L-Tetrahydropalmatine Induces a Negative BOLD Signal in the Nucleus Accumbens and Orbitofrontal Cortex in Heroin-Dependent Rats

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Introduction. The functional MRI (fMRI)method has demonstrated that cocaine-cues induce a set of mesolimbic cortical networks in the brain of cocaine users. Moreover, the cue-induced craving rating scores were significantly correlated with the positive BOLD signal changes in regions of the anterior medial orbitofrontal cortex (BA 11) and the subcollosal cortex (BA 25). If this positive BOLD signal could serve as a biomarker for drug craving, a medication that can specifically act on these regions with a negative BOLD signal could extinguish the drug craving, thereby preventing cocaine-seeking or -taking behaviors. A present fMRI study demonstrated that the Chinese herb extract, L-Tetrahydropalmatine (L-THP), would have therapeutic potential for anticraving.

Materials and Methods. ***Rat preparation.*** Thirteen naïve Sprague-Dawley rats (90-110 g, male) were treated with heroin in nine days using a progressive schedule (2 mg/kg daily for the first three days; 4 mg/kg daily for the second three days; and 8 mg/kg daily for the third three days). These rats became heroin dependent as evidenced by behavioral changes induced by naloxone. ***FMRI Experiments*****:** fMRI scanning was performed within 24 hours after the last daily injection of heroin. Under urethane anesthesia (1.2 g/kg), all rats received tracheotomies and were artificially ventilated with a 30% O_2/air mixture at a tidal volume of 5 ml and respiration frequency of 70 Hz to maintain stable physiological levels. Body temperature was monitored during scanning and maintained at 37 ± 1°C with a water-circulated heating pad. A femoral vein and artery were cannulated (PE 50) for drug delivery and monitoring of arterial blood gas levels, respectively. After surgery, rats were paralyzed with gallamine (250 mg/kg, iv) and an additional dose of 0.2-0.3 g/kg of urethane was administered prior to fMRI scanning. FMRI experiments were performed on a Bruker Biospec 3T/60 cm scanner using a custom-built RF birdcage volume coil (1.5" diameter × 2" length), inserted into a custom-made local gradient coil. To minimize motion artifacts, each rat head was anchored to the fixture of the RF coil with a clamping device consisting of a bar inserted under the hard palate and affixed to a nose clamp. To standardize slice anatomical locations across different rats, a medial sagittal Rapid Acquisition with Relaxation Enhancement (RARE) anatomical image (TR = 1000 ms, TE = 19 ms, matrix size 256 × 256, FOV = 3.5 cm) was obtained from each animal before functional scanning. On this slice, the interface between hard and soft palates is easily recognized and was employed as a starting point for the first imaging slice (approximately 2.2 mm from Bregma). Six 2-mm thick coronal slices were 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 = 2 s, TE = 27.2 ms, bandwidth 125 kHz) was used for functional imaging. ***Experimental Design*****:** The rats were divided into three groups. The first received a 0.1 mg/kg heroin treatment 5 min into a 25-min scan. The second group received a sham treatment with the same conditions as the first. The third group received 40-mg/kg L-THP treatment 5 min into a 65 - min scan. 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 area under the curve (AUC). Significance was set at $p < 0.05$ throughout.

Results. The present report focuses on the results from the L-THP treated group. As shown in Figure 1, L-THP induced a significant BOLD signal reduction (about 12±5%, n=3) in both the right and left sides of the NAC core and shell regions, as well as the obitofrontal cortex in the heroin-dependent rats. The time course of L-THP in the NAc showed a long-lasting effect. In addition, it is intriguing that L-THP has a very high spatial specificity. It is known that these regions contain rich D3-receptor distribution. It is hypothesized that the negative BOLD signal may be a result, in part, from the antagonistic binding of L-THP with D3-receptors in the region. To test this hypothesis, the L-THP was sent to NovaScreen (http://www.novascreen.com/). The latter confirmed that the L-THP was actively bound to D3-receptors when a concentration of 1.0E-5 of L-THP was employed; the Kd (M) being 0.9E-9 of [3H]7-OH-DPAT, and Ki (M) being 1.42E-9 of (+/-)7-OH-DPAT HBr. [Note: the testing compound of 7-OH-DPAT is a selective D3 receptor agonist (Kd<1 nM). Commercial profile testing reported by NovaScreen showed that L-THP also significantly binds to D1 and D2 receptors, weakly binds to adrenergic alpha 1A and 2A receptors, as well as serotonin, 5HT1A, 5HT1D, 5HT4, and 5HT7 receptors. No other significant bindings were found among 70 receptor profile testing.

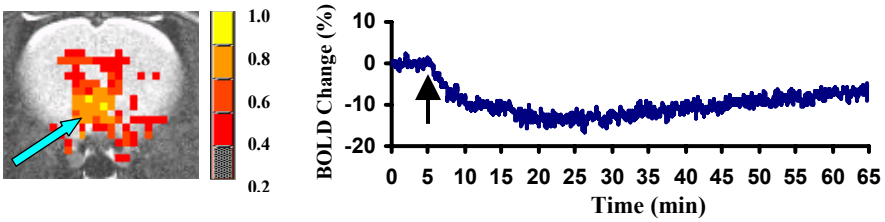

Figure 1. Left, the map of Cross correlation coefficients (CC = 0.22, P <0.0001) upon L-THP administration (40 mg/kg), the green arrow points to the NAC region. Right, the time course of L-THP in the region of NAc. The black arrow points to the time L-THP was i.v. administrated.

Discussion and Conclusion. L-THP significantly induced a negative BOLD signal in the region of the NAc and the OFC in heroin-dependent rats. The long lasting effect of L-THP in these regions suggested potential therapeutic efficacy. Limited binding effects of L-THP to the other receptors indicate less possible side effects or addictive potential. These results suggest that drug cue-induced positive BOLD signal can be suppressed by administering L-THP to extinguish the drug craving. Therefore, L-THP will have a high potential in treating drug craving for heroin, cocaine, nicotine, in addition to food craving in obesity. Further clinical studies will be needed.

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