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Manuscript Number: _____

Manuscript Type: Article

Main Figures: 8

Supplementary Figures: 8

Supplementary Tables: _____

Supplementary Videos: _____

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	

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 #
+ -	1d	Each symbol represents the pearson's correlation coefficient between the STA and the VWA, separated by cell type.	page 7; par 1	N = 51 L2 = 4; L3 = 7; L4 = 15; L5st = 11; L5TT = 11; L6 = 3.	Defined as neurons that fired at least 1 AP during the course of the experiment (57/86) but 6/57 could not be unambiguously identified by cell type and were left out of the plot.	page 7; par 1	N/A	N/A			
+ -	1e	N/A - see text	page 7, par 2	N = 86	All neurons in the data set.	page 7, par 2	Dashed lines and shading represent +/- SD	34/ fig legend			
+ -	2a	analysis of variance (r^2) and neural variability (see text)	page 8, par 1	N = 86	All neurons in data set.	page 8, par 1	Solid and dashed lines represent moving average of data.	In Fig	N/A		
+ -	2c	linear regression	page 9, par 2	N = 86	All neurons in data set.	page 6, par 3	Solid lines represent least squares regression of data.	page 9, par 2	N/A		
+ -	3a	unpaired t-test	page 9,par3	N = 39	A subset of the data containing neurons from each layer.	page 9,par3	means, SDs, medians	page 9,par3	P < 0.001; P = 0.6451; P = 0.0113; P = 0.0417	11, 12, 39	Degrees of Freedom (df) = 76; for all tests.
+ -	5c	non-parametric two sided rank-sum test	page 37	N = 71; Adapted (L2 = 9; L3 = 14; L4 = 13; L5st = 16; L5TT = 11; L6 = 8) N = 68; Unadapted (L2 = 8; L3 = 14; L4 = 12; L5st = 16; L5TT = 11; L6 = 7)	Neurons that could be identified morphologically. Collected during two conditions, adapted (complex stimuli) and unadapted (simple stimuli).	page 11, par 3	Red error bars represent +/- SEM	37	p < 0.001; L2 p < 0.001; L3 p < 0.001; L4 p < 0.001; L5st p < 0.01; L5TT p = 0.032; L6	37	df = 16; df = 26; df = 23; df = 30; df = 20; df = 12;
+ -	5d	non-parametric two sided rank-sum test	page 37		"	page 11, par 3	"	37	p = 0.03; L2 p < 0.001; L3 p = 0.01; L4 p = 0.002; L5st p = 0.012; L5TT p = 0.07; L6	37	"
+ -	5e	non-parametric two sided rank-sum test	page 37		"	page 11, par 3	"	37	not significant (NS)	37	"

+ -	5f	non-parametric two sided rank-sum test	page 37		"	page 11, par 3	"	37	P = 0.041; L2 rest NS	37	"	
+ -	5g	non-parametric two sided rank-sum test	page 37		"	page 11, par 3	"	37	not significant (NS)	37	"	
+ -	5h	non-parametric two sided rank-sum test	page 37		"	page 11, par 3	"	37	not significant (NS)	37	"	
+ -	7c	two-sided sign test	page 14	N = 46; Uadapted N = 75; Adapted	Neurons where playback was performed either in the adapted (blue) or anadapted conditions.	page 14, par 1	means, SDs, slopes, r's	page 14, par 1	p < 0.0001; for both adapted and unadapted (left)	39	df = 44; Unadapted df = 73; Adapted	
+ -	7c	comparison of slopes test	page 14	left: N = 46; Uadapted N = 75; Adapted right: N = 33; Unadapted N = 36; Adapted	"	page 14, par 1	means, SDs, slopes, r's	page 14, par 1	p < 10 ⁻⁵ for pairwise comparisons, p = 0.005 for multi-whisker;	39	df = 119 (7c) df = 67 (7d)	
+ -	7d	two-sided sign test	page 14	left: N = 46; Uadapted N = 75; Adapted right: N = 33; Unadapted N = 36; Adapted	Neurons where playback was performed either in the adapted (blue) or anadapted conditions.	page 14, par 2	means, SDs, slopes, r's	page 14, par 2	p = 0.002, (blue and red) for pairwise comparisons, p < 0.0001 (blue); p = 0.36 (red) for multi-whisker;	39	df = 44 ; df = 73 ; df = 31 ; df = 34 ;	
+ -	8b	two-sided sign test		N = 17; Unadapted N = 13; Adapted			slopes	page 15, par 2	p = 0.02, 0.30	page 15, par 2		
+ -	S2a	linear regression	sup.	N = 86	All neurons in the data set.	9	Solid lines represent least squares regression of data.	sup.	N/A	sup.		
+ -	S2c	linear regression	sup.	N = 86	All neurons in the data set	9	Solid lines represent least squares regression of data.	sup.	p = 0.22, R = 0.02	sup.		

+ -	S2d	Mean, Standard Error	sup	L2 (N = 9), L3 (N = 14), L4 (N = 13), layer 5 slender-tufted neurons (L5st) (N = 16), layer 5 thick-tufted neurons (L5TT) (N = 11), and L6 (N = 8).	Neurons that could be identified morphologically.	11			N/A		N/A	
+ -	S4a	Linear Regression		N = 86	All neurons in the data set	9	solid line is unity and red line is the least squares regression of data.	fig legend	N/A		N/A	
+ -	S7a,b	two-sided sign test	fig legend	adapted state (N = 71) unadapted state (N = 68)	Neurons that could be identified morphologically and had data in both adapted and unadapted conditions.	12	Histograms shown	fig legend	p = 0.15 p = 0.06 p = 0.08 p < 0.01 p = .39 p = .43 p = 0.96 p = 0.29	Fig	df = 69 ; df = 66 ;	
+ -	S8a,b	Linear Regression	fig legend	N = 550	each data point represents a single trial observation of the optimal stimulus.	fig legend	solid red line is unity and black line is constant at 1.	Fig	p = 0.42 p = 0.29	Fig	N/A	

► Representative figures

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

If so, what figure(s)?

No

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 #)?

N/A - results not dependent on repetitions but rather on N or number of neurons.

► 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.

Yes. The goal was to obtain good quality recordings from a statistically sufficient sample size (~10) from each of six layers of cortex. Additionally, we needed to make sure neurons could be histologically recovered and morphologically identified. As a result, for some layers of cortex we have more than 10 neurons (~16) and for some we have less (~8). For all statistics, if data was less than 30 samples and/or not normally distributed, we used non-parametric statistics.

2. Are statistical tests justified as appropriate for every figure?
Where (section, paragraph #)?

Yes

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

Yes
 - 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 #)?

Yes. non-parametric tests were used in the event that normality could not be assumed.
 - 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 #)?

yes. Described in text with SD for each group.
 - d. Are tests specified as one- or two-sided?

two-sided.
 - e. Are there adjustments for multiple comparisons?

yes.

3. Are criteria for excluding data points reported?
Was this criterion established prior to data collection?
Where is this described (section, paragraph #)?

No data was excluded. Some neurons were left out if the histology could not be recovered or data could not be collected under certain conditions. All of these have been explicitly stated in the text or figure legends.

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 #)?

Stimuli were computer randomized as described in the Methods (Stimulus Presentation). Randomization of samples (cells/animals) was unnecessary.

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? Yes. Page 23.
Where (section, paragraph #)?
7. Is the species of the animals used reported? Yes. Page 23.
Where (section, paragraph #)?
8. Is the strain of the animals (including background strains of KO/transgenic animals used) reported? Yes. Page 23.
Where (section, paragraph #)?
9. Is the sex of the animals/subjects used reported? Yes. Page 23.
Where (section, paragraph #)?
10. Is the age of the animals/subjects reported? Yes. Page 26. (approximated by weight)
Where (section, paragraph #)?
11. For animals housed in a vivarium, is the light/dark cycle reported? N/A
Where (section, paragraph #)?
12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported? N/A
Where (section, paragraph #)?
13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)? N/A
Where (section, paragraph #)?
14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported? N/A
Where (section, paragraph #)?
- a. If multiple behavioral tests were conducted in the same group of animals, is this reported? N/A
Where (section, paragraph #)?
15. If any animals/subjects were excluded from analysis, is this reported? No animal were excluded unless data could not be obtained from them (i.e.: experiment terminated early).
Where (section, paragraph #)?
- 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](#).

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.

All of the data analysis was performed using custom written MATLAB routines. Nonetheless, these scripts can all be easily implemented and have been carefully explained in the methods section.

2. Is computer source code/software provided with the paper or deposited in a public repository? Indicate in what form this is provided or how it can be obtained.

No. We would be happy to share - researchers can contact our lab directly. The majority of these custom written MATLAB routines are commonly employed in electrophysiology labs and none are "central" to the methods or results in this paper.

▶ 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

None