Biomolecule-enhanced functional ultrasound imaging of the mouse brain

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Background, Motivation and Objective

Functional ultrasound imaging (fUS) provides a unique combination of spatial coverage, spatiotemporal resolution (100 µm, 25 ms) and compatibility with freely moving animals. However, deep and transcranial monitoring of brain activity, and the imaging of dynamics in slow-flowing blood vessels remains a challenge. To enhance fUS capabilities, we are developing biomolecular hemodynamic enhancers based on gas vesicles (GVs), genetically encodable ultrasound contrast agents introduced as ultrasound analogs to green fluorescent proteins [Bourdeau, Nature 2018]. Here, we assessed the performance of GVs as hemodynamic enhancers for transcranial fUS imaging of the mouse brain, demonstrating their ability to smoothly elevate functional contrast.

Statement of Contribution/Methods

fUS was implemented with a programmable ultrasound scanner connected to a 15 MHz probe. *In vitro* characterization was performed in a flow phantom (flow velocities from 5 mm/s to 50 µm/s) comparing GVs to microbubbles (MBs) embedded in a blood-mimicking fluid. fUS was performed at 1 Hz in head-fixed, lightly anesthetized mice over 5 minute-long trials. We recorded brain activity during light-evoked stimulation (470 nm blue LED, 3Hz flashing, 15s ON / 45s OFF) of deep subcortical structures called the lateral geniculate nuclei (LGN). In each animal, we administered a single 50 µL bolus of GVs at a $6x10^9$ GV/µL concentration via tail-vein injections. GV enhancement of fUS signals was compared to saline and MB single bolus injections at previously published doses [Errico, Neuroimage 2016].

Results/Discussion

In vitro results at a 2 mm/s flow velocity showed that GVs can sustain peak positive pressures up to 590 kPa, whereas MBs showed a stable SNR until 250 kPa. In addition, GVs were the most accurate reporter of flow velocity below 500 µm/s.

In vivo results demonstrated that GV-enhanced transcranial Doppler signal fluctuations were similar to the saline case (variance of 1.95 and 2.25 respectively), whereas fluctuations of the MB-enhanced transcranial Doppler signal were one order of magnitude higher (variance of 14.64). Findings in groups of N>4 mice indicated a statistically significant increase in fUS-recorded LGN activations with GVs compared to red blood cells alone (p-value = 0.046). On the contrary, saline and microbubble injections both degraded fUS-recorded LGN activations compared to red blood cells contrast (p-value = 0.006 and 0.113).

Together, these results demonstrate that GVs provide the most accurate performance as reporters of slow flow velocities, which is critical to mapping functional activations at the level of finer blood vessels, and serve as fluctuation-free hemodynamic enhancers of fUS signals. Further engineering for enhanced circulation and contrast should make GVs a preferred enhancer of fUS contrast.