A neuronavigation-guided clinical ultrasound system for blood-brain barrier opening at the bedside with real-time cavitation monitoring – pre-clinical evaluation in non-human primates with behavioral amelioration and immunogenicity

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Background (character limit: 1000): Blood-brain barrier (BBB) opening using focused ultrasound (FUS) is currently being tested in multiple clinical trials. Most trials are conducted with either an implanted transducer following craniotomy or a multi-element array for MRgFUS. We recently developed a clinical system to treat Alzheimer’s disease (AD) patients based on a neuronavigation-guided FUS transducer that can achieve non-invasive BBB opening while eliminating the need of on-line MRI. Previous studies in rodents treated with FUS have shown an acute inflammatory response in the murine brain, which is resolved within 7 days after treatment. Here, we investigated the immune response in non-human primates (NHPs) treated with our clinical FUS system. Behavioral testing through a visuomotor task was also performed to assess cognitive function following BBB opening. Our aim was to investigate the effects of clinically-relevant FUS exposure in an animal model which closely resembles the human brain.

Materials and Methods (character limit: 1500): A 0.25-MHz, single-element FUS (Sonic Concepts, Bothell, WA) transducer was developed for neuronavigation-guided clinical BBB opening. The transducer’s design was based on k-wave simulations, which provided the optimal combination of aperture size and radius of curvature. Human skull-induced beam aberrations and skull heating were estimated during therapeutic sonication (MI: 0.4-0.8), using a hydrophone and a thermocouple, respectively. Four NHPs were treated in the prefrontal cortex (PFC) and motor cortex using the single-element FUS transducer (fc: 0.25 MHz, Ppk-neg: 200 - 400kPa, MI: 0.4 - 0.8, PL: 10 ms, PRF: 2 Hz, duration: 2 min) and the FDA-approved dose of Definity microbubbles (10 μl/kg). Two NHPs were treated bilaterally (MI: 0.4 and 0.8 - left and right hemispheres), and were sacrificed at the acute (48-h post-FUS) and chronic (18-days post-FUS) timepoints. Real-time cavitation monitoring was performed using a 1.5MHz passive cavitation detector. The NHP brains were stained for Iba1 and CD68 to evaluate microglia spatial distribution. Two NHPs performed a transitive inference test daily for up to 4 weeks before FUS treatment and were then treated unilaterally (MI: 0.4 and 0.8 - left hemisphere). A new set of images were presented on each day, to ensure that performance was only due to inference capacity. Behavioral testing using touch panels in the NHP home cage was conducted daily for 3 weeks post-FUS to evaluate changes in touch accuracy and reaction times.

Results (character limit: 1000): Numerical simulations showed that our system can target the human midbrain (fig. 1a). Transcranial transmission through a human skull caused a lateral and axial shift of 0.5 ± 0.4 mm and 2.1 ± 1.1 mm, while the focal size decreased by 3.3 ± 1.4% and 3.9 ± 1.8% along the lateral and axial dimension, respectively (fig. 1b-d). We measured a maximum temperature increase of 0.16 ±
0.03°C at MI of 0.8 (fig. 1e). Clinically-relevant FUS treatments led to BBB opening volumes (fig. 2a) of 680 ± 236 mm³ at MI of 0.4 (n = 3), and 1413 ± 299 mm³ at MI of 0.8 (n = 3). The density of Iba¹⁺/CD68⁺ cells within regions exposed to FUS was higher than in non-treated areas only at the acute timepoint and MI of 0.8 (fig. 2b). By day 18 post-FUS, cell numbers were restored to baseline for both MI. The increased number of cells at MI of 0.8 was due to microglia migration towards the periphery of blood vessels, indicating a repair mechanism to restore homeostasis during BBB closing (fig. 2c). Average accuracy increased for both NHPs (figs. 2d and e), but significantly only at MI of 0.8. The reaction time increased at MI of 0.4, but significantly decreased at MI of 0.8.

Conclusions (character limit: 750): We developed a clinical setup for BBB opening based on a single-element transducer with neuronavigation guidance and real-time cavitation monitoring. Transcranial ultrasound propagation caused a moderate focal shift and distortion, while the skull heating was negligible. Our pre-clinical findings in NHPs demonstrated that FUS-induced immune response can be triggered at high MI but is reversible, while BBB opening may be associated with improvement in NHP cognitive performance, both in terms of touch accuracy and reaction time. The clinical setup described here has been granted an investigational device exemption (IDE G180140) by the FDA, to achieve non-invasive and targeted BBB opening at the bedside in 6 AD patients.

Take Home message (character limit: 280): Neuronavigation-guided FUS systems allow bedside brain treatments without the need of on-line MRI guidance and with minimal focal distortions or skull heating. Additionally, clinically-relevant FUS-mediated BBB opening may lead to a reversible immune response and cognitive improvement.

Biography of the presenting author (character limit: 1000): Antonios N. Pouliopoulos was born in Thessaloniki, Greece, in 1990. He received the B.Sc. degree in Physics from Aristotle University of Thessaloniki, Thessaloniki, Greece, in 2011. As a B.Sc. student, he conducted research in the University of Bologna, Bologna, Italy, and the European Synchrotron Radiation Facility in Grenoble, France. He earned the M.Sc. degree in Nanotechnology and Regenerative Medicine from University College London, London, United Kingdom, in 2013. In 2017, he obtained the Ph.D. degree in Bioengineering from Imperial College London. He is currently an associate research scientist in the Department of Biomedical Engineering at Columbia University, New York, NY, USA. His research interests include targeted drug delivery using ultrasound, microbubble dynamics in ultrasound therapy, and ultrasound therapy monitoring.
Figure 1: a) Numerical simulations of ultrasound propagation through a human skull. b) Focal volume change and shift following propagation through a human skull. c)-d) Beam transverse profile in free field and through a human skull. e) Skull heating at clinically relevant MI (0.4 - 0.8). Black line shows the temperature increase using a higher duty cycle (DC), as a positive control.
Figure 2: a) BBB opening in the prefrontal cortex of a NHP (MI: 0.8). b) Inflammatory cell density within the targeted area after FUS treatment. Green shaded region: baseline density in a non-treated area. c) NHP brain slices stained with Iba1 and CD68, showing migration of microglia. d) Daily and e) time-average accuracy and reaction time before and after FUS treatment. Dotted line: responses by chance. Data presented as mean ± standard deviation. *: p<0.05, ns: non-significant.