Non-invasive optogenetic activation with functional ultrasound

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Background, Motivation, and Objective

Focused ultrasound (FUS) can non-invasively facilitate gene delivery for optogenetics via blood-brain barrier (BBB) opening in mice. However, one of the primary methods for validating uptake of the viral vector into the target brain tissue involves sacrificing the animal and using histology to show the areas of activation in acute studies. Functional ultrasound (fUS) has been shown to non-invasively image brain activation-correlated blood flow changes in mice. This study demonstrates fUS can monitor successful FUS-facilitated optogenetic activation and localize the associated brain perfusion increase.

Methods

Adeno-associated viruses (AAV) encoding a red-shifted variant of the light-gated ion channel, channelrhodopsin (ChrimsonR-tdTomato, peak wavelength: 600nm), were delivered to the left hemisphere of the brain via a blood-brain barrier opening induced with FUS (1.5 MHz center frequency, 0.8 MPa peak-negative pressure, 10ms pulse duration, 5 Hz pulse repetition frequency) and co-administered circulating microbubbles (Pouliopoulos et al. 2019). The mice were allowed to recover for at least 10 days prior to the imaging, which allowed for adequate transduction of the virus and expression of ChrimsonR-tdTomato. A high intensity LED source (635 nm) and ultrasound imaging transducer (L22-14v, Vermon) were placed directly over the scalp of head-fixed and anesthetized mice (n =5; 2 control, 3 AAV). fUS was performed by acquiring a timeseries of power Doppler images (PDI) during the optical stimulation session involving a series of 10 second stimuli (10 Hz pulse repetition frequency, 10% duty cycle) and 60 second interstimulus interval. Coronal PDIs were generated by applying spatiotemporal filtering using singular value decomposition (SVD) on a stack of compounded plane wave images (200 compounded images, 500 Hz frame rate) on a Vantage system (Verasonics). Five fUS planes (Fig. 1a) were evaluated that spanned the location of the BBB opening (left hemisphere) that was validated against contrast magnetic resonance imaging (MRI; Bruker 9.4T). A correlation analysis was then performed between the stimulus pattern and the fUS time-series intensity data for each pixel (Fig. 1b) to localize correlated blood flow changes associated with neuronal activity.

Results/Discussion

MRI confirmed successful BBB opening in all five mice. Following the viral transduction period, fUS revealed significantly correlated blood flow changes (figure 1a) in all three FUS-AAV mice and no significant changes in the control mice, confirming neuronal activation following successful transduction and upregulation of ChrimsonR-tdTomato. Activation was consistent between imaging planes in the anteroposterior direction. Interestingly, the activation was found to be bilateral and not contained within the hemisphere of BBB opening, a likely result of activation of downstream brain circuits. Functional ultrasound allowed thus to monitor noninvasive optogenetic activation. Ongoing improvements in the PDI temporal resolution are expected to better elucidate the timing of hemispheric activation.

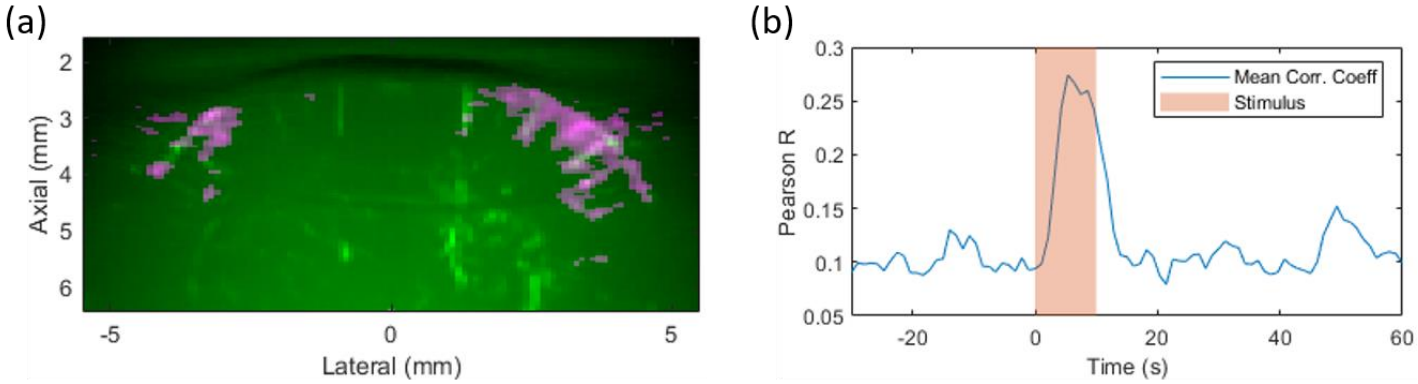

Figure 1: (a) Activity map overlaid on PDI (green) shows significantly correlated pixels (purple). (b) The mean Pearson correlation coefficient of the top decile of correlated pixels is shown (blue) with the location of the optical stimulus (red).