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Main Figures: 7

Supplementary Figures: 15

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
+ -	2b	unpaired T-test	results paragraph 3	19894 223	events from 21 neurons 16 neurons	results paragraph 3	mean +/- SEM 173+/- 16, 56+/17,	results para 3	p=1.8e-6	results para 3		

		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 para 1			22	neurons from 21 mice	results para 1	mean +/- SEM 14/-1.5	results para 1				
+ -	fig 1 h			226	events from 17 neurons	results para 2	mean +/- SEM 41+/- 0.3	results para 1 and fig 1h				
+ -	results para 1			22	neurons from 21 mice	results para 1	mean +/- SEM 7.9 +/-1.0	results para 1				
+ -	results para 1			22	neurons from 21 mice	results para 1	mean +/-SEM 2.8 +/-0.26	results para 1				
+ -	fig 1i			226	events from 17 neurons	results para 2	mean +/- SEM 56 +/-4	results para 1 and fig 1i				
+ -	fig 2b	unpaired t test	results para 3	19894 223	events from 21 neurons 16 neurons	results para 3	mean +/- sem 0.43+/-0.04 0.92+/- 0.03	results para 3	p=4.9e-4	results para 3		
+ -	fig 2h	unpaired t test	results para 4	6	neurons	results para 4	mean +/- SEM 8.6 +/- 1.4, 7.8 +/-1.7	results para 4 and fig 2h	p=0.45			
+ -	fig 2i	paired t test	fig 2i	6	neurons	fig 2i	error bars are mean +/- SEM 4.1+/-1.2 0.8+/-0.2	fig 2i	p=0.0289	fig 2i		
+ -	fig 2j	paired t test	fig 2j	6	neurons	fig 2j	error bars are mean +/- SEM 183.2+/-41 63.5+/-33.5	fig 2j	p=0.0026	fig 2j		
+ -	fig 3e	paired t test	fig 3 e	8	neurons	fig 3e	distribution	fig 3e	p=0.039	fig 3e		
+ -	fig 3f	paired t test	fig 3f	8	neurons	fig 3f	distribution	fig 3e	p<0.05	fig 3f		
+ -	fig 3g	paired t test	fig 3g	8	neurons	fig 3g	error bars are mean +/- SEM 3.8+/-0.8 1.7+/-0.5		p=0.04	fig 3g		
+ -	fig 3h	paired t test	fig 3h	8	neurons	fig 3h	error bars are mean +/- SEM 63.4+/-6.8 27.8+/-6.0		p=0.02	fig 3h		
+ -	fig 3i	paired t test	fig 3i	8	neurons	fig 3i	error bars are mean +/- SEM 2.3+/-0.5 0.5+/-0.2		p=0.02	fig 3i		

+ -	fig 3n	paired t test	3n	6	neurons from 6 slices	fig 3n	error bars are mean +/- SEM 54+/-7 vs 120 +/-25 120+/-25 vs59+/-9 50+/-11vs 151 +/-16 151+/-16 vs 60 +/-10	fig 3n	p=0.03, p=0.058, p=0.0009, p=0.003	fig 3n		
+ -	result ls para 5	paired t-test	metho ds	8	neurons	results para 5	mean +/- SEM 29.6+/-1.6, 28.5 +/-1.3	result s para 5	0.49			
+ -	Fig 4c	bootstrappe d permuation	results para 7 and metho ds	17	neurons	na			p=1e-4	results para 7 and fig legend fig 4		
+ -	fig 4d	paired t-test	fig 4d	17	neurons	na	error bars are mean +/- SEM	fig 4d	p=.0.034, 0.024, 0.041	fig 4d		
+ -	fig 4e	paired t-test	fig 4e	17	neurons	na	error bars are mean +/- SEM	fig 4e	p=0.005, 0.044, 0.041, 0.032, 0.002	fig 4e		
+ -	fig 4f	paired t-test	fig 4f	17	neurons	na			p=0.042, 0.038, 0.043, 0.027, 0.040	fig 4f		
+ -	fig5e			22	events from 6 neurons	results para 9	mean+/-SEM 138+/-23	Fig 5e				
+ -	resul ts para 9			6	neurons	resutls para 9	mean +/-SEM 12.7+/-2.2	result s para 9				
+ -	Fig 5f	paired t-test	fig 5f	6	neurons	results para 9	pre vs post mean +/-SEM 0.8+/-0.2 6.9+/-1.0	result s para 9 and fig 5f	p=1e-9	results para 9		
+ -	fig 5g	paired t-test	fig 5g	6	neurons	results para 9	pre vs post mean +/-SEM 0.6+/-0.2 1.7+/-0.4	result s para 9 and fig 5g	p=1e-4	results para 9		
+ -	fig 6g	paired t-test	metho ds	14	neurons	results para 10	pre vs post mean +/- SEM 1.3+/-0.4 7.2+/-0.6	result s para 10 and fig 6g	p=2.5e-11	results para 10		
+ -	fig 6h	paired t-test	metho ds	14	neurons	results par 10	pre vs post mean +/- SEM 0.9+/-0.3 1.7+/-0.2	result s para 10 and fig 6h	p=7.4e-8	results para 10		
+ -	6g	paired t-test	metho ds	6	neurons	results par 12	pre vs post mean +/- SEM 1.1+/-0.3 1.9+/-0.4	result s para 12 and fig 6g	p=0.007	results para 12		
+ -	6g	unpaired t-test	metho ds	14	neurons	results par 12	Plat vs APs mean +/- SEM 7.2+/-0.6 1.9+/-0.4	result s para 12 and fig 6g	p=4.7e-9	results para 12		
+ -	6h	unpaired t-test	metho ds	14	neurons	results par 10	Plat vs APs mean +/- SEM 1.7+/-0.2 0.8+/-0.1	result s para 10 and fig 6h	p<0.03	fig 6h		

+ -	6h	paired t-test	methods	6	neurons	results par 10	pre vs post mean +/- SEM 0.7+/-0.1 0.8+/-0.1	results para 10 and fig 6h	p=0.48	results para 12		
+ -	6k	ANOVA	results para 11	14	neurons	results para 11	error bars are mean +/- SEM	fig 6k	F=0.2425	fig 6k		
+ -	7e, g	paired t-test	results para 13	19	neurons	fig 7 legend	error bars are mean +/-SEM var pre post 2.4+/-0.1 8.0+/-0.8	fig 7g	p=8.5e-9	results para 13		
+ -	7 f, h	paired t-test	results para 13	19	neurons	fig 7 legend	error bars are mean +/-SEM kurtosis pre post 0.36+/-0.08 1.0+/-0.2	fig 7h	p=3.2e-5	results para 13		
+ -	7g	unpaired t-test	fig 7 legend	13, 12	neurons	fig 7 legend	error bars are mean +/-SEM var PC, post 8.8+/-1.4 8.0+/-0.8	fig 7g	p=0.43	reults para 14 and fig 7g		
+ -	7g	unpaired t-test	fig 7 legend	10, 12	neurons	results para 13	error bars are mean +/-SEM var silent var pc 8.8+/-1.4 2.0+/-0.2	results para 13	p=4.8e-7	results para 13		
+ -	7h	unpaired t-test	fig 7 legend	10, 12	neurons	fig 7 legend	error bars are mean +/-SEM kurt silent pc 1.7+/-0.4 0.2+/-0.1	fig 7h	p=1.2e-3	fig 7h		
+ -	7h	unpaired t-test	fig 7 legend	13, 12	neurons	fig 7 legend	error bars are mean +/-SEM kurt post and pc 1.0+/-0.2 1.7+/-0.4	fig 7g	p=0.061	fig 7h		
+ -	7c	unpaired t-test	results para 14	13,12	neurons	results para 14	mean +/-SEM power 1.6+/-0.2	results para 14	p=0.31	results para 14		
+ -	8e	unpaired t-test	fig 8 legend	16	neurons	fig 8 legend	mean +/-SEM	fig 8e	p=1e-4	fig 8 legend		
+ -	8d	unpaired t-test	fig 8 legend	16	neurons	fig 8 legend	mean +/-sem	8d	p=0.0004 (pre post) p=0.017 post inside post outside	fig 8 legend		
+ -	Fig S2	paired t-test	fig s2h	14	neurons	fig s2 legend	error bars are mean +/-SEM 191±1.5 vs 137±16 193±1.1 vs134±1.2	Fig s2h	p2e-3 thetain p1e-4 APs	fig S2h		
+ -	Fig s3g	unpaired t-test	Fig s3g	28, 73	events	fig s2 legend	error bars are mean +/- SEM 4.5+/-0.4 vs 33+/-2	fig s3g	p1e-28			
+ -	fig s5b	mann-whitney Utest	fig s5 legend	17, 13	neurons	fig s5 legend	error bars are mean +/- SEM 1.5+/-0.3 vs 0.38 +/-0.1	fig s5b	p=0.0016	fig s5b		
+ -	fig s5d	mann-whitney Utest	fig s5 legend	17, 10	neurons	fig s5 legend	error bars are mean +/- SEM 0.068+/-0.015 vs 0.013+/-0.006	fig s5d	p=0.009	fig s5d		
+ -	fig s8	mann whitney u test paired	fig s8 legend	8	neurons	fig s8 legend	error bars are mean +/-SEM 0.15+/-0.02 vs 0.10+/-0.013	fig s8c	p=0.015	fig s8 legend		

+ -	fig s9	paired t-test	methods	8	neurons	results para 5	error bars are mean +/-SEM 8.3±1.8 vs 5.6±1.6	fig s9	p=0.01	fig s9c		
+ -	fig s9	paired t-test	methods	8	neurons	results para 5	error bars are mean +/-SEM 1.3±0.20 vs 0.97 ±0.1	fig s9	p=0.05	fig s9d		
+ -	fig s9	paired t-test	methods	8	neurons	results para 5	error bars are mean +/-SEM 5.7±0.91 vs 3.9 ±0.55	fig s9	p<0.01	fig s9e		
+ -	fig s11d	unpaired t-test	results para 10	16,14	neurons	results para 10	error bars are mean +/-SEM 416±7 vs 385±15	fig s11d	p=0.068	fig s11d		
+ -	fig s11d	unpaired t-test	results para 10	16,14	neurons	results para 10	error bars are mean +/-SEM 0.83±0.04 vs 0.92 ±0.03	fig s11d	p=0.079	fig s11d		
+ -	fig s11e	unpaired t-test	results para 10	22,14	neurons	results para 10	error bars are mean +/-SEM 7.2±0.6 vs 7.9±1	fig s11e	p=0.46	fig s11e		
+ -	fig s11e	unpaired t-test	results para 10	22,14	neurons	results para 10	error bars are mean +/-SEM 2.8±0.26 vs 1.7 ±0.2	fig s11e	p=0.001	fig s11e		
+ -	fig s11e	unpaired t-test	results para 10	21,14	neurons	results para 10	error bars are mean +/-SEM 54±1.1 vs 59±3.1	fig s11e	p=0.09	fig s11e		
+ -	fig s11f	unpaired t-test	results para 10	22,14	neurons	results para 10	error bars are mean +/-SEM 12.7±2.2 vs 15.4 ±2.1	fig s11f	p=0.22	fig s11f		
+ -	fig s11f	unpaired t-test	results para 10	21,14	neurons	results para 10	error bars are mean +/-SEM 173±16 vs 182±19	fig s11f	p=0.28	fig s11f		
+ -	fig s11f	unpaired t-test	results para 10	21,14	neurons	results para 10	error bars are mean +/-SEM 57±1.4 vs 64±2.9	fig s11f	p=0.067	fig s11f		
+ -	fig s12	paired t-test	methods	14	neurons		error bars are mean +/-SEM 66.3±2.6 vs 68.8 ±3.3	fig s12e	p=0.3	fig s12e		
+ -												
+ -												

Representative figures

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

   If so, what figure(s)?

   yes representative fills of neurons in Figures 1a, 4a, 4j, s8

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 #)?

   No - each neuron was not necessarily filled, but that does not affect the interpretation of the result. There are clear "n" numbers for every experiment which demonstrates how many neurons were successfully recorded from regardless of the status of the intracellular fill.

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.

   no, we have not attempted this analysis given the difficulty in knowing the population mean and standard deviation for our measures. We have, however, reported measures from a population size that is substantially larger than any other study using similar techniques. If for example we use the experimental mean and st deviation for plateau duration shown in fig 1g to calculate sample size, a sample size approximating 200 is given for a 95% confidence. The number of plateau measured in our experimental manipulations approach this number (184 for figure2 and 146 for figure 3). A statement is included in the methods

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

   Where (section, paragraph #)?

   overall we have examined the distributions of our measures and where they were found to be normal (eg fig 1g,h) we used a two-sided t-test to make comparisons. Otherwise the appropriate test was used (eg Mann-Whitney U test in Sfig 5). We state this in either the figure, figure legend or the text.

   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 #)?

      In most cases yes but there are exceptions. See above.
      This is described in the methods section.

   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,
      yes
      the variance of the data is listed in each section.

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

      two sided

   e. Are there adjustments for multiple comparisons?

      if necessary

3. Are criteria for excluding data points reported?

   Was this criterion established prior to data collection?

   Where is this described (section, paragraph #)?

   no.

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 #)?

   This does not appear to be applicable, since all of our manipulations are done on a trial by trial basis. A statement about randomization was added to the methods

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 #)?	there is a statement about blinding in the methods
6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included? Where (section, paragraph #)?	yes, methods first sentence
7. Is the species of the animals used reported? Where (section, paragraph #)?	yes, methods
8. Is the strain of the animals (including background strains of KO/ transgenic animals used) reported? Where (section, paragraph #)?	yes methods
9. Is the sex of the animals/subjects used reported? Where (section, paragraph #)?	the sex of animals is included in the methods
10. Is the age of the animals/subjects reported? Where (section, paragraph #)?	yes methods
11. For animals housed in a vivarium, is the light/dark cycle reported? Where (section, paragraph #)?	no
12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported? Where (section, paragraph #)?	no
13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)? Where (section, paragraph #)?	no but experiments were done during dark cycle
14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported? Where (section, paragraph #)?	The surgical and behavioral training protocols are listed in the Surgery and training schedule section of the methods.
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 #)?	n/a

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)?

no

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

n/a

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.

All custom algorithms used for data analysis are clearly described in the methods section.

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.

n/a

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 #)?

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