Mapping Neuropharmacological-Evoked BOLD Signals in Rat Brain under Isoflurane Anesthesia: A Feasibility Study

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Introduction: BOLD (blood oxygenation level dependent) fMRI technique is a noninvasive procedure that has been extensively used for localizing and studying the brain activities in human and in animals [1, 2]. With this technology, specific neuronal functions of the brain can be mapped reliably in terms of organization and operation. Most BOLD-fMRI of animals has been performed under general anesthesia to minimize motion and stress-related artifacts. However, using anesthesia will also suppress the cerebral metabolism and blood flow, thus limits the full potential of BOLD fMRI. In most fMRI studies on rats, α-chloralose has been used for anesthetics, as it is generally considered to have minimum effects on functional responses in comparison with other methods [3]. However, using α-chloralose, the animals must be mechanically ventilated during the entire scanning, it is relative difficult to maintain the stable physiological conditions for animals over a long duration.

The aim of this study was to develop a robust and yet reliable technique for mapping BOLD fMRI signals on rat challenged with neuropharmacological drugs [4]. Isoflurane anesthesia was chosen because it is simple to setup, yet, the animal's physiological conditions are stable during spontaneous breathing. A multi-shot SE-EPI (spin echo echo-planar imaging) sequence was also implemented to measure the BOLD signal changes in anesthetized rats.

Methods: In total, 13 Sprague-Dawley rats (300-350 g) were used in this study. Animals were initially anesthetized with 3% isoflurane in O_2 at a rate of 3L/min and were maintained at 1.5% isoflurance at 1L/min during image acquisition via a face mask. The tail vein was cannulated with a 25–gauge needle for intravenous drug infusion. The needle was attached to tubing that run outside the magnet to allow infusion of drugs without re-positioning. SE-EPI sequence was used for all the fMRI studies. To reduce image artifacts due to susceptibility effects and increase the spatial resolution, 4-segment images were acquired with the following parameters: bandwidth 200khz, repetition time 3000 ms, effective echo time 50 ms, field of view 25.6 mm with a 128x128 matrix, corresponding to an in-plane resolution of 0.2x0.2 mm^2. Eight contiguous slices covered the whole rat brain with slice thickness of 2 mm, spaced 0.1 mm apart. To assess drug evoked BOLD signal changes in rat brain after the injection, the total imaging time was 40 minutes and total number of images were 200. Following 5-min of baseline image acquisition after saline injection, D-amphetamine was manually injected over a 1-min interval; and images were continuously acquired for the rest of the time course. The data were analyzed off-line using functions in SPM2. After motion correction, the time-series images were Gaussian smoothed to increase statistical analysis robustness. Function maps were then generated by applying a voxel-wise Student's t-test between the images pre- and post-injection. The threshold of statistical significance was set at $P<0.05$. The function maps were overlaid on the corresponding high resolution images.

Results: Figure 1 shows the significant BOLD signal changes in rat brain, using 1.5% isoflurance anesthesia, with an acute challenge of D-amphetamine (3mg/kg, i.v.). The signal extended from cortex to cerebellum, with the most significant changes in cortical regions. Higher dose induced responses at deeper and center structures (data not shown). The time-course of BOLD signal percentage changes (Figure 2) showed that the peak time was approximately 3 min after infusion of 3 mg/kg D-amphetamine, and different structures generated different drug-uptaking related neuronal responses.

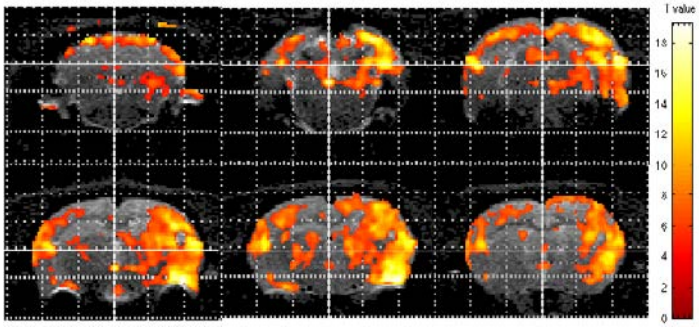

Figure 1: BOLD signal change following D-amphetamine challenge

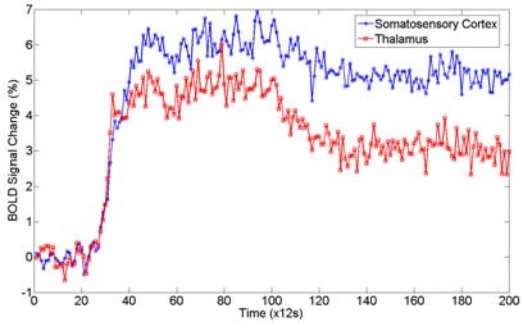

Figure 2. BOLD signal change time course.

Discussion: Isoflurane has been known to suppress neuronal activities [7, 8] and thus would reduce BOLD responses related to functional changes. α-chloralose has therefore been widely used for anesthetics in fMRI studies in animals. As shown in this study, it is possible to measure BOLD signal responses under isoflurane-anesthetized conditions, and its concentration may be critical in order to generate accurate measurements. Different levels of isoflurane mixture were tested while keeping O_2 at a constant rate of 2L/min. Reliable BOLD contrast changes were detected at 1.5% isoflurane. At lower concentrations, the respiration rates of the animals were not stable after drug infusion, and severe motion artifacts were detected. As one would expect, the dosage of amphetamine has also significant effects on the BOLD response, both dynamically and in spatial extents. Our study showed that, as the dosage of amphetamine increased to 4 mg/kg, BOLD responses increased dramatically. Overall, this study showed that, the multi-shot SE-EPI protocol, in combination with isoflurane anesthesia, is a simple, yet sensitive method for functional imaging in rats.

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