Effect of Pulse Length on Neuro-immune Response after Short-pulse Theranostic Ultrasound-mediated Blood-Brain Barrier Opening

Alec J. Batts[1], Rebecca L. Noel[1], Alina R. Kline-Schoder[1], Robin Ji[1], & Elisa E. Konofagou[1,2]
[1]Department of Biomedical Engineering, [2]Department of Radiology, Columbia University, New York, NY, USA

Introduction

- Focused ultrasound (FUS)-mediated BBB opening is a non-invasive, safe and reversible strategy for targeted drug delivery to the brain
- Disruption of neuro-immune homeostasis implicated by long-pulse FUS-mediated BBB opening to potentiate immunotherapeutic responses in AD
  - Clearance of amyloid beta pathology via increased microglial phagocytic activity using mechanical index (MI) ~0.4 [1],[2],[3]
- Theranostic ultrasound (TUS)
  - Single, multi-element imaging array technique for combined therapy and treatment monitoring [4], [5]
  - Ultra-short pulse lengths (USPL) enable synchronized BBB opening and power cavitation imaging (PCI) with the same transducer during a single sonication [6]
  - Exhibits faster reinstatement of BBB relative to long-pulse FUS [7], [8]
  - Previously characterized safety at MI of 0.4 [5], but elevated MI to 0.82 in this study to facilitate greater range of drug delivery including AAVs [8]

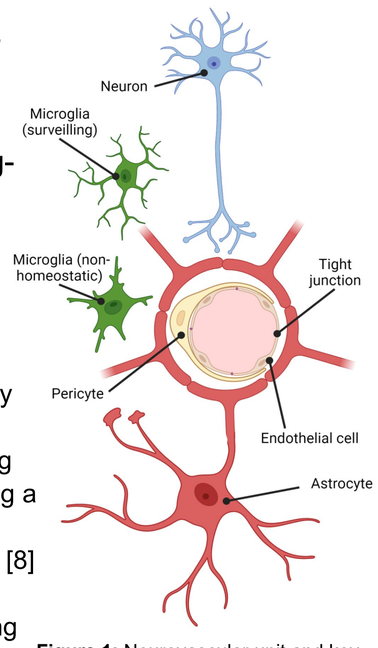

Figure 1: Neurovascular unit and key cell types in neuro-immune response

Objectives

- **Evaluate the effect of TUS and USPL on stimulation of the neuro-immune system via examination of microglia and astrocyte responses**
- **Determine the relationship between PCI pixel intensity and induced neuro-immune response**
- **Characterize reversibility of TUS-mediated erythrocyte extravasation and restoration of neuro-immune homeostasis after BBB opening**

Methods

Theranostic Ultrasound-mediated BBB Opening

- 6-8 week C57BL/6J mice (n=2/USPL/timepoint) underwent bilateral sonications with the rapid alternating steering angles (RASTA) pulse sequence for simultaneous BBB opening and PCI

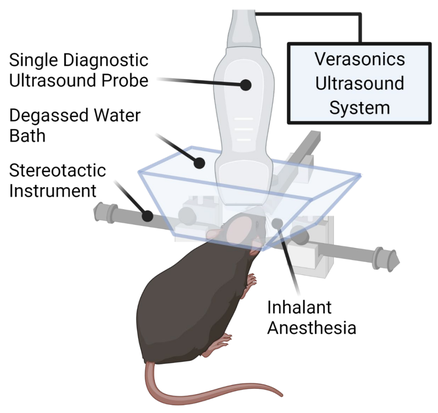

Figure 2: Schematic of TUS experimental apparatus

Table 1: TUS-mediated BBB opening parameters

Imaging Transducer	**P4-1 (ATL, Philips)**
Transmit Frequency	1.5 MHz
Bandwidth (-6 dB)	1.5 MHz – 3.5 MHz
Elements	96
Focal Depth	35 mm
Steering Angle	**± 3.72 deg**
Pulse Length	**1.5, 5, 10 cycles**
# of Sonications	2, Bilateral, simultaneous
Sonication Duration	2 min
Pulse Repetition Frequency	1000 Hz [5]
Peak Negative Pressure (derated)	**1.0 MPa**
Mechanical Index (MI)	**0.82**
Microbubbles	3.6e8 MBs/mL Polydisperse, made in-house

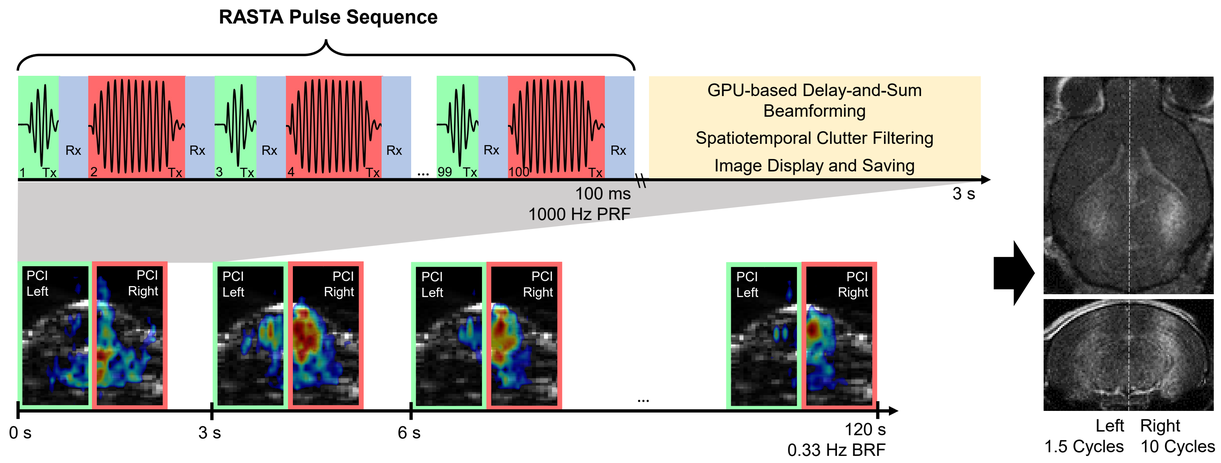

Figure 3: Overview of TUS-mediated BBB opening procedure. Left: schematic of RASTA pulse sequence and image processing pipeline. Right: representative contrast enhanced T_1-weighted MRI depicting bilateral BBB openings with target-specific opening volumes

Power Cavitation Image Analysis

- Cumulative PCI pixel intensity quantified from ROI on summed PCI map corresponding to imaging resolution afforded by each USPL
- Cumulative PCI pixel intensity was correlated with IBA1 and GFAP fluorescence

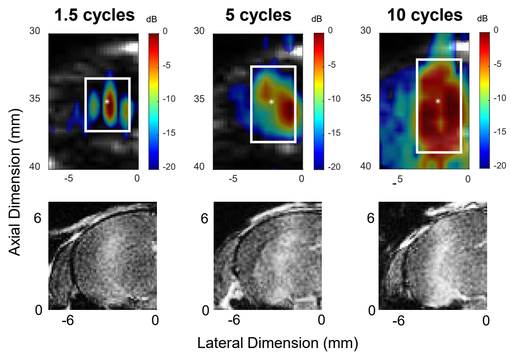

Figure 4: PCI maps and MRI depicting modulatory ability of TUS RASTA and USPL. Top: Representative summed PCI maps for each USPL overlaid onto B-mode images. Cumulative PCI pixel intensity was quantified from an ROI corresponding to the imaging resolution afforded by each USPL (white box indicates ROI for each USPL, white asterisk indicates coordinates of focus). Bottom: contrast-enhanced T_1-weighted MRI acquired 30 minutes after TUS-BBBO depicting increased BBBO volume with USPL

Histological Preparation and Analysis

- Brains were sectioned consecutively in the coronal orientation, where odd-numbered sections were stained with H&E, and even numbered sections were stained with IBA1/GFAP to register H&E images to IBA1/GFAP images

References

[1] A. Kline-Schoder, IUS 2022
[2] G. Leinenga et al., Sci. Transl. Med., 2015
[3] J. Jordão et al., Exp. Neurol., 2013
[4] R. Ji et al., IUS 2019
[5] A. Batts et al., IEEE Trans. Biomed. Eng., 2022
[6] M. Burgess et al., Phys. Med. Biol., 2018
[7] S. Morse et al., Radiology, 2019
[8] A. Batts et al., IUS 2021
[9] R. Noel et al., SFN 2022

Acknowledgements

This study was funded in part by the National Institutes of Health (R01EB009041 and R01AG038961) and the Focused Ultrasound Foundation.

Results

Incidence of reversible erythrocyte extravasation and disruption of neuro-immune homeostasis increased in a USPL-dependent manner

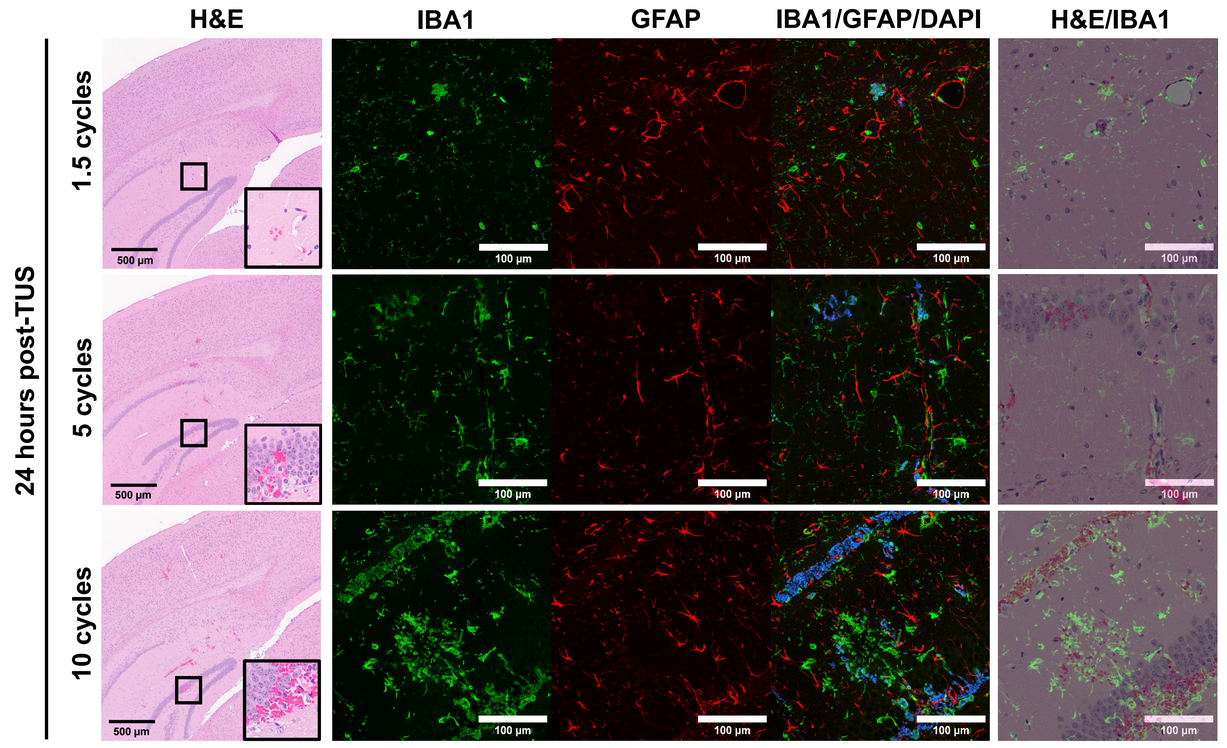

Figure 5: Histological evaluation of safety and neuro-immune activation 24 hours post-TUS. Inset in H&E images is 130 µm wide.

Erythrocyte extravasation and microglial aggregation improved in a USPL-dependent manner by 96 hours post-TUS, while astrocyte GFAP expression increased across all USPL

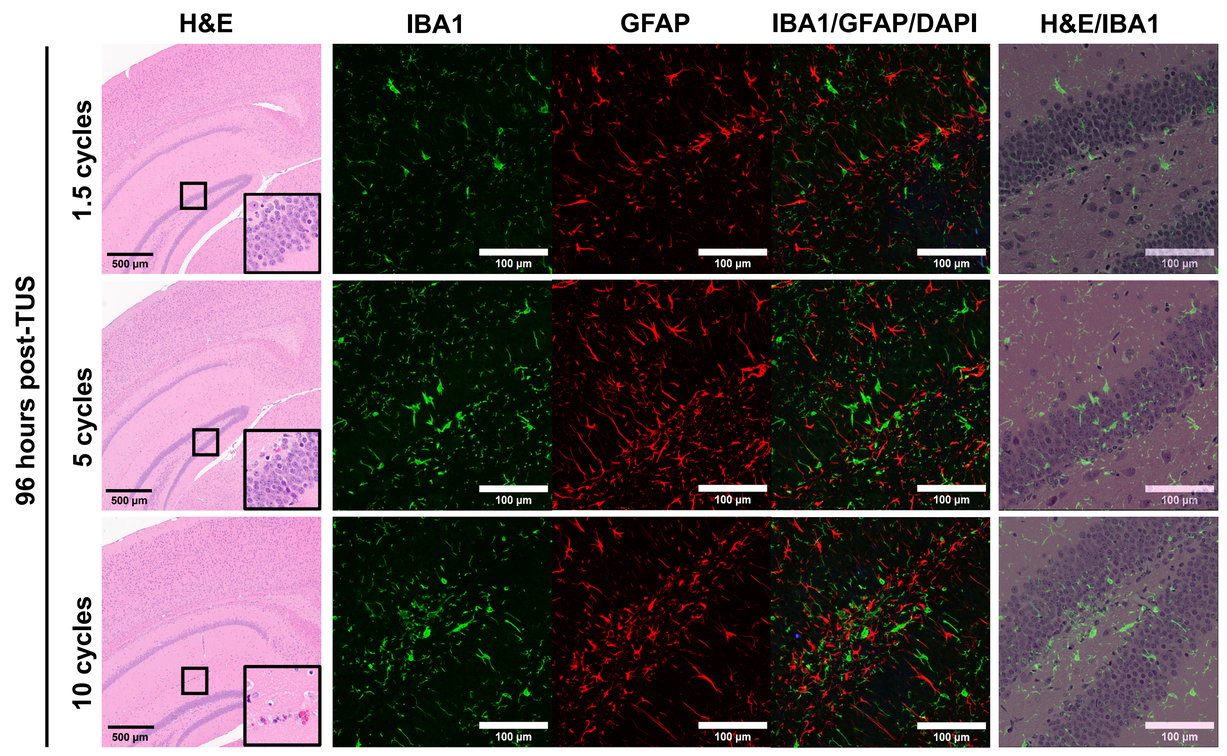

Figure 6: Histological evaluation of safety and neuro-immune response 96 hours post-TUS. Inset in H&E images is 130 µm wide.

IBA1 and GFAP responses were associated with increases in PCI pixel intensity

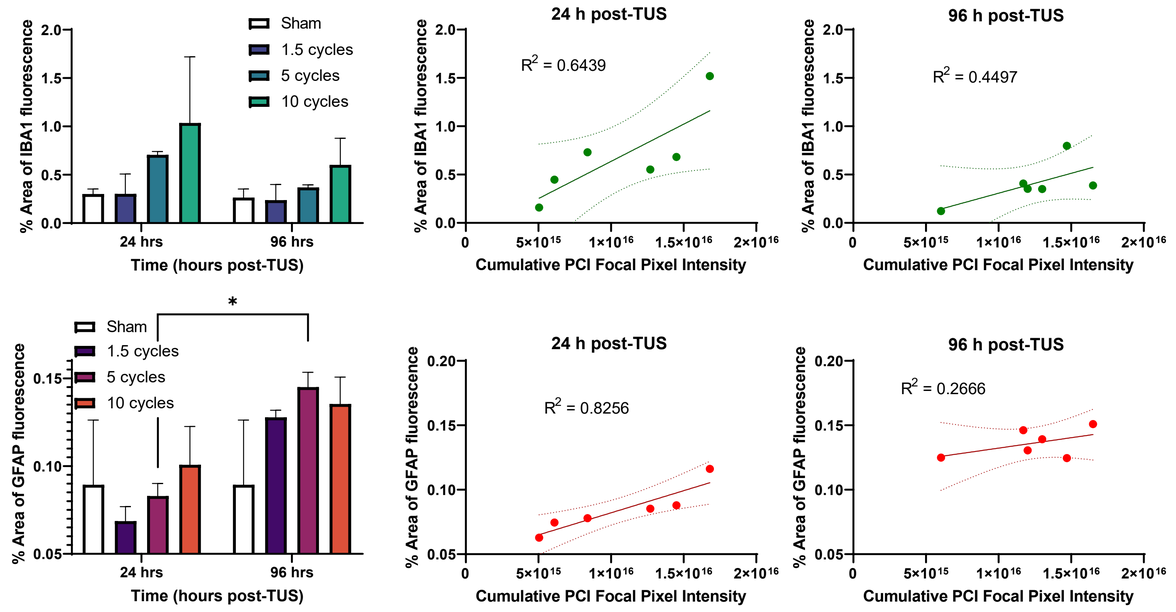

Figure 7: Quantification of temporal differences in fluorescence area for microglia and astrocytes across USPL and correlation with PCI. Top: quantification of IBA1 fluorescence area 24 h and 96 h post-TUS, and correlation with focal pixel intensity of PCI acquired during sonication. Bottom: quantification of GFAP fluorescence area 24 h and 96 h post-TUS and correlation with focal pixel intensity of PCI. Graphs on left analyzed with two-way ANOVA with Sidak multiple comparisons test (*p<0.05). Graphs on right analyzed with standard linear regression. Solid line indicates line of best fit, while dashed lines indicate 95% confidence interval.

Conclusions & Future Work

- **TUS-mediated BBB opening induced non-homeostatic microglia and astrocyte responses in a USPL-dependent manner which were associated with increases in PCI pixel intensity**
- **TUS-mediated BBB opening elicited a temporal delay in astrocyte response relative to initial disruption of microglia homeostasis [9]**
- **TUS-induced histological damage and associated microglia aggregations improved 96 hours post-BBB opening across all USPLs evaluated**
- **Future work includes characterization of cavitation regime induced by TUS, further optimization of transmit parameters to ensure safety, and translation to large animal models**

For more research pertaining to characterization of microglia response after focused ultrasound, see: **Kline-Schoder et al., IUS 2022**
For research pertaining to focused ultrasound-mediated BBB opening in Alzheimer's Disease, see: **Noel et al., IUS 2022**
For more information regarding theranostic ultrasound technology and translation, see: **Ji et al., IUS 2022**

ajb2305@columbia.edu