Direct Observation of Heroin-Induced Cerebral Blood Flow Change in Rats

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Introduction: Recently, pharmacological MRI (phMRI) using BOLD contrast has become a powerful non-invasive tool to assess the efficacy and side effects of drugs *in vivo*. However, confounding factors such as arterial blood pressure and respiration perturbation affect the interpretation of BOLD signal changes. In addition, discrepancy in baseline signal between normal and diseased conditions also limits the application of the BOLD method in drug development, especially for treatment of pathological conditions (1). Significant evidence has been presented that CBF is a faithful indicator of neuronal activation during functional task under various baseline conditions. Therefore, direct measurement of CBF in drug studies would provide more valuable information. In this study, a two-coil arterial spin labeling (ASL) technique (2) was implemented on a 3 Tesla MRI system. Direct CBF measurements were validated using the hypercapnea and forepaw stimulation paradigms. This technique was applied to study cerebral blood flow changes induced by heroin in a rat model.

Methods: Perfusion imaging using ASL was implemented on a Bruker Biospec 3T/60 system. The spin labeling was delivered through a second RF channel using a 5 mm diameter "Figure-8" RF coil, located at the neck of the rat. Images were acquired using a custom-built saddle RF excitation coil 5 inch in length and 5 inch in diameter, and a one-turn, 2 cm diameter surface receive coil. The RF coil set was inserted into a homemade cylindrical local gradient coil, which, at 100 A, produced gradient fields of 21.30 Gauss/cm, 20.83 Gauss/cm, and 41.20 Gauss/cm in the X, Y, and Z directions, respectively. Gradient-echo EPI time series were collected with alternating on/off tagging states. The imaging parameters were: TR = 2500ms, TE = 27.2 ms, tagging duration of 2000 ms. The tagging efficiency used for perfusion calculation was 0.85. The imaging slice was selected at interaural 9.2 mm.

Animal preparation: Six male Sprague-Dawley rats (250-350g) were anesthetized with isoflurane 1-2% (v/v) vaporized into a mixture of O_2:air (1:1) during surgery. The right femoral vein and artery were cannulated for drug or saline delivery and monitoring mean arterial blood pressure (MABP), respectively. After tracheotomy, rats were ventilated with room air using a rodent ventilator to maintain normal blood gases and pH. The body temperature of the rats was maintained at 37 ± 1^{O}C by pumping warm cycling water through a blanket. After surgery, anesthesia was switched from isoflurane to α-chloralose with initial dose of 80mg/kg, followed by 40mg/kg every hour during fMRI. Additionally, 250 mg/kg/hr gallamine i.v. was administered for each rat during fMRI experiments to minimize motion.

Hypercapnea: Two rats were used in the hypercapnea study. Experimental paradigm included 3 min of breathing room air, followed by 4 min of 7.5% CO_2, and 5 min of room air. Average CBF during hypercapnea and normocapnea were compared.

Forepaw Stimulation: Two rats were used in this study. Two needles were inserted into the left forepaw s.c.: one between digits 2 and 3, and the other between digits 4 and 5. Square wave electrical pulses of 0.3 ms duration at 1.5 mA were delivered through the needles at a repetition rate of 3Hz. The experimental paradigm included 150 sec of rest followed by 6 cycles of 50sec on / 100 sec off stimulation.

Heroin injection: Two rats were used in this study. Intravenous injection of 0.1mg/kg heroin was delivered at 5 min after the start of the 25 min scan. CBF time series were fitted to a differencial exponential (DiffExp) model using AFNI (3). CBF percentage changes were calculated for each voxel. Four regions of interest were selected: prefrontal cortex (PFC), cingulate (CING), caudate and putamen (Cpu), and parietal cortex (PRC).

Results: Global CBF increase was observed with hypercapnea, with the increase higher in cortical regions (GM) than in the corpus collosum (WM) (Data not shown). This is consistent with previous observations by other investigators (4). Fig. 1a shows the stimulation-induced CBF activation map overlaid on an EPI image, using a correlation coefficient threshold of 0.21 ($p<0.02$). ΔCBF time series of a representative voxel in the active region is shown in Fig.1b. The average ΔCBF in the region is 60%, consistent with previous findings. Fig.2a shows the CBF map in an ROI covering the prefrontal, parietal and cingulate cortices, caudate and putamen, overlaid on a high resolution anatomical image. Fig 2b shows the CBF time series in the ROI covering caudate and putamen. Table 1 compares the heroin-induced relative peak ΔCBF in four different brain regions.

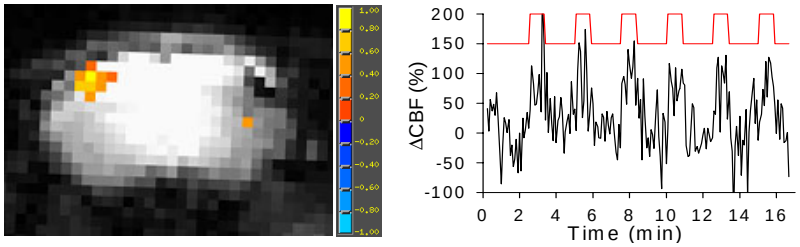

Fig.1 Forepaw stimulation: (a) ΔCBF activation map overlaid on an EPI image. (b) ΔCBF time series from a representative activated voxel. The box car function in red shows the timing and duration of the stimulation.

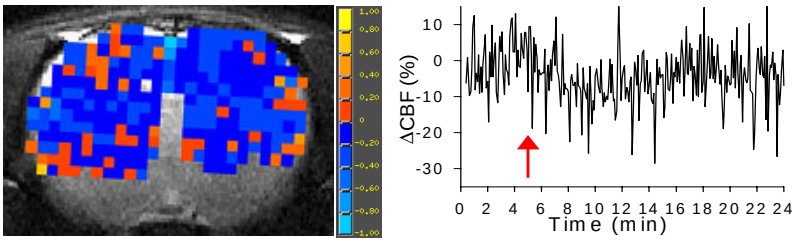

Fig 2. Heroin injection: (a) peak ΔCBF map overlaid on the anatomical image. (b) ΔCBF time series in caudate and putamen. The arrow indicates time of iniection.

Table 1. Comparison of heroin-induced CBF changes in different brain regions.

ROI	PFC	CING	Cpu	PRC
Number of voxels	55	14	115	127
ΔCBF(%)	-11.9	-15.85	-8.74	-7.46

Discussion: Successful CBF measurements using two-coil ASL were previously reported at 9.4 T and 4.7 T on rodents. The potential limitation to transfer this technique to mid-strength scanners (e.g., 3 T) was contrast-to-noise ratio. Here, direct measurement of CBF was performed at 3 T, and validated using the hypercapnea and forepaw stimulation paradigms. The ΔCBF measured at 3 T during forepaw stimulation is comparable to that at 9.4 T (5). The implementation of this CBF measurement technique at 3 T is significant for translational research from animal models to human studies, as most high-field human fMRI studies are performed at this field strength.

It has been shown previously that heroin induces negative ΔBOLD and ΔCBV in sub-cortical brain regions, possibly due to μ-receptor induced direct inhibition (6). Using the two-coil ASL technique, reduction of blood flow was directly observed in four different brain regions when heroin was injected. This observation is consistent with BOLD and CBV findings, without being confined by different baseline conditions. In addition to drug studies, application of this method can be generalized to the evaluation of other pathological conditions such as stroke, head trauma or aging.

References: 1) Brown GG, *et al.*, *JCBFM*, (23) 829-837, 2003; 2) Silva AC, *et al.*, *MRM* (33) 209-214, 1995; 3) Cox RW, *et al.*, *MRM*. 33:230-236, 1996; 4) Sicard K, *et al.*, *JCBFM*, (23) 472-481, 2003; 5) Doung TQ, *et al.*, *MRM* (43) 383-392, 2000; 6) Xi Z, *et al.*, *MRM* (48) 838-843, 2002.

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