Identify the "single unit" of neurovascular coupling by single-vessel fMRI and optogenetics

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Target Audience Scientists who are studying high resolution fMRI, cerebrovascular imaging, neurovascular coupling and optogenetics.

Purpose In contrast to the conventional fMRI studies, we have pushed the spatiotemporal limit to detect the single vessel fMRI signal in a sampling rate of 100ms [1]. To see through the large voxels of the rat brain, the single-vessel fMRI method make it possible to decipher the contribution to fMRI signal from distinct vascular components directly. This method has made fMRI more suitable to study the neurovascular coupling mechanism [2]. To further the mechanistic study, we combined single-vessel fMRI with optogenetics [3]. The goal of this work is to initiate the neurovascular in a cell-type specific manner so that we can analyze the neurovascular events in a "single unit" mode, similar to the single unit recording for neural responses by *in vivo* electrophysiology. In the present work, we compared the sensory and optical stimulation evoked BOLD and CBV fMRI signal from layer 4/5 in the rat somatosensory cortex. Penetrating venules and arterioles in the deep layer cortex were directly mapped with MRI based on inflow effects of blood and deoxy/oxy hemoglobin susceptibility differences. The BOLD and CBV fMRI signal detected by single-vessel fMRI was specifically overlapped with penetrating arteriole and venule voxels. The light-driven fMRI signal was primarily detected in the penetrating vessels surrounding the fiber optic tips inserted into the deep layer cortex. The time course of the light-driven fMRI signal shows similar temporal pattern to the vascular propagation detected by sensory stimulation-evoked fMRI response. This work provides the basic platform for future light-driven single-vessel fMRI studies on neurovascular coupling.

Methods All images were acquired with a 14.1 T/26cm horizontal bore magnet (Magnex), interfaced to an AVANCE III console (Bruker) and equipped with a 12 cm gradient set, capable of providing 100 G/cm with a rise time of 150 us (Resonance Research). A transreceiver surface coil with 6mm diameter was used to acquire fMRI images. Line-scanning fMRI: A 2D FLASH sequence was applied to map the fMRI signal with the following parameters: TE 4ms (CBV)/16ms (BOLD), TR 100ms; matric 96x64, slice thickness, 400μm for 14T, in plane resolution, 100x100μm. As previously described [4], the single k space line was acquired for each image of the block-design stimulation pattern. The on/off stimulation trials were repeated for the number of phase-encoding steps. The field of view (FOV) along the phase-encoding direction was kept to only cover the cortical regions of interest. To reduce the potential aliasing effect along the phase-encoding direction, two saturation slices were applied to null the signal out of the FOV as previously established for line-scanning fMRI [5]. The 2D FLASH slice image was reconstructed from the reshuffled k space data with 100ms sampling rate. Single vessel multiple gradient echo (MGE) imaging: to detect individual arterioles and venules, a 2D-MGEsequence was used with the following parameters: TR: 50ms, TE, 2.5, 5, 7.5, 10, 12.5, 15ms; flip angle 40°, matrix: 192x128, in-plane resolution: 50μmx50μm, 400 μm thickness for 14T. The single vessel map is acquired by averaging the MGE images acquired from the second echo to the forth echo, where the venule voxels showed as dark dots due to fast T2* decay, but arteriole voxels remain bright dots due to the in-flow effect [1]. Electrodes were placed on the forepaw to deliver a 2.5mA pulse sequence (2s, 300μs duration repeated at 3Hz). ChR2 was expressed by AAV5 virus in the barrel cortex with CaMKII promoter for optical stimulation (2S, 50ms duration repeated at10HZ). Fiber optic (50μm) was inserted into the deep layer cortex for optical stimulation. CBV-weighted signals were obtained after intravenous administration of 15 mg Fe/kg dextran coated iron oxide (Biopal, MA). Sensory stimulation-evoked fMRI data were acquired from 3 rats and light-driven data were acquired from 2 rats.

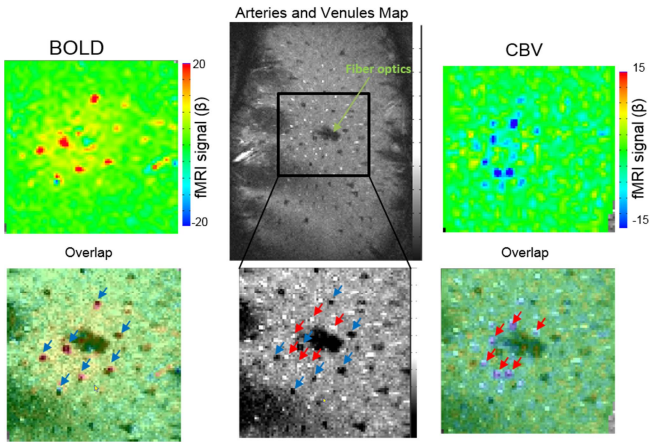

Fig 1. The light-driven BOLD and CBV fMRI maps by fiber optic mediated optical stimulation in the deep layer cortex. The overlay image is the single vessel map (red arrow, arterioles as bright spots. blue arrow, venules as dark spots).

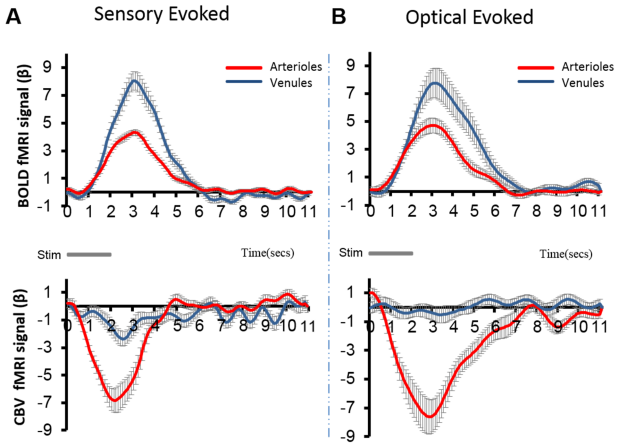

Fig 2. BOLD and CBV weighted time courses of venules and arterioles. **A**. Sensory evoked BOLD (Arterioles=43, Venules=46 from 3 rats) and CBV (Arterioles=27, Venules=30 from 2 rats) time courses. **B**. Sensory evoked BOLD and CBV time courses (Arterioles=14, Venules=27 from 2 rats). Error bars represent standard error of the mean.

Results Fig 1 shows light-driven peak BOLD signal primarily overlapped with the venule voxels (blue arrow). In contrast, the peak CBV signal overlapped with the arteriole voxels (red arrow). The sensory stimulation evoked single-vessel fMRI maps were previously reported [1] (data not shown). Fig 2. Individual arteriole and venules were directly identified based on the single-vessel map of the activated cortex. The time course of the BOLD/CBV fMRI signal from individual vessels were analyzed as the function of time after 2s electrical or optical stimulation. The arteriole BOLD signal is weaker than venule BOLD signal, but with early onset, consistent to previous report [6].

Conclusion The fMRI signal evoked by either electrical or optical stimulation shows similar temporal vascular propagation pattern. This work indicates that fiber-optic mediated light-driven fMRI signal can be alternative way to study the neurovascular coupling in the deep layer cortex. It makes it possible to link the vascular signal to specific cellular sources along the neuron-glia-vessel network.

Reference 1. Yu et al. ISMRM (2014). 2. Logothetis, Nature, 453:869-878 (2008) 3. Lee et al. Nature, 465:788–792 (2010) 4. Silva & Koretsky, PNAS, **99**:15182-7 (2002) 5. Yu et al. Nature Methods, **11**: 55-8 (2014). 6. Yu et al. NI, 59:1451-60, (2012)