

Supplementary information, Data S1 Supplementary methods.

Measurement of fluorescence emission and excitation spectrum with two-photon microscopy in HEK-293 cells

HEK-293 cells (200,000 cells/well in 24-well plate) were transiently transfected with individual retrovectors (diluted 1:200 with culture medium) encoding mCherry, Cerulean or Venus by using lipofectamine (Invitrogen, Carlsbad, CA). Thereafter, the cells were expanded to 35 mm culture dishes, fixed in 4 % paraformaldehyde and covered by a glass coverslip for examination under two-photon microscope (FV1000 system (Olympus)) equipped with a Nikon x40 water-immersion objective lens (NA 0.80). Images were taken at different excitation wavelengths (in nm): 760, 800, 850, 900, 940, 990. The laser power was adjusted to acquire identical gray value for the same group of cells across different excitation wavelengths. To obtain the emission spectra of the three fluorophores, we collected fluorescence light emitted by HEK-293 cells from 460 to 700 nm with 10 nm increment. The fluorescence intensity (F_{reading}) was calculated according to the following formula: $(F_{\text{reading}} - F_{\text{background}}) / (F_{\text{maximum}} - F_{\text{background}})$.

TH immunostaining of the OB and quantification of fluorescence signals

During cryostat sectioning of the OB, every 10th coronal section (thickness 40 μm) containing left and right side of the OB was collected to 96-well plate and annotated. Free floating sections from exactly the same coronal planes were incubated with blocking solutions (10 % goat serum, Dako, Glostrup, Denmark, in 1 % Triton-X 100 in PBS (PBST)) for one hour at room temperature and then overnight with anti-TH antibody (Millipore, 1:500 v/v) in blocking solution at 4° C. Then sections were twice rinsed in PBS, incubated with secondary antibody (Alexa 488 conjugated donkey anti-mouse IgG, 1:1000 in PBST, Invitrogen) for one hour at room temperature. Finally, the sections were washed three times in PBS, transferred to Superfrost Plus charged glass slides (Langenbrink, Emmendingen, Germany) and were mounted in Vectashield Mounting Medium (Vector Laboratories, Burlingame, CA). Sections were examined and documented with an inverted single-photon

fluorescent microscope (Olympus CKX41) equipped with a PixelFly Qe CCD camera (PCO AG, Kelheim, Germany). Brain sections from exactly the same coronal planes were aligned side by side and imaged with the same exposure time. The mean fluorescence value of the whole glomerular layer (I_{GL}) and the granule cell layer of the OB (I_{GCL}) was measured to calculate the background-corrected intensity of the GL ($I = I_{GL} - I_{GCL}$). The extent of TH expression in the odor-deprived OB was measured as the ratio of relative intensity of OD side to control side ($R = I_{OD}/I_{Control}$). Control group was composed of age and gender-matched, not odor deprived mice. All images were analyzed with ImageJ software (<http://rsb.info.nih.gov/ij/>).

Calculation of the angle between the Z axis and the radial axis of the bulb

To calculate the angle γ between the Z axis and the radial axis of the bulb, we defined the latter as a vector orthogonal to the surface of the dura. The surface of the dura was approximated by a plane surface. A vector orthogonal to the plane is defined as a cross product of two vectors lying within the plane. To define these two vectors let's consider 3 points P_1 , P_2 and P_3 with coordinates (x_1, y_1, z_1) , (x_2, y_2, z_2) and (x_3, y_3, z_3) , belonging to the same plane. The two vectors connecting them are defined as

$$a = P_1 \rightarrow P_2 = (x_2 - x_1, y_2 - y_1, z_2 - z_1) = (a_1, a_2, a_3) \text{ and}$$

$$b = P_1 \rightarrow P_3 = (x_3 - x_1, y_3 - y_1, z_3 - z_1) = (b_1, b_2, b_3).$$

Then the vector c , orthogonal to this plane, has coordinates c_1, c_2, c_3 , calculated as follows: $c_1 = a_2 \cdot b_3 - a_3 \cdot b_2$; $c_2 = a_3 \cdot b_1 - a_1 \cdot b_3$; $c_3 = a_1 \cdot b_2 - a_2 \cdot b_1$. For defining the plane of the dura we usually determined coordinates of 3 intersection points of the dura with image borders at different depths. Similar calculation holds true for the vector d , orthogonal to the second (horizontal) plane (i.e. the optical axis of the objective). The angle between the two orthogonal vectors c and d is described by the equation:

$$\cos \gamma = \frac{|c \cdot d|}{\|c\| \cdot \|d\|}$$

Thus, γ equals to:

$$\gamma = \arccos \left(\frac{|c_1 \cdot d_1 + c_2 \cdot d_2 + c_3 \cdot d_3|}{\sqrt{c_1^2 + c_2^2 + c_3^2} \cdot \sqrt{d_1^2 + d_2^2 + d_3^2}} \right)$$