Supplementary information

Spatiotemporal coding of natural odors in the olfactory bulb

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Methods and materials

Multichannel electrode

In this study, electrophysiological recordings from olfactory bulb (OB) were obtained through a 16-channel hand-made microelectrode (Fig. S1). This microelectrode consists of two parallel rows of eight microwires (formvar-coated nichrome wire of 38 μm coated diameter (AM system, #761500, WA, USA). The distance between microwires in a row varied from 200–300 μm, and the distance between the rows varied from 300–500 μm.

Animals, surgery and histology

Experiments were performed in accordance with a protocol approved by the Zhejiang University Animal Care and Use Committee. Seven male Sprague-Dawley rats (200–240 g) were implanted under anesthesia with chloral hydrate (intraperitoneal injection, 4 mL/kg). The microelectrode was stereotaxically positioned as: about 8.2 mm anterior to bregma and about 1 mm left lateral. Searching for M/T cells was done by advancing the microelectrode into OB in micro-sized steps with an oil hydraulic micromanipulator (Narishige Group, Japan). The identity of M/T cells was based on their large unit activity using electrophysiological monitoring (Kay and Laurent, 1999). When each electrode depth was adjusted at/near the level of M/T cell layer, the microelectrode was chronically fixed onto the rat's head by dental cement. Electrophysiological recordings were initiated 4–5 d after the surgery. At the end of experiment, perfusion-fixation with 4% formalin was performed to identify the recording regions in OB using immunostaining technique (Fig. 1).

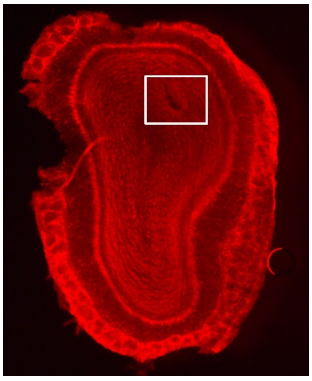

Fig. 1 Representative coronal OB section showing the implanting of electrode tip (white box).

Odor preparation and stimulation

All natural stimuli (banana, orange, pineapple, strawberry, rice, milk) were purchased from

local grocery stores. Rice was cooked before experiment. Fresh natural stimuli were prepared each experimental day. Spoiled natural stimuli were obtained by storing for one or more days at room temperature (25–30 °C) before being analyzed. Because the concentrations of natural odors sent by the olfactometer would be reduced, we manually placed a glass dish containing 5 g (fruits, rice) or 3 mL (milk) of stimuli in front of the rat's nose and stayed for 5 s. Within this 5 s, the rat itself determined the duration of odor sampling. The monomolecular odors (citral, isoamyl acetate) were diluted with odorless mineral oil. The concentration of used monomolecular odors is 1×10^{-3} mol/L that smells as strong as the natural odors. Monomolecular odors were also delivered manually: we placed a glass dish containing a piece of filter paper on which was deposited 0.3 mL odor in front of the rat's nose (1–3 cm) and stayed for 5 s.

All odor stimulation was conducted with freely behaving rats placed in a custom-made polymethyl methacrylate chamber (30 cm×25 cm×30 cm). After achieving odor response, we would refresh the chamber with fresh air and waited for 5 min to avoid habituation. Then, another trial would be started.

In vivo neural interface system for odor detection

By attaching the connector of microelectrode to pre-amplifier with headstage cable connected to OmniPlex Data Acquisition System (Plexon, Inc., Dallas, TX), electrophysiological investigation of neural activity could be achieved. The electrophysiological signals were recorded with reference to rat's skull screw which was amplified by 1000× gain, sampled at 40 kHz. Raw signals were saved for off-line analysis.

Data analysis

Neural activity from OB was recorded as a broadband signal (0.5–8000 Hz). Unitary and local field potential (LFP) signals were analyzed off-line by MATLAB (Mathworks, Inc.). Unitary activity (200–4000 Hz) was extracted by band-pass Butterworth filter. Once the distribution of spikes was achieved by peak value extraction method, each spike was timestamped. The time values were used to calculate firing frequencies and to construct raster plots. Single units were classified based on waveform shape, but we made no further attempt to distinguish between well isolated and intermixed waveforms. Student's *t*-test was used as an estimation for significance ($P<0.05$).

LFPs (1–100 Hz; θ oscillation, 1–12 Hz; β oscillation, 15–30 Hz; γ oscillation, 40–100 Hz) often reflect the activity of neural network within a volume of tissue. A larger oscillation represents a more coordinated M/T cell assembly near the electrode, while a smaller oscillation represents either a smaller or less precise assembly (Kay et al., 2009). LFPs were also obtained by band-pass Butterworth filter. Spectral analysis of LFPs were carried out by mtspectrumc or mtspecgramc function (Chronux script package, http://chronux.org). To quantify the similarity between the odor-evoked β oscillation, the cross-correlation between oscillatory waveform was calculated using the '*corrcoef*' function.

References

Kay LM, Beshel J, Brea J, et al., 2009. Olfactory oscillations: the what, how and what for. *Trends Neurosci*, 3**2**(4):207-214. https://doi.org/10.1016/j.tins.2008.11.008

Kay LM, Laurent G., 1999. Odor- and context-dependent modulation of mitral cell activity in behaving rats. *Nat Neurosci*, **2**(11):1003-1009. https://doi.org/10.1038/14801

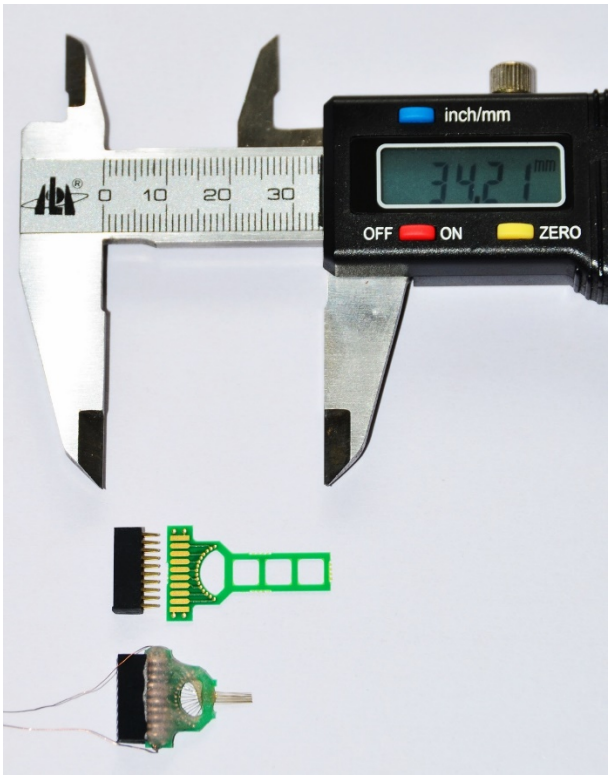

Fig. S1 **Sixteen-channel hand-made microelectrode. The 16-channel microelectrode consists of two parallel rows of eight microwires (formvar-coated nichrome wire of 38 µm coated diameter (AM system, #761500, WA, USA)). The distance between microwires in a row varied from 200–300 µm, the distance between the rows varied from 300–500 µm.**

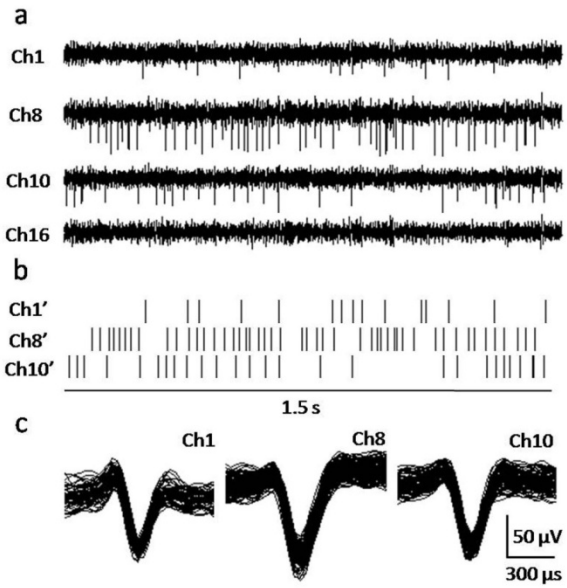

Fig. S2 **Discriminated unitary activity from four channels of microelectrode. (a) Spontaneous activity of single-unit (200–4000 Hz) from four channels (Ch1, Ch8, Ch10, and Ch16, *n*=16) of one microelectrode. (b) Raster plots of spike from three channels (Ch1', Ch8', and Ch10') shown in Fig. S2a. (c) Three recorded units could be reliably discriminated with spike waveforms.**

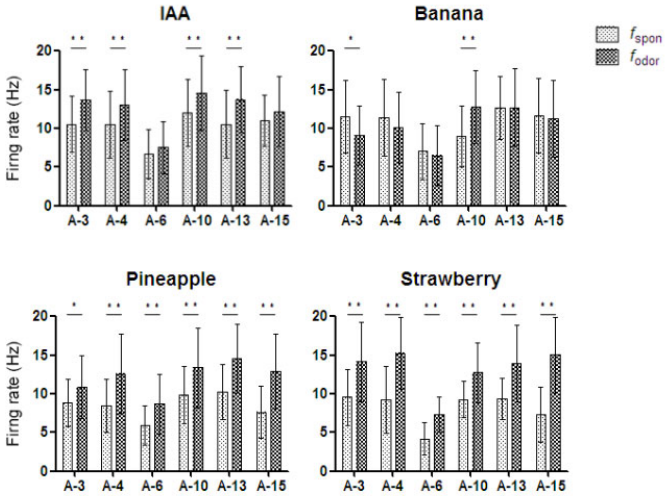

Fig. S3 Average odor-evoked responses of six putative M/T cells. f_{spon}: mean spontaneous firing rate before stimuli; f_{odor}: mean odor-evoked firing rate after stimuli. Data were presented as mean±standard deviation (SD), n>10. Significant differences are indicated by $^{**}P$<0.01 and $^{*}P$<0.05 using Student's t-test.

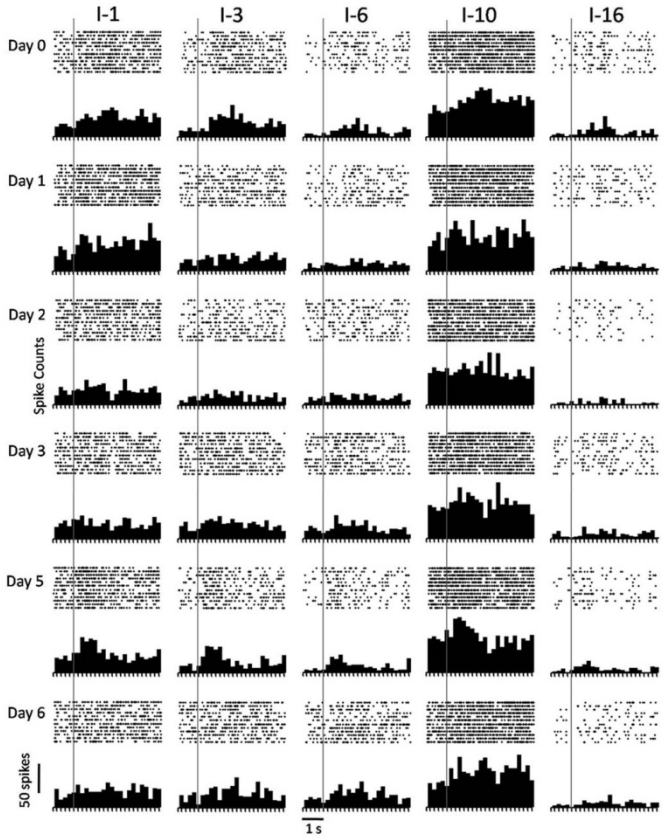

Fig. S4 Unitary activities in a conscious rat evoked by fresh and spoiled cooked rice. Rasters (top of each graph) and peristimulus time histograms (PSTHs; spike counts/bin, bin=0.2 s, bottom of each graph) show spike rates for five M/T cells (I-1, I-3, I-6, I-10, and I-16) recorded simultaneously in response to stimuli spoiled for different periods of time: 0–3 and 5–6 d. Twelve trials are displayed for each stimulus. The vertical lines indicate the odor onset time.

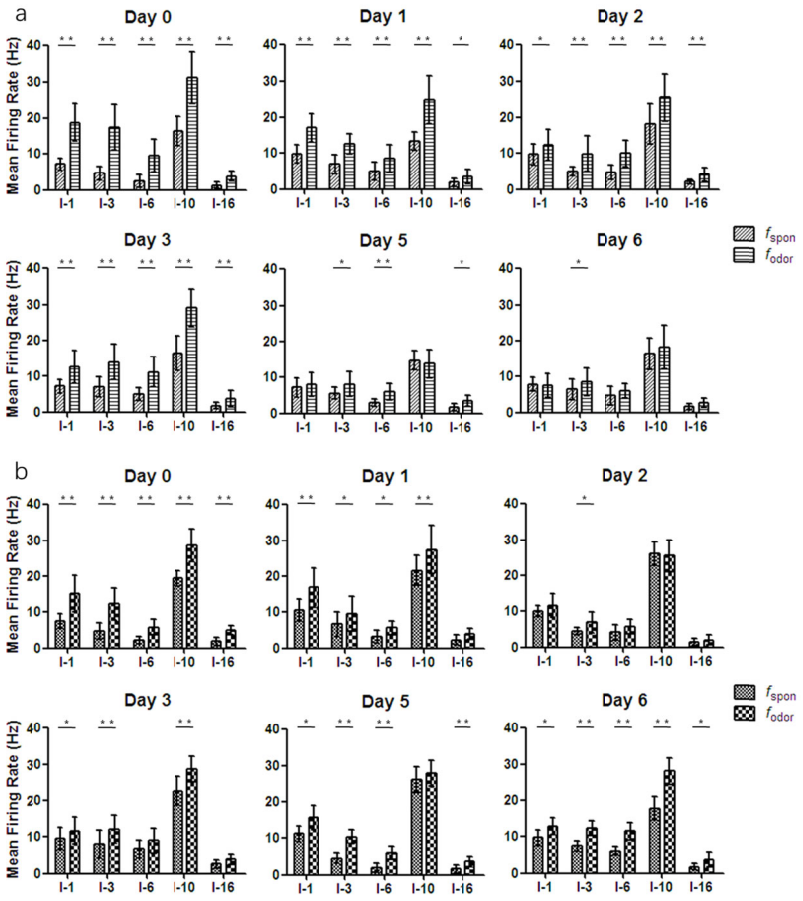

Fig. S5 Average response of five putative M/T cells (I-1, I-3, I-6, I-10, and I-16) to two stimuli spoiled for different time. (a) Average response to milk. (b) Average response to cooked rice. f_{spon}: mean spontaneous firing rate before stimuli; f_{odor}: mean odor-evoked firing rate after stimuli. Data were presented as mean±standard deviation (SD), n>10. Significant differences are indicated by $^{**}P$<0.01 and $^{*}P$<0.05 using Student's t-test.

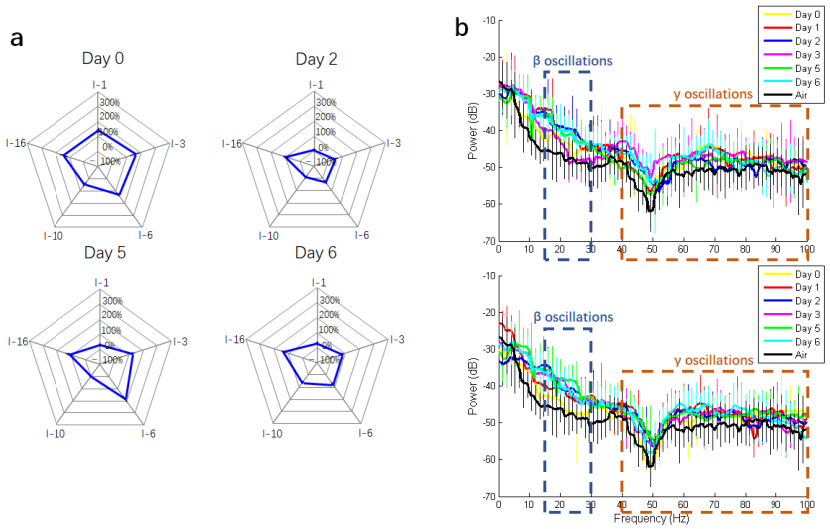

Fig. S6 (a) Odor-evoked polar plots of average response of five M/T cells from one rat to cooked rice at different days. The standard deviation (n>10) of firing rate change ratios for the five cells ranged between 8.2 and 32.6%. (b) Local field potentials (LFPs) evoked by two stimuli spoiled for different time. LFP spectral power before and during odor stimulation. The solid traces are average power spectrum. Vertical bars represent Jackknife error at P<0.01.

Table S1 Summary of spontaneously active statistics

Animal ID	Number of multiple units	Number of single units	Mean firing rate (Hz)	Mean peak-to-peak voltage amplitude (μV)
A	12	12	11.4±1.4 (range: 9.4–14.1)	69.1±8.3 (range:52.9–84.5)
B	8	10	17.0±3.0 (range: 11.0–20.7)	82.4±17.2 (range: 52.3–121.6)
C	7	9	17.9±1.8 (range: 15.2–20.6)	80.0±15.0 (range: 53.0–117.6)
D	7	10	23.6±2.3 (range: 20.4–27.0)	99.5±24.0 (range: 61.7–162.9)
F	10	11	6.1±1.3 (range: 4.0–9.0)	49.5±10.8 (range: 32.0–79.6)
G	4	4	3.2±1.4 (range: 2.0–5.7)	42.6±7.1 (range: 31.0–65.9)
H	4	6	8.7±2.8 (range: 5.7–13.5)	67.4±10.6 (range: 43.3–89.9)
I	11	13	6.9±5.9 (range: 1.0–21.6)	137.9±30.9 (range: 73.3–237.8)