperature rise on the brain surface were consistent, but there was more variation (R^2 : 0.49). The various symbols represent measurements obtained at different sessions for the four monkeys. Measurements and simulations were consistent over the different sessions for each monkey.

Figure 11.

Microphotographs showing H&E-LFB stained sections of two lesions. **(A):** Axial section showing the lesion from Monkey 2 at approximately two hours after sonication (left: 2.5 $\times$; right: 40 $\times$). A well-defined lesion core was observed with a sharp boundary. This core was pale-stained, and the neurons in the periphery were shrunken and edematous. Blood vessels within the lesion were congested. A larger area, indicated by the dotted yellow contour, contained dead or dying neurons and disorganized fibers. The circles shown on the 2.5 $\times$ view represent the dimensions of the thermal dose contours at 240 (red) and 18 (orange) CEM43 $^{\circ}$ C, which were obtained with MRTI acquired in a different imaging orientation. At this time, the dimensions of the lesion core was similar to the 240 isodose contour, and those of the larger area with dead neurons was consistent with the 18 CEM43 $^{\circ}$ C contour. **(B)** Sagittal section showing the lesion in Monkey 4 at 48h after sonication (left: 2.5 $\times$; right: 20 $\times$). The pale-stained lesion was edematous and lacked structure. Congested blood vessels were observed along with granulated fibers. The thermal dose contour obtained in MRI at 18 CEM43 $^{\circ}$ C is superimposed on the 2.5 $\times$ view. The accumulated thermal dose for this lesion was approximately 50 CEM43 $^{\circ}$ C. The tissue in some areas around large blood vessels (*) appeared relatively intact. A gap in the lesion was evident in other sections of this lesion and in MRI (Figure 5D); this gap may have been due to a large vessel evident in other sections. Bars: 1 mm (2.5 $\times$); 100 μ m (40 $\times$ and 20 $\times$).

Table 1

Parameters used in the simulations

Parameter	Water	Bone	Brain	Blood	Reference
Sound speed (m/s)	1480	See Figure 2	1560	-	(36,39,40)
Density (kg/m ³)	1000	2200	1030	1030	(40,41)
Absorption (dB/cm/MHz)	2.5×10^{-5}	See Figure 2	0.495	-	(36,40,42)
Specific heat capacity (J·kg/K)	4179	1440	3640	3660	(40)
Thermal conductivity (W/m/K)	0.615	0.43	0.534	-	(40)
Blood perfusion rate (1/s)	0	0	6×10^{-3}	-	(43)

Table 2

Temperature rise at the focus and on the brain surface

Frequency	Temperature rise normalized by energy			Temperature rise normalized by energy density	
	Focus (°C/kJ)	Brain surface (hottest, °C/kJ)	Brain surface (average, °C/kJ)	Brain surface (hottest, °C/kJ/cm ²)	Brain surface (average, °C/kJ/cm ²)
230 kHz (Monkeys 1–3)	2.6 ± 0.3	2.1 ± 0.3	1.0 ± 0.2	99 ± 11	47 ± 8.3
230 kHz (Monkey 4)	1.3 ± 0.3	1.3 ± 0.4	0.7 ± 0.2	72 ± 21	38 ± 10
670 kHz [§]	undetectable	3.6 ± 0.2	2.4 ± 0.2	200 ± 10 [*]	130 ± 10 [*]

[§]From reference (30)^{*} Assumed an outer skull area of 55 cm²

Table 3

Temperature rise at the focus and power levels associated with thermally-significant acoustic emissions

Monkeys	Subharmonic Emissions				Wideband Emissions			
	T (°C)	95% CI	Power (W)	95% CI	T (°C)	95% CI	Power (W)	95% CI
All	16.6	[14.1–23.3]	220.7	[165.4–582.9]	17.7	[14.8–24.7]	231.4	[173.4–634.2]
1–3	16.2	[12.7–23.8]	128.7	[102.0–275.4]	17.4	[13.7–25.4]	140.7	[110.0–368.8]