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Direct imaging of macrovascular and microvascular contributions to BOLD fMRI in layers IV–V of the rat whisker–barrel cortex

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ABSTRACT

The spatiotemporal characteristics of the hemodynamic response to increased neural activity were investigated *at the level of individual intracortical vessels* using BOLD-fMRI in a well-established rodent model of somatosensory stimulation at 11.7 T. Functional maps of the rat barrel cortex were obtained at $150\times150\times500$ μm spatial resolution every 200 ms. The high spatial resolution allowed separation of active voxels into those containing intracortical macro vessels, mainly vein/venules (referred to as macrovasculature), and those enriched with arteries/capillaries and small venules (referred to as microvasculature) since the macro vessel can be readily mapped due to the fast T2* decay of blood at 11.7 T. The earliest BOLD response was observed within layers IV–V by 0.8 s following stimulation and encompassed mainly the voxels containing the microvasculature and some confined macrovasculature voxels. By 1.2 s, the BOLD signal propagated to the macrovasculature voxels where the peak BOLD signal was 2–3 times higher than that of the microvasculature voxels. The BOLD response propagated in individual venules/veins far from neuronal sources at later times. This was also observed in layers IV–V of the barrel cortex after specific stimulation of separated whisker rows. These results directly visualized that the earliest hemodynamic changes to increased neural activity occur mainly in the microvasculature and spread toward the macrovasculature. However, at peak response, the BOLD signal is dominated by penetrating venules even at layers IV–V of the cortex.

Introduction

BOLD-fMRI has become one of the most common techniques to map brain function (Bandettini et al., 1992; Kwong et al., 1992; Ogawa et al., 1992). The BOLD contrast relies on detecting the hemodynamic response to changes in neural activity. Upon functional activation, the evoked neuronal/metabolic response increases the local release of vasoactive agents, causing vasodilation and local increases in cerebral blood flow (CBF) (Attwell and Iadecola, 2002; Attwell et al., 2010; Buxton, 2002; van Zijl et al., 1998). The increased inflow of oxygenated arterial blood leads to an increase in the oxy-to-deoxyhemoglobin ratio (HbO/Hb) along the local vasculature (Fox and Raichle, 1986; Malonek et al., 1997), producing a signal increase in T_2^*-weighted MR images (Ogawa et al., 1990; Thulborn et al., 1982). Thus, the spatial specificity of fMRI is limited by the spatiotemporal dynamics of the functional hemodynamic response (HRF) (Harel et al., 2006; Kim and Ugurbil, 2003; Ugurbil et al., 2003).

Previous measurements of the functional hemodynamic response to visual stimulation estimated the full-width-at-half-maximum (FWHM) of the BOLD spatial point-spread function (PSF) to be in the range of 1.7–3.9 mm in human subjects (Engel et al., 1997; Kim et al., 2004; Parkes et al., 2005; Shmuel et al., 2007; Turner, 2002; Yacoub et al., 2005) and 470 μm in the cat visual cortex (Duong et al., 2001). Recently, in studying the reorganization of the primary somatosensory cortex a PSF of approximately 300–400 μm FWHM was also reported (Yu et al., 2010). Thus, it is clear that the signal spread through the vasculature can limit the spatial specificity of BOLD fMRI. In particular, large draining veins on the surface lead to mislocalization of BOLD signals even at high magnetic field (Keilholz et al., 2006; Kim et al., 1994; Kim et al., 2004; Lai et al., 1993; Lu et al., 2004; Ugurbil et al., 2003). In contrast to BOLD fMRI, other methods, such as arterial spin labeling MRI or cerebral blood volume MRI, have been proposed to suppress the macrovascular effect (Bolan et al., 2006; Duong et al., 2001; Lu et al., 2003; Lu et al., 2004; Williams et al., 1992; Zhao et al., 2005). However, BOLD fMRI is still the most popular functional mapping method due to the robust signal and ease of acquisition. Two major strategies have been used to eliminate the contribution of large draining veins. One strategy relies on differentiating

Abbreviations: fMRI, functional magnetic resonance imaging; BOLD, blood oxygen level dependent; SI, primary somatosensory cortex; EPI, echo planar imaging; Hb, deoxyhemoglobin; HbO, oxygenated hemoglobin; SNR, signal-noise ratio; CNR, contrast–noise ratio; PSF, point spread function; HRF, hemodynamic response function; CBF, cerebral blood flow; CBV, cerebral blood volume.

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the intra/extra-vascular dephasing properties of spins surrounding large vessels, such as spin-echo sequences or phase-dependent elimination of voxels (Duong et al., 2003; Goense and Logothetis, 2006; Menon, 2002; Ugurbil et al., 2003; Zhao et al., 2004). Another strategy relies on measuring the spatial and temporal response of the BOLD HRF. By estimating the time-dependent PSF, the initial phase of the BOLD HRF has been reported to be more spatially specific than the later phase (Goodyear and Menon, 2001; Lee et al., 1995; Shmuel et al., 2007; Silva and Koretsky, 2002). However, due to the limited spatial resolution of typical fMRI experiments, there are no studies to analyze the time-dependent contributions from microvasculature and macrovasculature to BOLD signals in the deep cortex at the level of individual vessels with BOLD fMRI.

Previously, it has been reported that there is an early onset of positive BOLD signal changes in the deep somatosensory cortex of the rat ~600 ms after stimulation (Hirano et al., 2011; Silva and Koretsky, 2002). This early positive BOLD response is significantly shorter than the half-transit time of ~1.7 s from arteries to veins measured by in vivo optical imaging (Hutchinson et al., 2006; Masamoto et al., 2010), providing evidence that fMRI changes occur before the oxy-hemoglobin can enter large veins. Based on these previous results, it may be expected that analysis of the BOLD HRF in the interval of 0.6–1.7 s following stimulus onset will reduce the macrovascular contributions to the BOLD signal. In the present work, fMRI experiments at high spatial (150 x 150×500 μm) and temporal (200 ms) resolution were performed to investigate the spatiotemporal characteristics of the BOLD HRF in the time interval of 0.6–1.6 s following stimulation of the whisker pad in chloralose-anesthetized rats.

Material and methods

Animal usage

Fifteen male *Sprague–Dawley* rats were imaged at 8–9 weeks of age. The high spatiotemporal EPI images (200 ms TR) were acquired from eleven rats. Coronal EPI slices were acquired from 7 rats and horizontal EPI slices were acquired from 6 rats (Two among the eleven rats were imaged at both coronal and horizontal orientation). The EPI images (800 ms TR) were acquired from the other 4 rats.

Animal preparation for functional MRI

A detailed procedure is described in a previous study (Yu et al., 2010). To briefly describe the preparation procedure, rats were initially anesthetized with isoflurane. Each rat was orally intubated and placed on a mechanical ventilator throughout the surgery and the experiment. Plastic catheters were inserted into the right femoral artery and vein to allow monitoring of arterial blood gasses and administration of drugs. After surgery, all animals were given i.v. bolus of α-chloralose (80 mg/kg) and isoflurane was discontinued. Anesthesia was maintained with a constant infusion of α-chloralose (26.5 mg/kg/h). The rats were placed on a heated water pad to maintain rectal temperature at ~37 °C while in the magnet. Each animal was secured in a head holder with a bite bar to prevent head motion. End-tidal CO_2, rectal temperature, tidal pressure of ventilation, heart rate, and arterial blood pressure were continuously monitored during the experiment. Arterial blood gas levels were checked periodically and corrections were made by adjusting respiratory volume or administering sodium bicarbonate to maintain normal levels when required. An i.v. injection of pancuronium bromide (4 mg/kg) was given once per hour to reduce motion artifacts.

MRI image acquisition

All images were acquired with an 11.7 T/31 cm horizontal bore magnet (Magnex, Abingdon, UK) interfaced to an AVANCE III console (Bruker, Germany) and equipped with a 12 cm gradient set capable of providing 100 G/cm with a rise time of 150 μs (Resonance Research, MA). A custom-built 9 cm diameter quadrature transmitter coil was attached to the gradient. A 1 cm diameter surface receive coil or 4-coil cortical array with transmitting/receiving decoupling device was used during imaging acquisition. 2D gradient-echo EPI sequences were used for the fMRI studies. Setup included shimming, adjustments to echo spacing and symmetry, and B0 compensation. Using the four-array coil, a single shot sequence with a 128×64 matrix on a coronal slice was run with the following parameters: effective echo time (TE) 18 ms, repetition time (TR) 200 ms, bandwidth 278 kHz, flip angle 25°, field of view (FOV) 1.92×0.96 cm. Anatomical MRI images in the same orientation were acquired using a FLASH sequence with the following parameter: TE 5.5 ms, TR 450 ms, flip angle 30°, matrix 256×128. Using the single surface coil, a single shot sequence with a 64×64 matrix was run with the following parameters: effective TE 18 ms, TR 800 ms, bandwidth 138 kHz, flip angle 45°, FOV 0.96×0.96 cm. The slice thickness was 500 μm. To clarify the contribution of inflow effects on the BOLD signal changes on vessel voxels, a single shot sequence with 128×64 matrix on a horizontal slice was run with the following parameters: TE 18 ms, TR 1.6 s, flip angle 60°, FOV 1.92×0.96 cm (the same sequence setup was also applied with TR at 0.2 s with flip angle 25° and 0.8 s with flip angle 45°). According to the rat brain atlas by Paxinos (sixth edition), the coronal 2D slice was positioned to bregma −2.0 to −2.5 mm to cover the whisker barrel somatosensory area of the animals. The angle of the horizontal slice was set at 50° to the horizontal line and the slice center was set at 0.95 mm cortical depth to cover layers IV–V of the barrel cortex. Block design stimulation paradigm was applied in this study. For the EPI sequence with 200 ms TR, the paradigm consisted of 100 dummy scans to reach steady state; followed by 80 scans during rest, 20 scans (4 s) during electrical stimulation, and 80 scans during rest, which was repeated 8 times (880 scans were acquired overall). For the EPI sequence with 800 ms TR, the paradigm consisted of 20 dummy scans to reach steady state, followed by 20 scans during rest, 5 scans (4 s) during electrical stimulation, and 20 scans during rest, which was repeated 8 times (220 scans were acquired overall). Both block designs had a total experiment time of 2 min 56 s. For the EPI sequence with 1.6 s TR, the paradigm consisted of 20 dummy scans to reach steady state, followed by 10 scans during rest, 5 scans (8 s) during electrical stimulation, and 10 scans during rest, which was repeated 8 times (130 scans were acquired overall). 16–18 multiple trials were repeated for the block design with a TR at 200 ms and 6–8 trials were repeated for the block design with TRs at 800 ms and 1.6 s.

Whisker pad stimulation design

Electrical stimulation of the whisker pad was described in a previous study (Yu et al., 2010). A homemade electrode pad with five pins (one cathode in the center of a 5×5 mm square with four anodes at each corner) was used. The current delivered to the whisker pad covered a large area (5×5 mm), which could spread to activate a large barrel area. A World Precision Instruments stimulator (WPI, FL) supplied 2.5 mA, 300 μs pulses repeated at 3 Hz to the whisker pad upon demand. To stimulate different rows of the whisker pad, a two-pin electrode pad was designed with 3 mm distance between the two pins. Given the current spread of the 5-pin electrode, the electrical pulse was set at 0.75 mA when delivered by the two-pin electrode to match a similar electrical current level.

Imaging processing and statistical analysis

fMRI data analysis was performed using Analysis of Functional NeuroImages (AFNI) software (NIH, Bethesda) (Cox, 1996). First, a 2D registration function was applied to register EPI images to a template EPI for data acquired in the same orientation setup. The baseline level of EPI images was scaled to 100 and multiple runs of block design EPI time-courses were averaged for statistical analysis. To analyze the