Mapping Cocaine-Induced Brain Activation Using Manganese-Enhanced MRI

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Introduction: Cocaine is a potent psychostimulant that produces marked physiological and behavioral effects in both humans and experimental animals, including euphoria, enhanced alertness as well as increases in heart rate and blood pressure (1). The effects of cocaine on the cardiovascular and cerebral vascular systems raise questions whether and to what extent these vascular effects could potentially confound fMRI data that are based on the hemodynamic response after cocaine administration.

Manganese-Enhanced MRI (MEMRI) is an alternative approach to map neuronal activity (2,3). This technique is based on the fact that paramagnetic manganese ions (Mn^{2+}) enter synaptically activated neurons through voltage-gated calcium channels, and then remain inside the neurons for a long period of time (4,5). The accumulation of manganese in the activated neurons leads to a signal increase in T1-weighted images. Thus, unlike the fMRI techniques that detect hemodynamic response coupled to metabolic needs secondary to neuronal activity, MEMRI has the potential to directly map drug-induced neuronal activation without confounding effects from vasculature. In the present study, we applied this technique to map rat brain activation induced by acute intravenous administration of cocaine.

Materials and Methods: Twenty male SD rats (n=7 for saline; n=6 for 0.5 mg/kg cocaine; n=7 for 2 mg/kg cocaine) were anesthetized using α-chloralose with an initial dose of 50 mg/kg followed by an additional dose of 50 mg/kg each hour. The animals were mechanically ventilated. Rectal temperature, end tidal CO_2, O_2, arterial blood pressure were continuously monitored, and kept within normal ranges. After three baseline images were acquired, 1% manganese chloride ($MnCl_2$, 45 mg/kg) was continuously infused intravenously for about one hour. At 40 min after the onset of $MnCl_2$ infusion, first pass 25% mannitol (5-7 ml/kg) was injected via the right external carotid artery to disrupt the blood-brain-barrier (BBB). Either cocaine (0.5 or 2 mg/kg) or saline was administrated (IV) at 25 minutes after BBB disruption, experiment continued for about 45 min following cocaine or saline injection. Ten minutes before mannitol infusion, 2% isoflurane was delivered to animals to produce deep anesthesia. Isoflurane was stopped 10 min after the disruption of BBB. T1-weighted images were acquired during the entire period using a conventional spin echo sequence. Scan parameters: TR = 450 ms, TE =8 ms, matrix size = 128 × 128, FOV = 3.5 cm, slice thickness = 1 mm. Thirteen continuous slices were acquired with the central slice localized at the anterior commissure, which is about -0.36 mm from bregma. It took about 3 min to finish one scan. A customized program was developed to reconstruct images and display results in AFNI in a real time fashion.

Images from all animals were registered using AFNI software. Signal changes due to cocaine injection were quantified by (Sc-Sm)/Sm, where Sc is the average signal intensity of the last three data points after cocaine injection, and Sm is the average signal intensity of the last three data points after mannitol injection. Data from the cocaine groups and the saline groups were subject to a two-tailed student t-test to analyze the statistical significance of cocaine-induced change in MEMRI signals. $P \leq 0.05$ was considered significant.

Results: Following $MnCl_2$ infusion, signal intensities gradually increased within the skull muscle and all ventricles, with an average enhancement up to 120% by the end of infusion. There was no apparent signal enhancement within the cortical and subcortical brain regions. After the disruption of BBB by mannitol, signals rapidly increased in all brain regions. Figure 1 shows three representative time courses in the nucleus accumbens from three individual animals which received either saline, 0.5 mg/kg or 2.0 mg/kg cocaine. In each case, all signals were normalized to the data point immediately before cocaine or saline injection. There were 10 - 40% signal increases in the nucleus accumbens after mannitol infusion (marked by arrow "A"), and the then the signals reached a plateau within 20 min. The subsequent saline administration (marked by arrow "B") did not significantly alter MEMRI signals, while subsequent cocaine infusions dose-dependently increased MEMRI signals in the nucleus accumbens.

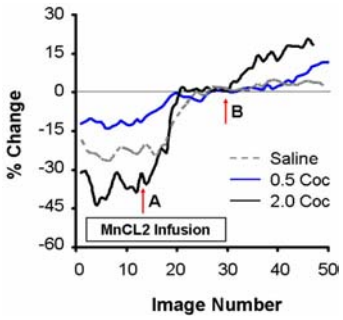

Fig. 1. Representative time courses in the nucleus accumbens from three animals after saline, 0.5 mg/kg or 2.0 mg/kg cocaine administration. Coc, Cocaine.

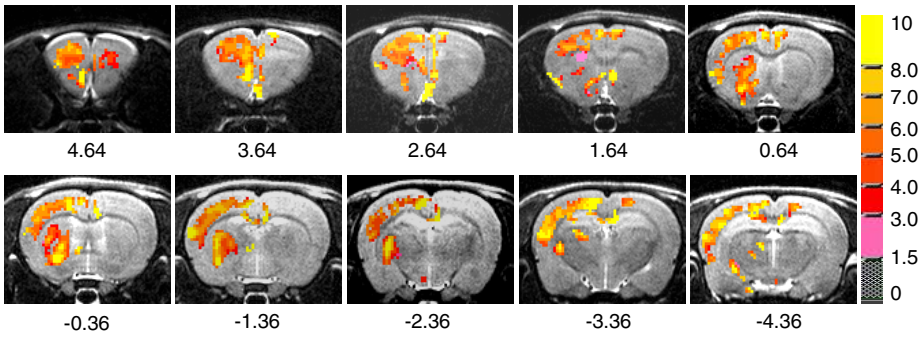

Fig. 2. T-statistical maps at 0.5 mg/kg of cocaine ($p = 0.05$).

Figure 2 shows t-statistical maps between the cocaine (0.5 mg/kg) and saline treatment groups. At 0.5 mg/kg, cocaine produced a significant increase in MEMRI signals in many brain regions, including the olfactory tubercle, nucleus accumbens (NAc), dorsal striatum, prefrontal cortex (PFC), cingulate cortex, somatosensory cortex and part of thalamus. Cocaine at a dosage of 2 mg/kg also activated hippocampus and amygdala (data not shown).

Discussions: 1) BBB disruption is required for manganese ion uptake into activated neurons. In most cases, we could only disrupt one side of the hemisphere, ipsilateral to the carotid artery side where mannitol was injected. Therefore, we could only detect cocaine-induced brain activations in one hemisphere where BBB was disrupted (Fig. 2). Although there were variations on the extent of BBB disruption from animal to animal, we did not see significant differences on the extent of BBB disruption between the cocaine groups and saline groups. 2) To further determine whether the observed MEMRI signals truly reveal changes in neuronal activity, we also conducted an MEMRI experiment using rat forepaw electric stimulation instead of the cocaine challenge. We observed significant MEMRI signal enhancement in the projected area of the somatosensory cortex. 3) Parrino et al. (6) mapped cocaine induced brain activation using the 2-DG technique. 0.5 mg/kg of cocaine significantly increased glucose utilization only in the mpfc and NAc, with more structures activated at 5 mg/kg. Here, we report substantially more activated structures at 0.5 mg/kg of cocaine. This may be due to sensitivity differences between 2-DG and MEMRI. A recent MEMRI mapping of cocaine activation (8) only reported signal increase in the NAc. However, the experimental protocol used in Ref. 7 was different from the one used in the present study.

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