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Reproducibility of odor maps by fMRI in rodents

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The interactions of volatile odorants with the ~1000 types of olfactory receptor neurons in the olfactory mucosa are represented in the olfactory bulb by glomerular spatial activity maps. If these spatial maps underlie the perceptual identification of odorants then, for a given organism, they must be both specific and reproducible. However, this intra-organism reproducibility need not be present between organisms because genetic and developmental studies of olfactory bulb wiring suggest that there is substantial variation between the glomerular arrangements of closely related organisms and even between the two bulbs in a given animal. The ability of functional MRI (fMRI) to record responses of the entire rodent olfactory bulb repeatedly within the same subject has made it possible to assess the reproducibility of odor-induced spatial activity maps both within and between subjects exposed to equivalent stimuli. For a range of odorants, representing multiple chemical classes, a level of fMRI reproducibility (at 7.0 T and 9.4 T) comparable or superior to other cortical regions was demonstrated. While the responses of different bulbs to the same odorant could be localized within the same broad regions of the glomerular sheet, the precise magnitude and topology of the response within those regions were both often highly variable. These results demonstrate the robustness of high-field fMRI as a tool for assaying olfactory bulb function and provide evidence that equivalent perceptual outcomes may arise from divergent neural substrates.

Keywords: Olfaction; fMRI; Reproducibility; Olfactory bulb; Rat

Introduction

The process of sensing of volatile odorants begin in the olfactory mucosa where millions of olfactory receptor neurons (ORNs) sample inspired air. A given mature mammalian ORN ultimately expresses only one of the ~1000 odorant receptor genes (Mombaerts, 2004), and all ORNs expressing the same receptor protein generally converge onto two glomeruli in each olfactory bulb (Ressler et al., 1994; Vassar et al., 1994). Mitral and tufted cells relay the integrated ORN signals from each glomerulus to higher perceptual centers (Pinching and Powell, 1971; Shipley and Adamek, 1984). Since every odor interacts uniquely with each receptor class, there is also a spectrum of glomerular responses that result in the formation of a stimulus-specific spatiotemporal activity pattern across the glomerular layer. Assays of these glomerular activity patterns have suggested that they encode physico-chemical information about peripheral olfactory stimuli, and that they correlate with behavioral outputs (Linster et al., 2001, 2002).

Because of the importance of glomerular activity patterns, many techniques have been used to reveal the rules of their generation (Imamura et al., 1992; Guthrie et al., 1993; Sallaz and Jourdan, 1993; Johnson et al., 2004; Rubin and Katz, 1999; Meister and Bonhoeffer, 2001; Wachowiak and Cohen, 2001; Fried et al., 2002; Bozza et al., 2004). Their putative functions necessitate that the activity patterns generated by repeated interactions with the same stimulus in the same animal be reproducible. Because one odor can stimulate a large fraction of glomerular space, the entire bulb must be studied. However, analysis of such intra-subject reproducibility at the entire bulb level has not been accomplished, mainly due to the limitations of previously available methods. Furthermore, when such comparisons have been made, they have been of an entirely qualitative nature—a fact that not only obscures our understanding of pattern differences but also makes it impossible to assess the relative utility of different imaging modality. In addition, genetic variability and environmental factors may cause the bulbs from different subjects to be neuroanatomically distinct. Significant work

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on the developmental aspects of the system has demonstrated that the projection of ORNs to the bulb is not absolutely fixed in relative or absolute space but is conserved between animals across broadly defined zones (Wang et al., 1998; Royal and Key, 1999; Strotmann et al., 2000; Potter et al., 2001; Feinstein and Mombaerts, 2004). There has been little evaluation of activity pattern differences at the bulbar level between animals for the same stimulation. Because the other global mapping tools need prolonged exposures (usually longer than 30 min), comparison of patterns elicited by short exposures has never been achieved.

High-field functional MRI (fMRI) is capable of measuring the responses at the level of individual glomeruli (Kida et al., 2002) across the entire bulb (Liu et al., 2004) at a time resolution superior to all the other whole bulb mapping techniques (Xu et al., 2000). In addition, its minimally invasive approach means that it can be used to measure the response of the same subject to multiple stimuli (Xu et al., 2003). Here, we have used these advantages of fMRI to assess the variability of bulbar responses to the same odorant both within and between subjects in order to test the hypotheses that the bulbar patterns of a given odor are highly reproducible between trials in a given subject and *only* globally similar between different subjects.

Our fMRI data revealed that the intra-subject glomerular patterns (of one bulb) are of a consistency comparable to that of visual (Rombouts et al., 1998; Miki et al., 2000) and somatosensory (Yetkin et al., 1996) representations in other early sensory processing regions of the brain. While substantial methodological contributions to test–retest reproducibility differences are difficult to rule out, these are not sufficiently great to result in intra-subject differences qualitatively greater than those observed within a smaller field of view using other methodologies (Wachowiak and Cohen, 2001; Bozza et al., 2004). Although the general features of the patterns from different animals are similar, there are significant differences between some animals, especially in terms of pattern intensity. This is expected given the conserved but broad projection zones of ORNs. Despite difficulties in quantifying the comparison of highly variable bulbar structures, examination of the representation of the same odorant in multiple animals lends support to the hypothesis that biological as well as methodological features underlie inter-subject differences in activity patterns.

Methods

Animal preparation

Details of preparation of the male Sprague–Dawley rat (250–350 g) subjects have been described (Yang et al., 1998). Briefly, subjects were anesthetized with 1–2% halothane, and the skull overlying the bulb was exposed. The subject was placed within a specially designed holder that stabilized the head to minimize motion artifacts and maintained body temperature with a warm water blanket. Anesthesia was switched to i.p. urethane (1.5 g/kg initial bolus, 0.1 g/kg/h maintenance), and the subject was placed into the magnet. Subjects were freely breathing. In some rats a femoral arterial catheter was used to measure blood pressure and physiology (pH, pCO_2, pO_2).

Odor preparation and delivery

The odorants (−) carvone, (+) carvone, amyl acetate (AA), ethyl acetate (EA), benzyl acetate (BA), and heptanal (Sigma, St. Louis, MO) were diluted in inert mineral oil (~500 ppm). They were connected to a Teflon and glass delivery system with a dead volume of ~20 ml and which delivered extra pure air (grade 0.1) over either the odorant solution or pure mineral oil at a rate of 5 l/min on its way to a glass funnel fitted over the subject's nose.

fMRI experiments

fMRI experiments were performed in modified horizontal bore spectrometers (Bruker AVANCE, Billerica, MA) operating at high magnetic field (7.0 T and 9.4 T). All scans were conducted with a circular radiofrequency ^1H surface coil (10 mm diameter) located over the skull immediately above the bulb. The static magnetic field was optimized on 10-mm coronal slices until the half-height line width of water was less than 20 Hz. Image contrast in all fMRI experiments was blood oxygenation level-dependent (BOLD) weighted, and data were collected with fast low-angle single shot (FLASH) gradient-echo imagining sequences with: field of view of 1.4 cm^2; image matrix of 64 × 64; slice thickness of 250 μm; flip angle of 15–30°; repetition delay of 500 ms, and echo time of 15–25 ms; and voxel size of 220 × 220 × 250 μm^3. Temporal resolutions were 32 s for entire bulb mapping. Each animal was also anatomically imaged using FLASH (Yang et al., 1998) with a voxel size of 110 × 110 × 250 μm^3. Sixteen dummy scans were carried out before fMRI data acquisition. Experiments were of a standard block design in which twenty-four scans of twenty slices consisted of eight pure air scans, four odor scans, and twelve pure air scans. In all cases, experimental trials were separated by a recovery period of at least 30 min, a duration sufficient for >90% recovery (Schafer et al., 2005).

Data analysis

Data were first processed using in-house Matlab (Natick, MA) algorithms. All data sets showing substantial movement artifacts (>25% of a pixel) by center-of-mass analysis were discarded. For each pixel, an average intensity was established for each stimulation period as well as for the flanking rest periods. A Student's t test generated t values reflecting the odorant-induced activation of each pixel. These t values were then graphically displayed as contour maps of olfactory bulb activity. The maps of sequential slices were combined into a single representation of activity across the entire olfactory bulb using OdorMapBuilder (Liu et al., 2004). All whole-bulb maps compared against one another were generated by the same operator to minimize inter-operator differences in identification of the glomerular layer. To measure the test–retest reproducibility, three analyses were performed: evaluation of the fractional activity overlap and determination of the spatial correlation coefficient (SCC) and normalized dot product (NDP). The first method is shown in Figs. 2b–e. Briefly, two activity maps from the same subject were thresholded such that the same fraction (20%) of their voxels was above threshold. These were then overlaid onto one another, and the fraction of voxels present in both over those present in either was determined. Unlike the fractional overlap, which only evaluates active voxels, the next method takes all pixels into consideration because even the pixels not significantly activated affect the spatial features of an activity pattern. Our method of