Origin of Heroin Induced Negative BOLD in the Rat Nucleus Accumbens: Oxygen Metabolism Assessment

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Introduction. Contrary to occasionally observed negative blood oxygenation level-dependent (BOLD) fMRI signals during a cognitive task, psychoactive-pharmacological challenge induced negative BOLD signals in subcortical regions are a common phenomena with an unknown mechanism (1). In this study, cerebral blood volume (CBV) and cerebral metabolic rate of oxygen utilization ($CMRO_2$) co-localized with negative BOLD signals during heroin (0.1 mg/kg) challenges was assessed at rat nucleus accumbens (Nacc), where γ-aminobutyric acid (GABA)-ergic interneurons predominate, by a transient-state biophysical model (2). Oxygen metabolism has long been used as a functional index for neural activation. $CMRO_2$ as well as CBV information was acquired in this study to interpret the negative BOLD neural origin. It is suggested that μ-receptor mediated direct inhibition on the GABAergic interneurons induced a decrease in oxygen metabolism and in CBV decrease and that is also the cause of heroin-induced negative BOLD in Nacc.

Materials and Methods. *Animal Preparation:* Eleven male Sprague-Dawley rats, weighing 300-350g, were used in fMRI studies. Seven underwent heroin (0.1 mg/kg) challenge and four underwent sham stimulation (saline). The details of animal preparation for fMRI study were described in Ref.2. Briefly, all rats were tracheotomized under urethane anethesia (1.2 g/kg) and artificially ventilated to maintain stable physiological levels. The right femoral vein and artery were cannulated for drug and saline delivery and monitoring mean arterial blood pressure (MABP) separately. A warm circular water blanket was used to maintain rat body temperature at 37±1^{O}C. *fMRI experiments:* fMRI experiments were performed on a Bruker Medspec 3T/60cm scanner using a custom-built, 2-inch long, 1.5-inch-diameter RF birdcage volume coil, inserted into an in-house-made cylindrical local gradient coil. In order to minimize motion artifacts, the rat head was immobilized within the RF coil using a bite bar. Galamine was administered intravenouly to further minimize motion. Anatomical localization acquired by RARE revealed Nacc at the interaural 11.2 mm plane of the rat brain. A single-shot, gradient echo EPI sequence was used for functional imaging with FOV=3.5 cm, slice thickness=2 mm, image matrix=64 x 64, giving an in-plane image resolution of 550 x 550 μm, TR=1 sec, TE=27.2 ms, and bandwidth=±62.5 kHz. Each rat underwent five 20-min scans. Experimental time schedule is shown in Figure 1. *Data analysis:* Voxel time courses in the BOLD (first and second scans) and CBV (fourth and fifth scans) experiments were analyzed by DiffExp model using AFNI 3DNLfim. Voxels were considered significantly activated with F test ≥ 11 (corresponding to $p<0.05$ for each voxel after Bonferroni correction). Two 14-voxel ROIs in both sides Nacc were defined in the interaural 11.2 mm slice. Voxel-wise $CMRO_2$ was calculated with significant voxel time courses of BOLD and CBV (2).

Results. CBV decreased as well as oxygen metabolism decreased in the Nacc co-localized with negative BOLD in Nacc (Fig.2). Statistical results (mean±SD) were shown in Table 1.

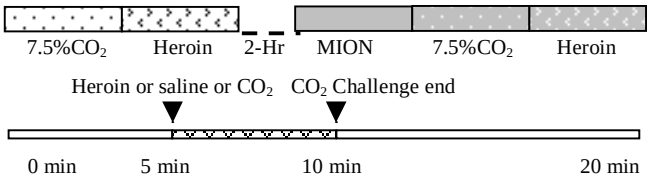

Fig.1 Experimental protocols were conducted sequentially with five scans, starting with hypercapnia challenge followed by a heroin challenge. After a two-hour pause, MION was infused and followed by second hypercapnia and heroin challenges. Each scan lasted 20 minutes; challenges were administered 5 minutes into the scan.

Table 1. Heroin-Treated vs. Saline Control (*: $p<0.05$)

	Heroin-Treated		Saline-Treated	
	Intensity	Voxels	Intensity	Voxels
BOLD	-3.9±4.5*	27.1±1.4*	-0.2±0.3	7.2±1.5
CBV	-3.0±4.0*	27.1±1.4*	0.3±0.4	5.6±3.5
$CMRO_2$	-6.2±7.7*	9.5±5.2*	0±0	3.0±2.9

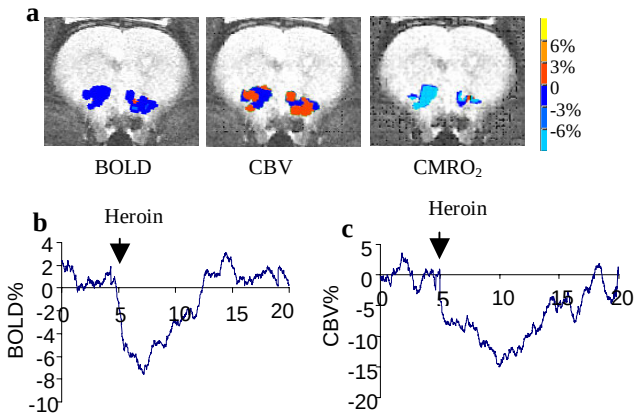

Fig. 2. Negative BOLD, decreased CBV and decreased oxygen consumption maps in Nacc were displayed in the upper row (Fig.2a) after heroin challenges. A decreased BOLD intensity and decreased CBV time course from one voxel (smoothed) after heroin infusion in Nacc were displayed in Fig.2b and Fig.2c, respectively. The arrows indicate time of heroin challenge in each scan.

Discussion. Nacc is a relatively homogenous structure dominated (>90%) by GABAergic cells with μ-receptors located on the afferent side of the targeted GABAergic neurons (3). Therefore Nacc was selected as a simple template to interpret the negative BOLD neural origin. A 6% decrease of oxygen metabolism coupled with decreased CBV was found in the Nacc during heroin infusion. This finding is comparable to a metabolic 8-10% decrease measured by autoradiographical techniques using [1-^{14}C]octanoate labeling (4). System or regional adminstration of morphine into the Nacc also showed decrease in CMRglu (5). Heroin acting on μ-receptor of GABAergic cells, inducing a direct inhibition could account for the negative BOLD signals in Nacc. This study also provides some evidence that pharmacologically-induced negative BOLD signals share the same mechanism of the positive BOLD: the signal sources are mainly coming from the neural afferent input. The developed transient-state biophysical model (2), specifically for calibrating the pharmacologically-induced non-steady state BOLD contrast, provides a way to interpret the complicated pharmacological BOLD signals.

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