

Corresponding Author: David Fitzpatrick

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# Main Figures: 6

# Supplementary Figures: 9

# Supplementary Tables: 0

# 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 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 #	
+ -	Results	Wilcoxon rank sum	Results paragraph 2	n = 9;836	all imaged somata and spines	Results paragraph 1	Median and IQR	Results paragraph 2		p = .88		z(843) = -.147
+ -	Figure 2C	Wilcoxon signed rank	Results paragraph 2	n = 9	all imaged cells	Results paragraph 2	Individual data points are shown in Figure 2C	N/A		p = .82		Signed rank = 25
+ -	Figure 3C	Wilcoxon signed rank	Results paragraph 3	n = 9	all imaged cells	Results paragraph 3	individual data points are shown in Figure 3C	N/A		p = .65		Signed rank = 27
+ -	Figure 3D	Pearson's correlation coefficient	Results paragraph 3	n = 9	all imaged cells	Results paragraph 3	individual data points are shown in Figure 3D	N/A		r = .66, p = .06		t(7) = 2.2934
+ -	Figure 3D	Pearson's correlation coefficient	Results paragraph 3	n = 9 cells	all imaged cells	Results paragraph 3	individual data points are shown in Figure 3D	N/A		r = -.0265, p = .946		t(7) = -.0701
+ -	Figure 3E	Wilcoxon rank sum	Results paragraph 3	n = 366; 470	all tuned spines	Results paragraph 3	data are shown as CDF in figure 3E	N/A		p = .6082		z(834) = .5126
+ -	Results	Pearson's correlation coefficient	Results paragraph 4	n = 9 cells	all imaged cells	Results paragraph 4	individual data points are shown in figure	N/A		r = .4532, p = .2206		t(7) = 1.3451
+ -	Figure 4c	Pearson's correlation coefficient	Results paragraph 4	n = 9 cells	all imaged cells	Results paragraph 4	individual data points are shown in figure	N/A		r = .7992, p = .0098		t(7) = 3.5179
+ -	Figure 5S	Wilcoxon signed rank	Results paragraph 5	n = 16	all patched cells	Results paragraph 5	individual data points are shown	N/A		p = 5.2896e-04		z(14) = -3.4656
+ -	Figure 4H	Pearson's correlation coefficient	Results paragraph 5	n = 16	all patched cells	Results paragraph 5	individual data points are shown	N/A		r = .3121, p = .2392		t(7) = .8692
+ -	Figure 5C	Pearson's correlation coefficient	Results paragraph 6	n = 146	all imaged dendrites with at least 3 active,tuned spines	Results paragraph 6	individual data points are shown	N/A		r = -.3022 p = .0002		t(144) = -3.8043
+ -	Results	Wilcoxon rank sum	Results paragraph 6	n = 43, n = 103	all imaged dendrites with at least 3 active,tuned spines	Results paragraph 6	individual data points are shown	N/A		p = .0035		z(144) = 2.9194
+ -	Figure 5D	Pearson's correlation coefficient	Results paragraph 6	n = 9	all imaged cells	Results paragraph 6	individual data points are shown	N/A		r = .7108, p = .0318		t(7) = 2.6736
+ -	Figure 5E	Pearson's correlation coefficient	Results paragraph 6	n = 9	all imaged cells	Results paragraph 6	individual data points are shown	N/A		r = .6998, p = .0359		t(7) = 2.5919
+ -	Figure 5F	Wilcoxon rank-sum	Results paragraph 6	n = 219; n = 136	in results paragraph 6	Results paragraph 6	data are shown as CDF	N/A		p = 1.5172e-12		z(353) = -7.0729
+ -	Results	Two sample Kolmogorov Smirnov test	Results paragraph 5	n = 9; n = 16	in results paragraph 5	Results paragraph 5	individual data points are shown in Figures 2D and 4H	N/A		p = .3808		D(23) = .3542

+ -	Figure 6E	Wilcoxon rank sum	Results paragraph 8	n = 30, n = n 21	in results paragraph 8	Results paragraph 8	distributions shown in figure	N/A	p = .042		z(49) = 2.033	
+ -	Results	Wilcoxon signed rank	Results paragraph 7	n = 9	in results paragraph 7	Results paragraph 7	median and IQR	results paragraph 7	p = .5703		signed rank = 17	
+ -	Figure 2D	Wilcoxon signed rank	Results paragraph 2	n = 9	all imaged cells	Results paragraph 2	All data points are in figure 2D	N/A	p = .0039		signed rank = 45	
+ -	Supplementary Figure 7b	Wilcoxon rank sum	Results paragraph 6	n = 72, 74	all imaged dendrites with at least 3 active, tuned spines	Results paragraph 6	distributions shown in figure	Results paragraph 6	p = .566		z(144) = 0.5734	
+ -	Supplementary Figure 8c	Wilcoxon rank sum	Results paragraph 7	n = 952, n = 1038	hotspots with small and large uniform dendritic events	Results paragraph 7	distributions shown in figure	Results paragraph 7	p = .09		z(1988) = 1.68	

## ► Representative figures

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

If so, what figure(s)?

Figures 1C-D, 5A, 6A, 6D, Supplementary Figures 2 and 3

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 values are reported throughout the text and in methods

## ► 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.

5 animals or greater is an accepted sample size for in vivo imaging in carnivores such as ferrets and cats.

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

Where (section, paragraph #)?

Yes, see Statistics section of methods

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

Yes, in the methods Statistics section

- 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 are used.

- c. Is there any estimate of variance within each group of data? Yes. Individual data points, cdf plots, or box plots, are shown.  
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? Two-sided.
- e. Are there adjustments for multiple comparisons? No.
3. Are criteria for excluding data points reported? Inclusion criteria are noted in the two photon imaging section of methods  
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. No method of randomization was used to assign subjects to experimental groups.  
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? The experimenter was blind to cellular location in the orientation preference map for spine imaging experiments; this is reported in the statistics section of the methods.  
If no blinding was done, state so.  
Where (section, paragraph #)?
6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included? Yes, methods paragraph 1.  
Where (section, paragraph #)?
7. Is the species of the animals used reported? Yes, in the methods animals paragraph  
Where (section, paragraph #)?
8. Is the strain of the animals (including background strains of KO/transgenic animals used) reported? N/A  
Where (section, paragraph #)?
9. Is the sex of the animals/subjects used reported? Yes, in the methods animals paragraph  
Where (section, paragraph #)?
10. Is the age of the animals/subjects reported? Yes, in the methods animals paragraph  
Where (section, paragraph #)?
11. For animals housed in a vivarium, is the light/dark cycle reported? Yes, in the methods animals paragraph  
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 #)?
2. Cell line identity
- a. Are any cell lines used in this paper listed in the database of commonly misidentified cell lines maintained by [ICLAC](#) and [NCBI Biosample](#)?
- 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](#).

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.

Analysis code was written using standard Matlab functions and MIJ.

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.

Analysis code is available on 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 #)?

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

Where (section, paragraph #)?

4. 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?

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 #)?

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 #)?

13. 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?

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

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

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 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