Tracing Neuronal Tract Between the Laminar Structures of Rat Olfactory Bulb Using Manganese Enhanced Magnetic Resonance Imaging

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Introduction: Recently there have been growing research interests on studying the olfactory function in human and animals using advanced neuroimaging techniques such as BOLD-based fMRI[1] and optical imaging[2]. Taking advantage of the fact that Mn^{2+} not only is a paramagnetic MRI T_1 contrast agent, but also can be transported as a calcium analog in the central nervous system, T_1-weighted manganese enhanced MRI is a powerful novel neuroimaging technique which can be used to trace neuronal tract and to study functional activation of brain[3,4]. Manganese enhanced MRI is especially suitable for studying the olfactory system because it employs T_1-weighted imaging, rather than T_2/T_2* weighted imaging used in BOLD-based fMRI, thus is not affected much by the susceptibility problem characterizing the olfactory system. In this study, manganese enhanced MRI with high spatial resolution was used to trace the neuronal tract between the laminar structures of the olfactory bulb in rat.

Materials and Methods: Male Wistar rats were anesthetized by i.p. injection of urethane (100 mg/ml saline solution; at a dose of 1.5 ml/100g body weight). 5 μl of 400 mM $MnCl_2$ saline solution was pipetted into the right naris of each rat using a precision microliter pipette. The rat was then transferred to an animal bed and placed in the magnet. Inside the magnet, the rat breathed room air voluntarily. MRI was performed on a Bruker Biospec 4.7 T/30 cm spectrometer equipped with a 20-cm diameter gradient insert. A 12-cm diameter Helmholtz volume coil was used for excitation and a 2.5-cm diameter surface coil for reception. Images were acquired using an inversion recovery spin echo sequence with a slice thickness of 0.8 mm, TR of 5 s, TE of 20 ms, TI of 450 ms, FOV of 1.5×1.5 cm^2, matrix size of 128×128 and number of averages of 4. The average T_1 of rat olfactory bulb at 4.7 T was found to be about 1 s. TI of 450 ms was chosen because it gave good contrast between the laminar structures. The in-plane spatial resolution of the images was 110 μm×110 μm. The averaged signal intensity of the entire left hemisphere of the olfactory bulb was used as a reference to normalize the signal intensities reported.

Results: Figure 1 show the images of a central transverse slice in the olfactory bulb from a representative rat. The images were obtained before, 5 hrs after and 15 hrs after the application of Mn^{2+}, respectively. The laminar structures of the olfactory bulb can be well distinguished in all three images. From lateral to medial, the layers in turn are olfactory nerve layer (ONL), glomerular layer (GL), external plexiform layer (EPL), granular cell layer (GCL) and subependamal zone (SEZ). The signal intensity of the left hemisphere of the olfactory bulb did not change significantly during the course of the entire experiment. The difference in image appearance between the left hemisphere and the right hemisphere of the olfactory bulb in Fig. 1b and c was caused by accumulation of the Mn^{2+} ions transported from the right naris. The signal intensities of all laminar structures in the right hemisphere of the olfactory bulb changed with time after Mn^{2+} application (Fig. 2). Except for the SEZ layer the signal intensity of which decreased continuously within the period of observation, the signal intensities of the other four layers all exhibited a biphasic inversion-recovery-like change (e.g., first decreased and then increased). The signal intensity changes in all the layers were the result of continuous T_1 decrease for tissue water caused by progressive Mn^{2+} accumulation in the layers.

Discussion and Conclusion: Mn^{2+} enhanced MRI has been used previously to trace neuronal tract and to study functional activation in the olfactory system of experimental animals. In this study, T_1-weighted inversion recovery MR imaging with high spatial resolution was used to observe the spatial pattern and the time course of Mn^{2+} transportation and accumulation in the laminar structures of rat olfactory bulb after intra-naris application of $MnCl_2$ solution. The results reveal that the transportation of exogenous Mn^{2+} in the olfactory bulb is from the outer layers to the inner layers, and is a slow process under non-stimulated condition. High spatial resolution Mn^{2+} enhanced MRI can be a powerful tool in studying dynamically the function of rat olfactory bulb at laminar level.

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References: [1] Kida I. et al. *Magn Reson Med* **48**: 570-76 (2002). [2] Rubin BD. et al. *Neuroscience* **4:**355-56(2001). [3] Lin YJ. et al. *Magn Reson Med* **38**: 378-88 (1997). [4] Pautler RG. et al. *NeuroImage* **16**: 441-48 (2002).

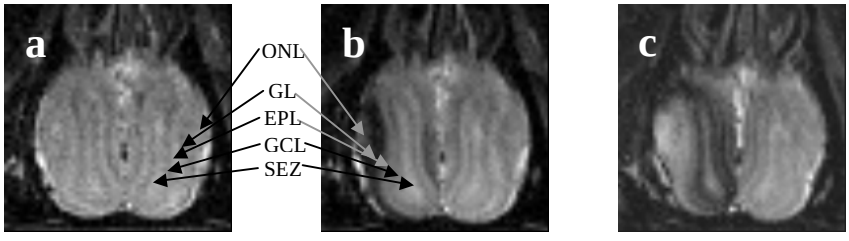

Figure 1 Transverse inversion-recovery images of rat olfactory bulb before (a), 5 hrs after (b) and 15 hrs after (c) intra-naris application of Mn^{2+}. The laminar structures are indicated by arrows.

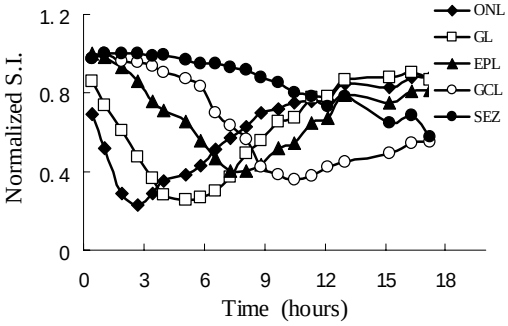

Figure 2 Time courses of normalized signal intensities (S.I.) of different olfactory bulb layers after intra-naris application of Mn^{2+}.