Supplementary Materials & Methods

Animals and initial preparation. All experiments were performed on 12 to 20 weeks old male C57BL/6J mice (Charles River France; for intrinsic imaging and tetrodes recording) and hemizygous *Omp*^{tm3Mom} (for *in vivo* 2-photon laser-scanning microscopy, referred as *Omp*-GFP in the text)[14]. All experiments were in accordance with the Swiss Federal Act on Animal Protection and Swiss Animal Protection Ordinance. Our experiments were approved by the university and the state of Geneva ethics committee.

A few days before performing any experiment, a custom made head-fixation system was mounted on the animal head. Mice were anesthetized with isoflurane (4% induction and 1-2% maintenance; Baxter, Baxter AG, Volketswil, Switzerland.). A local anesthetic, carbostesin (AstraZeneca, Zug, Switzerland), was subcutaneously injected before any incision, and the eyes were protected with artificial tears to prevent dryness. Body temperature was monitored and maintained at ~37 °C using a heating pad (FHC, Bowdoinham, ME). The skin overlaying the skull was removed and a custom made metallic head post was then fixed on the bone by embedding its base in dental cement (Omni-Etch Dentin, OmniDent, Switzerland). The rest of the skull was covered with dental cement excepting the OB. The bone overlying one of the two bulbs was carefully thinned to allow good light transmission and a dental cement well was constructed around it. To avoid direct contact of the thinned bone with air, a small amount of silicon elastomer (Kwik-Cast & Kwik-Sil, World Precision Instruments, USA) was applied.

A few days after recovery, we placed the animal in a tube and head-fixed it by screwing the head post into a home-made metal device fixed on the air table (**Fig. 1a**). The mice were trained in this restrained condition for 2-4 sessions (30 min each) over 2 days.

Odorants preparation and stimulation. All natural stimuli (banana, honey, ripe kiwi, coffee, oregano, strawberry, italian cheese (parmesan), swiss chocolate, wine rosé, nutmeg, rosemary, mint, thyme, cinnamon, tobacco, cardamom, cloves, vinegar, garlic, onion, pineapple, basil, lemongrass, apple, pear, sesame oil, black pepper, sesame seeds, ginger, milk, tomato, orange, black tea, lime, cumin, olive oil, and lemon) except mouse food, urine and feces were purchased from local grocery stores. C57Bl6 male mouse urine was collected and stored at –80 °C until use. The mouse feces were collected every day from male mice cages. Natural odorants were prepared fresh each experimental day (for example, fruits were freshly pressed and used for a few hours maximum). Either four grams of dry solid (for mouse feces: 1 g) or four milliliters (for mouse urine: 1 ml) of natural stimuli or 4 ml pure artificial odorant (including isoflurane) were placed in glass vials. They were indeed at natural partial pressure.

All monomolecular odorants (amyl acetate: AA, ethyl butyrate: EB, isoamyl acetate: IAA, eugenol: EU, citral: CI, carvone-: C-, 3-hexanone: 3-H, Acet: Acetophenone; Ger: Geraniol; Benz: Benzaldehyde; Cin: Cineol) were from Sigma-Aldrich. The molar concentrations of the monomolecular odorants (calculated based on the ideal gas law n = pV/RT) were:

Ethyl butyrate	0.63 mM
Amyl acetate	0.22 mM
Isoamyl acetate	0.28 mM

Eugenol	33 μM
Benzaldehyde	0.11 mM
Cineol	0.11 mM
Acetophenone	24 μM
3-Hexanone	0.76 mM
Carvone-(-)	22 μM
Geraniol	11 μM
Citral	11 μM

Odorants were delivered through a custom made olfactometer as described previously[2-3]. Odorants were delivered for 5 s (2 s after recording onset). An air flow passed through the vials containing the odorants. Depending on the experimental conditions, the odorants were differently diluted with clean dry air before being sent to the nose: 50% (**Figs. 1-2** and **Supplementary Figs. 1-3, 6**) dilution for natural odorants; 10% (**Fig. 3**), 5% (**Supplementary Figs. 5, 7-9**), 2% or 1% (**Fig. 3** and **Supplementary Figs. 6,9**) dilution for monomolecular odorants and isoflurane stimuli. The total flow was constant (0.4 l/min). To maintain a stable odor concentration during the entire stimulus application, we ensured that flows were stationary with a 5 s preloading before the odorant was delivered. For the experiments using natural products presented directly to the mouse (**Fig. 1**), the odorants were drawn up close to the snout for ~5 seconds with the help of a spoon containing 2 grams of solid natural products. For comparison, the concentrations of natural odorants sent by the olfactometer were reduced at least two fold (minimum of 2 times dilution in air due to the olfactometer design) to the concentrations reached when

presenting directly the product in the spoon, equivalent to the concentrations that a mouse would experience when being close to the odor source.

Intrinsic Optical imaging. For intrinsic signal imaging, the olfactory bulb was illuminated with red light at 700 nm (BP 20 nm) using a stable 100 W halogen lamp and a light guide system. Images were acquired at 5 Hz for 10 s (2s before, 5s during and 3s after the stimulus) using the Imager 3001F system (Optical Imaging, Mountainside, NJ) mounted both on a custom built macroscope (Navitar 17 mm, bottom lenses, Nikon 135 mm, upper lens; total magnification 7.9x). Images were acquired at 512 x 512 pixels and further 2 x 2 binned. The images were cropped to show the dorsal olfactory bulb. The blood vessel pattern was taken using green light (546 nm interference filter) at the beginning and the end of each experimental session, to assess the focus and to minimize drift. For the analysis, all the image were band passed filtered ($\sigma 1 = 2$; $\sigma 2 = 100$) and realigned by comparing the blood vessels pattern between images using a custom script running in Matlab (The MathWorks Inc; Natick, MA). Each odor was presented 4 times. For illustration, the images shown are the average of all the frames acquired during the stimulation (5 s) plus the frame acquired during the post-stimulus phase (3 s).

We analyzed the image time sequence, offering a better identification of individual glomeruli in single frame than in an average image (**Supplementary Figs. 2**,**3**). The glomerulus detection procedure (i.e. ovoid ROI exhibiting decrease in reflectance) was done on individual time frames by drawing regions of interest (the process was done independently by three experienced persons). We excluded every region that appeared

only in a single frame or that looked like blood vessels. For the unique ID glomeruli number (**Fig. 2d**) the overlapping glomeruli were removed using a script running in Matlab (The MathWorks Inc; Natick, MA). After calculating the average diameter of the glomeruli (103 μm, SE ±6.04), if two partially overlapping glomeruli had a distance between centers < 51.5 μm (half of the average diameter) they were counted as one; if instead the two glomeruli had a distance between centers > 51.5 μm, they were counted as different. All statistical testing were done in statistica or excel.

The number of activted glomeruli reported in this study may be an underestimate of the actual activation pattern as IOS imaging may not be sensitive enough to detect some weak glomerular responses or may not report all strongly activated glomeruli[16]. Furthermore as glomeruli strongly vary in size, small glomeruli often overlap with larger ones (**Supplementary Fig. 4**), limiting the possibility to discern all activated glomeruli.

Imaging in awake mice constrained us acquiring dorsal olfactory bulb activity. However, this limitation does not change our conclusions for several reasons: (1) OSNs expressing the same olfactory receptor converge on ~two glomeruli located in different parts of the olfactory bulb[17-18]. Therefore if one glomerulus is activated in the dorsal part, an equivalent glomerulus should be activated elsewhere on the OB surface as well. (2) Certain stimuli are known to activate more glomeruli in regions inaccessible to imaging[19-22]. Taken together, similar patterns should be triggered by odorants across the entire OB surface.

2-Photon laser-scanning microscopy. *Omp*-GFP mice were anesthetized with an intraperitoneal injection of 3.1 µl/g body weight of a mixture consisting of 60 µl Medetomidin (Dormitor®, Pfizer AG, Zurich, Switzerland; 1 mg/ml), 160 µl Midazolam (Dormicum®, Roche Pharma AG, Switzerland; 5 mg/ml) and 40 µl Fentanyl (Sintenyl®, Sintetica S.A., Mendrisio, Switzerland; 50 µg/ml). The bone overlying one hemibulb was thinned and gently removed, leaving the dura mater intact. A small drop of agarose 1% was added on top of the dura; a circular cover glass was then placed on top of the agarose and sealed with a thin layer of dental cement. After the surgery the animals were immediately transferred to the *2*-photon microscope for image acquisition.

Two-photon imaging was performed using a trimscope scanning head (LaVision BioTec GmbH, Bielefeld, Germany) mounted on a BX51WI microscope (Olympus, Switzerland). The specimen was illuminated with 915 nm light from a tunable pulsed Ti:sapphire femtosecond Chameleon laser with an approximate laser intensity of 50 mW (Coherent, Dieburg, Germany). A macroscopic image was initially taken under a 4X objective in order to obtain a global image of the entire bulb and eventually locate the extremities of the structure for the first z-stack. The bulb was then divided into successive square windows (field of view) of 500 x 500 µm with a spatial resolution of 970 x 970 pixels. All z-stacks were acquired with a 20X-0.95 N.A water immersion objective (Olympus, Switzerland). Emitted light was collected using a photomultiplier tube (Hamamatsu, Japan). Starting from the posterior and medial corner consecutive 200 µm thick z-stacks were acquired with a step size of 2 µm. Scanning and image acquisition were controlled using Imspector Pro software (LaVision BioTec GmbH, Bielefeld,

Germany). Subsequent image processing was performed in ImageJ (NIH, USA). The glomerulus counting procedure was done by delineating regions of interest (the process has been done independently by three experienced persons).