Using Manganese Enhanced MRI to Reveal Active Olfactory Circuitry in Response to Odorant Stimuli

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Introduction

Olfactory stimuli activate different combinations of olfactory sensory neurons (OSNs) in the olfactory epithelium. Since OSNs expressing the same odorant receptor (OR) project axons to the main olfactory bulb (MOB) and innervate the same set of glomeruli in a stereotypic fashion, the activation of OSNs elicited by odorants is transformed to a spatial pattern in the MOB, i.e., an odor map, in the glomerular layer (GL) [1]. Current methods (i.e., 2-deoxyglucose, optical imaging, or BOLD fMRI [2,3]) for mapping odorant activation in GL are limited by invasive procedures, field-of-view, or spatial resolution. Manganese enhanced MRI (MEMRI) is a new method for mapping neuronal function [4]. Manganese ion (Mn2+) can enter neurons through voltage-gated Ca-channel and be transported to projecting neurons. While many techniques that have been used to map the bulb struggle to resolve individual glomeruli, it may be possible to achieve glomerular level resolution with MEMRI. Previously, it has been shown that Mn2+ delivered to the nose of mice with an odor led to specific enhancement in the MOB due to accumulation in odor activated OSNs and then tracking of the Mn2+ to the MOB [5]. Neurons activated by an odorant take up Mn2+ at a faster rate than other neurons, therefore the fastest enhancement in the MOB represents the input activity into the glomerular layer. Since the tracing of Mn2+ is relatively slow the enhancement can be detected using high-resolution, dynamic MEMRI. Here we demonstrate a much simpler protocol than previously used and develop a dynamic imaging procedure that enables odor maps to be made from the glomerular layer of the bulb. Furthermore, it is demonstrated that individual glomeruli can be detected by comparison of MEMRI activation to anatomical images of the specific glomeruli expressing green fluorescent protein (GFP).

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 divided into four groups: no odor control (N = 7), octanal stimulation (N = 8), carvone stimulation (N = 5) and acetophenone stimulation (N = 6). To validate MEMRI, 8 rI7 transgenic mice were stimulated by octanal. After anesthetized by 5% isoflurane, 7-μL 10-mM $MnCl_2$ was injected into each nostril of the mouse by a micropipette. The animal was moved to a clean cage for exposing to one of the odors or clean air for 20 min, and then removed from the cage for MRI scans.

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 MOB were acquired every 20 min by 3D RARE (TR/TE = 300/10 ms; 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 [6]. 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 can be obtained by calculating the area under the curve. To generate an averaged odor map, MOB images of different animals were normalized to the same spatial dimension by 9-parameter 3D affine transform using AIR [7]. To reduce intensity variations between animals, the intensity of the activation map was normalized by the mean intensity in the central region of the MOB. The group averaged odor map was calculated by Student t-test with respect to the no odor control maps ($p < 0.05$). To visualize the activation in the GL, 2D flat odor maps were created by segmenting the GL and flattening using a method similar to Liu et al. [8].

Results

Fig. 1 shows the dorsal centered (dashed lines) average odor maps in the GL activated by acetophenone, carvone, and octanal, respectively. T-scores were calculated compared to no odor controls and indicate highly significant signal changes. The symmetrical activation patterns in the medial and lateral sides are consistent with a bilateral arrangement of similar glomeruli based upon the molecular map. Comparing the odors; acetophenone activates dorsal medial regions; carvone activates more ventral regions, and octanal more lateral regions. The activation detected by MEMRI was validated by rI7 transgenic mice in which specific individual glomeruli were genetically labeled with GFP [9]. Fig. 2 shows that MEMRI accurately detected the two rI7-glomeruli that expressing GFP in the specific mice studies. About 80% of the GFP expressing glomeruli in all rI7 mice studied were detected by MEMRI.

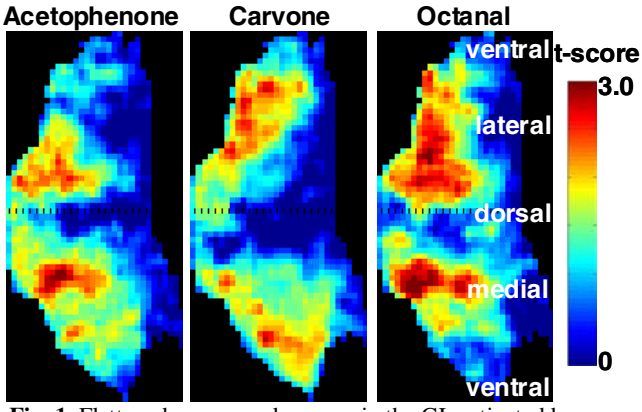

Fig. 1. Flattened average odor maps in the GL activated by acetophenone, carvone, and octanal, respectively.

Discussion and Conclusion

Dynamic MEMRI enabled odorant maps to be made from the GL of mouse MOB. Furthermore, odor activity in the mouse MOB could be detected at the resolution of a single glomerulus. The activation patterns in the GL by three odorants can be differentiated. Since Mn2+ can be transported to the projecting neurons, it has the potential to map the activity in the mitral cell layer. In conclusion, MEMRI can map activity at glomerular level in the mouse MOB. This non-invasive technique allows for repeated experiments in the same animal, thus will be useful in studies involving olfactory plasticity.

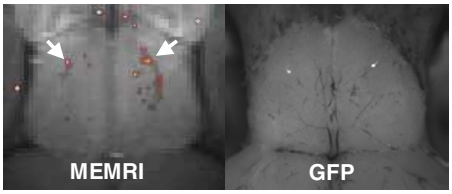

Fig. 2. MEMRI activation map (left) of the two GFP-expressing rI7 glomeruli that respond to a defined stimulus (arrows). On the right, a fluorescent image of the same bulb showing the two glomeruli.

Reference

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