Manganese Enhanced MRI Enables Identification of Individual Glomeruli in the Olfactory Bulb

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Introduction

In the olfactory system, the axons of olfactory sensory neurons expressing the same type of receptors converge to two specific glomeruli in each olfactory bulb (OB). The glomeruli are round-shaped structures, which are about tens of microns up to 200 microns in diameter in rats, and are where the axons of olfactory sensory neurons have synapses with the mitral cells in the OB [1]. The glomerulus and neurons connected with it is regarded as a fundamental information processing module [2]. Traditionally, glomeruli are identified by histological staining such as cytochrome oxidase [3]. MRI a day or two after systemic administration has been used to visualize cytoarchitectural features in the brain, such as layers in the cortex, hippocampus, and olfactory bulb [4, 5]. The higher sensitivity and spatial resolution available with high field MRI may make it possible to further delineate smaller neural units within a functional layer. In this study, we investigated the potential of manganese enhance MRI (MEMRI) to detect individual glomeruli in the rat OB.

Methods

All animal work followed the guidelines of the Animal Care and Use Committee of NINDS. Adult male Sprague-Dawley rats (body weights 200 – 350 g) were used. After anesthetized by 5% isoflurane, 176 mg/kg, 120-mM $MnCl_2$ solution was infused thru the tail vein at a rate of 2.25 mL/h by a syringe pump (Cole-Parmer Instrument, IL). During $MnCl_2$ infusion, the anesthesia was kept light between 0.5 – 1.2 % and the body temperature was maintained by a water bath. After infusion, rats were kept on a warm water bath until fully awake and were returned to cages for free access to food and water.

One day after $MnCl_2$ infusion, rats underwent MRI scans. The rat was orally intubated, connected to a mechanical ventilator, and secured in a stereotaxic holder. Images were acquired on an 11.7 T/31 cm horizontal magnet (Magnex Scientific Ltd., Abingdon, UK) interfaced to a Bruker Avance console (Bruker Medical GmbH, Germany). A homemade 9-cm birdcage coil was used for homogeneous RF transmission and a 1-cm surface coil on top of the OB was used for signal reception. The T_1-weighted (T1w) image was acquired by 3D RARE (TR/TE = 300/12 ms; 50-μm isotropic resolution). To visualize the blood vessels, 20 mg/kg MION was injected into the tail vein after the T1w scan. Then 3D gradient echo images (TR/TE/FA = 50/3.5/25°; 50-μm isotropic resolution) were acquired. The anesthesia, isoflurane, was kept between 1.5 – 2.0 % and the body temperature was maintained by a temperature controlled water bath.

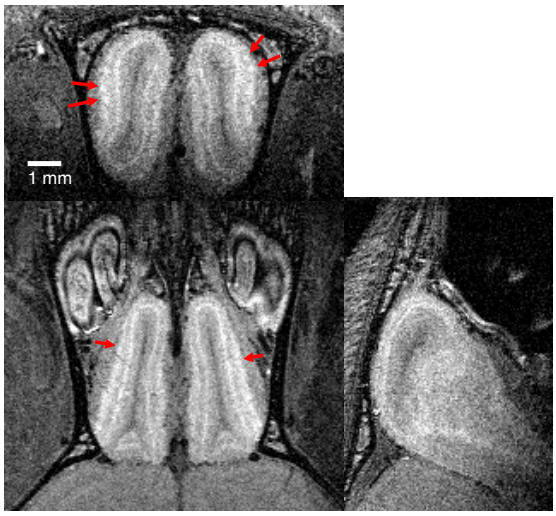

Fig. 1. T1w MRI of a rat OB at 24hr after systemic Mn2+ infusion. Some spots (arrows) in the glomerular layer could be individual glomeruli.

Results and Discussion

Fig. 1 shows three sections from the 3D T1w image of a rat. In the coronal section, the glomerular and mitral cell layers were enhanced as previously reported. Inside the glomerular layer, several round-shaped spots could be observed on the lateral sides (arrows in Fig. 1). The sizes of the spots are about 2 to 3 pixels wide, which corresponds to 100 to 150 microns. Compared to the atlas, this contrast could be due to individual glomeruli.

A number of artifacts could produce apparent contrast differences. The detected Mn^{2+}-enhanced spots could be confounded by the artifacts from blood vessels. Since the arteries have shorter effective T_1 due to fast blood flow, they could appear as high intensity spots in the T1w image. A thick flow saturation slab was placed caudally to the imaging slab. Comparing T1w images with and without flow saturation, the signal intensity in large arteries was reduced, however, the saturation slab had no significant effects in the glomerular layer (data not shown). Therefore, the enhanced focal regions in the glomerular layer were not due to flow in arteries.

Another potential artifact is that the darker boundaries around the spots could be caused by venous vessels which have lower signal due to shorter T_2 caused by deoxyhemoglobin. To reduce this effect, high oxygen levels were used as previously described [6], with no significant effects on contrast. In addition, the exact position of blood vessels was identified from gradient echo images after infusion of MION as an intravascular contrast agent. Compared with the T1w image, some vessels corresponded to boundaries of the enhanced regions indicating that these regions of higher contrast may have been created by presence of veins (blue arrows in Fig. 2). However, there were still many Mn^{2+}-enhanced spots that did not have blood vessels nearby (red arrows in Fig. 2).

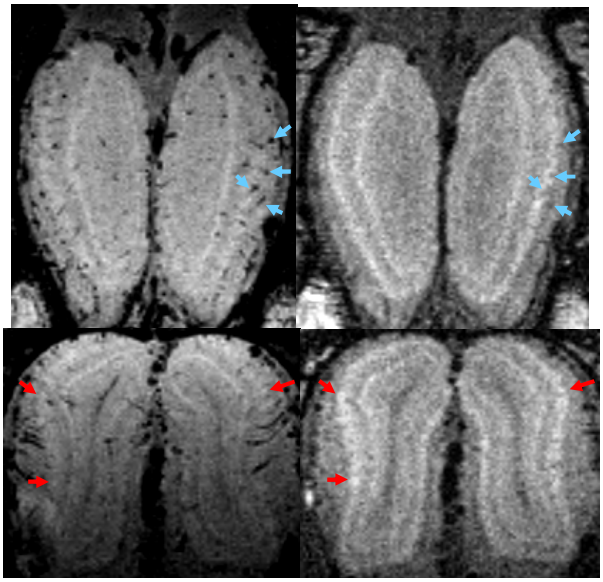

Fig. 2. Gradient echo images after IV infusion of MION (left column). Compared to T1w images (right column), some dark boundaries were created by blood vessels (blue arrows) but some spots (red arrows) didn't have vessels nearby.

In summary, we demonstrated that contrast can be detected in very high resolution MEMRI of the rat olfactory bulb that being caused by individual glomeruli in the bulb. To the best of our knowledge this is the smallest functional mammalian neuronal unit that has been imaged with MRI.

Reference

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