Determination of Odor Concentration Dependent Responses in Olfactory Circuitry using Manganese Enhanced MRI

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Introduction

Olfactory stimuli activate different combinations of olfactory sensory neurons in the olfactory epithelium. Since olfactory sensory neurons expressing the same odorant receptor project axons to the main olfactory bulb and have synapses with mitral cells in the same set of glomeruli in a stereotypic fashion, the activation of olfactory sensory neurons elicited by odorants is transformed to a spatial pattern in the bulb, i.e., an odor map, in the glomerular layer (GL) [1]. Since glomerular and mitral cell activity represent presynaptic and postsynaptic responses, respectively, mapping the functional connectivity in the GL and mitral cell layer (ML) will facilitate the understanding of the neural circuits in this first stage of olfactory processing. In our previous study, manganese-enhanced MRI (MEMRI) has been demonstrated to be able to map neural information flow in the GL and ML of mouse olfactory bulb induced by an odorant [2]. Here, the functional connectivity of the two layers in the bulb was evaluated by MEMRI under different odorant concentrations.

Methods

All animal work followed the guidelines of the Animal Care and Use Committee of NINDS. Adult male C57B/L6 mice (body weights 20 – 32 g) were used. After anesthetized by 5% isoflurane, 7-μL 10-mM $MnCl_2$ was injected into each nostril of the mouse by a micropipette. After waked up, the animal was moved to a modified polycarbonate vacuum-desiccator for odorant stimulation (Scienceware, Bel-Art Products, Pequannock, NJ). Amyl acetate with 10, 100, or 1000 folds dilution in mineral oil was delivered through a custom designed olfactometer. To minimize habituation of the sensation, pulse-like stimulation paradigm was used with 10 s odorant followed by 50 s of clean air and repeated for 20 min. Then the mouse was removed from the chamber for MRI scans. The same mouse was repeated simulating by different concentrations every 3 weeks after Mn^{2+} was cleared from the olfactory bulb. The order of the concentration given each time was pseudo-randomized across mice.

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 RF transmission and a 1-cm surface coil was used for signal reception. Time series T_1-weighted MR images covering the olfactory bulb were acquired every 6 min by 3D MP-RAGE (TR/TE/TI = 2000/2.1/1500 ms; FA = 12°; 100-μm isotropic resolution). The anesthesia (1 – 1.5% isoflurane) was delivered through a nosecone and the body temperature was maintained by a temperature controlled water bath.

Images of each mouse were realigned by SPM to reduce sub-voxel movement [3]. Since neurons activated by an odorant would uptake Mn2+ faster than other neurons, the integral of the signal time course can be used to represent the level of activity. An odorant activation map of individual animal was obtained by calculating the area under the curve. To generate an averaged odor map, olfactory bulb images of different animals were normalized to the same spatial dimension by 9-parameter 3D affine transform using AIR [4]. To reduce intensity variations between animals, the intensity of the activation map was normalized by the mean intensity in the central region of the bulb. To visualize the manganese enhancement in the GL and ML, 2D flat odor maps were created by segmenting both layers and flattening using a method similar to Liu et al. [5].

Results

Fig. 1 shows the dorsal centered (dashed lines) average odor maps induced by amyl acetate at concentrations of 10, 100, and 1000 folds dilution in the GL (upper row) and ML (lower row). In each layer, the enhancement patterns were generally similar and area enhanced by manganese was larger with increasing odorant concentration. This is in line with current knowledge that more glomeruli are recruited at higher odorant concentrations [6, 7]. Taking a threshold at 3x standard deviation, the increase in area size in the ML saturated faster than the GL (Fig. 2). This indicates that the functional connectivity between GL and ML is decreasing as odorant concentration increases.

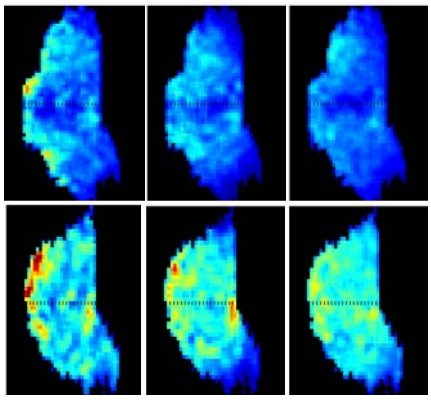

Fig. 1. Averaged odor maps in the GL (upper row) and ML (lower row) stimulated by amyl acetate with 10, 100, and 1000 folds dilution (from left to right).

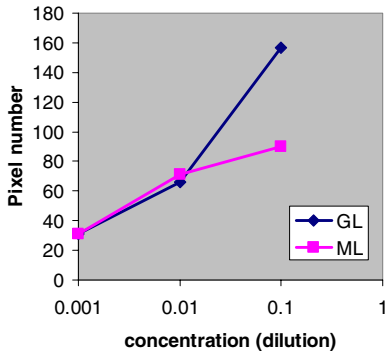

Fig. 2. Enhancement area vs odorant concentrations.

Discussion

Consistently varied enhancement in the same mouse stimulated by different odorant concentrations shows that odor mapping by MEMRI is highly reproducible. Different trends of enhancement levels and patterns in the GL and ML were observed. The GL seemed to respond more linearly with odorant concentrations, while the ML saturated. From the signal changes detected in the GL the 1000-fold dilution is probably the lowest that can be detected with the present MEMRI technique. These results set the useful limits of odorant concentration to study functional connectivity in the olfactory bulb using this MEMRI technique.

Reference

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