Targeted Blood-Brain Barrier Opening by Focused Ultrasound Improves Spatial Memory in Wild Type Mice

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Background & Motivation

- Focused Ultrasound (FUS) paired with systemically administered microbubbles (MB) is capable of opening the Blood-Brain Barrier (BBB) in a targeted, noninvasive, and transient manner
- Therapeutic applications of FUS+MB treatment focus primarily on its ability to enhance drug delivery to the central nervous system (CNS) via BBB opening (BBBO)[1]
- FUS+MB alone has been shown to ameliorate Alzheimer's Disease (AD) pathology and improve spatial memory in transgenic AD mice, and these studies attribute the observed spatial memory enhancements to the synchronous pathological improvements[2]
- Recent studies suggest that repeated FUS+MB-induced BBBO may elicit spatial memory improvements in wild type (WT) mice[3]
- The extent of FUS+MB-induced spatial memory improvement has yet to be fully characterized and the biological mechanism by which it occurs is poorly understood

Objective & Hypothesis

- Objective: Characterize the temporal persistence of spatial memory enhancement in WT mice following FUS+MB treatment
- Hypothesis: FUS+MB-induced BBBO in the hippocampus can enhance spatial memory in WT mice

Materials & Methods

- Experimental Overview
  - Four, weekly FUS+MB-induced BBBO sessions were conducted bilaterally targeting the hippocampi
  - 1-2 week rest period (separate cohorts tested 1 and 2 weeks prior to final sonication)
  - 1 week Morris Water Maze (MWM) behavioral testing
- Animal Specifications
  - WT (C57BL/6J) Male
  - 24 weeks old at first sonication
  - One week post-FUS cohort
    - $n_{FUS} = 10$, $n_{sham} = 10$
  - Two weeks post-FUS cohort
    - $n_{FUS} = 8$, $n_{sham} = 9$
  - Sham cohort underwent equivalent treatment without activating FUS transducer
- Contrast-Enhanced T1-weighted Magnetic Resonance Imaging (MRI)
  - Intraperitoneal Gadolinium (Gd) injection administered immediately following final sonication
  - Contrast-enhanced T1-weighted MRIs acquired to confirm BBBO
- Ascend™ 400MHz WB 9.4T Bruker MRI; Avance™ III HD electronics
- Behavioral Testing (MWM)
  - 4 days trial training, 1 day probe trial
  - Latency time to reach Target quadrant and % time elapsed per Target, Opposite, Adjacent A and Adjacent B quadrants recorded in probe trial (Figure 4)

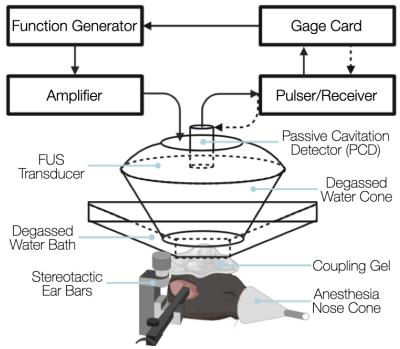

Figure 1: FUS Schematic.

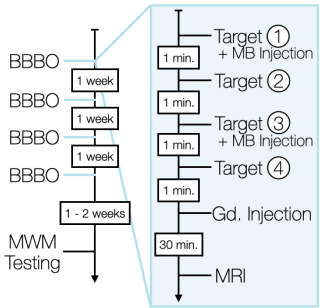

Figure 2: Experimental Timeline.

FUS Parameters	
Center Frequency	1.5 MHz
Peak Negative Pressure	450 kPa
Microbubbles	Polydisperse
	8E8 bubbles/mL
Pulse Length	10,000 cycles
Pulse Repetition Frequency	5 Hz
Sonication Duration	60 s.

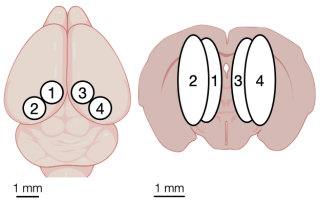

Figure 3: Sonication Hippocampal Targeting.

Results

BBBO confirmed by contrast-enhanced MRI

- BBBO confirmed in FUS+MB-treated cohorts by Gd-enhanced T1-weighted MRI imaging
- Hippocampal targeting confirmed with z-stacked axial and coronal images (Figure 5)

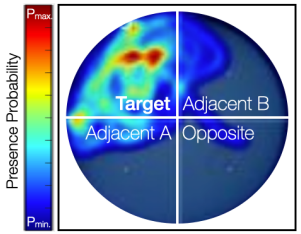

Figure 4: Representative heat map of subject trajectory in probe trial.

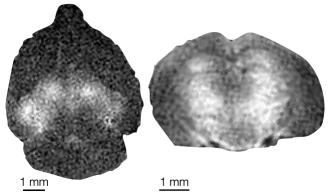

Figure 5: Representative contrast-enhanced, T1-weighted MRIs showing BBBO.

WT FUS+MB-treated mice demonstrate preference for target quadrant in MWM probe trial

- Target quadrant preference one week post-BBBO is statistically significant compared to Opposite ($P < 0.002$), Adjacent A ($P < 0.0001$) and Adjacent B ($P < 0.0081$) quadrants (one-way ANOVA) (Table 1) (Figure 6A)
- Target quadrant preference two-weeks post-BBBO is significant compared to Adjacent B ($P < 0.0369$) but preference for the Target compared to Opposite and Adjacent A quadrants is not statistically significant (one-way ANOVA) (Table 1) (Figure 6B)

MWM Testing Delay	Target Quadrant (% time)	Opposite Quadrant (% time)	Significance
1 week	43.27 ± 8.69	13.77 ± 5.11	$P < 0.0002$
2 weeks	38.09 ± 16.94	15.85 ± 7.3	ns

Table 1: Average percent time elapsed in Target and Opposite quadrants in cohorts tested one and two-weeks post-BBBO.

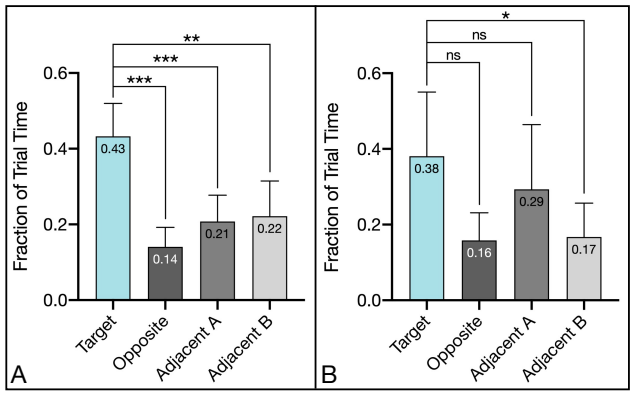

Figure 6: Fraction of total probe trial time spent in each quadrant of MWM test chamber by FUS+MB-treated mice. (A) FUS+MB-treated mice demonstrate significant preference for Target compared to all other quadrants in MWM probe trail one week after BBBO. (B) FUS+MB-treated mice demonstrate preference for Target compared to all other quadrants two weeks after BBBO, with significance only compared to Adjacent B quadrant.

Results

FUS+MB-treated mice out-perform sham mice with shorter latency times to reach platform region on average in MWM probe trial

- Both FUS+MB-treated cohorts, tested at one and two-weeks post-BBBO, consistently demonstrate shorter average latency time to reach the platform zone in the MWM probe trial (not statistically significant by unpaired t test) (Table 2) (Figure 7)
- The variance of the average Treatment and Sham latency times at both time-points differ significantly by F test statistic

MWM Testing Delay	FUS+MB Latency (s)	Sham Latency (s)	F test Significance
1 week	11.18 ± 8.45	24.00 ± 19.62	$P < 0.0282$
2 weeks	10.33 ± 3.57	17.28 ± 9.11	$P < 0.0244$

Table 2: Average latency time to reach platform zone in Target quadrant in MWM probe trial.

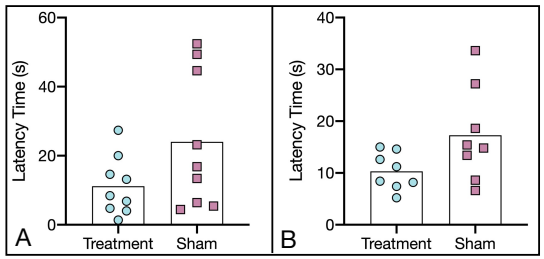

Figure 7: FUS+MB-treated cohorts demonstrate shorter average latency time to reach platform zone in Target quadrant in MWM probe trial. (A) FUS+MB cohort tested one week post-BBBO reached the target platform zone faster than sham with a shorter average latency time. (B) FUS+MB cohorts tested two weeks post-BBBO demonstrated faster average latency to target platform zone compared to sham.

Conclusions & Discussion

- Repeated FUS+MB-induced BBBO enhances spatial memory in WT mice with variable significance at different testing delay time-points
  - Target quadrant preference; one vs. two-week testing delay
    - FUS+MB-treated mice one week post-BBBO → statistically significant preference for Target compared to all other quadrants
    - FUS+MB-treated mice two weeks post-BBBO → preference for the Target compared to all other quadrants, not statistically significant for all quadrants
  - Latency to platform zone; treatment vs. sham
    - Both FUS+MB-treated cohorts, tested one and two-weeks post-BBBO, achieve shorter average latency times to platform zone compared to sham; improvement is not significant by unpaired t test
    - Treatment cohort performance at both one and two weeks post-BBBO is more consistent than sham, with a significantly smaller variance by F test statistic
- The observation of FUS+MB-induced memory enhancement in AD pathology-free, WT mice, suggests that some other biological mechanism, beyond disease pathology reduction, is responsible for previously reported and observed spatial memory improvements
- The reduction in statistical significance observed at the two-week time-point suggests that the mechanism responsible for these enhancements may be transient and revert to baseline, no longer contributing to enhanced learning, at longer time-points
- Future work will involve functional MRI of the brain post-BBBO to elucidate the cellular and network-level activations that result from FUS+MB treatment

References

[1] Pardridge, William M. 2003. "Blood-Brain Barrier Drug Targeting: The Future of Brain Drug Development." *Molecular Interventions* 3(2):90–105, 51.
[2] Leinenga, Gerhard, and Jürgen Götz. 2015. "Scanning Ultrasound Removes Amyloid-β and Restores Memory in an Alzheimer's Disease Mouse Model." Science Translational Medicine 7(278):278ra33-278ra33.
[3] Karakatsani, Maria Eleni. 2020. "Quantitative Analysis of the Focused Ultrasound-Induced Blood-Brain Barrier Opening with Applications in Neurodegenerative Disorders." Columbia University.

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