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ODOR REPRESENTATION IN THE OLFACTORY BULB UNDER DIFFERENT BRAIN STATES REVEALED BY INTRINSIC OPTICAL SIGNALS IMAGING

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Abstract—The olfactory system responds to the same stimulus with great variability according to the current state of the brain. At the levels of multi-unit activity and local field potentials, the response of the olfactory bulb (OB) to a given olfactory stimulus during a state of lower background activity is stronger than the response that occurs during higher background activity, but the distribution pattern of activity remains similar. However, these results have only been established at the individual neuron and neuron cluster scales in previous studies. It remains unclear whether these results are consistent at a larger scale (e.g., OB regions); therefore, intrinsic optical signals imaging was employed in the present study to clarify this issue. The basal brain states of rats were manipulated by using different levels of anesthesia. Under a state of low basal brain activity, the intensity of the activity pattern elicited in the dorsal OB by a given odorant was significantly higher than that under high basal brain activity, but the topography was highly similar across different brain states. These results were consistent across the levels of individual neurons, neuron clusters, glomeruli, and the OB regions, which suggest that the OB contains as yet unknown neural mechanisms that ensure the high-fidelity representation of the same olfactory stimulation under different brain states.

Key words: brain states, intrinsic optical signals, olfactory bulb, optical imaging, odor representation.

INTRODUCTION

One of the brain's basic functions is to ensure an accurate and precise perception of the ever-changing external world. This function is important not only for daily life and survival but also for higher brain functions, such as decision-making, planning, judgment, and the establishment of preferences that are proper for a given situation (Li et al., 2011). However, the brain itself is constantly modulated by both external and internal factors, such as circadian rhythms; aging; physical, physiological, and psychological factors; arousal; and metabolic conditions (Shulman et al., 2007; Fontanini and Katz, 2008; Tsuno et al., 2008; van Eijsden et al., 2009; Zhu et al., 2009). Therefore, determining how the sensory perception of the same stimulus is represented by the corresponding sensory system under different brain states is important for understanding the brain mechanisms that underlie sensory representations, which is attracting lots of attentions from the relevant fields (Li et al., 2011; Wilson et al., 2011; Kato et al., 2012; Vincis et al., 2012).

Olfaction is crucial for animals to survive in early life, to find food and mates, to recognize prey and predators, and to communicate with con-species (Xu, 2001). To achieve these tasks, the olfactory system must possess the capacity to discriminate among the thousands of odors that occur in nature via accurate representation in neural systems (Doucette and Restrepo, 2008; Restrepo et al., 2009; Doucette et al., 2011). The olfactory bulb (OB) is the first relay station in the olfactory system and is essential for coding, processing and transmitting odor information (Shipley and Ennis, 1996; Mori et al., 1999; Schoppa and Urban, 2003). Olfactory sensory neurons that express the same odorant receptor protein converge onto output and local neurons in a couple of glomeruli of the OB. According to various studies, a single given odor can activate a subset of the ~2000 glomeruli that are situated in the OB of rodents, and a given glomerulus can be activated to different levels by many odorants (Xu et al., 2000a,b, 2003; Soucy et al., 2009). Highly activated glomeruli frequently cluster together to form modules (also termed foci or domains) that constitute the most observable feature of an odor map (Xu et al., 2000a,b; Mori et al., 2006; Wilson and Mainen, 2006; Johnson and Leon, 2007; Soucy et al., 2009). The role of odor maps as the neural basis for perceptual discrimination of both odor quality

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Abbreviations: 2-DG, 2-deoxyglucose; BS, burst suppression; BSR, burst suppression ratio; CC, correlation coefficient; EEG, electroencephalography; HBBA, high basal brain activity; IOS, intrinsic optical signals; LBBA, low basal brain activity; OB, olfactory bulb; ROI, region of interest..

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and odor intensity is supported by ever-increasing experimental data (Uchida et al., 2000; Linster et al., 2001; Xu et al., 2003; Takahashi et al., 2004; Igarashi and Mori, 2005).

Odor maps in the OB of rodent have been studied extensively using various methods, including 2-deoxyglucose (2-DG) imaging (Johnson et al., 1999), c-fos mapping (Guthrie et al., 1993), functional magnetic resonance imaging (fMRI) (Xu et al., 2000b), intrinsic optical signals (IOS) imaging (Rubin and Katz, 1999) and optical imaging using exogenous activity-dependent tracers (Wachowiak and Cohen, 2001; Spors and Grinvald, 2002; Bozza et al., 2004; Fletcher et al., 2009). The results of these studies have led to a consensus that the patterns of the odor map are conserved when the OB responds to the same odor stimulus, although some minor modifications of the maps occur when the same odor is presented in differing concentrations, durations, intervals or other properties that are unrelated to the nature of the odor itself (Rubin and Katz, 1999; Xu et al., 2000b; Wachowiak and Cohen, 2003; Schafer et al., 2005).

Odor perception by the olfactory system is strongly modulated by the ever-changing brain states (Fontanini and Katz, 2008; Li et al., 2010, 2011). The representation of information regarding odors in the OB across different brain states has been extensively investigated using electrophysiological recordings (Adrian, 1950; Rinberg et al., 2006; Gervais et al., 2007; Fuentes et al., 2008; Wilson and Yan, 2010). Our previous studies indicated that in two resting states with significantly different baseline activities (both of which occur under relatively deep anesthesia), the correlation of local field potentials between the two OBs of the same animal were affected significantly (Li et al., 2010). However, the absolute levels and distribution patterns of neural activity elicited by the same stimulus did not change significantly across the two states (Li et al., 2011). This invariability of absolute neuronal activity and representation for a specific event was proposed to comprise a mechanism to ensure that the peripheral olfactory information sent to higher olfactory cortices maintains high fidelity under different operational states of the brain (Hyder and Rothman, 2011; Li et al., 2011). These studies, which used multiple unit and local field potentials, focused on the activities at the level of individual neurons or a cluster of neurons in the OB. However, the effects of brain states on the representation of the same odor at a larger scale, i.e., the patterns of odor map, remain largely unknown.

To evaluate the effects of brain state on a larger scale, we used IOS imaging, which can monitor large areas of the dorsal OB simultaneously with high spatial and temporal resolutions. Consistent with previous studies, high and low basal brain activity (HBBA and LBBA) in rats were generated by manipulating the anesthesia depth (Li et al., 2010, 2011, 2012). Odor representations in the glomerular layer were compared across the two brain states.

EXPERIMENTAL PROCEDURES

Animal preparation

All animal procedures were approved in advance by the Wuhan Institute of Physics and Mathematics, the Chinese Academy of Sciences (No. 00012092). Twenty-eight Sprague–Dawley rats (200–380 g) were divided into two groups and anesthetized with intraperitoneal injection of either chloral hydrate (initial: 350 mg/kg) or pentobarbital sodium (initial: 70 mg/kg), respectively. After being positioned prone on a stereotaxic holder, the scalp of each animal was retracted to expose the skull and bregma. The bone over the OB was thinned using a dental drill (Fine Science Tools, Foster City, CA, USA) and then carefully removed to provide a clean imaging window. The imaging window was then filled with 2% agarose (0815, low melting-point, Amresco) that was dissolved in artificial cerebrospinal fluid and finally covered with a glass coverslip. Anesthesia was maintained such that pinching the hindlimb produced no withdraw reflex during surgery. Body temperature of the animal was maintained at 37 °C and the respiration rate was monitored by recording chest wall movements using a piezoelectric device.

Electroencephalography (EEG) and generation of different brain states

For the EEG recording, a stainless-steel screw was threaded into the bone above the parietal cortex (4 mm posterior from the bregma, ±2.5 mm lateral from the midline). Another screw was threaded into the bone above the cerebellum for reference. The EEG signals were amplified (×2000) and digitized at 1000 Hz using an electrophysiological system (BL-420F, TME, China). The raw EEG data were band-pass filtered through 0.1–70 Hz and downsampled to 200 Hz prior to analysis.

Because two levels of baseline activity must be reliably generated to illustrate the response to odorant stimulation under different brain states, in the current study, we altered global brain activity and basic metabolism by varying the dose of the anesthetic, which was consistent with the procedures we reported previously (Li et al., 2010, 2011, 2012). Briefly, after animal surgery (~1 h after initial anesthesia), a supplementary dose of 30% of the original anesthetic was administered until the EEG recordings showed distinct iso-electrical lines, known as burst suppression (BS) pattern. This deeper anesthesia state was termed low basal brain activity (LBBA). To quantitate this phenomenon, the BS was recognized as periods lasting longer than 0.5 s during which the EEG amplitude was lower than 15 μV. The percentage of time per 60 s epoch that the EEG spends in suppression was defined as BS ratio (BSR) (Rampil, 1998; Vijn and Sneyd, 1998). When the anesthesia level decreased over time, the EEG recordings showed less of the BS pattern, and more high-frequency, high-amplitude signals (“burst”). This state of reduced anesthesia coupled with a significant BSR reduction was termed high basal brain activity (HBBA). In our study, by adjusting the doses of the anesthetics, the BSRs of the two brain states were maintained at 60.0 ± 5.0% for LBBA and 5.0 ± 1.0% for HBBA, respectively. Generally, the time required to reach HBBA from LBBA was approximately 25–40 min. The actual time required for this change varied among animals. In some cases, 2–3 cycles of LBBA and HBBA were generated to evaluate the changes in the baseline of IOS and the response under the two brain states.

Odor stimulation

The odorants used in this study were iso-amyl acetate, ethyl butyrate, hexanal, acetophenone and benzaldehyde (all from Sigma–Aldrich). The animals breathed freely during the