Levo-Tetrahydropalmatine Modulates Activities in Dopaminergic Circuits of Naïve Rat Brain

X. Liu[1], Z. Yang[2], J. Xie[1], Q. Yin[1], and S-J. Li[1]

[1]Biophysics, Medical College of Wisconsin, Milwaukee, Wisconsin, United States, [2]Beijing Institute of Basic Medical Science, Beijing, China, People's Republic of

Introduction

L- tetrahydropalmatine (*l*-THP), a D_1, D_2 receptor antagonist purified from traditional Chinese herb *Stephanie* [1], has long been used as an analgesic and anti-anxiety agent in China. Recent study has demonstrated that *l*-THP can significantly attenuate heroin craving and relapse in heroin addicts [2]. Animal behavior experiments also demonstrated this compound can inhibit cocaine's rewarding effect in terms of self-administration, reinstatement, and brain stimulation reward [3]. Being such a promising treatment for addiction, the baseline action sites and neuronal effect of *l*-THP, however, have yet to be fully understood. To further understand neuropharmacological mechanisms of *l*-THP, here, we employed high-field pharmacological MRI (phMRI) to detect activation induced by acute *l*-THP administration in naïve rat brain.

Materials and Methods

Animal materials and preparations: Twelve naïve male Sprague-Dawley **(**SD) rats weighting 300~350g were randomized into 4 *l*-THP dosage test groups: 5mg/kg, 10mg/kg, 20 mg/kg, and 40 mg/kg groups. Three of these above rats were also randomized and chosen to undergo MRI data acquisition for saline (control) group. With 20% urethane (1.2 g/kg) anesthesia, 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 an Insight surface receiver coil (Worcester, MA) were used for MR imaging. Midbrain aqueduct was chosen as 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 same geometry and sequence but shorter TR of 3000 ms, longer TE of 12.5 ms, single average and smaller matrix size of 64x64. Either various dose of *l*-THP or saline (0.2 ml injected within a minute each time) was injected at the fifth minute into the 60-minute (150 repetitions) phMRI scan. **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. Percentage change in the area under the curve (AUC%) of all voxels in each *l*-THP group were compared with those in the saline group. Two-tail Student's *t* test was employed for *l*-THP activation analysis. One-way analysis of variance (ANOVA) method was applied to differentiate the dose responses of voxelwise AUC% values in various *l*-THP dosage groups. Clustering was performed at threshold of F >5.67 (P <0.01 after Bonferroni correction) with minimum cluster size of 8 voxels. For ROI analysis, FSL software was used to register all rats' AUC% images onto the reference template (anatomic images of one chosen rat). MIVA software (NeuroImaging, Worcester, Massachusetts) was employed to segment the reference template into 43 ROIs (demonstrated as in Figure 1E). Least-square linear regression were further applied to evaluate the dose-response of regional-wise AUC% values with statistical significant threshold of P <0.05.

Results

For all dose groups, *l*-THP injection induced negative BOLD signal (AUC% significant decreases) compared with that in the saline group within five minutes after *l*-THP injection (P <0.05), which lasted at least until 40 minutes after injections. When each group voxelwise AUC% was compared with that in the saline group, different doses of *l*-THP show different activation maps, (as in Fig. 1A-1D, P <0.05). After ROI segmentation, ANOVA test reveals 16 ROIs mainly related to dopaminergic circuits, such as nucleus accumbens, substantia nigra, basal ganglia, prefrontal cortex, hippocampus, hypothalamus, thalamus to be significantly (P <0.01) *l*-THP dose-dependent. The regional AUC% values of most dose-dependent ROIs are positively correlated with the *l*-THP dose (demonstrated for nucleus accumbens, NAc as in Fig. 2).

Discussion and Conclusion:

Acute *l*-THP administration dose-dependently reduced region-specific BOLD signals of dopaminergic circuit in naïve rat brain. Combined with the fact that *l*-THP behaves as a non-selective D_1, D_2 receptor antagonist and also binds with D_3 receptor, our results suggest *l*-THP acts on the dopaminergic system and affects the corresponding projected areas. As drug addiction relies heavily on the mal-adaptation of the dopaminergic system, interactions of multiple dopamine receptors of *l*-THP imply its therapeutic potential for drug addiction. Lower doses of *l*-THP activated fewer brain regions, and therefore may have fewer side effects. Further study will be desired to investigate the mechanism for its negative BOLD responses and detailed modulatory effect on drug addiction.

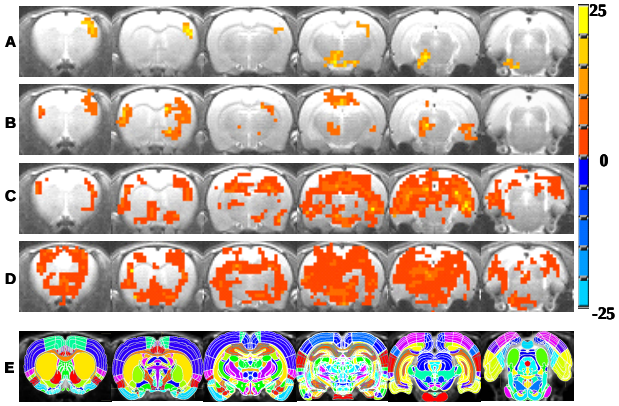

Fig. 1 Activation *t*-maps (P <0.05) of different dosages of *l*-THP induced BOLD signal changes (AUC%) overlaid with corresponding reference template of RARE images. Positive *t* value means the AUC% in THP group is significant less than that in saline group. **1A-1D** are AUC% activation maps of 5 mg/kg, 10mg/kg, 20mg/kg, and 40mg/kg *l*-THP group sequentially. **1E:** ROI reference image segmentation with MIVA software.

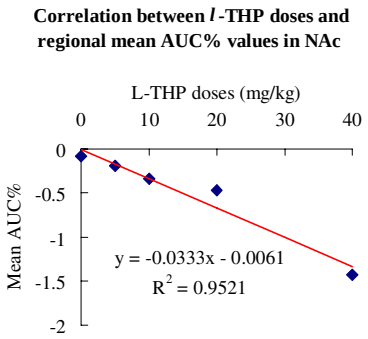

Fig. 2. Dose-dependent activation of *l*-THP in the nucleus accumbens (NAc) (P< 0.05).

References:

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