Negative BOLD Signals Correlate with Synchronized Delta Oscillations Induced by Heroin in the Nucleus Accumbens of the Rat Brain

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Introduction. A recent study demonstrated that positive hemodynamic signals measured by optical imaging correlate tightly with synchronized gamma oscillations measured simultaneously with the use of implanted microelectrodes in anesthetized adult cats with visual stimulation (1). This result has strengthened a hypothesis that the positive blood oxygen level dependent (BOLD) signal is correlated to a change in local field potentials reflecting many postsynaptic depolarizations from excitatory neurotransmission (2). It is not clear, however, how negative BOLD signals are linked to evoked potentials. In the present study, a rat model was employed and the measurements of local EEG changes in the nucleus accumbens (NAc) were performed by systemic administration of heroin. Under the same experimental conditions, a negative BOLD signal in the nucleus accumbens was robust. It is hypothesized that the negative BOLD signal and the synchronized delta oscillations are correlated.

Materials and Methods. ***Animal Preparation*:** Eighteen male Sprague-Dawley rats weighing 250-350 g were used. ***FMRI Experiments*:** FMRI experiments were performed on a Bruker Biospec 3T/60 cm scanner. A 2-mm thick coronal slice, centered approximately 2.2 mm from Bregma was acquired. A single-shot, gradient-echo echo-planar imaging sequence (FOV = 3.5 cm, image matrix = 64 × 64 giving an in-plane image resolution of 550 × 550 μm, TR = 2000 ms, TE = 27.2 ms, bandwidth 125 kHz) was used for functional imaging. ***Experimental Design*:** A 45-min scan was acquired with intravenous drug treatment during scanning. The rats were evenly divided into three groups. The first received a 0.1 mg/kg heroin treatment 5 min into the scan. The second group received naloxone (10 mg/kg) pretreatment 5 min before scanning. The third received two treatments: 0.1 mg/kg heroin treatment 5 min into the scan and naloxone (10 mg/kg) treatment 10 min into the same scan. All sham treatments for multiple drug injections were performed and showed little effect, as described previously (13). After the experiments, the rats were euthanized by disconnection of the ventilation tubing. All animal protocols were approved by the Laboratory Animal Safety Committee of MCW. The heroin was licensed and obtained from NIDA. ***fMRI Data Analysis*:** The BOLD fMRI signal in each voxel was fitted with a nonlinear differential exponent model, according to its pharmacological and functional responses using AFNI v2.2 software. Voxels were considered significant based on a goodness-of-fit F-test ≥10, (corresponding to P<0.001 after the Bonferroni correction). Significant drug effects were determined using a Student's t-test based on changes in voxel numbers and AUC. Significance was set at $p < 0.05$ throughout. ***EEG Study*:** To study the mechanisms of the heroin-induced negative BOLD signal in the NAc, we performed experiments outside the scanner to measure changes in local neural activity in the NAc before and during heroin administration (0.1 mg/kg IV.). In five rats, heroin was administered twice, 45 min apart. In another five rats, heroin was infused the second time after treatment with naloxone (10 mg/kg, IV). Saline, the vehicle for heroin, was infused as a control in five more rats. The experimental conditions of anesthesia, ventilation, animal preparation and physiological maintenance were the same as for the fMRI studies. Local EEG (field potential) was measured in the NAc using concentric bipolar semi-microelectrodes (tip contact diameter 0.1 mm, upper contact diameter 0.25 mm, contact separation 0.25 mm). Two electrodes were placed stereotaxically, one in each hemisphere. There was no hemispheric difference in the results.

Results. Fig. 1A shows that naloxone has no effect on the BOLD signal in the NAc; Fig. 1B shows the synchronized delta signal after heroin injection; Fig. 1C shows a negative BOLD signal after heroin administration. Figs. 1 D and 1E show the synchronized delta signal oscillations and negative BOLD signal after heroin administration, respectively, and such signal changes were reversed upon administration of naloxone, a μ-receptor antagonist. The right panel of Fig. 1 shows power distribution of EEG signal under saline, heroin, and naloxone administrations. The dominant frequency is the delta band after heroin.

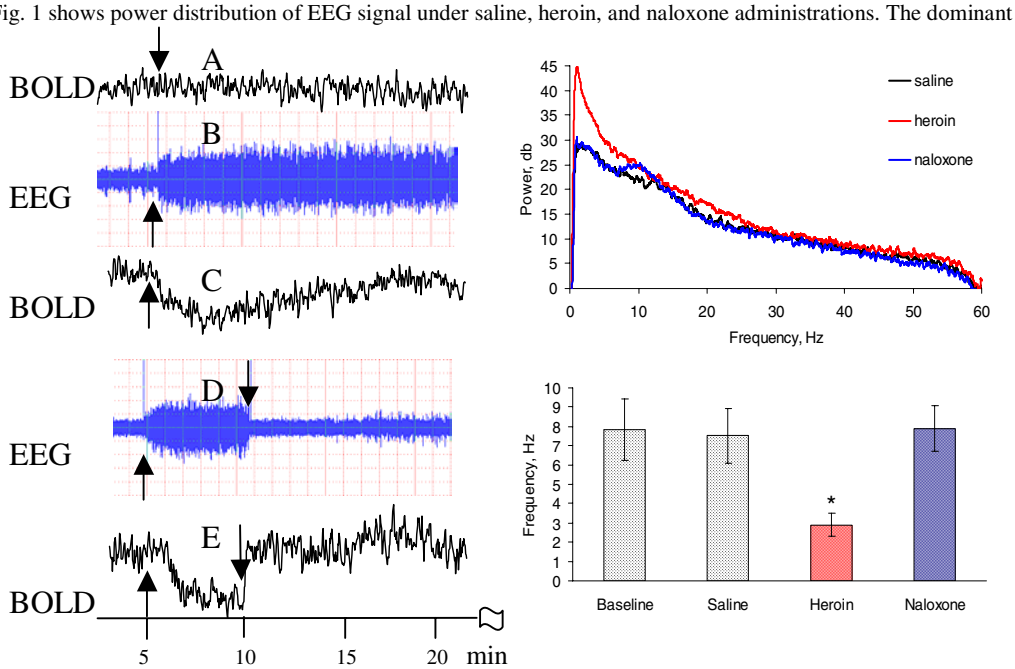

Figure 1. (Left panel) The BOLD and EEG signal time courses in the NAc region under different administrations of naloxone and heroin. (Right panel) Effect of heroin modulation on the power and frequency of field potentials in the NAc of the rat. Top: Field potential frequency distributions after the sequential infusions of saline, heroin and naloxone. Frequency spectra were obtained by Fast Fourier Transform (FFT) and the spectra were smoothed by a moving average (100 points = 5 Hz window at a sampling frequency of 500 Hz). Note the robust enhancement of low frequency power by heroin and the reversal of this effect by nalaxone. Bottom: Mean power frequency calculated from the spectra from all experiments. Also shown are data from baseline condition prior to saline infusion. Heroin decreased mean power frequency from approximately 8 Hz at baseline to 3Hz - a change that was significant at the p<0.0001 level. Naloxone fully reversed the effect of heroin on mean power frequency.

Discussion and Conclusion: We created a rat model to test our hypothesis that the negative BOLD signal is tightly correlated with the delta oscillations. The delta oscillations are similar to those evident during sleep-type activity. Since a low heroin dose predominantly activates μ-receptors, as indicated by the completed reversal by its antagonist naloxone, and the NAc contains more than 90% GABAergic spiny neurons, the observed negative BOLD signal is correlated with neural inhibitory activity, as indicated by the synchronized delta oscillations.

Reference: 1. Niessing et al., Science 309, 948-951, 2005. 2. Logothetis et al., Nature 412, 150-157, 2001. **Acknowledgement:** This work was supported by NIH Grants **DA10214** and **EB01820**.