MEMRI and BOLD analyses of the olfactory perception system in response to odorant stimuli in mice

Hirotsugu Funatsu[1], Sosuke Yoshinaga[1], Haruna Goto[1], Makoto Hirakane[1], Shigeto Iwamoto[1], and Hiroaki Terasawa[1]

[1]*Department of Structural BioImaging, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan*

Introduction: Living organisms receive information from their environment through various sensory organs. The surrounding chemical signals contribute to survival and species preservation especially for animals that rely heavily on chemoreception for gathering environmental information. Odors are chemical signals that regulate a wide range of social and sexual behaviors in many animals. They are assimilated by the nostrils upon air intake, and bind to selective olfactory receptors in the olfactory epithelium [1] (Fig. 1). The chemical signals induced by the receptor activation are transmitted through the olfactory sensory neurons to glomeruli, which are located in the peripheral zone of the olfactory bulb, and to higher-order brain areas. The spatial distribution patterns of the glomeruli are reportedly different between odor substances [2].

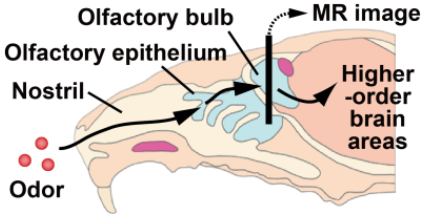

Fig. 1 Olfactory system for odor perception in mice.

The aim of this study is to reveal the olfactory neural circuitry from the olfactory bulb to the higher-order brain areas that recognize and discriminate the odor substances that activate the olfactory system in mice. Examples of such substances are muscone from musk deer to attract females [3] and Z5-14:OH from male mice to attract females [4]. The experiments are based on manganese-enhanced MRI (MEMRI), which has high spatial resolution, and blood oxygenation level-dependent (BOLD) analysis, which has high temporal resolution on a real-time basis.

Methods: MRI experiments were performed with a 7.0 Tesla Bruker Biospec 70/20 scanner and a mouse brain 2-channel phased array surface cryogenic coil (Bruker BioSpin). The experiments were performed on adult male C57BL/6 mice (8–10 weeks old). In the MEMRI experiment, a $MnCl_2$ solution was injected into a nostril of the mouse, anesthetized with 5% isoflurane. After awakening, the mouse was exposed to diluted muscone for 20 minutes. Under 2% isoflurane anesthesia, 3D-RARE images of the olfactory bulb (FOV = 0.80 x 1.00 x 0.64 cm, marix = 80 x 100 x 64, resolution = 100 x 100 x 100 µm, TR/TE = 350/8.6 ms, NEX = 2, RARE factor = 2, and scan repetition time = 32 min) were acquired for 4.5 hours after the odor stimulation. The integral of the signal time course of each voxel was used for analyses with the ImageJ software, to identify the activated regions. In the BOLD experiment, 2D-FLASH images (FOV = 1.28 x 1.28 cm, marix = 64 x 64, resolution = 200 x 200 µm, slice thickness = 320 µm, number of slices = 15, TR/TE = 468.8/16 ms, NEX = 1, flip angle = 40°, and scan repetition time = 30 sec) were acquired for 50 minutes under urethane anesthesia (i.p. 1.5 g/kg initial; 0.1 g/kg/h supplemental). In the middle of the repetitive scans, the mouse was exposed to diluted muscone for 5 minutes. Throughout the experiments, the mice were kept at 37±1°C by a heating pad, and respiration was monitored. The BOLD signal intensity and the T-score were analyzed with the SPM8 software with Matlab, to identify the activated regions.

Results: After the $MnCl_2$ aqueous solution was injected into the right nostril of the mouse, it was exposed to muscone for the MEMRI experiment. As a result, the signal intensity of the right olfactory bulb was gradually enhanced in a time-dependent manner during the four hours of observation after the odor stimulation (Fig. 2b, left and middle columns). The signal enhancement was larger in the olfactory bulb of the mouse stimulated by muscone than that of the control mouse without muscone stimulation (Fig. 2b, middle column). The signal enhancement was especially pronounced in the peripheral zone of the olfactory bulb. In the BOLD experiment, part of the olfactory bulb had a higher signal intensity in response to the muscone stimulation (Fig. 2b, right column). In addition to the lateral peripheral region of the olfactory bulb (arrowheads 1), which was also identified by the MEMRI analysis, the dorsomedial (arrowheads 2) and ventral (arrowhead 3) peripheral regions were identified by the BOLD analysis.

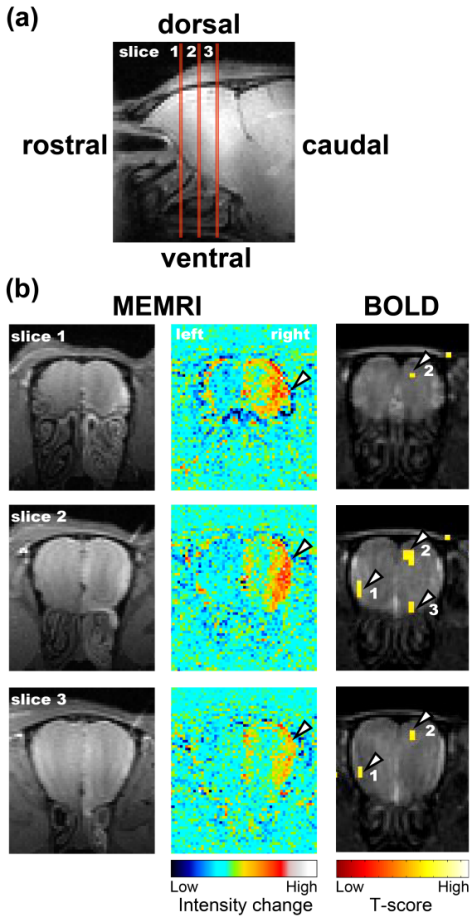

Fig. 2 MEMRI and BOLD activation maps. (a) Sagittal view of the locations of acquired slices. (b) Left column: MEMRI images of the $MnCl_2$-injected mouse without muscone stimulation. Middle column: MEMRI activation maps showing enhanced signal intensity in response to muscone stimulation. Right column: BOLD activation maps showing enhanced signal intensity in response to muscone stimulation.

Discussion: Signal enhancement in the peripheral zone of the olfactory bulb was observed in response to muscone stimulation in both the MEMRI and BOLD analyses. The peripheral zone includes the glomeruli, which receive the chemical signals from the olfactory epithelium [1]. The muscone stimulation appeared to increase Mn^{2+} uptake and accumulation within the glomerular cells. The dorsomedial and ventral peripheral regions identified by the BOLD analysis coincided with the regions identified by an immunohistochemical analysis using c-Fos [3]. The odor-evoked activation on a longer time-scale can be visualized by the combined use of real-time BOLD and longer time-scale MEMRI techniques.

Conclusion: We successfully revealed the olfactory bulb regions activated by muscone stimulation using MEMRI and BOLD.

Perspective: We plan to advance the MRI analyses to the whole brain regions, using odor substances such as Z5-14:OH, muscone, and muscone-mimicking compounds from cosmetic industries, with similar odors but considerably different structures.

References: [1] Touhara, K. and Vosshall, L.B., *Annu. Rev. Physiol.* **71**, 307–332 (2009), [2] Uchida, N. *et al., Nature Neurosci.* **3**, 1035–1043 (2000), [3] Shirasu, M. *et al., Neuron* **81**, 165–178 (2014), [4] Yoshikawa, K. *et al., Nature Chem. Biol.* **9**, 160–162 (2013)