Anesthesia Effects on BOLD and rCBV Responses Induced by L-tetrahydropalmatine

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Introduction

PhMRI studies in rodents are often conducted under general anesthesia to improve image quality by reducing head motion artifacts; also, it minimizes the stress induced by restraint. However, the anesthetic agent may cause reductions in baseline metabolism and neural activity, alterations in regional cerebral blood flow (CBF), or even uncoupling between blood flow and metabolism. Interactions with the anesthetic agent may affect the phMRI response to the drug of interest. This complicates the interpretation of the data. L-tetrahydropalmatine (*l*-THP), purified from Chinese herb *Stephanie*[1], was recently demonstrated to be effective in attenuating heroin craving and relapse in heroin addicts.[2] Also, it inhibited cocaine's rewarding effects in animal models [3]. We investigated the *l*-THP-induced BOLD and rCBV brain responses using three anesthesia conditions (isoflurane, urethane, and medetomidine) at high field to assess the confounding effects and interaction of different anesthetics on *l*-THP.

Materials and Methods

Animal materials and preparations: A total of 42 naïve male Sprague-Dawley (SD) rats weighting 300~350g were used in this study. Rats were randomized evenly into 3 anesthetic condition groups: 1.2 g/kg I.P. injection of urethane; 1.4-1.6% isoflurane in 3:7 O2 and N2 mixture; and finally 0.1mg/kg medetomidine bolus subcutaneous injection followed by o.1mg/kg/h tail vein pump infusion. Eight rats in each anesthetic group were further divided into 2 *l*-THP dosage subgroups (5mg/kg and 20mg/kg) for the BOLD response study. One subgroup of animals received a vehicle (0mg/kg) injection scan before the *l*-THP injection scan. The remaining 6 rats in each anesthetic group were used for rCBV measurement (n= 4 for *l*-THP, and n= 2 for vehicle control). 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 the 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. However, we used shorter TR of 3000 ms, longer TE of 12.5 ms, single average and smaller matrix size of 64x64. Either various doses of *l*-THP or vehicle were injected at the fifth minute into the (150 repetitions) 60-minute scan for BOLD evaluations. Additional phMRI scans were acquired with Monocrystalline iron oxide nanocolloid (MION) (10 mg Fe/kg, IV) injection at the fifth and 0 or 20mg/kg IV *l*-THP injection at the 15th minute into a 45-minute scan for rCBV study. **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 each THP group was compared to those in the vehicle group. Two-tail student's *t* test was employed for *l*-THP activation analysis. Two-way analysis of variance (ANOVA) method was applied to differentiate the anesthetic agent-*l*-THP dose interactions. Clustering was performed at the threshold of $F > 5.67$ ($P < 0.01$ after Bonferroni correction) with a minimum cluster size of 2 voxels.

Results

For both 5 and 20 mg/kg groups, *l*-THP injection induced small regions of positive BOLD activations under isoflurane anesthesia, positive and negative activations under medetomidine anesthesia, and consistently negative activations under urethane anesthesia (data not shown here). A dose of *l*-THP showed its primary effect in the hypothalamus, caudate putamen (CPu), insula, retrosplenium, and temporal cortices. The three anesthetic agents showed their effect in the cingulate cortex, insula, hypothalamus, CPu, globus pallidus, amygdala (Amy), retrosplenium, and parietal cortex, ventral tegmented area (VTA), and Raphe nucleus. Anesthesia and *l*-THP doses showed an interaction effect in the broader regions of cingulate cortex, insula, somatosensory cortex, hypothalamus, thalamus, CPu, Amy, retrosplenium, and parietal cortex, midbrain, and Raphe nucleus (Fig 1). *L-THP* induced spatially similar rCBV activation maps; all three maps cover most regions of dopaminergic, serotonergic, as well as noradrenergic neural circuits. Maps acquired under isoflurane and medetomidine anesthesia contained negative and positive activation areas, while the rCBV activation map obtained after administering urethane anesthesia consisted of primarily negative areas (Fig. 2).

Discussion and Conclusion:

Isoflurane, combined with the BOLD and rCBV results, revealed the smallest activation areas. Urethane evidenced the largest activation; however, all the areas were negative. The results for Medetomidine were in the middle. The anesthetic agents and the *l*-THP dose demonstrated interactions in the broad regions, thereby creating BOLD activation. The relatively small responses of BOLD changes under isoflurane may be due to the lower evoked field potential and/or higher baseline CBF under isoflurane anesthesia. Similar rCBV activation regions were found under all three anesthesia conditions, but only urethane revealed dominantly negative rCBV responses, which may account partially for the dominantly negative BOLD responses found under urethane anesthesia. The multiple channel inhibitory effects may be the underlying mechanism for urethane negative responses. Therefore, great caution should be taken in choosing the anesthetic agent and interpreting the phMRI data.

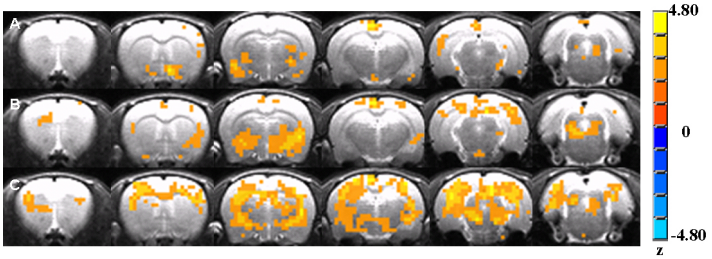

Fig. 1 *l*-THP two-way ANOVA test result of voxel-wise AUC% values in all (vehicle and *l*-THP) groups. **Row A,** dose-dependent maps among all groups; **Row B**, anesthesia agent dependent maps among all groups; **Row C**, dose-agent interaction maps among all groups. The z value is displayed according to the color bar shown on the right.

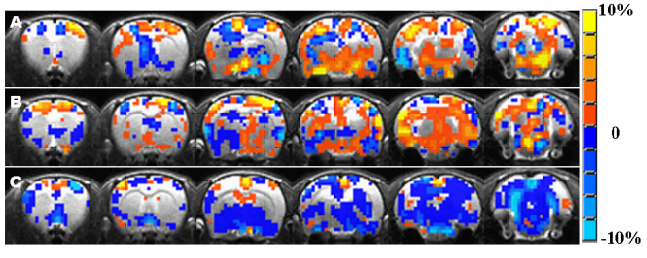

Fig. 2 Activation maps of *l*-THP induced AUC% of rCBV signal changes compared with those of vehicle injection, overlaid with RARE images. **Row A**, isoflurane anesthesia; **Row B**, meditomidine anesthesia; **Row C**,urethane anesthesia. rCBV AUC% values are showed in color in scale of the color bar on the right.

References:

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