

# Neural Substrate of Morphine Withdrawal Symptoms in Rat Revealed by Manganese-enhanced Magnetic Resonance Imaging

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**Introduction** Chronic morphine exposure induces dependence. Abstinence from the drug causes withdrawal symptoms. Many previous studies have investigated the neural substrate of morphine withdrawal symptoms. For example, fMRI has been used to measure brain activation during naloxone-precipitated morphine withdrawal [1]. Electrophysiological and microdialysis techniques have also been employed to study the neural activities in brain regions such as nucleus accumbens (NAc) during withdrawal [2, 3]. In this study, we employed manganese-enhanced MRI (MEMRI) technique to map accumulative brain activities in morphine-treated rat in a 24-hour period during spontaneous withdrawal.

**Materials and Methods** Male Sprague-Dawley rats, weighing 220-250g, were treated with escalating doses of morphine (n=11) or the same amount of saline (control group, n=9) for a total of 11 consecutive days (Fig. 1). For each morphine-treated rat, spontaneous withdrawal behaviors were recorded in a 30-min session at 36 hrs after the last morphine injection. All rats were then injected with a dose of MnCl<sub>2</sub> solution (0.12 M, 40 mg/100g, i.p.), and imaged 24 hrs later on a 7 T/20 cm Bruker Biospec scanner under 2% isoflurane anesthesia (in pure O<sub>2</sub>). T<sub>1</sub>-weighted images were acquired with a 3D FLASH sequence with flip angle 45°, TR 36 ms, TE 3.5 ms, FOV 3 cm × 1.5 cm × 2 cm, matrix size 128 × 64 × 48 and 32 averages. For voxel-based comparison, all images were first stripped out of the non-brain tissues pixels and corrected for image intensity nonuniformity artifacts. One representative dataset was chosen as the first template and resampled to an isotropic voxel size of 0.23 mm×0.23 mm×0.23 mm. The rest were co-registered to the initial template, followed by creation of an average template. Each raw dataset was then co-registered to the average template. The voxel-wise signal intensity was then normalized to the overall signal intensity of the whole brain. Finally, images were smoothed with a 0.46-mm FWHM Gaussian kernel before subjected to two-tailed independent samples t-test. Regions of interest (ROI)-based analysis was used to verify the results of voxel-wise comparison.

**Results:** All morphine-treated rats showed spontaneous withdrawals behaviors (i.e., bouts of erection, wet dog tremble, teeth shock, groom, drill, face-wash, deject and grid). Voxel-wise comparisons of manganese-enhanced signal intensity (VMI) between the morphine group and the control group revealed brain activation in anterior cingulate cortex (Cg), secondary motor cortex (M2), CA3 subfield of hippocampus, dorsal striatum (D-striatum), retrosplenial granular cortex (RS), shell subregion of NAc (AcbSh), core subregion of NAc (AcbC), central nucleus of amygdala (CeC), basolateral amygdaloid nucleus (BAL), central amygdaloid nucleus (CeM), anterior hypothalamic area, central (AHC) and scaphoid thalamic nucleus (SC) (Fig. 2, warm color), and brain inhibition in ventrolateral striatum (V-striatum) and lateral posterior thalamic nucleus (LP) (Fig. 2, cold color). These results were further verified by ROI-based analysis (Fig. 3).

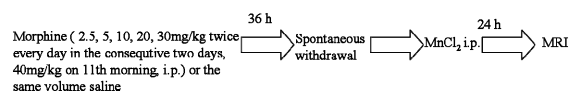
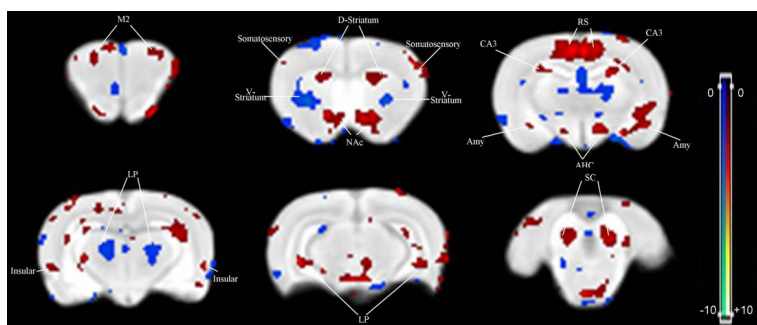


Figure 1. Experimental protocols

Figure 2. Morphine withdrawal-induced brain activation (warm color) / inhibition (cold color).  $p < 0.01$ .

**Discussion** Previous previous studies have demonstrated that suggested MEMRI can be used to study accumulative neuronal activity in awake, freely-moving animals [4]. In this study, we used the MEMRI paradigm

proposed by Yu et al to investigate the neural substrates associated with spontaneous morphine withdrawal. The results of voxel-based analysis demonstrated that morphine withdrawal is associated with activations of rewarding circuitry and other brain regions, including Cg, M2, CA3, D-striatum, RS, AcbSh, AcbC, CeC, BAL, CeM, AHC and SC. The activations of some of the brain regions have been reported in naloxone-precipitated withdrawal studies [1, 5, 6]. It is concluded that MEMRI can be used to measure brain activity associated with drug dependence/withdrawal in awake, freely-moving animals.

**Acknowledgements** Supported by grants from Natural Science Foundation of China (30870674 and 20921004) and Chinese Ministry of Science and Technology (2011CB707800).

**References** 1. Lowe et al. Neuroimage. 2002 Oct; 17(2):902-10. 2. Lintas et al. J Neurosci. 2011 Aug 3; 31(31):11172-83. 3. Nieto et al. J Neurosci. 2002 Feb 1; 22(3):1034-41. 4. Yu X et al. Nat Neurosci. 2005 Jul; 8(7):961-8. 5. Blackburn-Munro et al. Neuropharmacology. 2000 Jul 10; 39(9):1596-607. 6. Harris et al. Behav Brain Res. 2007 Jan 25; 176(2):251-8.

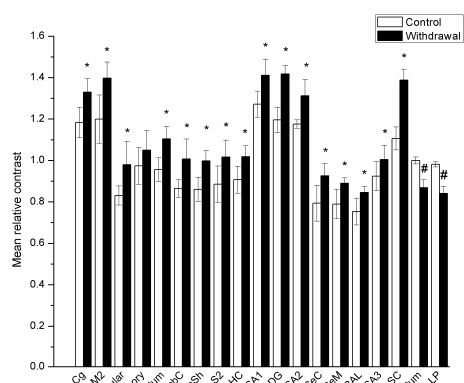


Figure 3. ROIs analysis of normalized manganese enhanced signal intensity enhancement. \*,# $p < 0.05$  compared to control.