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Spatiotemporal coding of natural odors in the olfactory bulb

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Smell that exists in the natural environment is composed of numerous odor molecules (Bushdid et al., 2014). The mammalian olfactory system can accurately identify environmental olfactory cues, including those related to food selection, recognition of conspecifics/predators, and emotional responses. Recent studies utilizing two-photon calcium imaging have demonstrated that odors, when present at their natural concentrations, elicit distinct patterns of neural activity within the olfactory system (Murthy and Rokni, 2017; Xu et al., 2020). However, knowledge of how food-related odors are coded in the olfactory system remains elusive.

Olfactory perception initially takes place in the mammalian olfactory system when odor molecules selectively bind to the corresponding odor receptors found in the mature olfactory sensory neurons (OSNs) situated in the olfactory epithelium (Buck and Axel, 1991; Zhuang et al., 2021). Subsequently, the OSNs transmit the odor information via their axons to the olfactory bulb (OB), where it undergoes encoding and processing prior to its transmission to the olfactory cortex (Lledo et al., 2005). The OB serves as the first center for the processing of olfactory information within the olfactory pathway. The mitral/tufted (M/T) cells, which are the main output neurons in the OB, possess the ability to generate odor-specific spatiotemporal patterns of spiking that are embedded within local field potentials (LFPs) (Mori et al., 1999; Losacco et al., 2020). The assessment of food freshness heavily relies on odor as a crucial parameter. Given the capacity of neural activities within the OB to reflect food-related information, we developed an in vivo neural interface system employing multichannel microelectrodes to capture the odor responses of the OB (Figs. 1 and S1). Then, we investigated the representation of natural odors released from fresh and spoiled food within the OB.

Diverse types of oscillation, categorized based on the frequency parameter, are observed in the OB. The θ (1–12 Hz) oscillation in the olfactory system has been confirmed to synchronize with the respiration cycle, also known as the respiratory rhythm (Zhuang et al., 2019). The β and γ oscillations in the 15–30 Hz and 40–100 Hz frequency ranges, respectively, are closely associated with olfactory coding and both play a crucial role in odor-discrimination (Kay et al., 2009). In order to investigate the odor-evoked LFP responses in the OB, we conducted an experiment using both natural and monomolecular odors (citral and isoamyl acetate). The natural odors utilized in this study encompassed the smell emitted from bananas, oranges, pineapples, strawberries, rice, and milk. These odors were characterized by their intricate composition of multiple molecules, capable of binding to various types of olfactory receptors.

The results revealed that different odors could evoke distinct oscillatory activities, with a notable enhancement in the power of LFP during odor stimulation. We then applied filtering techniques to isolate the θ, β, and γ frequency bands in the raw LFP data, and observed a significant increase in the amplitudes of β

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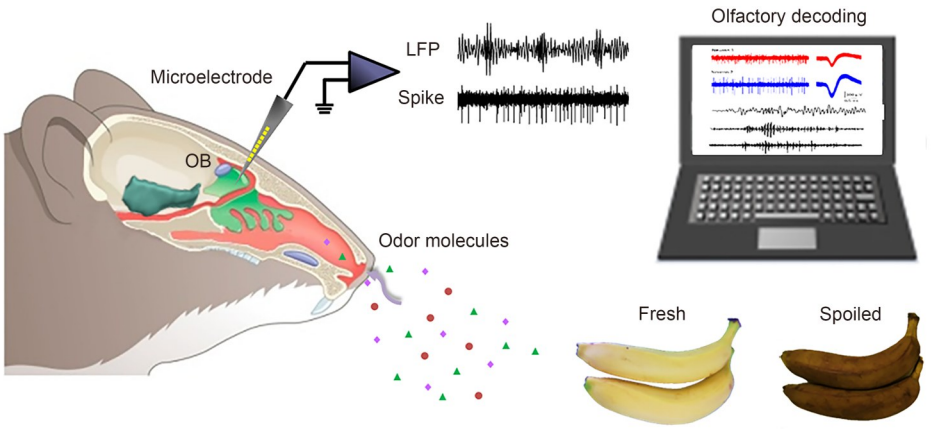

Fig. 1 Schematics of the in vivo neural interface system. Microelectrodes are used as a neural interface to read out food-related odor information from the olfactory bulb (OB). LFP: local field potential.

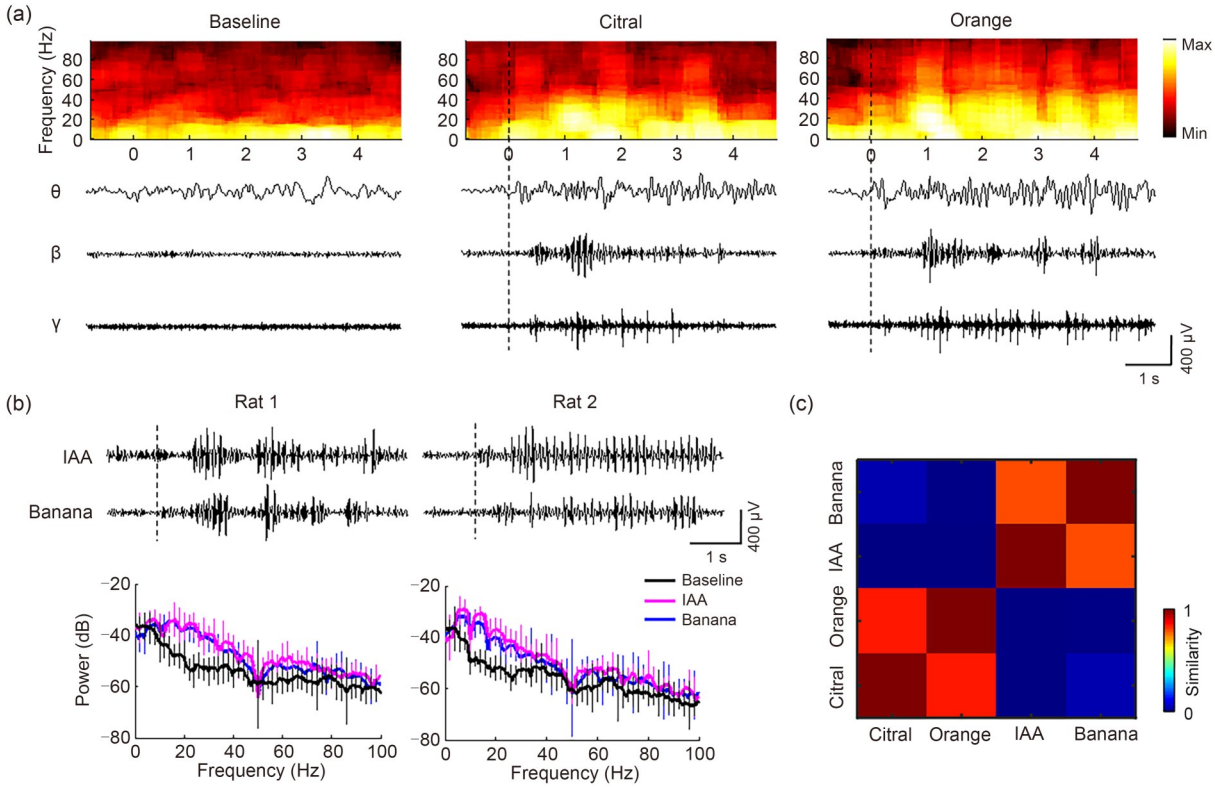

Fig. 2 Odor-evoked local field potentials (LFPs) in the olfactory bulb (OB). (a) LFPs evoked by citral and orange in a single rat. In each plot from top to bottom: the raw LFP (1–100 Hz) signal spectrogram, the representative filtered θ (1–12 Hz), β (15–30 Hz), and γ (40–100 Hz) oscillation signals. Signal power is represented using color scales. Dashed lines indicate odor onset time. (b) β oscillations evoked by banana and isoamyl acetate (IAA, $C_7H_{14}O$). LFP power spectra from two rats before and during odor stimulus. The average power spectra are indicated by solid traces. (c) Correlation matrix of response similarity between β oscillatory waveforms (n=3 rats).

and γ oscillations in response to the odors (Fig. 2a). Interestingly, natural and monomolecular odors that smell similar (citral and orange, isoamyl acetate and banana) elicited analogous β oscillations (Fig. 2), indicating that β oscillations displayed odor-specific waveforms.

While the responses of the OB to monomolecular odors have been extensively investigated, there is limited discussion regarding the responses to natural odors. Consequently, our analysis primarily focused on elucidating how the identity of natural stimuli was

encoded in the unitary activities. To achieve this, we employed an in vivo neural interface system to simultaneously record the extracellular potential of M/T cells. Before being exposed to the odor stimuli, the M/T cells exhibited varying levels of spontaneous activity (Fig. S2a and Table S1). Specifically, the spike firing mode (Fig. S2b) and the waveform display (Fig. S2c) recorded in the different channels were significantly different. Fig. 3a provides an illustration of six recorded M/T cells responding to four stimuli. The response of the cells can be categorized into three groups based on the changes in firing rate: excitatory, inhibitory, and non-responsive. For each cell, we calculated the mean spontaneous firing rate (f_{spon}) and the mean odor-evoked firing rate (f_{odor}) for each neuron cell (Fig. S3). Fig. 3b presents polar graphs depicting the average response as a function of the firing-rate change ratio $\Delta f/f_{spon}$ ($\Delta f=f_{odor}-f_{spon}$). The graphs exhibit distinctive shapes for each odor, suggesting that M/T cells have the capacity for odor discrimination.

According to the above findings, it was observed that distinct natural odors elicited odor-specific β oscillations and unitary activities. Moreover, related odors elicited comparable β oscillations but distinct unitary activities. Given that LFPs provide insights into neural activity across extensive areas of the OB and unitary activities were generated by individual units, the results suggest that the neuronal population of the OB may exhibit analogous response patterns to related odors, while individual units within the population manifest odor-specific responses to different types of odors. We conjectured that this phenomenon may provide an explanation for the OB coding mechanisms involved in related odor discrimination. These results demonstrated that the in vivo neural interface system was capable of discerning related odors. Therefore, further investigation was conducted to examine activity of the OB in response to natural odors emitted by fresh and spoiled food.

Figs. 4a and S4 show the unitary activities of five M/T cells following exposure to two distinct spoiled odor stimuli at varying time intervals. The mean spontaneous spikes and odor-evoked spikes were subsequently calculated (Fig. S5). The firing patterns

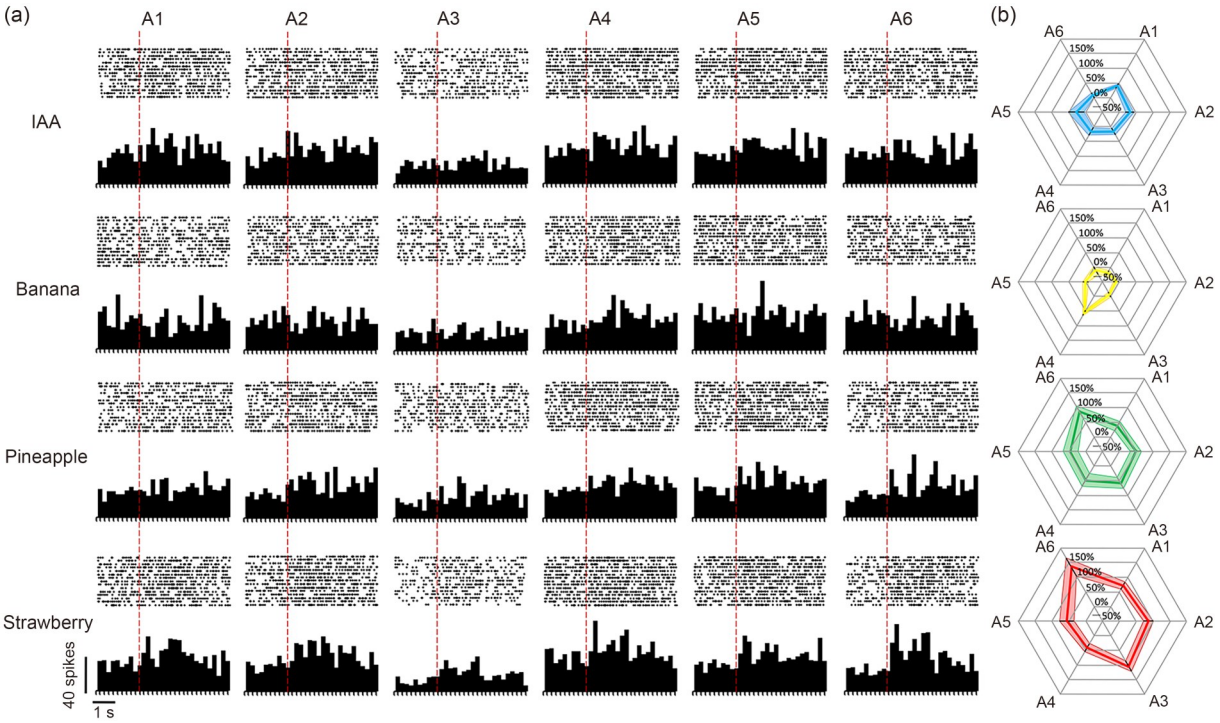

Fig. 3 Unitary activities evoked by different odors in the olfactory bulb (OB). (a) Rasters and peristimulus time histograms (PSTHs, bin=0.2 s) of six simultaneously recorded mitral/tufted (M/T) cells (A1–A6) from one rat when responding to isoamyl acetate (IAA), banana, pineapple, and strawberry. Fifteen trials are displayed for each odor. The vertical dash lines show the odor onset time. (b) Polar graphs of the six M/T cells' average responses in terms of $\Delta f/f_{spon}$. The standard deviation (n=15 trials) for the six cells ranged between 6.1% and 31.6%. f_{spon}: mean spontaneous firing rate; f_{odor}: mean odor-evoked firing rate; $\Delta f=f_{odor}-f_{spon}$.

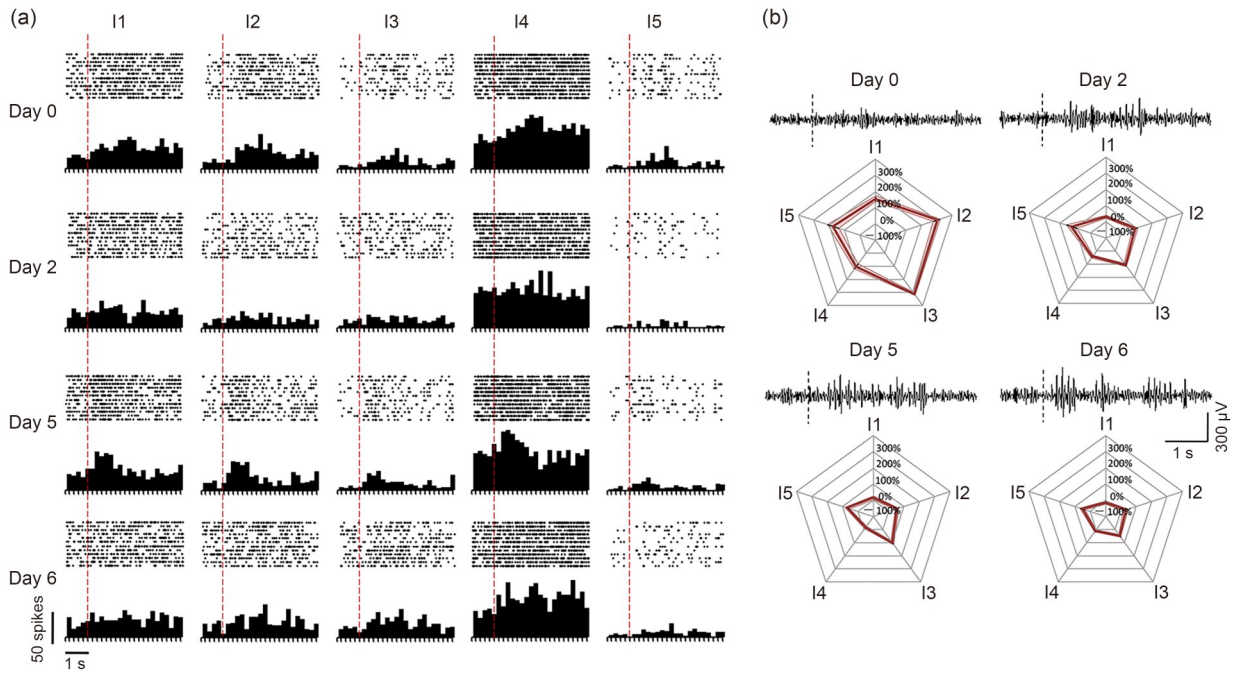

Fig. 4 Olfactory bulb (OB) responses to fresh and spoiled milk. (a) Unitary activities of five mitral/tufted (M/T) cells (I1–I5) from one rat in response to odor stimuli from food spoiled for different time periods. (b) Odor-evoked β oscillations and polar graphs of the five M/T cells' average responses to milk with different storage days. The standard deviation (n=15 trials) of firing-rate change ratios for the five cells was 4.1%−34.7%.

of the neuron cells were influenced by the composition and concentration of volatile compounds emitted from the natural food. Through the calculation of firing-rate change ratios, a notable disparity in the polar graph shapes elicited by fresh and spoiled stimuli was identified (Figs. 4b and S6a). Furthermore, it was discovered that stimuli derived from food stored for varying durations ("storage days") could evoke distinct β oscillations (Fig. 4b). In Fig. S6b, a clear increase in the power of β and γ oscillations was observed during odor stimulation. Various natural odors elicited odor-specific β oscillations and unitary activities, indicating that the composition of compounds in spoiled food differs based on the level of decomposition. The M/T-cell activity induced in the OB by different odors may partially reflect the freshness of food.

The assessment of food quality greatly relies on the odor perception of the mammalian olfactory system, which explains the extensive efforts to develop instruments mimicking olfactory capabilities. Significant progress has been achieved in comprehending the mechanisms underlying the detection, discrimination, and recognition of odor molecules within the olfactory system, owing to advancements in various academic disciplines such as biochemistry, genetics, cellular biology, and neurophysiology (Sankaran et al., 2012; Kim et al., 2022). These interdisciplinary studies have furnished a solid foundation for our research endeavors.

The integration of microelectrodes with the OB enables the extraction of olfactory information through the analysis of neural activity. A single M/T cell carries substantial information regarding stimuli, and thus a limited number of cells may be adequate for discerning multiple stimuli (Zhuang et al., 2015). Previous investigations have identified the significant involvement of β and γ oscillations in odor discrimination (Kay and Beshel, 2010; Lepousez and Lledo, 2013), yet conflicting findings have emerged regarding the relative importance of β and γ oscillations. In this study, we observed bursts of β and γ oscillations following odor stimulation, and it was determined that β oscillation bursts exhibited a more consistent, odor-specific oscillatory pattern compared to γ oscillation bursts, suggesting that β oscillation plays a more important role in odor discrimination.

Material and methods

Detailed methods are provided in the electronic supplementary materials of this paper.

Data availability statement

The authors declare that the data supporting the findings of this study are available within the paper and its supplementary information files. If any raw data files should be needed in another format, they are available from the corresponding author upon reasonable request.

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Author contributions

Liujing ZHUANG and Ping WANG conceived the research and designed experiments; Mengxue LIU and Nan JIANG performed the experiments and analysis; Yingqian SHI performed data analysis; Liujing ZHUANG and Mengxue LIU wrote the manuscript. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Liujing ZHUANG is a young scientist committee member for *Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)* and was not involved in the editorial review or the decision to publish this article. Mengxue LIU, Nan JIANG, Yingqian SHI, Ping WANG, and Liujing ZHUANG declare that they have no conflict of interest.

All animal studies were approved by the Institutional Animal Care and Use Committee and the Ethics Committee of Zhejiang University, Hangzhou, China (No. 19496).

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Supplementary information

Materials and methods; Figs. S1–S6; Table S1