Supporting Information

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SI Text

Identification of Recording Sites. On completion of the recording, a current of 1 mA with a duration of 20 s was applied to the recording site in the olfactory bulb (OB) through the recording and indifferent electrodes (1, 2). The animal was given a cardiac perfusion with physiological saline and followed by a mixture of 4% paraformaldehyde in 0.1 M phosphate buffer of pH 7.4. The brain was then removed from the cranium, fixed by immersion in 4% paraformaldehyde in a 0.1-M phosphate buffer, and stored in PBS solution containing 20% sucrose at 4 °C. After the brain was embedded in OCT (optimal cutting temperature) compound, 40-µm coronal sections were obtained with a cryostat. The tissue sections were mounted onto glass slides and stained using the Nissl method. The electrolytic lesion was examined under a light microscope. All recording electrodes were at the expected layers, as shown in Fig. S3.

Data Processing. The methods to identify neuronal spikes are similar to the previously reported (3, 4). Briefly, off-line analysis (Spike-2; CED) was used to create a spike template, which excludes artifacts of the stimulus and identifies high signal-to-noise ratio units from one to four neurons per microelectrode. The artifact-free spike template was then used to create a temporal history of the ensemble by converting to spiking frequency (ν)

with 0.25-s bins. The template created from the light anesthesia data were used to analyze the data from both light and deep anesthesia. Another template was created from deep anesthesia data and also used to analyze the data from both deep and light anesthesia. Results from both templates yielded consistent results that matched well ($\pm 10\%$ in amplitude and ± 0.1 ms in shape), suggesting that the template recognition process was not biased to specific anesthetic state nor were there any microelectrode movements during transition between the baselines (3, 4). Spike analysis from 29 recording sites provided 69 neurons in the neuronal ensemble.

Raw data [both local field potential (LFP) and multiunit spiking signals] from 4 s before to 6 s after the onset of the odor stimulus were selected for further process. These 10-s data were binned with a width of 0.256 s (512 sample points for LFP). Time courses of LFP, multiunit, or spiking rate were obtained for each recording. Spectrum analysis and calculation of the spectrum power of LFP and spike count of multiunit activity were performed using spike software (Spike-2; CED). After time-frequency transformation analysis (Fourier Transform, Hanning window) of the LFP, the following three frequency bands of LFP were filtered for further process: 12 to 32 Hz, 33 to 64 Hz, and 65 to 90 Hz. The selected three frequency bands are comparable to other OB studies (5, 6).

- Fontanini A, Bower JM (2005) Variable coupling between olfactory system activity and respiration in ketamine/xylazine anesthetized rats. J Neurophysiol 93:3573–3581.
- Hyder F, Rothman DL, Shulman RG (2002) Total neuroenergetics support localized brain activity: Implications for the interpretation of fMRI. Proc Natl Acad Sci USA 99:10771–10776.
- Murakami M, Kashiwadani H, Kirino Y, Mori K (2005) State-dependent sensory gating in olfactory cortex. Neuron 46:285–296.
 Neville KR, Haberly LB (2003) Beta and gamma oscillations in the olfactory system of the urethane-anesthetized rat. J Neurophysiol 90:3921–3930.
 - the urethane-anesthetized rat. J Neurophysiol 90:3921–3930.
 Rojas-Líbano D, Kay LM (2008) Olfactory system gamma oscillations: The physiological dissection of a cognitive neural system. Cogn Neurodyn 2:179–194.
- Smith AJ, et al. (2002) Cerebral energetics and spiking frequency: The neurophysiological basis of fMRI. Proc Natl Acad Sci USA 99:10765–10770.



Fig. S1. LFP signals of five odorants at nine recording sites (S1–S9) in the GCL. (*A* and *B*) The LFP signals under high brain state (HBS) and low brain state (LBS), respectively. Because the error bars of an odorant at a given site will overlap with the others, they are presented in horizontal orientation but with the same scale as in the *y* axis. Data are from nine rats, each odor with four to six repeats at each recording site.



Fig. S2. LFP and EEG recordings under different brain states. EEG of the parietal cortex and LFP of the GCL recorded in LBS (A) showing iso-electrical lines, and in HBS (B) with more high-frequency signals.



Fig. S3. Identifications of recording sites. The arrows mark the recording electrodes at the mitral cell layer (MCL) (A) and granular cell layter (GCL) (B), respectively. The thin mitral cell layer is within the two dotted white lines.

Table S1.	Effects of a	anesthetics and	recording	locations of	on the I	LFP sig	gnals in	LBS	and	HBS
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		GCL, chloral hydrate		GCL, pen	tobarbital	MCL, chloral hydrate		
		Rest	Activated	Rest	Activated	Rest	Activated	
12–32 Hz	LBS	0.46 (0.04)	2.72 (0.21)	0.61 (0.05)	2.12 (0.12)	0.76 (0.05)	2.27 (0.11)	
	HBS	1.00 (0.04)	2.87 (0.20)	1.00 (0.04)	2.27 (0.15)	1.00 (0.03)	2.15 (0.08)	
	t test	<i>P</i> < 0.001	P = 0.46	<i>P</i> < 0.001	P = 0.43	<i>P</i> < 0.001	P = 0.44	
33–64Hz	LBS	0.54 (0.05)	3.39 (0.20)	0.50 (0.04)	1.52 (0.10)	0.54 (0.02)	1.77 (0.05)	
	HBS	1.00 (0.04)	3.44 (0.18)	1.00 (0.04)	1.75 (0.10)	1.00 (0.02)	1.86 (0.05)	
	t test	<i>P</i> < 0.001	P = 0.67	<i>P</i> < 0.001	<i>P</i> = 0.16	<i>P</i> < 0.001	P = 0.20	
65–90 Hz	LBS	0.40 (0.02)	2.29 (0.05)	0.47 (0.02)	1.32 (0.08)	0.33 (0.01)	2.13 (0.06)	
	HBS	1.00 (0.03)	2.32 (0.07)	1.00 (0.03)	1.47 (0.08)	1.00 (0.02)	2.04 (0.03)	
	t test	<i>P</i> < 0.001	P = 0.80	<i>P</i> < 0.001	<i>P</i> = 0.23	<i>P</i> < 0.001	P = 0.23	

GCL, with chloral hydrate as anesthetic: data are from 12 rats, 12 recording sites for both LBS and HBS, each site repeated six times for each brain state. GCL, with pentobarbital as anesthetic: data are from 13 rats, 13 recording sites for both LBS and HBS, each site repeated four to six times for each brain state. MCL, with chloral hydrate as anesthetic: data are from 19 rats, 29 recording sites for both LBS and HBS, each site repeated four to six times for each brain state. MCL, with chloral hydrate as anesthetic: data are from 19 rats, 29 recording sites for both LBS and HBS, each site repeated four to six times for each brain state. Numbers in parentheses are SEs.

Table S2.	Statistical	comparisons	of	activities	patterns	across	the	nine	recording	sites	for
different o	dorants un	der different l	brai	in states							

Odorants

			ouorants				
	01	01	01	01	01	Ave	SD
HBS							
O1		-0.16	-0.19	-0.54	0.04		
02	-0.16		0.36	0.37	-0.40		
03	-0.19	0.36		-0.21	0.56		
04	-0.54	0.37	-0.21		-0.73		
05	0.04	-0.40	0.57	-0.73		-0.09	0.41
LBS							
O1		0.26	0.32	-0.60	0.33		
02	0.26		0.71	0.15	0.25		
03	0.32	0.71		-0.29	0.81		
04	-0.60	0.15	-0.29		-0.70		
05	0.33	0.25	0.81	-0.70		0.12	0.49
LBS/HBS							
	0.91	0.96	0.84	0.94	0.79	0.89	0.07

LBS: Pearson correlations among the activity patterns elicited by five odors (O1–O5) under LBS (the circle profiles in Fig. 2B). HBS: Pearson correlations among the activity patterns elicited by five odors under HBS (the filled circle profiles in Fig. 2B). HBS/LBS: Pearson correlations between the activity patterns of a given odorant under HBS and LBS (the circle and filled circle profiles in the same panel in Fig. 2B). The boldface small average correlation coefficients (Ave) and the large SD (SD) in the LBS and HBS sections indicate high site specificity for these odorants; the large average correlation coefficient and small SD in the HBS/LBS secton indicate that the selectivity is similar under these two brain states. Raw data are from Table S4.

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	Recording site										
	S1	S2	\$3	S 4	S 5	S6	S7	58	S 9	Ave	SD
LBS											
S1		-0.49	-0.59	0.24	-0.30	0.97	0.50	-0.06	0.25		
S2	-0.49		0.87	0.44	-0.25	-0.69	-0.11	0.68	0.63		
S3	-0.59	0.87		0.51	0.05	-0.75	-0.57	0.33	0.49		
S4	0.24	0.44	0.51		-0.58	0.07	-0.24	-0.03	0.45		
S5	-0.30	-0.25	0.05	-0.58		-0.19	-0.48	-0.12	-0.09		
S6	0.97	-0.69	-0.75	0.07	-0.19		0.46	-0.24	0.02		
S7	0.50	-0.11	-0.57	-0.24	-0.48	0.46		0.51	0.18		
S 8	-0.06	0.68	0.33	-0.03	-0.12	-0.24	0.51		0.77		
S9	0.25	0.63	0.49	0.45	-0.09	0.02	0.18	0.77		0.07	0.47
HBS											
S1		-0.11	-0.17	0.57	-0.11	0.75	0.14	-0.30	0.57		
S2	-0.11		0.88	0.52	-0.39	-0.29	-0.36	0.10	0.53		
\$3	-0.17	0.88		0.50	0.01	-0.59	-0.75	-0.32	0.18		
S4	0.57	0.52	0.50		-0.46	0.10	-0.30	-0.42	0.48		
S5	-0.11	-0.39	0.01	-0.46		-0.35	-0.55	-0.56	-0.61		
S6	0.75	-0.29	-0.59	0.10	-0.35		0.74	0.37	0.66		
S7	0.14	-0.36	-0.75	-0.30	-0.55	0.74		0.80	0.37		
S8	-0.30	0.10	-0.32	-0.42	-0.56	0.37	0.80		0.41		
S9	0.57	0.53	0.18	0.48	-0.61	0.66	0.37	0.41		0.06	0.48
HBS/LBS											
	0.98	0.99	0.98	0.99	0.98	0.99	0.98	0.96	0.99	0.98	0.01

Table S3. Statistical comparison of the activity patterns across the five odorants among the nine sites

LBS: Pearson correlations among all nine sites under LBS (data in columns of Fig. S1*B*). HBS: Pearson correlations among all nine sites under HBS (data in columns of Fig. S1*A*). HBS/LBS: Pearson correlations for the nine sites between HBS and LBS for the same odorant. The boldface small average correlation coefficient (Ave) and the large SD (SD) in the LBS and HBS sections indicate high site specificity for these odorants; the large average correlation coefficient and small SD in the HBS/LBS section indicates that the selectivity at a given site is similar under these two brain states. Raw data are from Table S4 and shown in Fig. S1.

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Tuble 54. The Err signals of anterent ouorants at anterent sites in thes and Es.	Table S4.	The LFP signals of different odorants at different sites in HBS and L	BS
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		O1	02	O3	O4	O5
S1	HBS	2.35 (0.33)	2.17 (0.28)	3.60 (0.50)	4.21 (0.38)	2.99 (0.58)
	LBS	2.37 (0.31)	2.79 (0.31)	3.31 (0.64)	3.67 (0.40)	2.64 (0.38)
	P (t test)	0.96	0.14	0.73	0.33	0.62
S2	HBS	4.08 (0.24)	3.46 (0.33)	6.17 (0.49)	2.15 (0.14)	4.64 (0.47)
	LBS	4.99 (0.45)	3.86 (0.41)	6.44 (0.62)	1.63 (0.14)	5.67 (0.48)
	P (t test)	0.07	0.44	0.73	0.01	0.14
S3	HBS	3.78 (0.41)	2.16 (0.19)	4.14 (0.49)	1.82 (0.17)	3.93 (0.55)
	LBS	3.54 (0.37)	2.47 (0.23)	3.40 (0.32)	2.30 (0.29)	3.60 (0.32)
	P (t test)	0.66	0.30	0.21	0.16	0.61
S4	HBS	1.81 (0.26)	1.74 (0.16)	4.04 (0.39)	2.97 (0.32)	4.43 (0.28)
	LBS	2.19 (0.33)	1.82 (0.25)	3.38 (0.33)	2.77 (0.44)	3.73 (0.40)
	P (t test)	0.38	0.77	0.20	0.71	0.16
S5	HBS	4.79 (0.51)	1.98 (0.25)	1.86 (0.16)	3.41 (0.52)	2.48 (0.28)
	LBS	5.03 (0.48)	2.60 (0.46)	2.34 (0.23)	3.33 (0.47)	1.91 (0.33)
	P (t test)	0.74	0.24	0.09	0.91	0.19
S6	HBS	2.18 (0.023)	3.79 (0.38)	4.54 (0.32)	5.44 (0.42)	2.40 (0.19)
	LBS	1.89 (0.02)	3.28 (0.46)	3.90 (0.33)	5.94 (0.46)	2.41 (0.24)
	P (t test)	0.31	0.40	0.17	0.42	0.97
S7	HBS	2.56 (0.37)	5.46 (0.47)	4.09 (0.51)	4.62 (0.47)	2.75 (0.23)
	LBS	3.06 (0.46)	4.88 (0.57)	4.60 (0.53)	3.98 (0.35)	3.28 (0.25)
	P (t test)	0.40	0.43	0.49	0.28	0.13
S8	HBS	3.08 (0.35)	5.02 (0.38)	4.19 (0.60)	3.17 (0.39)	2.56 (0.31)
	LBS	4.25 (0.58)	4.47 (0.43)	5.48 (0.53)	3.25 (0.47)	3.61 (0.55)
	P (t test)	0.09	0.34	0.12	0.89	0.10
S9	HBS	3.08 (0.09)	3.34 (0.14)	4.10 (0.22)	3.48 (0.14)	3.23 (0.20)
	LBS	3.52 (0.15)	3.24 (0.17)	4.07 (0.21)	3.36 (0.15)	3.38 (0.23)
	P (t test)	0.02	0.62	0.89	0.55	0.64

Recording at each site (1–9) is repeated for four to six times for each odorant (O1–O5). Numbers in parentheses are SEs. O1 to O5 are ethyl propionate, ethyl butyrate, ethyl valerate, heptanal, and 2-heptanone. Because the baseline activities at HBS and LBS are similar to those in Table S1, they are omitted here.

Table S5.	Effects of	odor	concentration	on	LFP	signals
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		12–32 Hz			33–64 Hz			65–90 Hz		
		LBS	HBS	t test	LBS	HBS	t test	LBS	HBS	t test
C1	Rest	0.62 (0.05)	1.00 (0.06)	<i>P</i> < 0.001	0.48 (0.03)	1.00 (0.03)	<i>P</i> < 0.001	0.54 (0.03)	1.00 (0.03)	P < 0.001
	Activated	2.15 (0.18)	2.44 (0.12)	P = 0.20	1.77 (0.17)	1.99 (0.08)	P = 0.22	1.47 (0.14)	1.56 (0.05)	P = 0.56
C2	Rest	0.62 (0.03)	1.00 (0.07)	<i>P</i> < 0.001	0.56 (0.03)	1.00 (0.04)	<i>P</i> < 0.001	0.50 (0.01)	1.00 (0.02)	<i>P</i> < 0.001
	Activated	2.73 (0.15)	3.24 (0.19)	P = 0.06	2.78 (0.22)	2.96 (0.15)	<i>P</i> = 0.52	1.75 (0.07)	1.74 (0.10)	<i>P</i> = 0.92
C3	Rest	0.60 (0.06)	1.00 (0.07)	<i>P</i> < 0.001	0.51 (0.03)	1.00 (0.04)	<i>P</i> < 0.001	0.49 (0.01)	1.00 (0.02)	P < 0.001
	Activated	3.22 (0.19)	3.51 (0.20)	P = 0.30	3.75 (0.15)	4.15 (0.25)	<i>P</i> = 0.19	3.16 (0.08)	3.22 (0.16)	P = 0.70
Pearso	on correlation	0.989	0.928		0.993	0.999		0.965	0.949	

The LFP signals are significantly correlated with odor concentration, with an averaged coefficient of 0.97 for six possible conditions. C1, C2, and C3 are the air stream flowing over pure odorants and then diluted by 26-, 11-, and 6-fold (or 3.8%, 9.1%, and 16.7%); anesthetics, chloral hydrate; data are from seven rats, 14 recoding sites, each site with four to six repeated exposures.