

## **Odorant Mapping Datasets, overview and file organization.**

Credits: S. Burton (data collection, analysis), A. Brown (analysis), M. Wachowiak (analysis), T. Rust (software).

### **Parent manuscript:**

Mapping odorant sensitivities reveals a sparse but structured representation of olfactory chemical space by sensory input to the mouse olfactory bulb.

Shawn D. Burton<sup>1</sup>, Audrey Brown<sup>1</sup>, Thomas P. Eiting<sup>1</sup>, Isaac A. Youngstrom<sup>1</sup>, Thomas C. Rust<sup>1</sup>, Michael Schmucker<sup>2</sup>, Matt Wachowiak<sup>1</sup>

<https://www.biorxiv.org/content/10.1101/2022.05.11.491539v2>

## **Methodology**

Imaging data were obtained from OMP-IRES-tTA mice (gift from R. Yu, Stowers Institute) crossed with the tetO-GCaMP6s reporter line (Jax# 024742), leading to GCaMP6s expression in all olfactory sensory neurons. In isoflurane-anesthetized mice, we imaged responses to each odorant (185 compounds plus 2 blank and solvent controls) simultaneously across both dorsal OBs under epifluorescence illumination, and using artificial inhalation at 3 Hz to ensure uniform odorant sampling across trials and animals and to eliminate OSN activation by the anesthetic. Imaging data was collected with a cooled CCD camera (RedShirtImaging LLC) at 25 Hz and 256 x 256 pixel resolution using Neuroplex software.

Odorant delivery was controlled using a novel odorant-delivery device described in Burton et al., 2019 (<https://pubmed.ncbi.nlm.nih.gov/30657873/>), with the educator configuration to ensure thorough mixing of odorant with the carrier stream. Odorants were presented in banks of twelve, cycling through each bank for 3 - 5 trials per odorant (8 - 10 sec intertrial interval), in random order, under computer control. Odorant was presented for 2 seconds per trial.

## **Data processing pipeline**

To generate odorant response matrices, repeated trials were first averaged, then  $\Delta F$  'maps' generated by subtracting baseline fluorescence averaged across 1 second before odorant presentation from the mean of seconds 2 – 3 after odorant onset. Regions of interest (ROIs) were generated from maximal projections of all odorant response maps using an initial automated segmentation routine followed by manual adjustments and selection. All data processing was done using custom Matlab code.

Initial response matrices were generated by averaging the  $\Delta F$  pixels within each ROI and for each odorant. However, the epifluorescence configuration and the high density of activated glomeruli required an additional segmentation step to eliminate spread of fluorescence signals to adjacent ROIs due to scattered or out-of-focus light. Segmentation was performed manually by visual inspection, and signals arising from

adjacent ROIs, as well as a lack of focal signal in a given ROI, were set to zero for the respective ROI-odorant pair. Segmentation was performed blind to odorant identity.

## Data organization – files.

**'odormappingdata\_simple\_06\_2021.mat'** file.

Variables:

**allrespmatrices\_dF.** Structure array consists of odorant response matrices and ROI positions for each OB. Variables are indexed in the following order: (1) omp111L, (2) omp111R, (3) omp112L, (4) omp112R, (5) omp113L, (6) omp113R, (7) omp114L, (8) omp114R. All odorants are in the same order for all response matrices (see 'odornameslist' variable).

ROI positions indicate centroid of each ROI, after visual registration by aligning the midline and caudal sinus. Units are microns, reference (zero) is midline (for 'Xpos') and caudal sinus (for 'Ypos'). Note that the Xposition for ROIs from the left OB is negative relative to midline.

**allconcs\_prep.** Calculated final delivered concentration of each odorant, indexed in the same order as response matrices and odornames list, for each mouse, in mols/L. Note that concentrations estimated from vapor pressure, calibrated air dilution, and liquid dilution, assuming ideal behavior.

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

**'odormappingdata\_fullinfo\_06\_2021.mat'** file.

This file contains more complete information for response matrices for each of the eight OBs, including underlying  $\Delta F$  maps. Data for each OB are contained in a separate structure. The fields for each structure are:

**ORxxxx.Reflmage.** Image (256 x 256 pixels) of mean baseline fluorescence of the imaged area.

**ORxxxx.RespMatrix.** Segmented response matrix for all ROIs vs. odorants. Identical data as in 'allrespmatrices\_dF' for the corresponding OB (see above).

**ORxxxx.OdorList.** Identical to 'odornameslist' (above).

**ORxxxx.ROIPos.** Centroids for each ROI in X (med-lat) and Y (rostr-caud); identical to above (see 'allrespmatrices\_dF', ROIPositions variable).

**ORxxxx.Maps.**  $\Delta F$  map for the indexed odorant, saved as a cell array (use 'cell2mat' command to convert to numeric array for display).

**ORxxxx.Reg.** Information about scaling and registration of OB to reference OB, using midline and caudal sinus as reference landmarks. pixelsize units are microns per pixel.

**ORxxxx.MetaData.** Reference data containing parameters used to generate maps and output values to response matrices.