Supplementary Figure 1:

**a.** Olfactory circuit diagram and models of axon targeting. a, Olfactory sensory neurons (OSNs) expressing the same receptor project from distributed locations in the olfactory epithelium (OE) to spatially invariant glomeruli in the olfactory bulb (OB) (left panel). Approximately 20-50 homotypic mitral and tufted (MT) neurons receive input from a glomerulus. MT axons innervate 5-7 cortical centers including the anterior olfactory nucleus pars externa (AON pE), pars principalis, (AON pP) and the anterior piriform cortex (aPC). Glomerular representations in the cortex (right panel). In the AONpE, axons preserve the gross spatial relationships of MT neurons in the OB (models I and II). However, anterograde and retrograde mapping studies indicate that small OB regions innervate broadly distributed regions of the AON and PC. But, these studies do not address mapping with reference to glomerular input. Therefore, projections of individual glomeruli map might map to multiple locations but remain segregated from other glomeruli (model I) or glomerular inputs might mix (model II). Our studies support model II and identify diverging axons in the AON pE (dotted blue line). Model II would expand the potential integrative capacity of target neurons. b, Mapping of homotypic MT neurons: Homotypic MT neurons are electrically coupled and fire synchronously in response to the same sensory stimuli. As a result, one model of connectivity would predict that homotypic neurons would "wire" together, which might help to amplify or correlate weak signals (model I). Alternatively, homotypic MT neurons could wire differently (model II). Our studies show that homotypic MT neurons rarely branch together, supporting model II. This organization would also expand the potential integrative capacity of target neurons and would predict a complex or potentially stochastic means to diversify neurons to produce diversity in branching and potential connectivity.
Supplementary Figure 2:

a. **SEPARATED (S1)**

b. **CO-INJECTED (CO 1)**

- **OB**
  - SIN-G = 26
  - SIN-Y = 28

- **AON**

- **PC**

C. 

D. 

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Supplementary Figure 2:
Supplementary Figure 2: Analyses of axon organization the AONpE and PC.

a-b, To determine whether the topographic map in the OB is maintained in the axonal projections of MT neurons to the cortex, individual OBs received either separated or co-localized injections of SIN-G (green) and SIN-Y (yellow) viruses. The distribution of labeled neurons in the OB and their axons in the AON pE and aPC are shown for a separate (S1, a) and a co-injected (CO 1, b) sample. Segregation of green and yellow axons is observed in the AON pE of S1 but not in the aPC. In CO 1, green and yellow axons from neighboring neurons mix in the AON pE and aPC suggesting that the topography of injections is maintained in the AON pE, but is lost in the aPC. 

c-d, Inverted single channel images of S1 (a, c) and CO 1 (b, d) confirm this finding. SIN-G axons are visible only in the green channel (green boxes), while SIN-Y (red+green) axons are visible in both the green and red channels (red boxes). 

e-f, In total, three animals received separate injections (S1, S2, S4) and one animal was co-injected (CO 1). In a fifth animal, SIN-G and SIN-Y were injected in the same site while SIN-R (red) was injected in a distant site. The distribution of co-injected SIN-G and SIN-Y axons in this animal was recorded (CO 3). In addition, this set of co-injected SIN-G and SIN-Y axons was treated as a single group and compared with the segregated SIN-R axons (S3). Two independent analyses were used to quantify the extent of axon mixing in the AON pE and aPC of each sample. Sector Analysis (e, left): In schema, green and yellow lines represent labeled axon segments. Each sector is 128 square pixels. Blue and red dots denote segregated vs. mixed sectors respectively. Proximity Analysis (f, left): In the schema, the red dot represents the center of the axon segment used to measure its relative proximity to “same” and “different axons (gray lines). The % mixed sectors (e, right) and % total “mixed” axons (f, right) were quantified for the AON pE and aPC of each sample. Significant segregation was observed only in the AON pE for separate injections (Student’s t-test, *p = 0.03 (f), *p = 0.02 (g)). Error bars: 95% confidence. Proximity analyses were performed on pooled sections for each sample so error bars are omitted but significant.
differences between the groups are shown in Figure 1 (b). Scale bars: 100 µm. Blue is TOTO-3 nuclear staining.

**Supplementary Movie 1| Three-dimensional reconstruction of a single neuron.**
This QuickTime movie, generated using Neurolucida software, shows the axonal projections of a single non-EG mitral neuron (Fig. 2c) traced from the OB into the olfactory cortex until the collaterals off of the main axon in the LOT became too weak to trace reliably (~Bregma 1.2). This encompasses the entirety of the AON pE and pP (purple) and the majority of the anterior PC (blue), along with part of the olfactory tubercle (orange). The tenia tecta is labeled (yellow) and may be a target of some dorsomedially projecting collaterals. Pairs of EG and MOR28 glomeruli are labeled with squares (green). Many collaterals project deep within the brain, especially in the aPC. These would be obscured in ventrally imaged flattened brains.
Supplementary Figure 3:

**Supplementary Figure 3 | Cell morphologies and intrabulbar projections of mitral and tufted cells from EG and non-EG glomeruli.** The mitral cell layer contains many cell types of which only three innervate single glomeruli: internal tufted cells (iT), the canonical type I mitral cells (M-I), and type II mitral cells (M-II). To classify MT neurons we examined the distribution of lateral dendrites, somatic morphology and intrabulbar axon branching of each neuron in our sample set. The above panel showcases all the single neurons analyzed in this study. This does not include the paired neurons (Supplementary Figure 8). The gray outline represents the external plexiform layer between the cell body and the associated glomerulus. The primary dendrite and main axon (with bulbar collaterals) are highlighted in magenta. Lateral dendrites are blue. Dendritic processes were traced to at least 100µm from the cell body. Green circles denote EG glomeruli and gray indicate non-EG glomeruli. The dotted gray lines separate M-I cells from putative M-II cells based on the absence or presence of a bulbar collateral. No other reliable differences were observed in the cell morphology or extent of lateral dendrites among the mitral cell types. Consequently, M-I and M-II cells were grouped together as M neurons for all quantitative analyses. iT cells, on the other hand, displayed a distinct oblong somatic morphology and relatively superficial lateral dendrites in comparison to mitral cells. In addition, the characteristic intrabulbar axon collateral used to define iT cells is shown here alongside the symmetric glomerulus. “||” indicates several 100 µm. All mitral cell layer neurons target to the same gross regions of the AON and PC and neither projects selectively to the OT, in contrast to the external tufted neurons.
Supplementary Figure 4:

a-c, Brains with sparsely labeled neurons were sectioned and every section was imaged using automated confocal scanning. Axons and branches were visible in serial coronal sections through the OB (top row), AON (middle) and PC (bottom) (green: GFP, blue: TOTO-3 nuclear staining).

d, A reference brain was generated by imaging an age-matched mouse of the same genotype as all mice used to trace neurons (except the sample neuron in Fig. 2c). These mice were genetically altered to express GFP in two sets of glomeruli, (EG and mOR-28-EGFP in green).

e, Traced neurons were “loaded” into the reference brain in the context of three linked cross-sections through the AON, aPC and OT that contained nuclear staining (TOTO-3, white).

f-g, Green glomeruli and landmark clusters of nuclei in the three brain regions were used to place the neurons in the reference brain along the anterior posterior axis, followed by rotation to produce the best overall alignment.

h, Traced neurons were aligned to compare patterns of axon branching in the AON pE and pP (magenta), anterior PC (blue) and OT (red). The alignment can be best viewed in a three dimensional projection but is presented here as a collapsed screenshot. 

Axes: Blue, A-P; green, V-D; red, L-M
Supplementary Figure 5:

**a.**

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**b.**

![Bar graph](image5)

**Supplementary Figure 5 | Flattened three dimensional tracings of single MT cells aligned to the reference brain and branch analysis of EG neurons.**

**a.** The entire traced axons of individual mitral and tufted neurons (whose cell bodies and intrabulbar projections are shown in Supplementary Figure S2) are in black. Neurons extensively innervate the AON (pE and pP, purple), PC (blue) and occasionally extended arbors into the olfactory tubercle (OT, red). The arrows in the lower left corner apply to all traces. Green points to dorsal, red to medial and blue to posterior.

**b.** The numbers of branches per neuron were plotted individually for each EG (red) or non-EG MT (gray) neuron. Comparisons of groups revealed no significant differences.
Supplementary Figure 6 | Analyses of mitral and tufted neurons. a-b, Trimmed cortical projections of mitral (n = 13) and tufted (n = 6) neurons were aligned in the reference brain. The AON (pE and pP) is shown in purple and the anterior PC in cyan/blue. Axes: Blue, A-P; green, V-D; red, L-M c-d, Total numbers of dorsal (D) and ventral (V) primary axon branches per neuron in each region for mitral (red) or internal tufted cells (gray) were compared for each neuron (c) and displayed as averages of the percentages of the total number of primary branches for that neuron (d). Error bars are 95% confidence intervals. No differences between subtypes were observed in any region. e, Pairs of fully traced neurons were aligned in the reference brain and the distance between each axon terminal and its nearest neighbor in the other neuron was computed. The average distance for all terminals in each mitral-mitral pair (M-M, n=20) and mitral-tufted pair (M-T, n=22) are plotted with 95% confidence intervals (e, left). An identical analysis was performed on the same pairs using branch nodes in place of axon termini (e, right). No significant differences were detected (Student’s t-test).
Supplementary Fig 7:

a. Neuron 2

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b. Neuron 2

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d. Average distance (µm)

* p = 0.03

e. SKEW

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Class 1 = Skew > 1
Class 2 = Skew < 1
**Supplementary Figure 7 | Axon end proximity analyses.** 

* a, To validate this analysis we constructed a test set of neurons that should differ predictably in their terminal overlaps as follows. We began with a fully traced neuron (FULL). We then computationally removed its most distal axon branches (TRIM). Next we used matrix-based rotation to skew the entire neuron at its center point (OFFSET). Finally, we computationally rotated this neuron by 180 degrees along its A-P axis (FLIP). We would predict that FULL vs. TRIM would exhibit more similarity than FULL vs. OFFSET and that OFFSET or FULL vs. FLIP would be highly dissimilar. The distance of each axon terminal to its nearest neighbor terminal in the comparison neuron was computed and the number of termini falling into each of ten equally sized distance bins were plotted (based on the maximum distance/10). Similar neurons should have the majority of points fall in into the smallest bins and be skewed to the left (i.e. TRIM vs FULL). Neurons with few similarities should exhibit a more normal distribution (i.e. OFFSET vs. FLIP). Results of the test set comparisons confirm this. For identical neurons all distances are zero, which is plotted as a gray square. 

* b, This analysis was applied to the neurons traced in Supplementary Figures S3 and S5. These include four neurons associated with the EG glomerulus (EG1-3, EG4-T) and three associated with non-EG “random” glomeruli (N1-T, N2, N3). Two cells were tufted (EG4-T and N1-T) while the rest were mitral neurons. Each pair was compared twice. The starting neuron is listed on the Y axis and the comparison neuron across the top. To compare classes of neurons, the shapes of the distributions were classified using the skew function (in Microsoft Excel). Class I = skew >1, indicating a skew to the left and more similarity in endpoint proximity (CI). Class II = skew <1 indicating a more normal distribution and less similarity, based on the control data sets (CII). 

* c, The validity of this analysis is supported by analyses showing that pairs of neurons in class I possess significantly more overlapping branches at the point of exit from the main axon than do neuron pairs in class II (using results of a previous analysis). 

* d, In addition, neurons of class I show a significantly shorter average end distance than neurons of class II. 

* e, Yet, no differences in skew or class exist between matched EG neuron pairs (EG-EG) or unmatched pairs (NON) or between matched mitral cells (M-M) and unmatched mitral vs. tufted pairs (M-T). Skew is shown in (e), class in (b). For (c-e) Error bars are 95% confidence intervals. Significance was assessed by a Student’s t-test.
Supplementary Figure 8:
Supplementary Figure 8 | Same animal homotypic and heterotypic neuron pairs from six different animals. a-e, Injections of SIN-G and SIN-Y viruses into the OR-EG glomerulus occasionally resulted in multiply-labeled neuron sets in which 2-3 neurons innervated an OR-EG glomerulus (HOM, n = 6 pairs) or in which pairs of neurons innervated different nearby glomeruli (HET, n = 7 pairs). The cell bodies of two homotypic pairs (HOM1, 2; a,b) and two heterotypic pairs (HET5, 6; c,d) are shown in collapsed coronal sections of raw image data. In another animal, (HOM3/HET3, e), four neurons (2 EG and 2 non-EG) are labeled. Raw data are colored as: SIN-G (green neuron), EG glomerulus (green glomerulus), SIN-Y (yellow neuron), nuclei (blue based on TOTO-3 staining). Neurites extending 50-200 µm were traced (for a-d) in Neurolucida and overlaid on the raw images (HOM: green, red, yellow HET: red and white lines). White arrows represent the point at which the primary dendrite enters the associated glomerulus. Images were rotated in 3D to obtain the most optimal perspective. Consequently, scale bars could not be assigned to these images. f-h, Partial axon reconstructions of homotypic and heterotypic pairs in the same animal were mapped to the AONpE, AON pP and PC. f, Trimmed cortical traces of the four neurons in HOM3/HET3 are shown (black trace corresponds to white neuron in panel e). Cortical regions: AON pE: white left, AON pP: gray, PC: white right. g-h, Multiple pairwise comparisons of homotypic and heterotypic pairs of neurons reveal extensive diversity in targeting. Trimmed traces of neuron pairs were rotated in three-dimensional space to identify overlapping primary branches (dark gray arrows). Consequently, some overlaps are not apparent in these flattened representations. HOM1 and HOM2 are the traces corresponding to the neuron pairs in panel a and b. In HOM4, three EG neurons were labeled in one animal and are shown as pairwise alignments (g, bottom row). In HET 5, one neuron in the pair innervates the EG glomerulus (green) while the other does not. The pair HET 6 contains neurons with distinct glomerular inputs but nearly overlapping soma, from a more caudal region of the OB. The homotypic pair (g, HOM3) and each heterotypic pair are shown for HOM3/HET3 (h, HET3 top and middle). The length of the main axon for each pair is 2.7 mm (HOM 1), 2.9 mm (HOM 2), 2.0 mm (HOM3/HET3), 2.4mm (HOM 4), 1.9 mm (HET 5) and 2.5 mm (HET 6).
Supplementary Figure 9:

a. AONpE

b. AONpP

c. PC

d. % Total varicosity per terminal

% En-passant varicosity per terminal
Supplementary Figure 9 | Axon termini are associated with swellings and fine axon branches possess internal varicosities in the olfactory cortex. a-c, We imaged GFP-labeled neuron tufts in the AONpE, pP and anterior piriform cortex (PC) to identify axon termini and observed terminal swellings and internal varicosities that are consistent with these being sites of synaptic contacts. Representative images of these sections are shown as inverted grayscale images of collapsed serial sections with GFP as black and gray (~ 100-200 µm along the anterior-posterior axis). The main axon is the thick black line in each section (~ 100-200 µm in AP). Red circles indicate a representative set of focal swellings at the termini of individual axon tufts in the three regions. Green boxes highlight internal varicosities along the axonal arbor. We do not observe varicosities along the main axon, where synapses are not thought to form. d, We calculated the number of total termini in each section and asked how many of these exhibited swellings. This is plotted as % total varicose termini. In all three regions, ~90% of ends exhibited swellings (error bars are s.e.m). e, We also counted the total number of en-passant varicosities in each section and plotted this as a percentage of total axon ends. Different sections were more variable by this measure, potentially due to difficulties in counting in complex regions (a range of ~ 0.5 to 3 internal varicosities per end with an average of ~2, plotted as 200% in e).