Supplementary Methods

Animals and odor stimulation Adult (> 3 months old) zebrafish were bred and kept at 26 – 27 °C on a 13/11 h light/dark cycle. Wild type strains Ab/Ab, Ab/TÜ, TÜ/TÜ (n = 30) and crosses of these strains with transgenic strain HuC:YC[1] (n = 57) or with other transgenic strains (n = 18) were used. The transgenic line, HuC:YC[1], expresses the fluorescent protein, yellow cameleon 2.1 (YC)[2], under the control of a fragment of the HuC promoter. Previous analyses demonstrated that in the adult zebrafish OB, HuC:YC is expressed selectively in MCs[3]. YC fluorescence did not change in response to odor stimulation and was used exclusively as an anatomical marker.

Experiments were performed in an explant preparation of the intact brain[4,5]. Briefly, fish were cooled to 4 °C and decapitated in artificial cerebrospinal fluid (ACSF)[6]. After removal of eyes, jaws, and bones covering the ventral telencephalon, the preparation was placed in a flow chamber and slowly warmed up to room temperature.

Odors were delivered through a constant flow directed at the ipsilateral inflow naris using a computer-controlled, pneumatically actuated HPLC injection valve (Rheodyne, Rohnert Park, CA) as described[5]. From amino acids of the highest available purity (Fluka, Neu-Ulm, Germany), stock solutions (1 or 10 mM) were prepared at least every 10 days, frozen, and diluted to a final concentration of 10 µM before the experiment. The minimum inter-stimulus interval was 90 seconds to avoid adaptation. The standard stimulus set included 16 amino acids used in previous studies[4,7,8] and a blank, which did not evoke responses. Odors were applied in a pseudo-random sequence. One stimulus was applied before and after each stimulus series to control for stability of response patterns.

Electrophysiology Recordings were performed with Axoclamp 2B (Axon Instruments) or SEC-05 (npi electronics) amplifiers in bridge mode and digitized at 10 kHz using National Instruments data acquisition boards and sofware written in IgorPro (Wavemetrics). Loose-patch recordings were performed as described[4] using patch pipettes (15 MOhms for MCs and 25 MOhms for INs) filled with ACSF. Fluorescein was added to visualize the pipette and target it onto neuronal somata under 2-photon optics.

Juxtacellular recordings[9] were used to inject defined current pulses into individual neurons. Patch pipettes were filled with intracellular solution containing (in mM) 130 K-gluconate, 10 Na-gluconate, 10 Na-phosphocreatine, 4 NaCl, 4 Mg-ATP, 0.3 Na-GTP, 10 HEPES (pH 7.25) and fluorescein. After establishing a low-resistance seal on a soma, brief suction or negative current pulses were applied to partially disrupt the plasma membrane so that action potentials of ~10 mV were recorded under high-noise conditions[9]. The spontaneous firing rate did usually not change noticeably after membrane disruption. Otherwise, a small negative holding current was injected to keep the spontaneous firing rate close to its original value. In some cases, neurons were hyperpolarized to prevent spontaneous spiking. Ca^{2+} signals evoked by current injection or odor stimulation decreased during the first 10 – 15 minutes, indicating that disruption of the plasma membrane resulted in a washout of the Ca^{2+} signal. Data sets used for quantitative analyses therefore included only the first response to current injection from each neuron. Whole cell recordings were avoided because the washout effect was even faster. No decay of Ca^{2+} signals over time was observed in the loose-patch recordings.

For comparison to simultaneously recorded Ca^{2+} signals, AP trains of individual neurons were converted to a continuous firing rate signal by convolution with a Gaussian. σ corresponded to the image frame duration (128 ms in most cases) unless noted otherwise. Firing rates were then

binned within the frame time intervals of image acquisition and the average firing rate before stimulation was subtracted.

Dye injection Fifty µg of Rhod-2-AM, Oregon Green 488 BAPTA-1-AM, or Fluo-5F-AM (Molecular Probes) were dissolved in 16 µl of DMSO/Pluronic F-127 (80/20; Molecular Probes), diluted 1:10 in ACSF, and loaded in a patch pipette (~4 – 6 MOhms). The pipette was introduced into the OB and the dye was ejected by pressure application. Beginning of dye uptake was monitored under fluorescence optics and became visible within seconds. Pressure was adjusted to achieve a slow increase in labeling intensity. To label INs, the pipette was advanced through the dura mater and 1 - 2 injections were placed at different locations below the glomerular/MC layer at a depth of ~250 – 300 µm. To label MCs, the dura mater was removed and 7 – 10 smaller injections were placed in the glomerular/MC layer of the lateral OB. Injections were terminated when a certain brightness level was reached, usually after 2 – 10 min for MCs and 30 – 40 min for INs. Multiple injections ensured that the indicator was loaded efficiently within a large tissue volume.

Tissue damage was assessed using SYTOX Green (Molecular Probes). This dye crosses the plasma membrane of necrotic cells and, upon binding to DNA, produces green fluorescence. A stock solution of 5 mM SYTOX Green in DMSO was diluted 1:10 in water and further diluted 1:100 in ACSF or in ACSF containing rhod-2 and DMSO/Pluronic as during dye loading. When SYTOX Green alone was injected into the deep layers of the OB following the protocol used for rhod-2 labelling of INs, 14 ± 7 labeled cells (mean ± SD; n = 3 OBs) were detected along the trajectory of the pipette. Injection of SYTOX Green together with rhod-2 and DMSO/Pluronic resulted in green fluorescence in 18 ± 6 (mean ± SD; n = 3 OBs) along the pipette's trajectory. Subsequent killing of neurons by strong laser illumination quickly produced green labeling in

neurons within, but not outside, the illuminated area, confirming that SYTOX Green detects necrotic cells. These results indicate that cell death caused by the injection pipette was minimal, and that the dye and solvents caused little or no additional damage.

Two-photon imaging Fluorescence imaging was performed with a custom 2-photon microscope[10] equipped with a mode-locked Ti:Sapphire laser (Mira900, 100 fs, 76 MHz; 830 - 850 nm; pumped by a 10 W Verdi laser; Coherent), a 20 x, N.A. 0.95 objective lens (Olympus), external detection optics, Hamamatsu R6357 photomultiplier tubes, and custom software. Laser intensity was adjusted to minimize photobleaching in each focal plane. To separate fluorescence emission of rhod-2 andYC, emitted light passed through an interference filter (515/30 nm) onto a first photomultiplier. The light reflected by the interference filter passed through a second filter (610/75 nm) onto a second photomultiplier. To separate fluorescence emission of rhod-2 from Oregon Green 488 BAPTA-1 or Fluo-5F, a slightly longer-wavelength red emission filter (645/75) was used.

Time series of fluorescence images were converted to image series representing the relative change in fluorescence in each pixel after stimulus onset ($\Delta F/F$). Most image series were acquired at 128 ms/frame and contained 128 x 256 pixels. In some cases (Fig. 2a, c), images were acquired at 32 ms/frame and traces were filtered using a Gaussian filter with $\sigma = 32$ ms. To explore the temporal resolution of TDCa imaging, images with 8 x 64 pixels were acquired at 8 ms/frame. To measure responses of INs to the set of 16 odors, images containing 256 x 256 pixels were acquired at 256 ms/frame to obtain a large field of view. Time values represent the starting time of frames. In analyses of odor-evoked activity patterns, $t = 0$ designates stimulus onset, which was determined as the first time bin in which a response was observed. Because it is

unclear when the response started within this time bin, the first time bin (128 or 256 ms) was omitted from the analysis.

For quantitative analysis of response patterns, somata of individual neurons were outlined manually and pixel values were averaged prior to ΔF/F calculation. In HuC:YC transgenic fish, Ca^{2+} signals from MC somata were identified based on the co-localization of YC fluorescence in the green emission channel (Fig. 1b). Most INs were imaged in layers below the glomerular/MC layer where no HuC:YC-positive neurons are found[3] and are therefore likely to be granule cells. Somata of INs were identified by their circumscribed Ca^{2+} signal, from the raw Rhod-2 fluorescence, or both. The data sets used for analysis of odor response patterns evoked by the 16 stimuli included 1313 MCs in 9 OBs and 8009 INs in 4 OBs. Within each OB, responses were measured in 5 – 14 (mean ± SD, 9.5 ± 2.8) different optical sections, separated usually by ≥ 15 μm. The imaged volume therefore covered much of the amino acid-sensitive lateral (MCs) or central (INs) subregion of the OB. The number of MCs recorded in each optical section was lower than that of INs because the density of MCs is lower.

Decay time constants of Ca^{2+} transients were determined by fitting a single exponentials to the Ca^{2+} signal trace. Fits were performed during periods when neurons fired no APs following periods of spiking activity. Data analysis was performed using routines written in Matlab and IgorPro.

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