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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
+ -	1d	Wilcoxon signed-rank test	Results para 2 & 3	22, 41, 55	neurons from 6 mice	Fig. legend	z-score	Fig. legend	p < 0.001	Results para 2 & 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 #
+ -	3c	Wilcoxon signed-rank test	Results para 5	19	neurons from 5 mice	Fig. legend	z-score	Fig. legend	p < 0.001	Results para 5	-	-
+ -	4e	One-way repeated ANOVA	Results para 6	5	mice	Results para 6	error bars are mean +/- SEM	Fig. legend	p = 0.0004	Results para 6	F (2, 8) = 25.34	Results para 6
+ -	4d	Wilcoxon signed-rank test	Results para 6	57	neurons from 4 mice	Results para 6	z-score	Fig. legend	p < 0.001	Results para 6	-	-
+ -	7c	unpaired t-test	Results para 9	17	sessions	Fig. legend	dashed lines are mean + s.d.	Fig. legend	p = 1.3e-6	Results para 9	t (32) = 5.94	-
+ -	7d	unpaired t-test	Results para 9	11	mice	Fig. legend	error bars are mean + SEM	Fig. legend	p = 0.696	Results para 9	t (20) = 0.40	-
+ -	7e	unpaired t-test	Results para 9	11	mice	Fig. legend	individual mice	Fig. legend	p = 0.364	Results para 9	t (20) = 0.93	-
+ -	7f	unpaired t-test	Fig. legend	11	mice	Fig. legend	individual mice; error bars are mean +/- SEM	Fig. legend	p = 0.0042	Fig. legend	t (20) = 3.23	-
+ -	S6d	Wilcoxon signed-rank test	Fig. legend	32	neurons from 6 mice	Fig. legend	error bars are mean + SEM	Fig. legend	p = 5.52e-6 p = 0.313 p = 0.029	Fig. legend	z = 4.54 z = 1.01 z = -2.19	- - -
+ -	S6f	Wilcoxon signed-rank test	Fig. legend	26	neurons from 5 mice	Fig. legend	error bars are mean + SEM	Fig. legend	p = 0.001 p = 0.790 p = 0.501	Fig. legend	z = 3.29 z = 0.27 z = -0.67	- - -
+ -	S9b	unpaired t-test	Fig. legend	5	mice	Fig. legend	error bars are mean + SEM	Fig. legend	p = 0.604 p = 0.021	Figure	t (8) = 0.54 t (8) = -2.87	- -
+ -	S9d	ANOVA (3 section x 2 group)	Fig. legend	3	mice	Fig. legend	error bars are mean + SEM	Fig. legend	p = 0.45	Figure	F (1, 4) = 0.70	Fig. legend

Representative figures

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

   If so, what figure(s)?

   Yes.
   Fig.1a, Fig.4b, Fig. 5a,b and Supplementary Fig. 9c.

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.
   Reported as the N number in 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.	Yes. Methods section: "Electrophysiological Data Analyses" subsection: "Key analyses on electrophysiological data were based on the sample size of 191 MnR neurons from 6 mice. These numbers are generally considered as sufficient in conducting statistical analyses." Methods section: "Statistical analyses" subsection: "Sample sizes were based on the authors' experience, previous similar studies and preliminary experiments performed in our lab."
2. Are statistical tests justified as appropriate for every figure? Where (section, paragraph #)?	Yes. Statistical tests used for all figures are appropriate as described in the subsection "Statistical analyses" of the Methods section.
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. The assumptions of normality and sphericity were tested. When they do not meet, analyses that do not depend on the assumptions were used. This is clearly described in the subsection "Statistical analyses" of 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, s.d. or s.e.m. were reported in all mean analyses in the Results section of the main text and figures (Figure legends 2-5 & 7; Supplementary Figure legends 6 & 9). Yes, the variances are similar between groups (Figure 7; Supplementary Figure 9) but it hasn't been described.
d. Are tests specified as one- or two-sided?	All tests are two-sided, but they have not been specified.
e. Are there adjustments for multiple comparisons?	Yes, all post-hoc tests are adjusted 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 #)?	Yes. Methods section: "Electrophysiological Data Analyses" subsection: "Low-frequency firing neurons of 0.2 Hz or less were excluded from the study's analyses due to insufficient number of spikes." This criteria or similar was pre-established. Some mice were found either dead/sick or failed in recording single neuron activities in the MnR due to surgery issues. These mice never entered the dataset and thus were not considered an "exclusion".
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 #)?	Methods section: "Fear conditioning and optical stimulation procedures" subsection: "Mice were randomly assigned into 2 groups, which received the ChR2-EYFP and EYFP-Control virus injections, respectively".

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 #)?	Yes. Methods section: "Fear conditioning and optical stimulation procedures" subsection: "Pairs of mice, consisting of an experimental (ChR2-EYFP) mouse and a control (EYFP only) mouse, had gone through the following procedure at the same time in two separate chambers. The assignments of the experimental and control mice between the two chambers were counterbalanced among pairs; thus although the experiment was not a blind one, it was conducted in a highly systematic manner." Methods section: "Fear conditioning and optical stimulation procedures" subsection: "Freezing scores of the computerized system were verified by a human rater blind to the manipulation".
6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included? Where (section, paragraph #)?	Yes. Methods section, page 1, paragraph 1
7. Is the species of the animals used reported? Where (section, paragraph #)?	Yes. Methods section, page 1, paragraph 1
8. Is the strain of the animals (including background strains of KO/ transgenic animals used) reported? Where (section, paragraph #)?	Yes. Methods section, page 1, paragraph 1
9. Is the sex of the animals/subjects used reported? Where (section, paragraph #)?	Yes. Methods section, page 1, paragraph 1
10. Is the age of the animals/subjects reported? Where (section, paragraph #)?	Yes. Methods section, page 1, paragraph 1
11. For animals housed in a vivarium, is the light/dark cycle reported? Where (section, paragraph #)?	Yes. Methods section, page 1, paragraph 1
12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported? Where (section, paragraph #)?	Yes. Methods section, page 1, paragraph 1
13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)? Where (section, paragraph #)?	Yes. Methods section: "Fear conditioning and optical stimulation procedures" subsection: "kept on a 12 h light/dark cycle (lights on at 06:00 A.M.). "
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 #)?

No.

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

Where (section, paragraph #)?

No.

a. How were the criteria for exclusion defined?

Where is this described (section, paragraph #)?

N/A

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 #)?

N/A

Reagents

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

Yes.

a. Is antibody catalog number given?

Where does this appear (section, paragraph #)?

Yes.
Methods section: "Immunohistochemistry" and "RNA in situ hybridization" subsections.

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

Where does this appear (section, paragraph #)?

Methods section: "Immunohistochemistry" and "RNA in situ hybridization" subsections.

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

Where (section, paragraph #)?

N/A

a. Were they recently authenticated?

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

N/A

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.

Matlab scripts.

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 #)?

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?

   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