Direct Imaging of Distinct Vascular Elements Detected by BOLD and Iron Oxide Based Blood Volume Using High Resolution fMRI of the Rodent Whisker Barrel

Xin Yu[1], Benjamin Porter[1], Stephen Dodd[1], Afonso Silva[1], and Alan Koretsky[1]
[1]*NINDS, NIH, Bethesda, MD, United States*

Introduction Interest in determining the spatial specificity of fMRI signals with respect to neuronal activity continues to drive NMR research [1]. A meaningful issue concerns localizing BOLD and cerebral blood volume (CBV) changes to specific vascular elements. Efforts have been made to separate large vessel effects from small vessel effects on BOLD by modeling the time course of the signal and through developing different MRI sequences [2,3]. In addition, a number of studies have used arterial spin labeling approaches and oxygen sensitivity of perfluorocarbons to separate the arterial and venous CBV changes [4,5]. It is well accepted that arterial blood volume changes occur in arterioles due to control by smooth muscle and there is increasing interest in the role of capillary volume changes in controlling regional blood flow. The balloon model of fMRI time courses highlights the venous contribution to CBV changes [3]. None of these MRI studies have directly imaged the different vascular elements involved. Recently, high resolution BOLD fMRI allowed for the separation of active voxels into those containing intracortical venules and those enriched with arteries and capillaries [6].
CBV with exogenous iron oxide particles use the loss of MRI signal due increased susceptibility in areas with increased blood volume to make detailed MRI maps. We predicted that contrasting high resolution BOLD and CBV fMRIs would allow us to delineate the different vascular components. The present study compared the time-dependent spatial patterns of high resolution BOLD and CBV signal in layer 4/5 of the whisker-barrel cortex of anesthetized rats. Results showed distinct voxels activated with CBV based fMRI that did not overlap BOLD fMRI activated voxels. Consistent with earlier work, the CBV signal started as early as 0.5s after stimulus onset. The early onset and the spatial separation from BOLD active voxels lead us to assign the CBV active voxels to the dilation of arterioles.

Methods fMRI was performed in 4, α-chloralose anesthetized rats. For each rat, CBV fMRI was performed directly following BOLD fMRI. CBV weighted signals were obtained after intravenous administration of 15 mg Fe/kg dextran coated iron oxide (Biopal, MA). Detailed experimental procedures have been described previously [7]. Briefly, all images were acquired with an 11.7T/31cm horizontal bore magnet (Magnex, UK) interfaced to an AVANCE III console (Bruker) and equipped with a 12 cm gradient set (Resonance Res. MA). A custom-built 9 cm inner diameter transmitter coil was used for transmit and a custom-built surface coil was used for receive employing a transmit/receive decoupling device. A 2D EPI sequence was run with the following parameters: TE/TR 18/800ms (BOLD), ~10ms/800ms (CBV); matrix 192x64 (BOLD), 150x60 (CBV); BW ~300kHz, flip angle 40°. For increased temporal resolution, the FLASH sequence acquired each k space line per image during an fMRI block design paradigm and repeated for the number of phase-encoding steps required to make an image [8]. The FLASH-fMRI sequence used the following parameters: TE ~4ms (CBV)/16ms (BOLD), TR 100ms; matrix 80x32, BW ~20kHz, flip angle 22°. In both sequences, a 5-pin electrode pad was placed on the whisker pad to deliver a 2.5mA pulse sequence (300μs duration repeated at 3Hz). The coronal 2D slice (in plane resolution 150x150μm, 500μm thickness) covered the barrel S1 areas based on the Paxinos atlas. The horizontal slice angle was set at 50° and the slice center was set at 0.75mm cortical depth to cover layers 4/5. EPI block design was 8 epochs of 4s on/16s off, and 3 epochs of 2s on/16s off for FLASH-fMRI. AFNI software was used for image analysis [6].

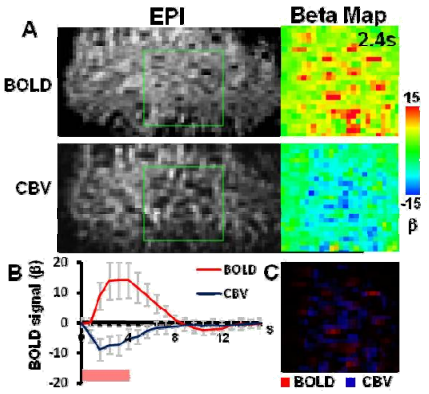

Fig 1. BOLD/CBV functional maps acquired by EPI sequence. **A**. BOLD/CBV EPI images and functional beta maps. **B**. Mean hemodynamic response function (HRF) of ROIs defined in the overlapped functional maps shown in **C** (red: BOLD, blue, CBV)

Results Fig 1A shows representative BOLD and CBV EPI images and functional maps of a slice. Low signal voxels in the EPI image were attributed to venules. Both BOLD and CBV maps punctuate activation. Fig 1B shows time courses from the punctuate active voxels in BOLD and CBV functional maps. This graph shows most of the response was in the punctuate areas, and the CBV started and peaked earlier than the BOLD signal. Consistent with a previous report [6], active areas on the BOLD functional map (2.4s after stimulation) corresponded primarily with low signal intensity areas attributed to venules. Interestingly, the BOLD punctuate activations did not overlap with the punctuate area in CBV (Fig 1C). A FLASH-fMRI sequence with short acquisition times was used to minimize large field distortions induced by iron oxide particles in the EPI images, and our method of obtaining k-space enabled a much higher temporal resolution than the EPI. Fig 2B shows the BOLD and CBV functional maps of the same slice. Active voxels in the BOLD functional map do not overlap with the active voxels in CBV functional map (Fig 2C inset). The CBV signal also has a much earlier onset (~0.5s) than the BOLD signal (Fig 2C). The early onset of the CBV and lack of co-registration with BOLD signals suggests the arteriole origin of CBV signal in these active voxels.

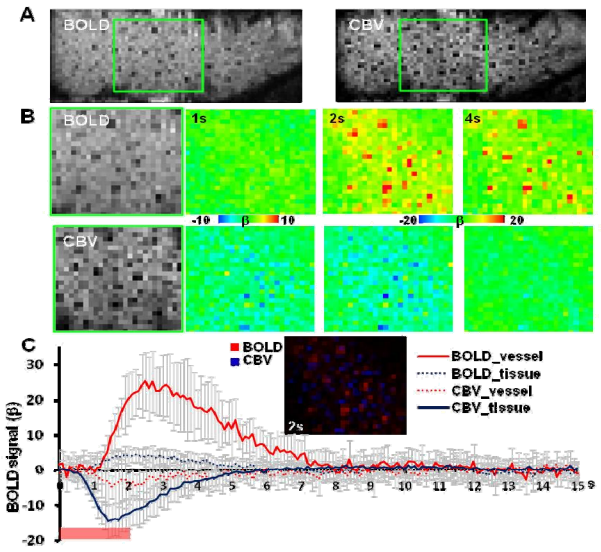

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Fig 2. Functional maps with FLASH-fMRI sequence. **A**. fMRI-FLASH BOLD/CBV images. **B**. BOLD/CBV functional beta maps at 1,2,4 s after stimulus onset. **C**. HRF of ROIs defined in the functional maps (inset, red, BOLD, blue, CBV)