Changes of Orbitofrontal Activities Induced by Morphine Administration and Withdrawal Observed by Manganese-Enhanced Magnetic Resonance Imaging in Rats

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Introduction Previous studies have shown that the orbitofrontal cortex (OFC), together with its connections with the sensory and limbic systems, plays an important role in a number of behavioral aspects of drug abuse, including reinforced anticipation of drug reward, craving for drugs, conditioned responses and impairments in judgment that could influence decision-making [1-3]. In cocaine abusers, hyperactivity of the OFC is observed during early withdrawal and shortly after last use of the drug, and is positively correlated with the intensity of craving [1,2]. In contrast, decreased activity of the OFC is frequently detected in cocaine-addicted subjects during protracted withdrawal and without drug stimulation, and which has been shown to be associated with reduction in the availability of dopamine D2 receptors in striatum [1]. In view that few previous investigations have examined the changes in the OFC activity elicited by opiate addiction and withdrawal, manganese-enhanced magnetic resonance imaging (MEMRI) was used in this study to measure the functional activities of the OFC after chronic morphine administration and during the early stage of sudden withdrawal.

Materials and Methods Male SD rats weighing 200-250g were injected with either morphine hydrochloride (10 mg/mL solution, 10 mg/kg body weight) or saline (i.e., control) twice a day (i.e., separated by a 12hr interval) for a total of 12 days, followed by a period of detoxification, or withdrawal, during which drug administration was ceased. At different stages of morphine administration (i.e., 1d, 6d, and 12d, 40 minutes after last morphine injection) and withdrawal (i.e., 1d, 3d, and 5d), the rats were anesthetized with pentobarbital (40 mg/kg) and stereotaxically injected with 200 nL of 80 mM $MnCl_2$ saline solution into the right OFC (AP, 3.7 mm; ML, -2.4 mm; DV, -4.6 mm). Five hours after $MnCl_2$ injection, the rats were anesthetized with ketamine (40 mg/kg), perfused by 10% formalin solution, and decapitated. The heads were stored in 10% formalin solution for 6-7 days before MRI measurements. Coronal T_1-weighted images were acquired on a Bruker Biospec 4.7 T/30 cm spectrometer using an inversion recovery spin echo sequence with FOV 3.0×3.0 cm^2, matrix size 128×128, slice thickness 0.8 mm, TR 3 s, TE 14 ms, and TI 600 ms. The in-plane spatial resolution of the images was 230 μm×230 μm. Regions of interest (ROI) were drawn in the OFC ipsilateral to Mn^{2+} injection, and in the contralateral OFC. The signal intensity ratios between the ipsilateral and contralateral OFC were calculated and compared among the different groups using one-way ANOVA and a two-sided Post Hoc Dunnett's test.

Results Figure 1 shows representative inversion recovery images of the OFC obtained from the rats at different stages of morphine administration and withdrawal. It is clear from the images that, in general, the average signal intensity of the hemisphere ipsilateral to Mn^{2+} injection was higher than that of the contralateral hemisphere, due to the T_1- shortening effect of Mn^{2+} injected. However, the degree of signal enhancement in different rats varied. The rat abstained from morphine for 1 day (Fig. 1e) showed the least signal enhancement, while the rats abstained from morphine for 3 and 5 days (Fig. 1f and g) appeared to have higher signal enhancement than control. The intensity ratios of the ipsilateral OFC to the contralateral OFC for different groups were shown in Fig. 2. Compared to the control rats, the rats in all morphine administration groups displayed somewhat lower signal intensity ratios, but the differences did not reach statistical significance as revealed by the Dunnett's test. The rats abstained from morphine for 1 day showed significantly lower signal intensity ratio ($p<0.05$, Dunnett's test) than control, while the rats examined 3 days and 5 days into the withdrawal period showed statistically insignificant higher signal intensity ratios.

Discussion and Conclusion As an analog of Ca^{2+}, the rate of synaptic uptake of Mn^{2+} and the rate of Mn^{2+} transportation along the axons are thought to be able to reflect the level of neuronal activity [4,5]. It has been shown by MEMRI that somatosensory stimulation as well as glutamate and dopamine challenges increase the uptake of Mn^{2+} by brain tissues [4-6]. In this study, MEMRI was used to measure the changes in the OFC activity induced by chronic morphine administration and sudden withdrawal. Mn^{2+} was injected directly into the OFC, and the brain was perfused 5 hrs later to remove Mn^{2+} in the blood vessels and that in the extracellular space. If the Mn^{2+} ions left in the brain after perfusion were located in the intracellular space only, the amount of Mn^{2+} ions in brain tissue should be determined by the uptake rate of Mn^{2+}, and therefore can be used to reflect the level of brain activity. During chronic morphine treatment, the OFC activity appeared to be lower than control. When abstained from the drug, the rats showed significantly decreased OFC activity at 1d, but followed by recoveries at 3d and 5d. The dynamic changes of the OFC activity during morphine administration and withdrawal, to some extent, agree with those found for human cocaine-addicts [1-3].

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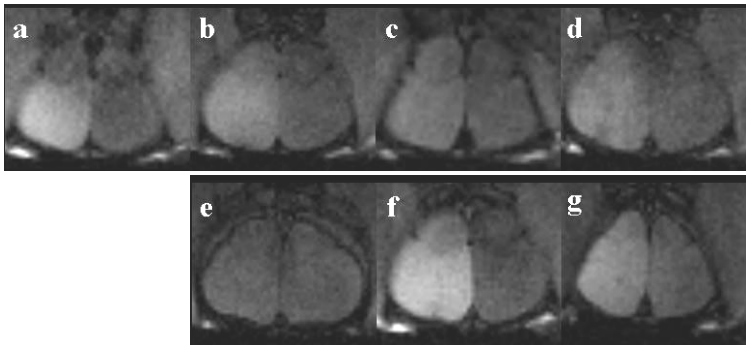

Figure 1. Inversion recovery T_1-weighted images of the OFC 5 hrs after Mn^{2+}injection. (a) control rat; (b-d) rats had morphine administration for 1, 6, and 12 days, respectively; (e-g): rats 1, 3 and 5 days into the withdrawal period, respectively.

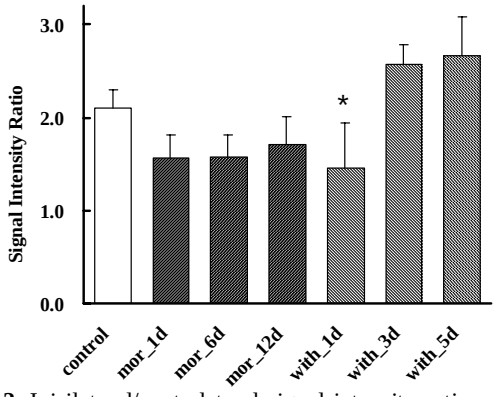

Figure 2. Ipisilateral/contralateral signal intensity ratios of the OFC for the control, morphine administration (mor) and withdrawal (with) groups. *$p<0.05$ compared to the control group by Dunnett's test.

References [1] Volkow, ND et al, *Cereb Cortex*, 2000, **10**:318-25. [2] London, ED et al, *Cereb Cortex*, 2000, **10**:334-42. [3] Volkow, ND et al, *Neuropharmacology*, 2004, **10**:318-25. [4] Duong, TQ et al, *Magn Reson Med*, 2000, **43**:383-92. [5] Lin, YJ et al, *Magn Reson Med*, 1997, **38**:378-88. [6] Aoki I, et al. In the 12th Annual Meeting of International Society for Magnetic Resonance in Medicine, 2004. Kyoto, Japan. p19.