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Main Figures: 5

Supplementary Figures: 8

Supplementary Tables: 1

Supplementary Videos: 2

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 (section, e.g. Results, & paragraph number).
- 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 paragraph 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; 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?	SECTION & PARAGRAPH #	EXACT VALUE	DEFINED?	SECTION & PARAGRAPH #	REPORTED?	SECTION & PARAGRAPH #	EXACT VALUE	SECTION & PARAGRAPH #	VALUE	SECTION & PARAGRAPH #	
example 1a	one-way ANOVA	Fig. legend	9, 9, 10, 15	mice from at least 3 litters/group	Methods para 8	error bars are mean +/- SEM	Fig. legend	p = 0.044	Fig. legend	F(3, 36) = 2.97	Fig. legend	
example results, para 6	unpaired t-test	Results para 6	15	slices from 10 mice	Results para 6	error bars are mean +/- SEM	Results para 6	p = 0.0006	Results para 6	t(28) = 2.808	Results para 6	
+ 1				Representative examples from 21 mice								

TEST USED		n			DESCRIPTIVE STATS (AVERAGE, VARIANCE)		P VALUE		DEGREES OF FREEDOM & F/t/z/R/ETC VALUE		
FIGURE NUMBER	WHICH TEST?	SECTION & PARAGRAPH #	EXACT VALUE	DEFINED?	SECTION & PARAGRAPH #	REPORTED?	SECTION & PARAGRAPH #	EXACT VALUE	SECTION & PARAGRAPH #	VALUE	SECTION & PARAGRAPH #
+ -	2c		1056, 1302, 1477	boutons from 3 and 5 mice, respectively	Results para 2	error bars are SEM (standard error of mean)	figure legend				
+ -	2f,g		1302, 1477	boutons from 5 mice, before and after AO correction	Results para 2						
+ -	3		10129	Representative data from 3, 6, and 5 mice	Fig. legend	dark gray shade in b and error bars in c indicate SEM	figure legend				
+ -	4a,d, g,j,m		11697	L1 boutons from 19 mice	Results para 5						
+ -	4b,e, h,k,n		6076	L2/3 boutons from 17 mice	Results para 5						
+ -	4c,f,i ,l,o		10129	L4 boutons from 14 mice	Results para 5						
+ -	4p-t		1239	L4 neurons from 3 mice	Results para 4						
+ -	4u-y		1279	L2/3 neurons from 6 mice	Results para 4						
+ -	4z- dd		1637	L5 neurons from 5 mice	Results para 4						
+ -	5c,f,i ,l,o		AS=3092 DS=2816	AS & DS L1 boutons from 19 mice	total in histogram						
+ -	5d,g, j,m,p		AS=1488 DS=1359	AS & DS L2/3 boutons from 17 mice	total in histogram						
+ -	5e,h, k,n,q		AS=2945 DS=2778	AS & DS L4 boutons from 14 mice	total in histogram						
+ -	5s-w		AS=571 DS=452	AS & DS L4 neurons from 3 mice	Results para 10						
+ -	5y-cc		AS=656 DS=401	AS & DS L2/3 neurons from 6 mice	Results para 11						
+ -	5ee-ii		AS=688 DS=294	AS & DS L5 neurons from 5 mice	Results para 12						

▶ Representative figures

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

If so, what figure(s)?

Figure 1, 2, 3, 5

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

If so, where is this reported (section, paragraph #)?

Yes. Sample sizes (number of mice) for each experiment above were stated in corresponding figure legends.

► Statistics and general methods

1. Is there a justification of the sample size?

If so, how was it justified?

Where (section, paragraph #)?

Even if no sample size calculation was performed, authors should report why the sample size is adequate to measure their effect size.

We systematically characterized the representational/tuning properties of very large numbers of thalamic boutons (~28,000) and cortical neurons (>1000 for each type) in the input and output layers of a neural circuit. Our sample sizes are similar to or larger than those generally employed in the field.

2. Are statistical tests justified as appropriate for every figure?

Where (section, paragraph #)?

We used statistical tests (ANOVA) that are widely used in the field on our data.

- a. If there is a section summarizing the statistical methods in the methods, is the statistical test for each experiment clearly defined?

Statistical tests used for tuning curve analysis were described in "tuning curve analysis" section of Methods (Paragraph 17); For all figures with descriptive statistics, the presentations were defined in their figure legends.

- 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 (section, paragraph #)?

ANOVA test was used to determine whether the responses of neurons/boutons to different stimulus orientations are significantly different, following the standard practice of the field.

- 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 (section, paragraph #)?

Not tested.

- d. Are tests specified as one- or two-sided?

One-sided ANOVA

- 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?

Where is this described (section, paragraph #)?

All data were analyzed and reported, according to criteria established prior to data collection. The details on our criteria were described in "Image processing and analysis" and "Tuning curve analysis" sections of the Methods (paragraph 15-18).

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.

Where does this appear (section, paragraph #)?

Visual stimuli were presented in a pseudorandom sequence, as described in "Visual stimulation" section of the Methods (paragraph 9).

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, state so.
Where (section, paragraph #)?
- N/A
6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included?
Where (section, paragraph #)?
- Yes.
Methods, paragraph 1.
7. Is the species of the animals used reported?
Where (section, paragraph #)?
- Yes.
Throughout Results section and figure legends.
Methods, paragraph 2.
8. Is the strain of the animals (including background strains of KO/transgenic animals used) reported?
Where (section, paragraph #)?
- Yes.
Throughout Results section and figure legends.
Methods, paragraph 2.
9. Is the sex of the animals/subjects used reported?
Where (section, paragraph #)?
- Yes.
Methods, paragraph 2.
10. Is the age of the animals/subjects reported?
Where (section, paragraph #)?
- Yes.
Methods, paragraph 2.
11. For animals housed in a vivarium, is the light/dark cycle reported?
Where (section, paragraph #)?
- No.
Animals were maintained on a reversed light/dark cycle, 12hr each.
12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported?
Where (section, paragraph #)?
- No.
Animals were housed in groups of 1- 5 before the cranial surgery. After the surgery, the animals were housed in pairs or alone.
13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)?
Where (section, paragraph #)?
- N/A
14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported?
Where (section, paragraph #)?
- N/A
- a. If multiple behavioral tests were conducted in the same group of animals, is this reported?
Where (section, paragraph #)?
- N/A
15. If any animals/subjects were excluded from analysis, is this reported?
Where (section, paragraph #)?
- Data from all animals were used in analysis.

a. How were the criteria for exclusion defined?

N/A

Where is this described (section, paragraph #)?

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

N/A

Where is this described (section, paragraph #)?

▶ Reagents

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

N/A

a. Is antibody catalog number given?

N/A

Where does this appear (section, paragraph #)?

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

N/A

Where does this appear (section, paragraph #)?

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

N/A

Where (section, paragraph #)?

a. Were they recently authenticated?

N/A

Where is this information reported (section, paragraph #)?

▶ Data deposition

Data deposition in a public repository is mandatory for:

- Protein, DNA and RNA sequences
- Macromolecular structures
- Crystallographic data for small molecules
- 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](#).

We encourage publication of Data Descriptors (see [Scientific Data](#)) to maximize data reuse.

1. Are accession codes for deposit dates provided?

N/A

Where (section, paragraph #)?

▶ Computer code/software

Any custom algorithm/software that is central to the methods must be supplied by the authors in a usable and readable form for readers at the time of publication. However, referees may ask for this information at any time during the review process.

- | | |
|--|---|
| 1. Identify all custom software or scripts that were required to conduct the study and where in the procedures each was used. | Custom Matlab scripts for data analysis, custom LabVIEW codes for data acquisition |
| 2. If computer code was used to generate results that are central to the paper's conclusions, include a statement in the Methods section under " Code availability " to indicate whether and how the code can be accessed. Include version information as necessary and any restrictions on availability. | Matlab data analysis script is not provided with the paper, but the reference to the method was provided ("Image processing and analysis" section of Methods). We will be happy to share the code upon request. |

▶ Human subjects

- | | |
|---|-----|
| 1. Which IRB approved the protocol?
Where is this stated (section, paragraph #)? | N/A |
| 2. Is demographic information on all subjects provided?
Where (section, paragraph #)? | N/A |
| 3. Is the number of human subjects, their age and sex clearly defined?
Where (section, paragraph #)? | N/A |
| 4. Are the inclusion and exclusion criteria (if any) clearly specified?
Where (section, paragraph #)? | N/A |
| 5. How well were the groups matched?
Where is this information described (section, paragraph #)? | N/A |
| 6. Is a statement included confirming that informed consent was obtained from all subjects?
Where (section, paragraph #)? | N/A |
| 7. For publication of patient photos, is a statement included confirming that consent to publish was obtained?
Where (section, paragraph #)? | 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?
 - a. If yes, is the number rejected and reasons for rejection described?
Where (section, paragraph #)?
2. Is the number of blocks, trials or experimental units per session and/or subjects specified?

Where (section, paragraph #)?
3. Is the length of each trial and interval between trials specified?
4. Is a blocked, event-related, or mixed design being used? If applicable, please specify the block length or how the event-related or mixed design was optimized.
5. Is the task design clearly described?
Where (section, paragraph #)?
6. How was behavioral performance measured?
7. Is an ANOVA or factorial design being used?
8. For data acquisition, is a whole brain scan used?
If not, state area of acquisition.
 - a. How was this region determined?
9. Is the field strength (in Tesla) of the MRI system stated?
 - a. Is the pulse sequence type (gradient/spin echo, EPI/spiral) stated?
 - b. Are the field-of-view, matrix size, slice thickness, and TE/TR/flip angle clearly stated?
10. Are the software and specific parameters (model/functions, smoothing kernel size if applicable, etc.) used for data processing and pre-processing clearly stated?

11. Is the coordinate space for the anatomical/functional imaging data clearly defined as subject/native space or standardized stereotaxic space, e.g., original Talairach, MNI305, ICBM152, etc? Where (section, paragraph #)?

N/A

12. If there was data normalization/standardization to a specific space template, are the type of transformation (linear vs. nonlinear) used and image types being transformed clearly described? Where (section, paragraph #)?

N/A

13. How were anatomical locations determined, e.g., via an automated labeling algorithm (AAL), standardized coordinate database (Talairach daemon), probabilistic atlases, etc.?

N/A

14. Were any additional regressors (behavioral covariates, motion etc) used?

N/A

15. Is the contrast construction clearly defined?

N/A

16. Is a mixed/random effects or fixed inference used?

N/A

a. If fixed effects inference used, is this justified?

N/A

17. 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?

N/A

18. If the threshold used for inference and visualization in figures varies, is this clearly stated?

N/A

19. Are statistical inferences corrected for multiple comparisons?

N/A

a. If not, is this labeled as uncorrected?

N/A

20. Are the results based on an ROI (region of interest) analysis?

N/A

a. If so, is the rationale clearly described?

N/A

b. How were the ROI's defined (functional vs anatomical localization)?

N/A

21. Is there correction for multiple comparisons within each voxel?

N/A

22. For cluster-wise significance, is the cluster-defining threshold and the corrected significance level defined?

N/A

▶ Additional comments

Additional Comments