Intra- and inter-subject reproducibility of olfactory bulb activity patterns in the rodent by fMRI

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INTRODUCTION

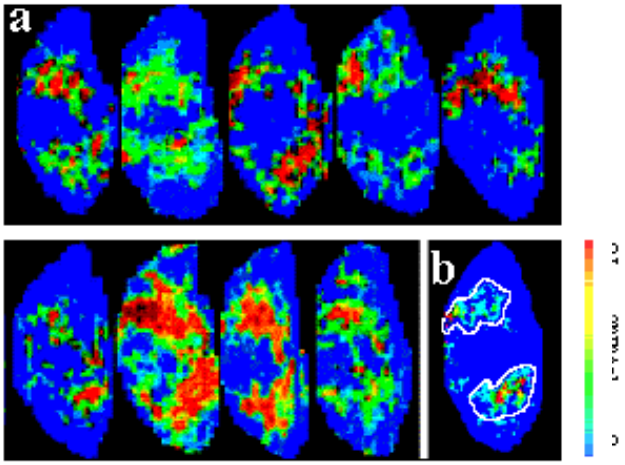
The first processing center in the mammalian olfactory system is the olfactory bulb (OB) in which ~2000 neuropil spheroids called glomeruli encode the input from ~1000 types of olfactory receptor neurons, each specific for a particular chemical feature [1]. A number of imaging modalities have been used to simultaneously assess the activity of many of these channels and have provided insight into the way in which volatile odorants are represented in the OB [2,3]. However, there has been little evaluation of the functional differences between animals or the extent to which a given animal's response is conserved over multiple trials. Here we have used high-resolution fMRI to quantitatively evaluate the similarity of patterns produced by the same subject to a repeated stimulus and to compare the responses of different subjects to the same stimulus.

EXPERIMENTAL

Male Sprague-Dawley rats under urethane anesthesia were stimulated with iso-amyl acetate or carvone (-) in this study. All data were acquired on a modified 7T Bruker Biospec. Imaging experiments were performed using fast low-angle single-shot (FLASH) gradient-echo sequence. T_1-weighted FLASH anatomical images have resolution of 110x110x250 μm^3. Each fMRI experiment contained a series of 24 T_2^*-weighted FLASH images (resolution = 220x220x250 μm^3). The mean image of the pre-stimulation "baseline" images was subtracted from the "stimulation" images on a pixel-by-pixel basis to generate student *t*-maps, which were overlaid onto the corresponding anatomical images to locate the activated region in the OB. Activity maps of the entire glomerular layer were constructed with an algorithm that selects and integrates the glomerular data from multiple slice representations of the entire OB [4].

RESULTS AND DISCUSSION

Carvone (-) has a minty quality and is easily and consistently recognized by rodent subjects. OB activity maps from the same subject were highly conserved from trial to trial using this odorant at the same concentration and exposure duration. Figure 1 shows whole glomerular maps of a subject exposed twice to carvone (-). A quantitative comparison of the extent of pixels activated by both trials (yellow in Fig. 1) for multiple odorants yielded values from 48 to 60%. While this reproducibility level may appear to be low, it is well within expectations from other modalities as well as the fact that the entire activity pattern in the entire glomerular sheet is used to compute the reproducibility level within a subject.



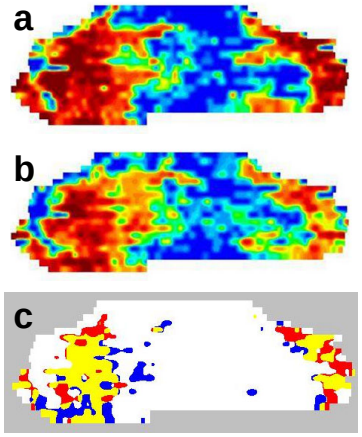


Fig. 1 The activity pattern of carvone (-). Concentration, 4 μM; duration, 2 minutes. (a) trial one, (b) trial two, (c) overlap of (a) and (b) with equivalent thresholds. The yellow pixels in (c) represent regions activated in both trials.

Fig. 2 The activity patterns of iso-amyl acetate in multiple rats (4 μM; 2 minutes). (a) The equivalently scaled patterns from nine OBs (b) the average pattern from eighteen OBs – white boundaries indicate the most highly conserved regions.

In contrast, there was more substantial variability when a given odorant was tested in multiple subjects. Figure 2 shows the response of multiple animals to an equivalent exposure of iso-amyl acetate, an odorant with a fruity quality that is commonly used in olfactory experiments. While there is considerable variability in the fraction of the OB involved and the intensity of that involvement (Fig. 2a), when the images are equivalently scaled and averaged it becomes clear that there are certain shared regions of activity (Fig. 2b). This is consistent with genetic studies that show that olfactory receptor neuron projections often vary greatly between individuals but still localize to broadly defined regions [5].

In conclusion, the consistency of intra-subject patterns and the variability of inter-subject patterns suggest that the primary causes for variations in the responses to identical stimuli are neuroanatomical. These findings help to validate the use of fMRI in the study of olfaction and recommend caution in comparing patterns from different animals.

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