**Combination of lateral beam steering and axial focusing significantly increases theranostic ultrasound mediated blood-brain barrier opening volume and viral gene delivery *in vivo***

## Background, Motivation and Objective

Simultaneous blood-brain barrier opening (BBBO) and real time high-resolution power cavitation imaging (PCI) can be achieved using a repurposed diagnostic phased array operating with custom short pulse sequences and electronic beam steering, a technique called theranostic ultrasound (ThUS). Previously, elongating ThUS pulse length at a fixed acoustic peak-negative pressure (PNP) was shown to increase delivery of agents ranging from 540 Da gadodiamide to 4 MDa adeno associated viruses (AAV), at the expense of short-term, reversible microhemorrhage in mice. This study aims to increase viral gene delivery while minimizing risk for microhemorrhage by combining ultra-short pulses with electronic beam steering and focusing to elicit widespread BBBO throughout the murine brain during a single ThUS sonication.

## Statement of Contribution/Methods

BBBO with synchronized real time PCI was achieved using a customized Verasonics script for the ThUS phased array (P4-1, ATL, Philips) where bursts consisting of 100 interleaved focused transmits (1.5 MHz $f_c$, 1.0 MPa PNP, 1.5 cycle PL, 1.0 kHz PRF, 6e8 MBs/mL, 2 min sonication) deployed at distinct focal coordinates defined by a steering angle and a focal depth, and alternating between hemispheres, were repeated every 3 seconds in anesthetized C57BL/6J mice. Two sonication paradigms were used to determine the effect of increasing the number of focal regions on BBBO volume and delivery of AAV9-hSyn-GFP at a dose of 1.1e11gc/mouse; the first consisted of a single focal region on one hemisphere (green asterisk in Fig. 1A) and three focal regions on the other hemisphere (red asterisks in Fig. 1A) in the same mouse brain, while the second was used to demonstrate the feasibility of whole-brain BBBO using nine focal regions (magenta asterisks in Fig. 1B). Contrast-enhanced $T_1$-weighted MRI and fluorescence microscopy were used to confirm the BBBO volume 30 minutes post-ThUS, and GFP transgene expression 3 weeks post-ThUS, respectively.

## Results/Discussion

Increasing the number of distinct focal regions from one to three per hemisphere elicited a significant 1.78-fold increase in the volume of BBBO (Fig. 1A, I), while PCI confirmed increased cavitation dose on the right hemisphere relative to the left hemisphere (Fig. 1C). Using nine distinct focal regions elicited BBBO throughout nearly the entire brain (Fig. 1B), yielding significant 5.54-fold and 3.11-fold increased BBBO volume associated with increased PCI cavitation dose and spatial coverage (Fig. 1D) relative to the single or triple focal region paradigms, respectively. Fluorescence microscopy of hemispheres exposed to three foci revealed increasing number of sites where neuronal GFP transduction was observed, particularly in the midbrain (Fig. 1F) and cortex (Fig. 1H), relative to a single focal region (Fig. 1E, G). A significant 1.66-fold increase in the total number of transduced cells per unit area was also observed with the triple focal region paradigm relative to a single focus (Fig. 1J). This study demonstrates the first whole-brain BBBO and PCI capabilities using a spatially fixed diagnostic phased array with a single sonication and microbubble injection.

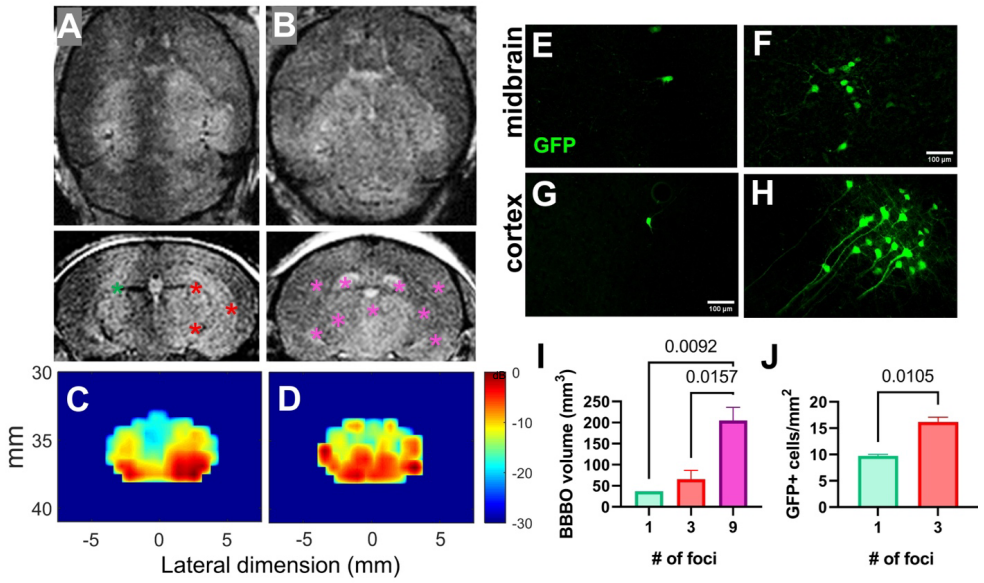


Figure 1