Temporal hemodynamic responses of BOLD fMRI in the rat brain related to electric forepaw stimulation

D. Yang[1], Z. Xie[1], J. Goodman[1], A. Burkholder[2], and N. Poy[2]
[1]BioImaging COE, Pifizer Global Research & Development, Groton, CT, United States, [2]WW Comparative Medicine, Pifizer Global Research & Development, Groton, CT, United States

Introduction

Evidence of pathways of brain connectivity associated with a stimulus under normal physiological conditions can be detected and measured using fMRI. Previous published studies of electrical forepaw stimulation (EFS) were focused on BOLD signal detection in the related cerebral somatosensory cortex (1-3). In the present study we employed the BOLD technique to investigate the temporal hemodynamic responses of the brain to the EFS. We also combined BOLD with an ASL technique to depict the temporal hemodynamic responses of the brain associated with electrical stimulus.

Methods

We evaluated 15 normal male SD rats on a Bruker 4.7T/40 MRI. All animals were initially anesthetized with 3% isoflurane on 100% O_2 for surgical preparations and mechanically ventilated (52 Breaths/min) through tracheal intubation. Prior to completion of tail vein and femoral artery cannulation, 70 mg/kg α-chloralose Intraperitoneal (IP) was given and isoflurane was gradually reduced to 0% during the next half hour. Animals were maintained during imaging with a mixture of O_2 and N_2 gas (3:7) and with 30 mg/kg/h (IV) infusion of α-chloralose. Muscle relaxation was achieved by pancuronium bromide, 1mg/kg IV bolus/30 min). Body temperature was monitored via rectal probe and maintained at 37.4±0.6ºC by a water heated bed and a water heated blanket. Direct blood pressure was monitored during fMRI studies and arterial blood gas samples obtained before and after fMRI studies to ensure normal physiological conditions and PO_2 level. Following high resolution anatomical MR imaging in the coronal orientation, single-shot EPI imaging for BOLD fMRI was performed under the following parameters: TR/TE/NAQ=1000ms/10ms/2; FOV= 32mm×32mm; matrix size= 64×64; slice thickness= 2mm; 3-5 slices without gap; center slice was placed at 5 mm posterior to the rhinal fissure; 180 repetitions with 6 minutes total scan time. Regular pattern of EFS (1min30sec off, 30sec on, 2min off, 30sec on; 1min30sec off) applied to the right or left forepaw via two needles at the following parameters: 3Hz; 0.3ms; 1.5-2.5mA. In two of the rats, single slice ASL was performed 10–15 min following BOLD fMRI at center slice position using following parameters: RARE continuous arterial spin labeling with TR/TE/Rare factor/labeling time= 2501.7ms/8ms/16/2s; 8 minutes total scan time. CBF maps were generated every 30s. During the ASL studies, EFS (5min off, 30 sec on, 2min30sec off) was applied to the rats. AFNI (Analysis of Functional NeuroImages) was used for fMRI data analysis. BOLD images were smoothed using a Gaussian kernel with 0.8 mm FWHM. General linear modeling was performed on each subject. Activated regions were identified with the threshold set to $p< 0.01$ and cluster size > 5 pixels. Serial CBF maps were generated in MATLAB.

Results

The neurovascular response in the contralateral somatosensory cortex was observed in 11 rats. The response was not observed in four rats that had an abnormal blood gas physiology (PO_2< 100mmHg). Depending on stimulus strength in each experiment, the BOLD signal was detected between 3 and 7 mm posterior to the rhinal fissure with the main signal located 3 mm posterior to the rhinal fissure (Fig.1), A higher electric current increased the number of pixels in which statistically significant BOLD signal changes were observed, suggesting that a larger brain area was involved in activation and/or that blood circulation was higher in response to the stronger stimulus. We were also able to observe a response in the ipsilateral cortex 6 – 60 seconds after the EFS (Fig.2-3). Furthermore, BOLD signal and location was correlated with CBF maps in the two rats in which ASL was performed (Fig.3), though after EFS, the ipsilateral BOLD signal increased sooner than the CBF signal.

Discussion

Previous studies of EFS have shown distinct patterns of activation of the sensorimotor cortex using the BOLD technique (1-3). In this study, we found that hemodynamic responses of the BOLD signal was not only stimulus strength (electrical current) dependent, but also time dependent. The BOLD signal enabled us to detect delayed brain activation, which spread out to the ipsilateral sensorimotor cortex from the expected contralateral somatosensory cortex. Moreover, this finding of temporal hemodynamic responses of the BOLD signal was correlated with CBF maps collected following ASL in two rats. fMRI can monitors the combined or integrated effect of neuronal interactions and reflects activity of the true neural circuitry, which can act as a key translational technology for CNS research and provide a bridge from human to animal and back again (4). Although more work needs to be done to elucidate the physiology underlying the observed spatio-temporal response, initial results are certainly intriguing.

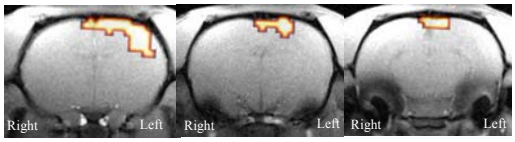

Fig.1. Three-slice BOLD fMRI experiment. Statistical parametric map was superimposed on high-resolution FLASH images (2 mm slice thickness) for right forepaw stimulation. The slices are located 3 (left image), 5 (middle image), 7-mm (right image) posterior to the rhinal fissure. Positive BOLD response (red/yellow) are observed in contralateral forepaw (left) areas of the primary somatosensory cortex and extended to adjacent areas.

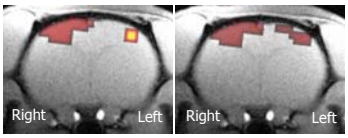

Fig.2. Statistical parametric map of BOLD fMRI experiment was superimposed on high-resolution FLASH images (2 mm slice thickness) for left forepaw stimulation. The slices are located 3-mm posterior to the rhinal fissure. Positive BOLD response (dark red) are observed in right primary somatosensory cortex (left image) and spread out to left primary somatosensory cortex area at same slice with setting of 6-sec delay times (right image).

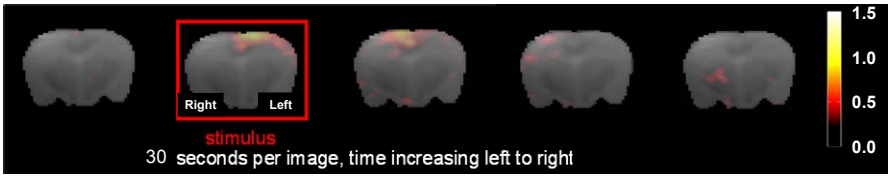

Fig.3. Local CBF response to electric stimulus. The time course ASL images show local CBF changes before, during (red frames) and after electric stimulation on single slice (same rat with middle slice position shown in Fig.1). The color bar shows relative increase in CBF from baseline values for each pixel within the same animal.

References: 1. Weber R et al (2006). NeuroImage, 29: 1303-1310. 2.Van Camp N et al (2006). NMR Biomed, 19: 10-17. 3.Kida I et al (2008). Neuroscience Research, 62: 25-31. 4.Borsook D et al (2006). Nature Rev. Drug Discove., 5: 411-424