Tracing Olfactory Neural Circuits in vivo using 3D Manganese enhanced MRI

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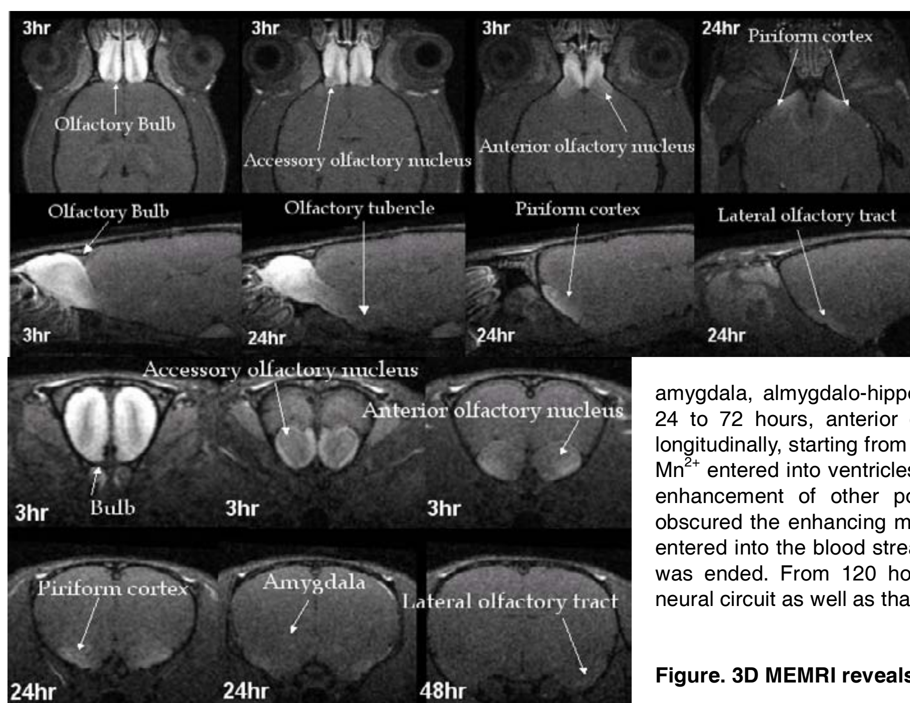
Purpose

In rodents, olfactory system consists of olfactory receptor neurons connected through the bulb to cells whose axons project through the lateral olfactory tract to regions of the olfactory cortex including the anterior olfactory nucleus, olfactory tubercle, and piriform cortex as well as the amygdala and entorhinal cortex (1). To investigate this olfactory system, classic tract tracing studies with radioisotopes of metal ions and functional MRI using BOLD (blood oxygenation level dependent) technique have been done, but low resolution of those studies have limited the comprehensive understanding of olfactory neural circuit (2,3). Manganese enhanced MRI (MEMRI) is a new method for mapping neuronal function(4). Manganese ion (Mn^{2+}) can enter neurons through voltage-gated Ca^{2+}-channels(5) and be transported to the projecting neurons in anterograde direction by a microtubule dependent manner (4). In this work, we developed a protocol using MEMRI by which $MnCl_2$ is delivered directly into the nasal cavity and the animal is briefly exposed to an odorant enabling us to map and trace the pathway of neuronal activation as it appears and evolves over time across the entire olfactory bulb and to the associated regions in olfactory cortex thereby localizing regions respond to an odorant stimulus in the cortex.

Methods

Animal Preparation: For MRI, female Sprague-Dawley rats (160-200g) were anesthetized with 2% isoflurane mixture into oxygen-enriched medical air with a facemask and the rectal temperature was maintained at 36 ± 1°C. To investigate the time course of distribution of Mn^{2+} (40μl for each nostril,120 mM) along the olfactory neural network in normal rat, a series of MRI was performed before, and 1-hr, 3-hr, 24-hr, 48-hr, 72-hr, 96-hr, 120-hr, 7-day, and 14-day after administration of Mn^{2+} through intranasal injection (n=3).
Odor delivery: The entire odor delivery system was composed of two separated covered acryl boxes, in one of which gauzes coated with vanillin (*4-hydroxy-3-methoxybenzaldehyde*) and glycerol mixture (1:10) were attached in the uppermost wall. For prevention of olfactory habituation, fully awakened rat was putting into two separated covered acryl boxes alternatively for 30 seconds, a total of 20 minutes.
In vivo MRI: All *in vivo* MRI were carried on a 4.7T/30 MRI System (Bruker-Biospin, Fallanden, Switzerland) equipped with a 20 cm gradient set capable of supplying up to 100mT/m in 200 μsec rise-time. A birdcage coil (72 mm i.d.) (Bruker-Biospin, Fallanden, Switzerland) was used for excitation, and actively decoupled from a 20 mm diameter saddle-shaped surface coil (homebuilt), which was used for receiving the signal for brain imaging. High-resolution 3D Manganese enhanced MRI was obtained using a fast spin-echo T_1-weighted MRI sequence (TR/TE= 300/12.6 ms, NEX =1, Matrix=256/128/64, 155 μm 3D isotropic resolution).

Results and Discussion



After Mn^{2+} was injected intranasally, we observed bright high signal enhancement in T_1-weighted MR images for up to 2 weeks in normal rats. At 1 hr post-Mn^{2+} application, the whole nasal turbinates showed signal enhancement. At 3 hrs, four layers of the olfactory bulb and accessory and anterior olfactory nucleus could be readily distinguished. The contrast enhancement proceeded from the outer olfactory nerve layer and glomerular layer into the deeper layers such as granular layer of the olfactory bulb causing the final laminar enhancement as time going on. Enhancement in lateral olfactory tract, olfactory tubercle and piriform cortex could be detected at 24 hrs after Mn^{2+} application. From 48 to 72 hours, the maximum signal enhancement was maintained and amygdala, almygdalo-hippocampal transitional zone could be distinguished. From 24 to 72 hours, anterior communicating fiber was thoroughly seen, which runs longitudinally, starting from olfactory bulb, ending in front of 3rd ventricle. At 96 hours, Mn^{2+} entered into ventricles, enhancement of subependymal layer of ventricles and enhancement of other portions of brain parenchyma was started, which fact obscured the enhancing margin of olfactory neural circuit and suggested that Mn^{2+} entered into the blood stream, at that time point detection of olfactory neural circuit was ended. From 120 hours to 2 weeks, the enhancement degree of olfactory neural circuit as well as that of other part of brain parenchyma was decreased.

Figure. 3D MEMRI reveals functional olfactory neural circuit in rats *in vivo*.

Conclusion

We have succeeded in imaging and tracing the pathways of functional olfactory neural circuits in rats for investigations of functional connections of the olfactory neural system using MEMRI.

References

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