**Algorithm for determining functionally-identifiable glomeruli and their diagnostic odorants.**

Our odorant mapping datasets reveal narrow tuning and high sensitivity of glomeruli to their putatively ‘characteristic’ odorants, suggesting that many dorsal glomeruli can be identified based simply on their response to a single diagnostic odorant delivered at low concentration. We applied a working algorithm to identify odorants that can easily and reliably identify the same putative glomerulus across animals, first by screening for sparse but reliable responses across the 8 imaged olfactory bulbs (OBs), and then by screening for consistency in the response spectra of each putatively-identified glomerulus across the total 185-odorant panel.

More specifically, we screened each odorant using highly conservative criteria for sparseness and reliability. The sparseness criterion required that no more than two glomeruli be activated after thresholding responses at 50% of the peak response in any of the 8 imaged olfactory bulbs. The reliability criterion required that the odorant activate at least one glomerulus in at least 6 of 8 OBs, and in at least one bulb in all 4 imaged mice. 80 of the 185 odorants passed this initial screen, and are all included in the list of diagnostic odorants.

For each of these 80 odorants, we then: 1) identified the glomerulus most strongly activated by that odorant in each of the 8 OBs, and 2) compared the 185-odorant response spectrum of those glomeruli, in order to test the ability of single diagnostic odorants to reliably identify the same glomeruli (exhibiting the same odorant response fingerprint) across OBs and animals. For 19/80 odorants, the strongest (or only) activated glomerulus identified the glomerulus with the highest-correlated response spectrum in 100% of comparisons, with a median correlation coefficient in response spectra across OBs of 0.95 ± 0.04 (mean ± st. dev.). Relaxing these criteria slightly to allow for inherent variability in responsiveness across the odorant panel, to a cutoff of a match between the strongest-activated and most-correlated glomerulus in 80% of OB comparisons, and a median correlation coefficient across OBs of >0.8, yielded 22 additional diagnostic odorants (41 odorants total) that identified 26 unique glomeruli.

We note that visual inspection of response maps indicates that numerous additional glomeruli are likely to be easily identified with a slight relaxation of cutoff criteria or a slight increase in complexity of the algorithm.

Notably, while spatial location was not used as a diagnostic criterion, glomeruli identified with this approach appeared in a similar location in the dorsal OB, with a spatial jitter consistent with that characterized for OR-defined OSN projections. The locations of each of the 26 identified glomeruli, referenced to the midline and caudal extent of the dorsal bulb, are included in the Matlab variables MLmean and RCmean described further below.

**Data organization:**

Variables in ‘ID gloms 05 2022.mat’ file:

**‘odornameslist’.** List of all 185 odorants (plus two vehicle controls), indexed in same order as odors in response spectrum matrices.

**‘allgloms\_odors\_sorted\_IDglomorder’**.

Structure containing info about identified glomeruli and diagnostic odorants, including additional odorants meeting criteria for sparseness and reliability, but not meeting cirteria for functional identification. Data should match Tables S2 and S3.

The fields of these structures are:

**numgloms50.** The number of glomeruli activated by each odorant after thresholding at 50% of max response. Numbers are in order of preparation (8 OBs total), indexed in the same order as in the 'allrespmatrix' Matlab file.

**maxgloms.** The identity of the max-activated ROI in each OB, indexed as above.

**odorname** (self-explanatory; the first two letters are a short identifier and can be ignored).

**odorindex**.Index of odorants in odornames list and in the response spectrum.

**erroratio**. Fraction of ‘errors’ in match between strongest-activated glomerulus in each imaged OB and most-correlated glomerulus response spectrum, of all possible pairwise comparisons.

**meancorr; median corr**. Mean and median correlation coefficient (Pearson’s r) in response spectrum for the strongest-activated glomerulus in each imaged OB for a given odorant, calculated across all pairwise comparisons.

**mean\_norm\_spectrum, median\_norm\_spectrum**. Mean odorant response spectrum for the ID’d glomerulus, normalized within each OB to the response to the given odorant and then averaged across all imaged OBs. Odorants are in the order given in the ‘odornameslist’ variable.

**mediolateral, rostrocaudal.** Positions of max-activated glomeruli in each OB, indexed as in 'maxgloms' field. Units are microns, reference (zero) is midline (for ML units) and caudal sinus (for RC units).

**MLmean, RCmean**. Mean position of each ID’d glomerulus, averaged across each imaged OB after visual registration by aligning the midline and caudal sinus. Refer to Figure for relative positions and scatter for all glomeruli.