Levo-Tetrahydropalmatine Treatment Attenuates Heroin-Priming Induced BOLD Responses in Heroin-Dependent Rats

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Introduction. Levo-tetrahydropalmatine (*l*-THP), purified from the Chinese herb, *Stephanie* [1], was recently demonstrated to be effective in attenuating heroin craving and relapse in heroin addicts; [2] it also inhibites cocaine's rewarding effects on animal models [3]. Despite this behavioral evidence, the treatment mechanisms of *l*-THP for drug addiction have yet to be elucidated. Here, we applied high-field pharmacological MRI (phMRI) on heroin-dependent rats with or without chronic *l*- THP treatment to determine the neurobiological mechanism of *l*-THP treatment effect on heroin dependency.

Materials and Methods. Animal materials and preparations: A total of 14 naïve male Sprague-Dawley (SD) rats weighing 300~350g were used in this study. Heroin-dependent models were built up with nine days of progressive heroin I.P. injections (2 mg/kg for 3 days, then 4mg/kg for 3 days, and finally 8mg/kg for 3 days). The heroin dependency was confirmed with nalaxone (4mg/kg I.P.) induced heroin withdrawal symptoms on the ninth day after the heroin injection. All heroin-dependent rats were randomized into two groups, the *l*-THP treated group (n=7) and the non-treated group (n=7). Rats then went through 6 days of extinction with no heroin injection, but 40mg/kg *l*-THP/ vehicle (saline) I.P. injection. On the 16th day, no treatment or vehicle was given, and phMRI scans were taken with the following preparations. With the anesthesia of 1.2 g/kg I.P. injection of urethane, the animal right femoral vein and femoral artery were cannulated for intravenous drug delivery and arterial blood pressure monitoring, respectively. The rats' core temperature was maintained at 37 ± 1°C with a water-pump driven temperature regulator. Tracheotomy and intubation were then performed for respiratory ventilation. Animal BP, ECG, respiration and blood oxygen saturation rate were also monitored and maintained within physiological range. **MRI procedures:** A 9.4 T spectrometer (Biospec Avance 94/31; Bruker, Germany) with a cylindrical volume transmit coil and Insight surface receiver coil (Worcester, Mass.) were used for MR imaging. Anterior commissure was chosen as a localizing landmark. High-resolution spin-echo rapid acquisitions with relaxation enhancement (RARE) axial anatomical images were acquired with TR= 5000 ms, TE= 11.3 ms, Number of average= 2, FOV= 35 mm x 35 mm, Matrix size= 128x128, Slice thickness= 2 mm, Number of slices= 6. Later, pharmacological MRI scans were taken with the same geometry and sequence, but shorter TR of 3000 ms, longer TE of 12.5 ms, single average and smaller matrix size of 64x64. Scan I was done with a priming injection of I.V. 0.1mg/kg heroin at 5 minutes into a 25-minute scan. Scan II was acquired with 40mg/kg *l*-THP I.V. injection at 5 minutes into a 35-minute scan. The time between scans 1 and 2 were approximately one hour. **Data Analysis:** AFNI v2.2 software was applied for major data analysis procedures. PhMRI time series of each voxel was spatially smoothed and fitted with a nonlinear *Beta* model according to the pharmacological and functional response character. The percentage change in the area under the curve (AUC%) of all voxels in the *l*-THP-treated group and the vehicle-treated group were compared and analyzed. A one-sample student's *t* test was employed for *l*-THP activation analysis in both groups. Statistical significant clustering was performed at threshold of goodness of fit value $F > 5.67$ (clusterwise $P < 0.01$) with a minimum cluster size of 2 voxels.

Results and Discussion. As in Figure 1, Row A shows the activation maps induced by a heroin priming injection in the sham-treated heroin-dependent rats. Both the reward and PFC control system, which are usually negatively activated, were activated. These included the NAc, PFC, ACC, M1, M2, insula, S1, retrosplenium, Amy, Cpu, and VTA. Row B shows the activation maps induced by the heroin priming injection in the *l*-THP-treated heroin-dependent rats. Only the NAc, ACC, and S1 were found to be negatively activated. Regional analysis showed that the responses were significantly less in the NAc, M1, M2, insula, retrosplenium, Amy, Cpu, and VTA in *l*-THP-treated group compared to results in the sham-treated group. Activation maps (Fig. 2) of acute *l*-THP injection in both groups showed that *l*-THP induced both positive and negative responses in mainly the cortical areas in the sham-treated group. The activation was in the similar area, but more negatively dominant in the *l*-THP-treated group. Regional analysis only found a significant difference in the retrosplenium between the results from the sham-treated and *l*-THP-treated groups (not shown).

These findings indicate that *l*-THP treatment can inhibit and attenuate heroin-priming induced BOLD responses in many of the addiction-related neuronal circuits. These results support our hypothesis that maladapted neuronal circuits with the treatment of *l*-THP were recovered, and became less sensitive to direct drug stimulation. An increased response to acute *l*-THP injection, though barely significant, was found in the *l*-THP-treated group, compared with that in the sham-treated group. This could be a result from the gradually occurring sensitization of chronic *l*-THP injection during the treatment process.

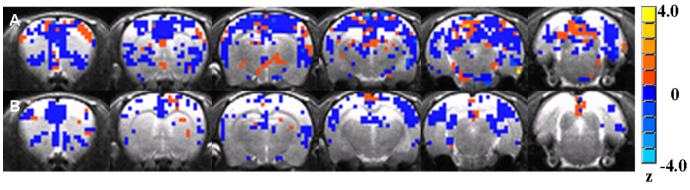

Fig. 1 Acute heroin-induced activation maps of saline treated heroin-dependent group (Row A) and l-THP treated heroin-dependent group (Row B). The z value is displayed according to the color bar shown on the right.

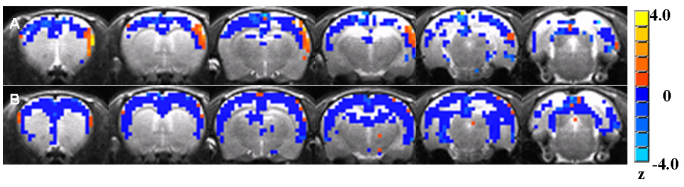

Fig. 2 Acute *l*-THP induced activation maps of saline treated heroin-dependent group (**Row A**) and *l*-THP treated heroin-dependent group (**Row B**). The z value is displayed according to the color bar shown on the right.

References:
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