Olfactory neural tract tracing by manganese-enhanced magnetic resonance imaging

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Introduction

The previous study using functional magnetic resonance imaging (MRI) suggests that the medial frontal cortex (mFC), which is considered as "visceral motor cortex" (1) and is little considered as one of the olfactory centers (2), responded well to olfactory stimulation with similar extent and response pattern in the ventrolateral primary olfactory cortices (*i.e.*, the piriform cortex, olfactory tubercle, etc.) (3). The present study was aimed to investigate neural projections from olfactory cortices and higher olfactory centers to mFC by using manganese-enhanced MRI (MEMRI), a newly developed *in vivo* neural tracing technique (4, 5). Injection of Mn^{2+}, a biological calcium analogue and paramagnetic tract tracing agent, allows highlighting specific brain areas that are active in MEMRI.

Materials and Methods

Male Wistar rats, weighing 300-450g, were used. Each rat was anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and mounted in a stereotaxic apparatus. $MnCl_2$ (100 mM, 0.2-0.5 μl) was ejected into one of several parts in the olfactory pathway with pressure by a syringe pump through glass micropipette (tip diameter, 20-40 μm) connected to Hamilton microsyringe. The site injected (left site) were the olfactory bulb, the ventrolateral frontal cortex, the lateral hypothalamic area, the basolateral nucleus of amygdala, the mediodorsal nucleus of thalamus, and the CA1 region of hippocampus. The MRI experiments were performed on a SMIS (Surrey Medical Imaging Systems Ltd., Guilford, U.K.) MRI system, consisted of a 4.7 T/400 horizontal superconducting magnet equipped with actively shielded gradient coils. T_1-weighted multislice spin-echo images were acquired at 8 h and 24 h after Mn^{2+} injection. Rats were re-anesthetized with 1.5% isoflurane in a mixture of 70% N_2O/30% O_2 gas. Body temperature was controlled at 37°C by circulating water. Their heads were fixed in a non-magnetic stereotaxic apparatus specially designed for MRI. A home-made volume coil (60 mm diameter) was used for both transmission and reception of radio frequency. T_1-weighted multislice spin-echo pulse sequence was used with the following parameters: TR = 500 ms, TE = 20 ms, FOV = 38.4 mm x 38.4 mm, matrix = 128 x 128, slice thickness = 1.0 mm, slice gap between center-to-center of consecutive slices = 1.5 mm, number of slices = 6-10, one average. Data were processed by home-made image analysis software.

Results and Discussion

Injection of Mn^{2+} into the olfactory bulb clearly enhanced T_1-weighted MR signals in the anterior olfactory nucleus, lateral olfactory tract, piriform cortex, and orbital cortex but not in the mFC (Fig. 1). Injection of Mn^{2+} into the ventrolateral frontal cortex (the lateral olfactory tract) enhanced the signals in the olfactory bulb, anterior olfactory nucleus, the lateral entorhinal cortex, the lateral hypothalamic area, and substantia nigra but not in the mFC. Signals in the mFC were enhanced by injection of Mn^{2+} into the amygdale (Fig. 2), the mediodorsal nucleus of thalamus, and the CA1 region of the hippocampus. These results suggest that the mFC receives inputs from high-order olfactory centers (*i.e.*, the amygdala, the mediodorsal thalamic nucleus and the CA1 of hippocampus) but not from the olfactory bulb and the ventrolateral primary olfactory cortices. MEMRI is proved to be useful technique to visualize neural connections in the olfactory pathway in rats.

References

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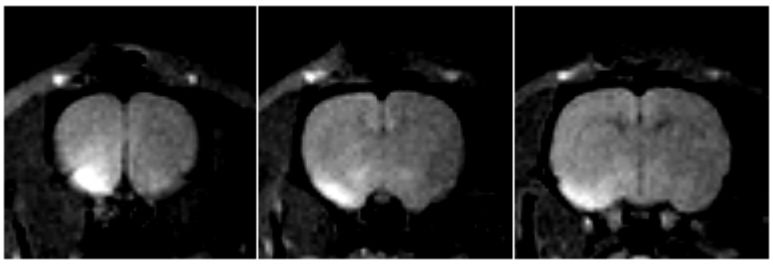

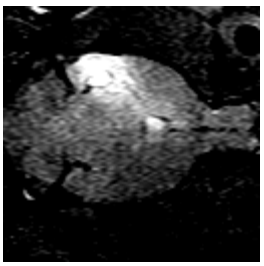

Fig. 1 (left) Examples of T_1-weighted images at 24 h after the injection of $MnCl_2$ into the olfactory bulb.
Fig. 2 (Right) An example of T_1-weighted image at 24 h after the injection of $MnCl_2$ into the amygdala.