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Supplementary Figures: 6

Supplementary Tables: 1

Supplementary Videos: 4

Reporting Checklist for Nature Neuroscience

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. For more information, please read Reporting Life Sciences Research.

Please note that in the event of publication, it is mandatory that authors include all relevant methodological and statistical information in the manuscript.

Statistics reporting, by figure

- Please specify the following information for each panel reporting quantitative data, and where each item is reported.
- Each figure legend should ideally contain an exact sample size (n) for each experimental group/condition, where n is an exact number and not a range, a clear definition of how n is defined (for example x cells from x slices from x animals from x litters, collected over x days), a description of the statistical test used, the results of the tests, any descriptive statistics and clearly defined error bars if applicable.
- For any experiments using custom statistics, please indicate the test used and stats obtained for each experiment.
- Each figure legend should include a statement of how many times the experiment shown was replicated in the lab; the details of sample collection should be sufficiently clear so that the replicability of the experiment is obvious to the reader.
- For experiments reported in the text but not in the figures, please use the page number instead of the figure number.

Note: Mean and standard deviation are not appropriate on small samples, and plotting independent data points is usually more informative. When technical replicates are reported, error and significance measures reflect the experimental variability and not the variability of the biological process, and it is misleading not to state this clearly.

		TEST USED		n			DESCRIPTIVE STATS (AVERAGE, VARIANCE)		P VALUE		DEGREES OF FREEDOM & F/t/z/R/ETC VALUE	
	FIGURE NUMBER	WHICH TEST?	PAGE	EXACT VALUE	DEFINED?	PAGE	REPORTED?	PAGE	EXACT VALUE	PAGE	VALUE	PAGE
example	1a	one-way ANOVA	4	9, 9, 10, 15	mice from at least 3 litters/group	4	error bars are mean +/- SEM	4	p = 0.044	4	F(3, 36) = 2.97	4
example	results, pg 6	unpaired t-test	6	15	slices from 10 mice	6	error bars are mean +/- SEM	6	p = 0.0006	6	t(28) = 2.808	6
+ -	2b	paired t-test	3	8	maps	3	error bars are mean +/- SEM		0.043 (volume) 0.0067 (post. frac)	3		
+ -	2c	Kolmogorov-Smirnoff	3	8	maps	3			0.0323	3		
+ -	2d	paired t-test	3	8	maps	3	error bars are mean +/- SEM		.0037 (thresh 0.5) 0.064 (thresh 1) 0.077 (thresh 2)	3		
+ -	3d		4	5	maps	4	error bars are mean +/- SEM	4				

		TEST USED		n			DESCRIPTIVE STATS (AVERAGE, VARIANCE)		P VALUE		DEGREES OF FREEDOM & F/t/z/R/ETC VALUE	
	FIGURE NUMBER	WHICH TEST?	PAGE	EXACT VALUE	DEFINED?	PAGE	REPORTED?	PAGE	EXACT VALUE	PAGE	VALUE	PAGE
+ -	3e	one-way ANOVA	4	5	maps	4	error bars are mean +/- SEM	4	$p < 0.05$ vs. all others	4		
+ -	4b	custom	4	5	maps	4			$p < 0.05$ compared to shuffled maps	4		
+ -	4d	custom	4	5	maps	4	observed versus shuffled maps	4	PSI ~= z all $p < 0.01$	4	PSI ~= z	4
+ -	4e	custom	4	5	maps	4			various (56 comparisons displayed)	4	PSI ~= z average of 5 maps	4
+ -				7	maps	6	mean +/- SEM	6	0.0045	6		
+ -	8b	custom	6-7	10	maps	6-7			various (110 comparisons displayed)	6-7	PSI ~= z average of 10 maps	6-7
+ -	8c	custom	6-7	N/A (simulation)	10,000 shuffled receptive fields	6-7			various (110 comparisons displayed)	6-7	CSI ~ = z	6-7
+ -	8d	custom	6-7	10	maps	6-7	position reflects glomerular closeness line color reflects receptive field similarity		0.63 (p-value associated with observed value given expectations from shuffled maps)	6-7		

Representative figures

1. Are any representative images shown (including Western blots and immunohistochemistry/staining) in the paper?

   If so, what figure(s)?

Yes. Every main figure contains at least one representative glomerular activity map illustrating the core findings.

2. For each representative image, is there a clear statement of how many time s this experiment was successfully repeated and a discussion of any limitations in repeatability?

   If so, on what page(s) is this reported?

There are multiple ways in which we repeated experiments. First, we generated activity maps for individual ex vivo preparations using 3 to 5 randomized, interleaved trials of every stimulus and control. This is shown in Figs. 1d-e and Fig. 5a-b and described in Methods on page 11 (second paragraph within "AOB GCaMP2 Ca2+ imaging"). We identified regions of interest (ROIs) in the tissue using a "response reliability index" (similar to a statistical z-score) to identify only statistically reliable parts of the tissue, and later verified that ROIs selectively responded to stimuli by comparing ROI responses to negative control Ringer's stimuli (statistical comparison: Wilcoxon rank sum test, $p < 0.05$, described in Methods on pp. 12-13).

The representative image maps in all figures, therefore, represent the statistically-reliable responses observed in a particular piece of tissue.

The total number of animals used (n = 10) is noted on p. 10.

2 animals were exposed to 10 uM of 11 sulfated steroids
3 animals were exposed to the same 11 steroids in addition to 1:100 BALB/c intact adult male and female urine
5 animals were stimulated with the same 13 stimuli above in addition to 1:100 juvenile male/female urine, 1:100 gonadectomized male/female urine, and 1:100 equivalent sulfatase treated and sulfatase control urine.

The numbers of repeated experiments for each Figure are:

Fig. 1: representative images from a single experiment illustrating within-experiment statistical comparisons used in the study
Fig. 2: n = 8 stated in Fig. 2 legend and p. 3
Fig. 3: n = 5 stated in Fig. 3 legend and p. 4
Fig. 4: n = 5 stated in Fig. 4 legend and p. 4
Fig. 5: n = 10 stated on p. 5 (along with descriptions of Fig. 6-8).
Fig. 6: n = 10 stated in Fig. 6 legend and on p. 6
Fig. 7: n = 10 stated on pp. 5-7
Fig. 8: n = 10 stated in Fig. 8 legend and on pp. 6-7

Statistics and general methods

1. Is there a justification of the sample size? If so, how was it justified? On what page(s)? Even if no sample size calculation was performed, authors should report why the sample size is adequate to measure their effect size.	There is no explicit justification of the sample size in the text. The n of 10 animals represents a similar number to other studies of glomerular maps in the accessory olfactory bulbs (Wagner et al 2006: n = 12, Belluscio et al, 1999: n = 22. The slightly smaller number of animals used is largely due to the large amount of data generated in each experiment, and the time needed to objectively identify glomeruli and evaluate maps. Also, since we generate 13-19 individual maps (each response to a stimulus represents one map) per experiment, we greatly increased the breadth of identified glomeruli evaluated per animal over previous reports. The animal-animal variability in glomerular maps has been noted by previous studies of AOB projections from vomeronasal neuron populations expressing the same receptor (Wagner et al, Belluscio et al, etc.). We intentionally show and measure this variability (Figs. 3 and 8 and Supplementary Fig. 1). One of the important findings of this paper is that despite this variability there are consistent patterns of relative (i.e. within-subject) glomerular spacings, which we show explicitly through example maps and our statistical measurements of glomerular spacing (Figs. 4 and 8).
2. Are statistical tests justified as appropriate for every figure? On what page(s)?	Fig 1: SRI/z-score p. 2 and p. 12 Fig. 2: in legend (b,d)Paired Student's t-test, (c) Kolmogorov-Smirnoff test Fig. 3: one-way ANOVA stated in legend Fig. 4: shuffle tests explained for (b, d, e) on pp. 13-14, Fig. 5: same as Fig. 1 Fig. 6: clustering methods on p. 13 Fig. 7: receptive field analysis on p. 14 Fig. 8: p. 6 and pp. 13-15
a. If there is a section summarizing the statistical methods in the methods, is the statistical test for each experiment clearly defined?	Yes. RRI/z-score on p. 12. Clustering p. 13. Spacing indices on p. 13-14. Isomap and spring embedding pp. 14-15.
b. Do the data meet the assumptions of the specific statistical test you chose (e.g. normality for a parametric test)? Where is this described?	Yes. When possible, we have utilized nonparametric methods. In cases where a parametric test (Student's t-test or ANOVA) are used, the data follow an approximately normal distribution.
c. Is there any estimate of variance within each group of data? Is the variance similar between groups that are being statistically compared? Where is this described?	Yes. Yes. This is shown directly whenever possible: Trial-trial variability for ROIs: in Figs. 1e, 5b Across-animal variability: Figs. 3 and 8 and SFig. 1
d. Are tests specified as one- or two-sided?	Yes.
e. Are there adjustments for multiple comparisons?	N/A

3. Are criteria for excluding data points reported? Was this criterion established prior to data collection? On what page(s) is this described?	Yes. Yes. We utilized automated, objective methods for identifying ROIs and measuring activities for these data sets (pp.12-13).
4. Define the method of randomization used to assign subjects (or samples) to the experimental groups and to collect and process data. If no randomization was used, state so. On what page(s) does this appear?	N/A
5. Is a statement of the extent to which investigator knew the group allocation during the experiment and in assessing outcome included? If no blinding was done, is a statement to this effect included? On what page(s)?	N/A
6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included? On what page(s)?	Yes. p. 10
7. Is the species of the animals used reported? On what page(s)?	Yes. p. 10
8. Is the strain of the animals (including background strains of KO/ transgenic animals used) reported? On what page(s)?	Yes. C57Bl/6 animals. p. 10
9. Is the sex of the animals/subjects used reported? On what page(s)?	Yes. Male subjects, various urine sources p. 10
10. Is the age of the animals/subjects reported? On what page(s)?	Yes. P60+ p. 10
11. For animals housed in a vivarium, is the light/dark cycle reported? On what page(s)?	Yes. Standard 12/12 light/dark cycle. p. 10
12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported? On what page(s)?	Yes. The animals were housed in cages of no greater than 5 mice. p. 10
13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)? On what page(s)?	N/A

14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported?

    On what page(s)?

    Yes. p. 10.

    a. If multiple behavioral tests were conducted in the same group of animals, is this reported?

       On what page(s)?

       N/A

15. If any animals/subjects were excluded from analysis, is this reported?

    On what page(s)?

    N/A

    a. How were the criteria for exclusion defined?

       Where is this described?

    b. Specify reasons for any discrepancy between the number of animals at the beginning and end of the study.

       Where is this described?

Reagents

1. Have antibodies been validated for use in the system under study (assay and species)?

   N/A

   a. Is antibody catalog number given?

      On what page(s) does this appear?

   b. Where were the validation data reported (citation, supplementary information, Antibodypedia)?

      On what page(s) does this appear?

2. If cell lines were used to reflect the properties of a particular tissue or disease state, is their source identified?

   On what page(s)?

   N/A

   a. Were they recently authenticated?

      On what page(s) is this information reported?

Data deposition

Data deposition in a public repository is mandatory for:

a. Protein, DNA and RNA sequences
b. Macromolecular structures
c. Crystallographic data for small molecules
d. Microarray data

Deposition is strongly recommended for many other datasets for which structured public repositories exist; more details on our data policy are available here. We encourage the provision of other source data in supplementary information or in unstructured repositories such as Figshare and Dryad.

1. Are accession codes for deposit dates provided?

   On what page(s)?

N/A

Computer code/software

1. Is there any custom algorithm/software that is integral to the study that has not been previously reported?

   If so, is this algorithm/software provided in a usable and readable form for the referees?

   Indicate in what form this is provided.

The custom clustering algorithm used to identify common response patterns across active regions (Figs. 3 and 6) is based on the previously-established mean-shift clustering algorithm (Comaniciu and Meer, 2002). Our modifications (T. Holy), which implement detection of cluster boundaries using local neighborhood statistics rather than an explicit guess about the number of clusters (e.g. k-means), has not yet been published, but is part of a manuscript in preparation that will be provided upon request (T. Holy).

Human subjects

1. Which IRB approved the protocol?

   Where is this stated?

N/A

2. Is demographic information on all subjects provided?

   On what page(s)?

N/A

3. Is the number of human subjects, their age and sex clearly defined?

   On what page(s)?

N/A

4. Are the inclusion and exclusion criteria (if any) clearly specified?

   On what page(s)?

N/A

5. How well were the groups matched?

   Where is this information described?

N/A

6. Is a statement confirming that informed consent was obtained from all subjects included?

   On what page(s)?

N/A

7. For publication of patient photos, is a statement confirming that consent to publish was obtained included?

   On what page(s)?

N/A

fMRI studies

For papers reporting functional imaging (fMRI) results please ensure that these minimal reporting guidelines are met and that all this information is clearly provided in the methods:

1. Were any subjects scanned but then rejected for the analysis after the data was collected?

N/A

   a. If yes, is the number rejected and reasons for rejection described?

      On what page(s)?

2. Is the number of blocks, trials or experimental units per session and/or subjects specified?

   On what page(s)?

N/A

3. Is the length of each trial and interval between trials specified?

N/A

4. Is a blocked design used?

   If so, is length of blocks specified?

N/A

5. Is an event-related design being used?

   If so, how was the design optimized?

N/A

6. Is the task design clearly described?

   Where?

N/A

7. How was behavioral performance measured?

N/A

8. Are any planned comparisons being used?

N/A

   a. Are they clearly described?

   b. Is an ANOVA used?

9. For data acquisition, is a whole brain scan used?

   If not, state area of acquisition.

N/A

   a. How was this region determined?

10. Is the field strength (in Tesla) of the MRI system stated?

N/A

a. Is the pulse sequence type (gradient/spin echo, EPI/spiral) stated?	
11. Is the software used for data processing and pre-processing clearly stated?	N/A
12. For any anatomical imaging, is the coordinate space defined?	N/A
13. How was the brain image template space, name, modality and resolution determined?	N/A
14. How were anatomical locations determined?	N/A
15. Is the statistical model and estimation method clearly described?	N/A
16. Were any additional regressors (behavioral covariates, motion etc) used?	N/A
17. Is the contrast construction clearly defined?	N/A
18. Is a mixed/random effects or fixed inference used?	N/A
a. If fixed effects inference used, is this justified?	
19. Were repeated measures used (multiple measurements per subject)?	N/A
a. If so, are the method to account for within subject correlation and the assumptions made about variance clearly stated?	
20. If the threshold used for inference and visualization in figures varies, is this clearly stated?	N/A
21. Are statistical inferences corrected for multiple comparisons?	N/A
a. If not, is this labeled as uncorrected?	
22. Are the results based on an ROI (region of interest) analysis?	N/A
a. If so, is the rationale clearly described?	
b. How were the ROI's defined (functional vs anatomical localization)?	
23. Is there correction for multiple comparisons within each voxel?	N/A
24. For cluster-wise significance, is the cluster-defining threshold and the corrected significance level defined?	N/A

Additional comments

Additional Comments