Functional Imaging of Olfactory Neural Network in vivo using Manganese Enhanced MRI: A Comparative in vivo Study between Normal and Anosmia Rat Models

Y. J. Yim[1], H. J. Lee[2], H-J. Kim[1], H-J. Dhong[2], S. K. Chung[2], K-H. Lim[3], K-S. Kim[4], and J. H. Lee[1]

[1]Radiology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of, [2]Otorhinolaryngology-Head and Neck Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of, [3]NMR Laboratory, Asan Institutes for Life Science, University of Ulsan, Seoul, Korea, Republic of, [4]Pediatrics, Asan Medican Center, University of Ulsan, Seoul, Korea, Republic of

Purpose

In the olfaction system, environmental chemicals are translated into neural sensory signals that are generated in the nasal olfactory epithelium, relayed through the olfactory bulb to the olfactory cortex, which then transmits signal information to higher cortical areas and limbic structures (1). Since olfaction plays an important role in survival and reproduction in many mammal spices, the development of *in vivo* imaging of olfactory neural network is important and will facilitate greatly a better understanding of neuro-functional connectivity. Manganese (Mn^{2+}) enhanced MRI (MEMRI) is a new emerging technique for imaging functional neural connectivity of olfactory system (2), which has a potential to be used as a tool to diagnose human olfactory dysfunction clinically, given that there is no objective tool available in modern medicine to diagnose human olfactory dysfunction. Therefore, this work was intended to investigate temporary and permanent olfactory dysfunction using MEMRI and to image and visualize the functional status and functional neuronal connections of temporary and permanent olfactory dysfunction compared with normal model.

Methods

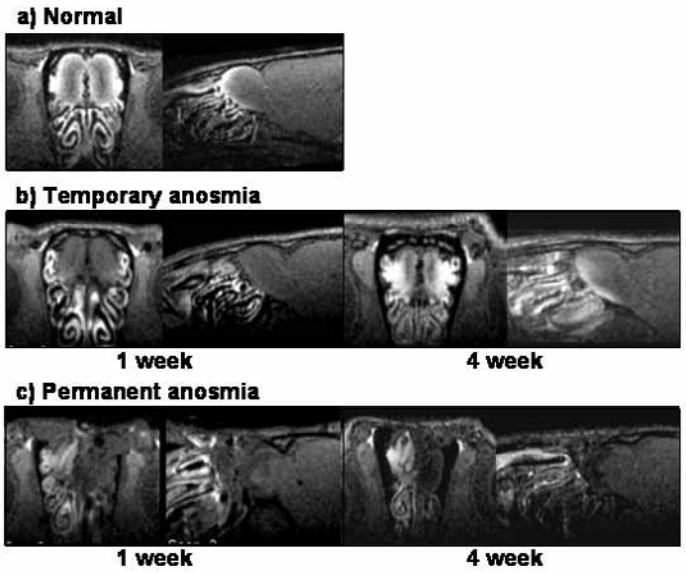
Animal Preparation: For MRI, female Sprague-Dawley rats (160-240g) were anesthetized with 2% isoflurane mixture into oxygen-enriched medical air with a facemask. To investigate the time course of distribution of Mn^{2+} along the olfactory neural network in normal rats (n=3), a series of MRI was performed before, and 1-hr, 3-hr, 24-hr, 48-hr, 72-hr, 96-hr, 120-hr, 7-day, and 14-day after intranasal administration of Mn^{2+} in the form of 120 mM of Mn^{2+} (40 μl for each nostril) (n=3). The reversibly damaged model (n=3) was made by injecting 3-methylindole (300 mg/kg) intraperitoneally 1 week prior to MRI, and a series of MRI was performed before, and 1-hr, 3-hr, 24-hr, 48-hr, 72-hr, and 4 weeks after intranasal administration of Mn^{2+} to investigate the restoration of damaged olfactory epithelium and neural network. For irreversibly damaged model (n=3), bulbectomy (surgical disconnection of olfactory bulb) was done 1 week prior to MRI, and then serial MRI was performed before, and 1-hr, 3-hr, 24-hr, 48-hr, 72-hr, and 4 weeks after intranasal administration of Mn^{2+} to investigate the irreversibility of surgically damaged olfactory neural network (n=3). After MRI, H&E (hematoxylin and eosin) staining and gross pathology were done for examination of olfactory neural network focusing on olfactory epithelium and olfactory bulb.

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 4.7 T/30 MRI System (Bruker-Biospin, Fallanden, Switzerland) equipped with a 20 cm gradient set capable of supplying up to 100 mT/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

In normal rats, signal enhancement in the olfactory bulb was observed in T_1-weighted MRI for up to 2 weeks after intranasal injection of Mn^{2+} (Fig. 1a). The nasal turbinates showed signal enhancement at 1 hr after injection, and the layers of the olfactory bulb, and accessory and anterior olfactory nucleus could be readily detected at 3 hr after injection. Enhancement in cortical areas could be detected at 24 hrs after injection, which was continued up to 72 hours. In contrast, the anosmia model of reversibly damaged olfactory epithelium showed signal enhancement only in the nasal turbinates at 1 week after injection of 3-methylindole and no other enhancement was observed in the olfactory bulb (Fig 1b), as shown in H&E staining that showed a nearly complete disappearance of olfactory epithelium. The follow-up MRI at 4 weeks showed the same signal enhancement pattern as observed in normal rats, and the H&E staining showed a complete recovery of normal arrangement of olfactory epithelium. In the irreversibly damaged anosmia model, MRI performed 1 week after bulbectomy showed signal enhancement only in the nasal turbinates, no other enhancement was observed in the disconnected olfactory bulb (Fig. 1c) and the H&E staining and gross pathology showed a complete disconnection of olfactory bulb. In this irreversibly damaged model, no signal enhancement in the disconnected olfactory bulb as well as any associated olfactory neural network continued for 4 weeks, as shown in the H&E staining and gross pathology.



Conclusion

We have succeeded in functional imaging of olfactory neural network *in vivo* in normal rat model, temporary and permanent anosmia rat models using manganese enhanced MRI. The current result suggests a promising future of the functional imaging of olfactory neural network *in vivo* using MEMRI for diagnosis of olfactory dysfunction.

Figure 1. MEMRI of olfactory bulb a) normal rat model, b) temporary anosmia model at 1 wk and 4 wk follow up, c) permanent anosmia model at 1 wk and 4wk follow up.

References

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