nature neuroscience

Corresponding Author:	Daniel O'Connor	# Main Figures:	7
Manuscript Number:	NN-A53650A	# Supplementary Figures:	10
Manuscript Type:	Article	# Supplementary Tables:	1
		# Supplementary Videos:	0

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 US	ED		n		DESCRIPTIVE STATS (AVERAGE, VARIANCE)		P VALUE		DEGREES FREEDON F/t/z/R/ETC	1&
	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
	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		D n			DESCRIPTIVE S (AVERAGE, VARIA		P VALU	JE	DEGREES FREEDOM F/t/z/R/ETC	1&
	FIGURE NUMBER	WHICH TEST?	SECTION & PARAGRAPH #	EXACT VALUE	DEFINED?	SECTION & PARAGRAPH #	REPORTED?	SECTION & PARAGRAPH #	EXACT VALUE	SECTION & PARAGRAPH #	VALUE	SECTION & PARAGRAPH #
+ -	2e	two-tailed sign test	fig. legend	17	single unit from 6 mice	fig. legend	errorbars are median ± interquartile range	result s, para 3	p = 0.55	fig. legend	sign = 4	
+ -	2d	two-tailed Wilcoxon signed-rank test	fig. legend	17	single unit from 6 mice	fig. legend	errorbars are mean +/- SEM	metho ds, statist ics	p = 0.38	fig. legend	signed rank = 58	
+ -	Зf	two-tailed sign test	fig. Iegend	17	multi-unit from 4 mice	fig. legend	errorbars are mean +/- SEM	metho ds, statist ics	p = 0.14	fig. legend	sign = 5	
+	3d	two-tailed Wilcoxon signed-rank test	fig. legend	17	multi-unit from 4 mice	fig. legend	errorbars are mean +/- SEM	metho ds, statist ics	p=0.0065	fig. legend	signed rank = 19	
+ -	3e	two-tailed sign test	fig. legend	17	multi-unit from 4 mice	fig. legend	errorbars are mean +/- SEM	metho ds, statist ics	p=0.63	fig. legend	sign = 7	
+ -	4d left	two-tailed sign test	fig. legend	12	multi-unit from 3 mice	fig. legend	errorbars are mean +/- SEM	metho ds, metho ds, statist ics	p = 0.039	fig. legend	sign = 2	
+ -	4d right	two-tailed sign test	fig. legend	9	multi-unit from 2 mice	fig. legend	errorbars are mean +/- SEM	metho ds, statist ics	p = 0.039	fig. legend	sign = 1	
+ -	4e left	two-tailed Wilcoxon signed-rank test	fig. legend	12	multi-unit from 3 mice	fig. Iegend	errorbars are mean +/- SEM	metho ds, statist ics	p=0.57	fig. legend	signed rank = 31	
+ -	4e right	two-tailed sign test	fig. legend	9	multi-unit from 2 mice	fig. Iegend	errorbars are mean +/- SEM	metho ds, statist ics	p=1.0	fig. legend	sign = 4	
+	4f left	two-tailed Wilcoxon signed-rank test	fig. legend	12	multi-unit from 3 mice	fig. Iegend	errorbars are mean +/- SEM	metho ds, statist ics	p=0.34	fig. legend	signed rank = 26	
+	4f right	two-tailed Wilcoxon signed-rank test	fig. legend	9	multi-unit from 2 mice	fig. legend	errorbars are mean +/- SEM	metho ds, statist ics	p=0.57	fig. legend	signed rank = 17	
+	4j left	two-tailed sign test	fig. legend	15	neurons from 3 mice	fig. legend	errorbars are mean +/- SEM	metho ds, statist ics	p=0.61	fig. legend	sign = 6	
+	4j right	two-tailed Wilcoxon signed-rank test	fig. legend	15	neurons from 3 mice	fig. legend	errorbars are mean +/- SEM	metho ds, statist ics	p=0.0003	fig. legend	signed rank = 3	

+	4k left	two-tailed Wilcoxon signed-rank	fig. legend	15	neurons from 3 mice	fig. legend	errorbars are mean +/- SEM	metho ds, statist	p=0.54	fig. legend	signed rank = 42	
	ien	test	legenu		Inice	legenu		ics		legenu		
+	4k right	two-tailed sign test	fig. Iegend	15	neurons from 3 mice	fig. Iegend	errorbars are mean +/- SEM	metho ds, statist ics	p=0.42	fig. legend	sign = 5	
+	4l left	two-tailed Wilcoxon signed-rank test	fig. legend	15	neurons from 3 mice	fig. Iegend	errorbars are mean +/- SEM	metho ds, statist ics	p=0.71	fig. legend	signed rank = 46	
+	4l right	two-tailed Wilcoxon signed-rank test	fig. legend	15	neurons from 3 mice	fig. Iegend	errorbars are mean +/- SEM	metho ds, statist ics	p=0.36	fig. legend	signed rank = 37	
+	5d left	two-tailed sign test	fig. Iegend	22	neurons from 17 mice	fig. legend	errorbars are mean +/- SEM	metho ds, statist ics	p=1.2e-4	fig. legend	sign = 2	
+	5e left	two-tailed Wilcoxon signed-rank test	fig. Iegend	22	neurons from 17 mice	fig. legend	errorbars are mean +/- SEM	metho ds, statist ics	p=0.13	fig. legend	signed rank = 80	
+	5e right	two-tailed Wilcoxon signed-rank test	fig. Iegend	22	neurons from 17 mice	fig. legend	errorbars are mean +/- SEM	metho ds, statist ics	p=0.64	fig. legend	signed rank = 112	
+	5f left	two-tailed sign test	fig. legend	22	neurons from 17 mice	fig. legend	errorbars are mean +/- SEM	metho ds, statist ics	p=0.04	results. para 13	sign = 5	
+	5f right	two-tailed sign test	fig. legend	22	neurons from 17 mice	fig. legend	errorbars are mean +/- SEM	metho ds, statist ics	p=1.1e-5	results. para 13	sign = 1	
+ -	6e	K-S test	fig. legend	167	axons from 4 mice	fig. legend	n/a	fig. legend	p=0.0078	fig. legend	K-S stat = 0.1796	
+	7c left	F statistic	fig. legend	14	neurons from 11 mice	fig. legend	individual observations are shown in fig.	n/a	p = 0.0012	fig. legend	F = 17.85	
+	7c right	F statistic	fig. legend	14	neurons from 11 mice	fig. legend	individual observations are shown in fig.	n/a	p = 0.0012	fig. Iegend	F = 17.86	
+	7d	F statistic	fig. legend	1746	neurons from 6 mice	fig. Iegend	all observations are histogrammed	n/a	p=2.0e-57	fig. legend	F = 274.8	
+	S2h	two-tailed sign test	fig. Iegend	23	neurons from 2 mice	fig. legend	errorbars are mean +/- SEM	metho ds, statist ics	p = 2.4e-7	fig. legend	sign = 0	
+	S3c S3d	two-tailed sign test	fig. legend	17 2091,	multi-unit from 4 mice inter spike interval	fig. legend	errorbars are mean +/- SEM	metho ds, statist ics	p = 0.049 p = 0.82	fig. legend	sign = 4 K-S stat = 0.023	
		K-S test two-tailed		1193	from 4 mice		n/a		F 0.02			
+	S3f	Wilcoxon signed-rank test	fig. legend	7	single unit from 4 mice	fig. legend	individual observations are shown in fig.	n/a	p=0.016	fig. legend	signed rank = 0	
+	S4d left	two-tailed Wilcoxon signed-rank test	fig. Iegend	21	neurons from 2 mice	fig. legend	individual observations are shown in fig.	n/a	p=0.61	fig. legend	signed rank = 101	
+	S4d right	two-tailed sign test	fig. legend	21	neurons from 2 mice	fig. legend	individual observations are shown in fig.	n/a	p=0.26	fig. legend	sign = 7	

+	S4e left	two-tailed sign test	fig. Iegend	21	neurons from 2 mice	fig. legend	individual observations are shown in fig.	n/a	p=0.66	fig. legend	sign = 9	
+	S4e right	two-tailed Wilcoxon signed-rank test	fig. legend	21	neurons from 2 mice	fig. legend	individual observations are shown in fig.	n/a	p=0.08	fig. legend	signed rank = 66	
+	S4f left	two-tailed sign test	fig. legend	21	neurons from 2 mice	fig. legend	individual observations are shown in fig.	n/a	p=0.11	fig. Iegend	sign = 6	
+ -	S4f right	two-tailed Wilcoxon signed-rank test	fig. legend	21	neurons from 2 mice	fig. legend	individual observations are shown in fig.	n/a	p=0.28	fig. legend	signed rank = 68	
	S5f	two-tailed sign test		12	neurons from 10 mice				p=0.00049		sign = 0	
+	S5g left	two-tailed sign test	fig. legend	22	neurons from 17 mice	fig. legend	individual observations are shown in fig.	n/a	p=0.29	fig. legend	sign = 8	
	S5g right	two-tailed Wilcoxon signed-rank test		22	neurons from 17 mice				p=6.9e-4		signed rank = 22	
+	S8a	F statistic	fig. legend	22	neurons from 17 mice	fig. legend	individual observations are shown in fig.	n/a	p=0.0075	fig. Iegend	F = 8.84	
+	S8b	F statistic	fig. legend	186	neurons from 1 mouse	fig. legend	individual observations are shown in fig.	n/a	p=1.5e-17	fig. Iegend	F = 89.49	
+	S8b	F statistic	fig. legend	372	neurons from 1 mouse	fig. legend	individual observations are shown in fig.	n/a	p=1.9e-17	fig. Iegend	F = 33.00	
+	S8b	F statistic	fig. legend	268	neurons from 1 mouse	fig. legend	individual observations are shown in fig.	n/a	p=7.9e-9	fig. Iegend	F = 35.55	
+	S8b	F statistic	fig. legend	245	neurons from 1 mouse	fig. legend	individual observations are shown in fig.	n/a	p=2.8e-5	fig. Iegend	F = 18.27	
+	S8b	F statistic	fig. legend	373	neurons from 1 mouse	fig. legend	individual observations are shown in fig.	n/a	p=1.1e-27	fig. legend	F = 140.4	
+	S8b	F statistic	fig. legend	302	neurons from 1 mouse	fig. legend	individual observations are shown in fig.	n/a	p=7.5e-5	fig. Iegend	F = 16.12	
+	resul ts, para 2	K-S test	results, para 2	1746	neurons from 6 mice	results, para 2	error bars are mean +/- SEM	metho ds, statist ics	p=2.7e-6	results, para 2	K-S stat = 0.088	
+	resul ts, para 4	two-tailed sign test	results, para 4	17	single unit from 6 mice	results, para 3	errorbars are mean +/- SEM	metho ds, statist ics	p=1.5e-5	results, para 4	sign = 0	
+	resul ts, para 4	two-tailed Wilcoxon signed-rank test	results, para 4	17	single unit from 6 mice	results, para 3	errorbars are mean +/- SEM	metho ds, statist ics	p=0.41	results, para 4	signed rank = 59	
+	resul ts, para 7	two-tailed Wilcoxon signed-rank test	results, para 7	17	multi-unit from 4 mice	results, para 6	errorbars are mean +/- SEM	metho ds, statist ics	p=0.0086	results, para 7	signed rank = 21	
+	resul ts, para 7	two-tailed Wilcoxon signed-rank test	results, para 7	17	multi-unit from 4 mice	results, para 6	errorbars are mean +/- SEM	metho ds, statist ics	p=0.59	results, para 7	signed rank = 65	

+ ts, - para 7	two-tailed Wilcoxon signed-rank test	results, para 7	17	multi-unit from 4 mice	results, para 6	errorbars are mean +/- SEM	metho ds, statist ics	p=2.9e-4	results, para 7	signed rank = 0	
+ ts, - para 12	two-tailed Wilcoxon signed-rank test	results, para 12	22	neurons from 17 mice	results, para 12	errorbars are mean +/- SEM	metho ds, statist ics	p=0.0049	results, para 12	signed rank = 34.5	
+ ts, - para 15	two-tailed sign test	results, para 15	19	neurons from 19 mice	results, para 15	errorbars are mean +/- SEM	metho ds, statist ics	p = 0.21	results, para 15	sign = 5	
+ ts, - para 21	F statistic	results, para 21	14	neurons from 11 mice	results, para 21	n/a		p=0.0096	results, para 21	F = 9.47	
 meth ods, rever sal pote ntial 	two-tailed Wilcoxon signed-rank test	metho ds, revers al potenti al	14	neurons from 11 mice	methods, reversal potential	errorbars are mean +/- SD	n/a	p = 0.49	methods , reversal potential	signed rank = 41	
+ ods, anes thesi a	two-tailed Wilcoxon rank-sum test	metho ds, anesth esia	10, 56	behaving sessions from 10 and 27 mice	methods, anesthesi a	errorbars are mean +/- SD	metho ds, anest hesia	p = 0.78	methods , anesthes ia	rank sum = 319	

▶ Representative figures

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

If so, what figure(s)?

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 #)?

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.

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

Where (section, paragraph #)?

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

Fig. 1e, Fig. 4a, Fig. 6b, Supp. Fig. 3b.

Images are schematics for experiments whose N values are reported in the legends and methods.

No statistical methods were used to predetermine sample sizes. Sample sizes are similar to what have been reported in the field. This is described in the Methods section.

Yes. We describe all the statistical tests in each figure legend, and Methods section titled 'Statistics'.

Yes. In Methods section titled 'Statistics'.

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 #)?

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 #)?

- d. Are tests specified as one- or two-sided?
- e. Are there adjustments for multiple comparisons?
- 3. Are criteria for excluding data points reported?

Was this criterion established prior to data collection?

Where is this described (section, paragraph #)?

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 #)?

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 #)?

6. For experiments in live vertebrates, is a statement of compliance with Yes, reported in Methods section ethical guidelines/regulations included?

Where (section, paragraph #)?

7. Is the species of the animals used reported?

Where (section, paragraph #)?

8. Is the strain of the animals (including background strains of KO/ transgenic animals used) reported?

Where (section, paragraph #)?

9. Is the sex of the animals/subjects used reported?

Where (section, paragraph #)?

10. Is the age of the animals/subjects reported?

Where (section, paragraph #)?

Yes. Described in Methods section titled 'Statistics'.

Yes, all data are reported with standard error of the mean or median with interguartile range. The variance is similar between groups. Dispersion is reported directly with the data. This is described in Method section "Data analysis".

Yes

No

Yes. This is described in multiple subsections titled "data preprocessing" under Methods.

No randomization was used. Stated in Methods section titled "Statistics".

No blinding was performed. Stated in Methods section titled "Statistics".

Yes, in Methods section titled "Mice".

11. For animals housed in a vivarium, is the light/dark cycle reported?

Where (section, paragraph #)?

12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported?

Where (section, paragraph #)?

13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)?

Where (section, paragraph #)?

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

Where (section, paragraph #)?

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

Where (section, paragraph #)?

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

Where (section, paragraph #)?

a. How were the criteria for exclusion defined?

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.

Where is this described (section, paragraph #)?

Reagents

- 1. Have antibodies been validated for use in the system under study (assay and species)?
 - a. Is antibody catalog number given?

Where does this appear (section, paragraph #)?

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

Where does this appear (section, paragraph #)?

Yes, in Methods section titled "Mice".

Yes, in Methods section titled "Mice".

Yes, in Methods section titled "Mice".

Mice were only used for the experiments reported here, as described in the Methods.

Behavioral testing is described fully in the Methods section titled "Behavioral task."

No animals were excluded.

N/A

N/A

N/A. No antibodies were used.

N/A

N/A

- 2. Cell line identity
 - Are any cell lines used in this paper listed in the database of commonly misidentified cell lines maintained by <u>ICLAC</u> and <u>NCBI Biosample</u>?

Where (section, paragraph #)?

- b. If yes, include in the Methods section a scientific justification of their use--indicate here in which section and paragraph the justification can be found.
- c. For each cell line, include in the Methods section a statement that specifies:
 - the source of the cell lines
 - have the cell lines been authenticated? If so, by which method?
 - have the cell lines been tested for mycoplasma contamination?

Where (section, paragraph #)?

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.

N/A. No cell lines were used.

N/A

N/A

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

1. Are accession codes for deposit dates provided?

Where (section, paragraph #)?

N/A			

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.
- 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.

N/A

N/A

Human subjects

- Which IRB approved the protocol?
 Where is this stated (section, paragraph #)?
- Is demographic information on all subjects provided? Where (section, paragraph #)?
- Is the number of human subjects, their age and sex clearly defined? Where (section, paragraph #)?
- Are the inclusion and exclusion criteria (if any) clearly specified? Where (section, paragraph #)?
- 5. How well were the groups matched?

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

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

Where (section, paragraph #)?

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

Where (section, paragraph #)?

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?	N/A
	Where (section, paragraph #)?	
2.	Is the number of blocks, trials or experimental units per session and/ or subjects specified?	N/A
	Where (section, paragraph #)?	
3.	Is the length of each trial and interval between trials specified?	N/A

N/A

N/A			
N/A			

- 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?
- 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 #)?
- 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 #)?
- How were anatomical locations determined, e.g., via an automated labeling algorithm (AAL), standardized coordinate database (Talairach daemon), probabilistic atlases, etc.?
- 14. Were any additional regressors (behavioral covariates, motion etc) used?
- 15. Is the contrast construction clearly defined?
- 16. Is a mixed/random effects or fixed inference used?

ble,	N/A
	N/A
	N/A
al)	N/A
/TR/	N/A
and	N/A
a : tion,	N/A
2	N/A
d tion,	
d ach	N/A
)	N/A
	N/A

N/A

April 2015

17. Were repeated measures used (multiple measurements per subject)?

N/A

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

- a. If so, are the method to account for within subject correlation and the assumptions made about variance clearly stated?
- 18. If the threshold used for inference and visualization in figures varies, is N/A this clearly stated?
- 19. Are statistical inferences corrected for multiple comparisons?
 - a. If not, is this labeled as uncorrected?
- 20. Are the results based on an ROI (region of interest) analysis?
 - a. If so, is the rationale clearly described?
 - b. How were the ROI's defined (functional vs anatomical localization)?
- 21. Is there correction for multiple comparisons within each voxel?
- 22. For cluster-wise significance, is the cluster-defining threshold and the corrected significance level defined?

Additional comments

Additional Comments

