

Resting state functional connectivity changes after MR-guided focused ultrasound mediated blood-brain barrier opening in patients with Alzheimer's disease

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ARTICLE INFO

Keywords:

Blood-brain barrier
Blood-brain barrier opening
MR-Guided focused ultrasound
Functional connectivity
Resting state functional MRI

ABSTRACT

MR-guided focused ultrasound (MRgFUS) can temporarily permeabilize the blood-brain barrier (BBB), non-invasively, to allow therapeutics access to the central nervous system. However, its secondary and potential neuromodulation effects are not well understood. We aimed to characterize the functional impact of MRgFUS BBB opening in human subjects, based on the phase I trial in patients with Alzheimer's disease. We analyzed for changes in bilateral frontoparietal networks in resting state functional MRI from five subjects after BBB opening in the right frontal lobe. We found a transient functional connectivity decrease within only the ipsilateral frontoparietal network that was recovered by the next day. Additionally, baseline to month three comparisons did not reveal any significant differences from matched-controls from the Alzheimer's Disease Neuroimaging Initiative. Overall, MRgFUS may transiently affect neurologic function, but the functional organization is restored at one day and remains unchanged at three months. This first in human data has implications for the development of MRgFUS as a drug delivery platform to pathologic brain tissue and potential use for non-invasive neuromodulation.

1. Introduction

Non-invasive brain delivery of targeted therapy has widespread applications treating neurological disorders, yet effective penetration of the blood-brain barrier (BBB) remains a limitation. Transcranial low-intensity focused ultrasound, in the presence of intravenous microbubbles, is an emerging technology to transiently increase BBB permeability (Hynynen and Jones, 2016). Mechanical stress on cerebrovascular walls exerted by sonicated microbubbles leads to reduced integrity of tight junctions and other membrane proteins expressed by endothelial cells (Cho et al., 2016; Sheikov et al., 2008). Secondary consequences of

this process including increased neurogenesis and altered blood oxygenation level dependent (BOLD) activity have also been reported in animals (Chu et al., 2015; Mooney et al., 2016; Todd et al., 2018). The latter observation is particularly interesting given the utility of non-invasive neuromodulation. So far, ultrasound, in most instances applied without BBB opening, has been studied for stimulating or inhibiting neural activity, but the underpinnings of these effects demand further investigations (Lee et al., 2016; Legon et al., 2014; Sato et al., 2018).

In this study, we aimed to characterize the secondary, functional effects of MR-guided focused ultrasound (MRgFUS) induced BBB opening

Abbreviations: BBB, blood-brain barrier; GLUT1, glucose transporter 1; MRgFUS, MR-guided focused ultrasound; FUS, focused ultrasound.

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<https://doi.org/10.1016/j.neuroimage.2019.06.060>

Received 12 December 2018; Received in revised form 13 June 2019; Accepted 25 June 2019

Available online 26 June 2019

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in human subjects for the first time. Our group recently demonstrated its safety and feasibility in an early phase clinical trial among five patients with mild-to-moderate AD (Lipsman et al., 2018). Apart from clinical examinations and neurocognitive scores, functional imaging is useful to assess the biological impact of the procedure. In particular, BOLD signals measured by functional MRI (fMRI) are a surrogate marker of neural activity and function (Logothetis and Pfeuffer, 2004). Correlations between distributed regions on resting state fMRI (rs-fMRI) have been shown to reflect the functional organization of the brain in healthy or diseased states (Badhwar et al., 2017). In the primary study, the BBB opening target was the right frontal lobe, an associative region of the brain. As such, we tested primarily in a seed-to-seed analysis whether the procedure led to alterations in the functional connectivity (FC) of the ipsilateral versus contralateral frontoparietal networks (FPN), followed by a seed-to-voxel analysis secondarily.

We also measured FPN and default mode network (DMN) FC changes in study subjects with comparison to matched-control data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) over the same timeframe. The DMN changes have been implicated in cognitive impairment, and proposed as a biomarker for early diagnosis and disease monitoring of AD (Palmqvist et al., 2017; Damoiseaux et al., 2012). The prefrontal cortex is an important associative center that mediates attention, working memory, and other executive functions (Yuan and Raz, 2014; Lowe et al., 2018). The rationale for this approach is to determine whether transient focal BBB opening can lead to chronic widespread changes in functional organization.

2. Material and methods

2.1. Study recruitment and procedures

Five patients (three men, two women) with a mean age of 66.8 (SD standard deviation 6.1) years and mean Mini-Mental Status Exam MMSE 22.2 (SD 2.3) were included in this rs-fMRI study. All rs-fMRI sequences were acquired in an exploratory fashion as part of a pilot study to investigate the safety and feasibility of MRgFUS induced BBB opening in the right frontal lobe of patients with mild-to-moderate AD. The study rationale was based on preclinical evidence showing 1) enhanced anti-amyloid antibody delivery after FUS and 2) reduced amyloid plaque after BBB opening alone. As a pilot, first-in-human study, the sample size was small, and a power justification was not performed. The primary results were published previously (Lipsman et al., 2018). All parts of the study were approved by Sunnybrook Health Sciences Centre research ethics board.

Each patient underwent MRgFUS twice, one month apart, with the second target volume prescribed to be twice the size of the first. Rs-fMRI was acquired at baseline, as well as immediately, one day, and approximately one week after each procedure (Fig. 1). Immediate timepoint rs-fMRI was acquired as soon as practically possible, which occurred on average 60.1 (SD 10.6) minutes after the last sonication. The last rs-fMRI was acquired three months after the first procedure. Data from this study may be made available upon reasonable request to the corresponding author.

2.2. MRgFUS procedure and fMRI acquisition

The transcranial BBB opening procedure was performed using ExAbate (220 kHz, InSightec, Haifa, Israel), which was coupled to a 3-Tesla MRI (Signa MR750; GE Healthcare, Milwaukee, Wis.) allowing for intraprocedural imaging with a body coil. BBB opening was targeted in the anterior right frontal lobe (MNI 18, 40, 22) measuring approximately 350 mm³ for the first procedure and double this for the second. BBB opening after sonication was visualized as gadolinium contrast enhancement on the T1-weighted image (described below). After radiological evidence of BBB opening, the subject was removed from the MRgFUS device and set up in the head coil anew for further MRI scanning.

Anatomical images were acquired using a 3D fast spoiled gradient echo (3D-FSPGR) sequence with 176 slices of 1 mm thickness. Scan parameters were TE = 2.94 ms, TR = 7.65 ms, and matrix size = 265 × 265. For the rs-fMRI acquisition, each subject was instructed to remain still with eyes closed. Sequence parameters were 200 temporal volumes of 40 slices with 3.6 mm thickness; TR = 2400 ms, TE = 30 ms; flip angle = 70°; and matrix size = 64 × 64. Follow-up rs-fMRIs were obtained with the same parameters. Of note, rs-fMRI immediately after the MRgFUS procedure was collected following gadolinium administration as the MRgFUS protocol required its injection for determining the procedure end-point.

A comparison group of ten AD patients was selected from ADNI (six men, four women), identifiers given in Table S1, with similar age (71.6 SD 6.6 years) and MMSE (23.5 SD 2.1) as our five subjects. The ADNI database (<http://adni.loni.usc.edu/>) was formed with the goal of measuring the progress of mild cognitive impairment to early AD with serial MRI and other imaging, clinical, and biological markers. Rs-fMRI scans at baseline and three-months follow-up were available for matched-control comparison. These sequences were acquired by Philips 3-Tesla MRI with parameters 140 temporal volumes of 48 slices with 3.3 mm thickness, TR = 3000 ms, TE = 30 ms, flip angle = 80°, matrix size = 64 × 64, and EPI factor of 59.

2.3. Data processing and statistical analysis

MRI data were pre-processed using SPM12 (SPM12, 2014) and the CONN toolbox, an SPM-based package, was used for the fMRI processing (Whitfield-Gabrieli et al., 2012). Pre-processing procedures consisted of motion correction, slice-time correction, functional realignment, co-registration to the anatomical image, normalization to standard space T1-weighted template image (Montreal Neurological Institute, MNI152 2 mm), removal of first three volumes for equilibration, and spatial smoothing with 8 mm Gaussian kernel. Further denoising of white matter and cerebrospinal fluid signals and 0.008–0.09 Hz bandpass filters were applied to elicit low frequency signals related to neuronal activity.

For seed-to-seed analysis, we computed FC by Pearson product-moment correlation between the spatially averaged BOLD signal time series of the regions of interest (ROIs) for each timepoint and followed this calculation with a Fisher's *r*-to-*z* transformation. The ROIs were the

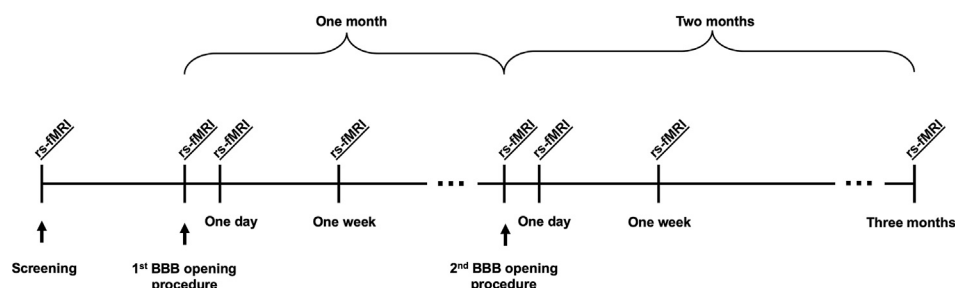


Fig. 1. Study outline and points of functional MRI acquisition.

right prefrontal cortex (right PFC, MNI 41, 38, 30), right posterior parietal cortex (right PPC, MNI 52, -52, 45), left PFC (MNI -43, 33, 28), left PPC (MNI -46, -58, 49), medial prefrontal cortex (mPFC, MNI 1, 55, -3), and posterior cingulate cortex (PCC, MNI 1, -61, 38). To assess the contribution of gadolinium contrast in the right frontal lobe to BOLD signals, specifically for the rs-fMRI collected immediately after the BBB procedure, we also included a control seed-to-seed correlation between

the right Circle of Willis (MNI -14, -4, -20, 2 mm radius) and right PFC (performed prior to denoising). Given that fMRI with gadolinium is atypical and to mitigate potential spurious FC results, this seed-to-seed calculation was meant to emulate an intravascular contribution by choosing an arterial signal.

We constructed a linear mixed model (IBM SPSS v23) to test for changes in FPN FC over time, albeit one missing data at one-day and

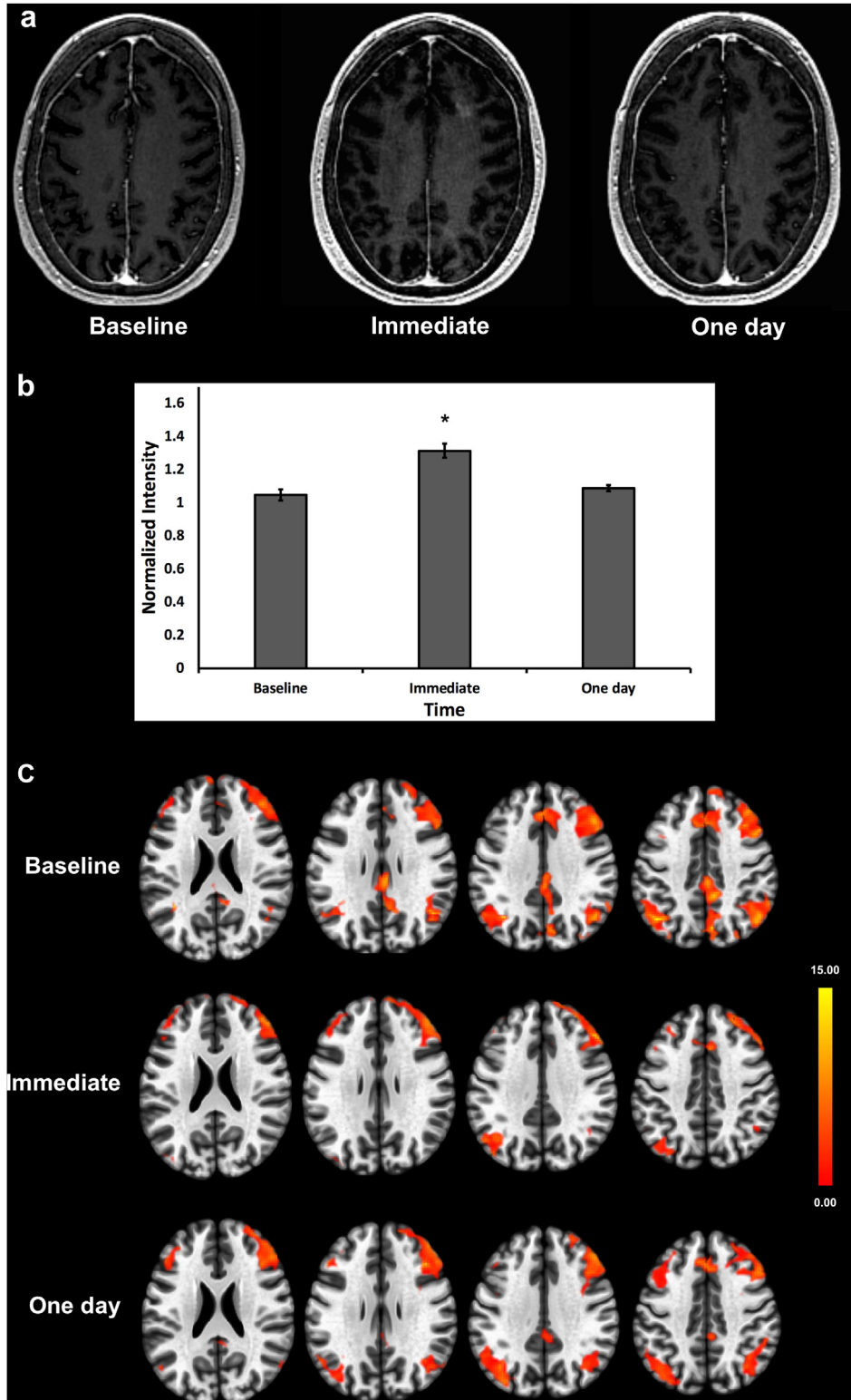


Fig. 2. (a) Demonstration of gadolinium contrast extravasation in the right frontal lobe on T1-weighted MRI (middle) as the result of MRgFUS procedure. T1-weighted plus contrast images from baseline and one day post procedure are presented for comparison. (b) Normalized intensities of the target volumes over time show a significant increase following MRgFUS (* $p < 0.001$), indicating transient increase in BBB permeability. (c) Functional connectivity maps from the seed-to-voxel analysis (ROI right PFC) of all patients overlapped on standard MNI space at three corresponding time points. The color bar denotes t-values.

three at one-week. Fixed effects parameters include hemisphere side and time, while subject was a random effect. Shapiro-Wilk test was performed to test normality. Further pairwise comparisons were performed, without correcting for multiple comparisons given the exploratory nature of the study, where the results are intended to inform future studies. P-values less than 0.05 were considered statistically significant. Finally, a seed-to-voxel analysis with the ROI in the right PFC (MNI 41, 38, 30) was performed to explore differences between baseline and subsequent rs-fMRIs. Significant results were reported with voxel-level height threshold p uncorrected <0.001 and cluster-extent FDR-corrected threshold $p < 0.05$.

To quantify the change in enhancement as the result of the procedure, we manually created ROIs corresponding to the target on normalized T1-weighted with gadolinium MR images. The mean intensity was extracted from the structural image masked with this ROI, and then normalized with that of the mirrored region.

3. Results

Increased BBB permeability in the sonicated volume was detected by contrast extravasation subsequent to all MRgFUS procedures ($p < 0.001$), with return to baseline intensity on MRI the morning after the procedure (Fig. 2) (Lipsman et al., 2018). BBB opening was achieved with an average maximum power of 4.7 (SD 1.8) Watts. The sonicated volumes are superimposed in Fig. S1, to demonstrate localized spatial coverage.

3.1. Short-term changes after MRgFUS

To determine the bearing of MRgFUS BBB opening on the FC between a target related and distant region, the FCs of the bilateral FPNs were plotted over time in Fig. 3 and analyzed in a linear mixed model. The main effects of side ($p > 0.2$) and time ($p > 0.1$) were not statistically significant. The linear mixed model did not support a different pattern in FPN FC over time by side (p value for interaction of time and side > 0.2). However, the data was likely underpowered for capturing such an effect. A direct comparison revealed a decrease in the right (ipsilateral) FPN FC during BBB opening BOLD sessions ($p = 0.004$, paired $t = -3.38$, $df = 9$), which was not observed in the contralateral non-sonicated side ($p = 0.70$, paired $t = 0.55$, $df = 9$). Furthermore, this change appeared to be temporary, with no statistically significant differences at one-day ($p = 0.30$, paired t -test) or one-week ($p = 0.32$, paired t -test).

The correlation between the right PFC and arterial signal did not appear to be different during BBB opening and baseline ($p > 0.9$, Fig. S2). In all, the evidence would support a short-term reduction in FC observed in the ipsilateral FPN secondary to the MRgFUS induced BBB opening. Subsequently, a seed-to-voxel analysis revealed the right ipsilateral PFC showed decreased connectivity (i.e. baseline $>$ immediate scans) with a cluster in the parietal cortex (MNI 30, -50, 46, p -FDR = 0.002), consistent with our *a priori* ROI-to-ROI analysis (Fig. 4). Comparisons to baseline at subsequent timepoints with seed-to-voxel analysis did not yield any statistically significant clusters.

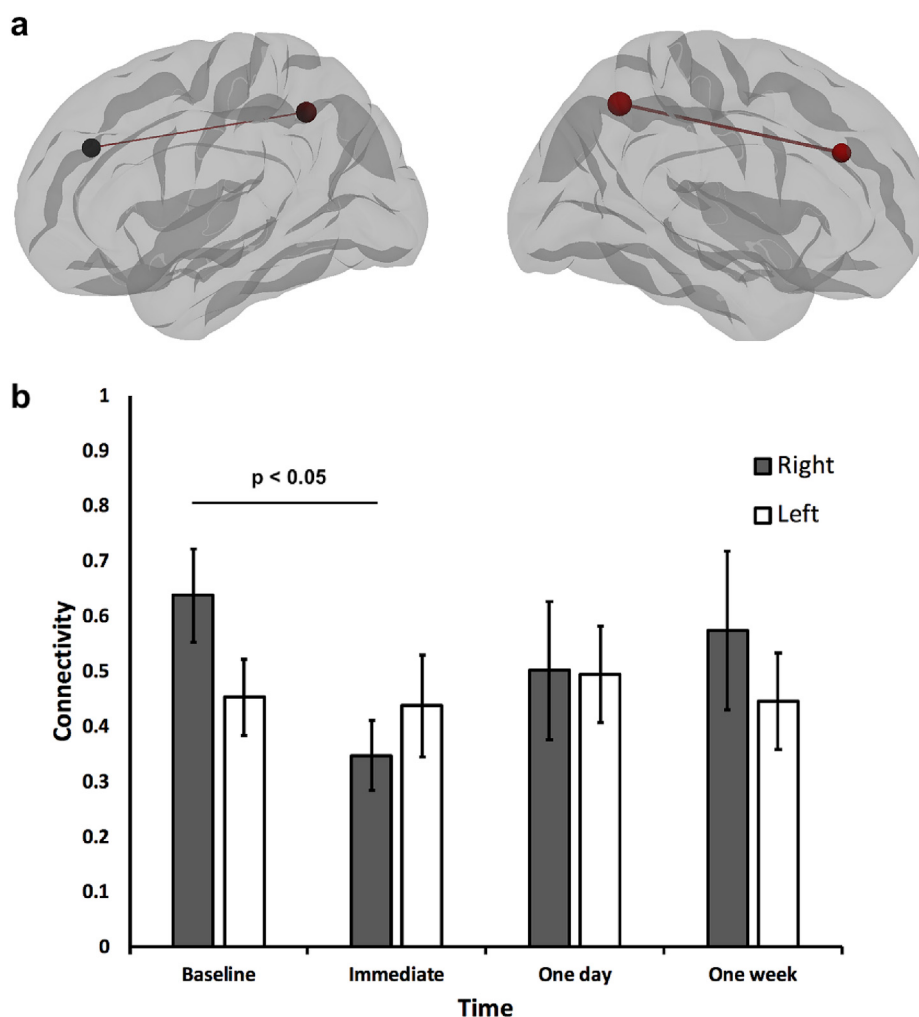


Fig. 3. (a) Schematic illustration of the right and left frontoparietal network, and (b) the functional connectivity at baseline and on follow-up after each MRgFUS procedure. Error bars indicate standard error of mean.

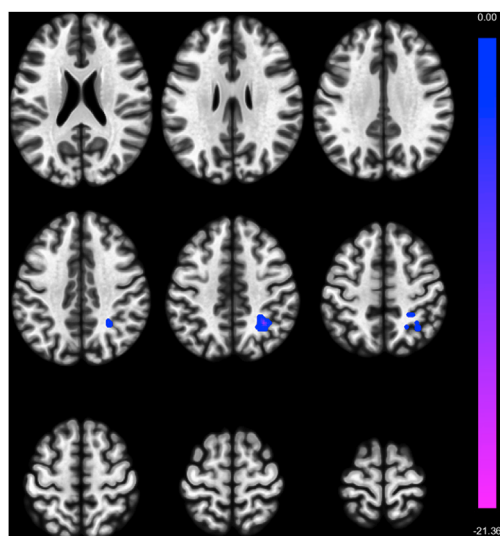


Fig. 4. Comparison of whole brain functional connectivity to right prefrontal cortex for immediately after MRgFUS to baseline. The colored areas, superimposed in MNI space, represent spatial regions of significant decrease, which appear isolated to the ipsilateral parietal cortex. The color bar denotes t-values.

3.2. Long-term changes after MRgFUS

We compared the FCs of the right ipsilateral FPN and DMN at the final visit to baseline to assess for any long-term changes after the MRgFUS BBB opening procedures (Fig. 5). The trajectories were additionally compared to AD controls. We noticed a decrease in average right FPN FC ($p = 0.10$), but this was not statistically different from what might be expected in patients with AD ($p > 0.9$). Furthermore, the FC of the DMN at three-months was unchanged relative to baseline, with a trajectory that seemed to differ from matched controls, although this did not meet statistical significance ($p = 0.06$).

4. Discussion

Transcranial application of FUS is now feasible for clinical use and is particularly attractive as a non-invasive and targeted brain therapy. With image-guidance and sub-millimeter spatial accuracy, the opening of the BBB with MRgFUS enables precise, individually tailored targeting. While the short-term safety and biological impact of the procedure have been studied in animal models (Chu et al., 2015; Mooney et al., 2016; Todd et al., 2018; Jordão et al., 2013; Leinenga and Götz, 2015), the

experience in human subjects is still young. This report is the first description of functional changes from MRgFUS BBB opening in humans. In this study, we assessed the FC changes over time after MRgFUS BBB opening in the white matter of the right frontal lobe. We hypothesized the FPN would be involved given its proximity to our target, and indeed found the FC of the ipsilateral, but not contralateral FPN was reduced after MRgFUS. The decreased connectivity was further observed preferential to the ipsilateral FPN in a seed-to-voxel analysis. Notably, ipsilateral FPN FCs were restored 24 hours later, which were temporally concurrent with BBB closure.

We showed, through frequent longitudinal follow-ups, these perturbations are transient even after repeated BBB opening. In fact, long-term over three months, FC trajectories in the FPN and DMN were not significantly different from matched controls. Decreased FC in the FPN may reflect progressive decline in organization or dysfunction of individual nodes, and in this case, is more likely contributed by the underlying disease than repeated MRgFUS procedures.

A previous animal study found microhemorrhages from high intensity ultrasound exposure corresponded with permanently depressed BOLD hemodynamic activation (Chu et al., 2015). FUS induced BBB opening can also initiate a transient inflammation response (McMahon and Hynynen, 2017; Kovacs et al., 2017). Our results suggest focal BBB opening did not result in long-term and widespread functional changes in the brain, and further support the safety of the procedure. Here, we were vigilant to deploy optimal and safe ultrasound parameters as defined by an acoustic feedback algorithm (O'Reilly and Hynynen, 2012). Furthermore, contrary to preclinical studies, we did not find a widespread reduction in FC, as our seed-based analysis showed an isolated cluster covering the ipsilateral parietal cortex. This discrepancy may be explained by differences in study subjects, targeting strategies, and brain structures. Certainly, strategies to directly test for deleterious events at a more granular level (e.g. cerebrospinal fluid sampling and molecular imaging) will be included in future trials.

An interesting implication of our findings is the potential of FUS to neuromodulate the brain. If FUS is indeed capable of neuromodulation in humans, it has several meaningful advantages over existing technologies such as deep brain stimulation and transcranial magnetic stimulation (e.g. non-invasiveness, deeper targeting, and better spatial resolution). Previous studies have been predominantly dedicated to acute stimulation and inhibition by ultrasound alone (Lee et al., 2016; Legon et al., 2014). However, the exact mechanism and protocol for neuromodulation have yet to be established (Sato et al., 2018). Two animal studies have shown that FUS at BBB opening parameters can temporarily reduce neural activity as measured by electromyography and fMRI, without histological evidence of tissue damage (Chu et al., 2015; Todd et al., 2018). Durability is another feature to consider in translation of FUS neuromodulation.

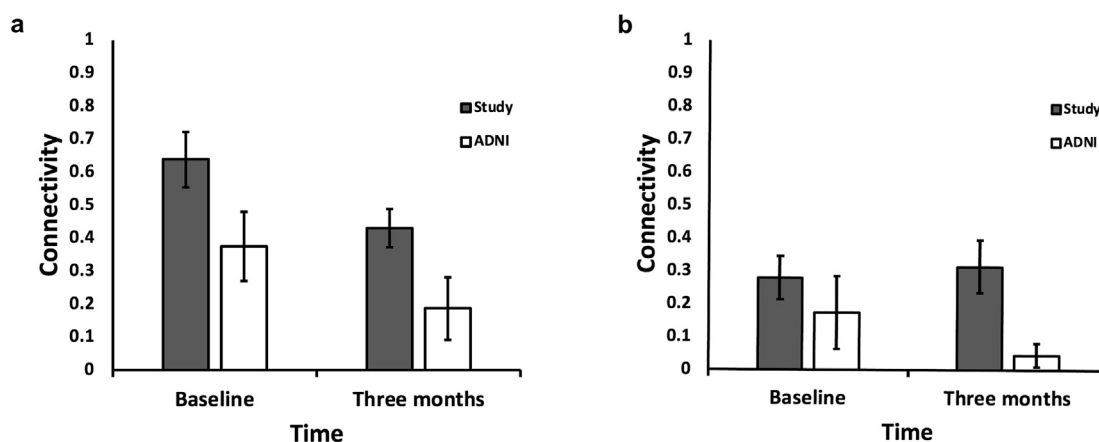


Fig. 5. Functional connectivity of (a) the right frontoparietal network and (b) default mode network over three months in study patients and ADNI patients matched for age and MMSE. Error bars indicate standard error of mean.

While Chu et al. found persistent changes in BOLD response seven days after ultrasound exposure, this was demonstrated only at a high mechanical index of 0.8 and accompanied by microhemorrhages (Chu et al., 2015). Because the parameters for BBB opening have been extensively characterized with a well-defined endpoint, this particular approach holds promise for future investigations.

We speculate our observations are primarily due to BBB opening or processes at the neurovascular unit as opposed to sonications alone. The mechanism for the latter also remains in question, but has been theorized to be related to the mechanical effects on cellular membranes (Tyler et al., 2018). The time course of FC reductions appears to match BBB permeabilization and extend beyond acute neuromodulation effects reported of sonications alone (Lee et al., 2016). Todd et al. also showed reductions in FC were correlated with the extent of BBB opening in rodents. Furthermore, arterial vasoconstriction and metabolic alterations upon ultrasound exposure may contribute to changes in neurovascular coupling (Raymond et al., 2007). In a previous study, FUS BBB opening significantly decreased the expression of GLUT1 transporters, which recovered by 24 hours (Yang et al., 2014). Other potential explanations include alterations in neuronal activity via mechanical effects (Chu et al., 2015) and neuronal microenvironment via microglial activation (McMahon and Hynynen, 2017), all of which function jointly as the neurovascular unit to regulate cerebrovascular function.

Several study caveats are worthy of discussion. First, our results stem from a small cohort of patients, but they are complementary to similar findings in animals. Second, rs-fMRI occurred after gadolinium injection, which was a necessary endpoint for this proof-of-concept study; this may have resulted in false-positive signals. However, we did not find a significant correlation between the target ROI containing gadolinium and the arterial signal, reducing the likelihood of such an error. Third, we chose to employ rs-fMRI because of its ease of implementation and performance. Undergoing the MRgFUS BBB procedure, which can last three to four hours, is typically fatiguing for patients with AD. Nevertheless, a task-based paradigm may be more powerful in detecting true functional changes, being hypothesis-driven rather than generating. While the study design and analysis were exploratory in nature, this experience serves as a foundation for future investigations in MRgFUS BBB opening as 1) a therapeutic delivery platform and 2) a tool for neuromodulation.

5. Conclusions

We found MRgFUS BBB opening transiently reduced resting-state FC of distributed brain regions involving the target. However, the functional organization was preserved relative to baseline at one-day, one-week, and three-month exams. This first human data has implications for the development of MRgFUS as a drug delivery platform and potential use for non-invasive neuromodulation. We have leveraged the results and limitations of this current study in designing the next phase trial for AD, which features expanded therapeutic brain targets such as the DMN. Future neuroimaging studies may be directed at the effect of the procedure on multimodal network activity during functionally relevant tasks, and along with animal studies, at identifying the underlying mechanisms using more invasive techniques available such as histology and two-photon microscopy.

Declaration of interests

NL, KH, and SEB have received an honorarium for serving on an expert steering committee on FUS in AD. KH is an inventor on intellectual property owned by Brigham and Women's hospital in Boston and Sunnybrook Research Institute in Toronto related to transcranial FUS technology. Other authors do not have conflict of interest to declare.

Funding sources

This study was supported by the Focused Ultrasound Foundation.

Acknowledgements

We wish to thank Ruby Endre, Garry Detzler, and Fred Tam for their technical assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.neuroimage.2019.06.060>.

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