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.

<table>
<thead>
<tr>
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<th>DESCRIPTIVE STATS (AVERAGE, VARIANCE)</th>
<th>P VALUE</th>
<th>DEGREES OF FREEDOM &amp; F/T/Z/R/ETC VALUE</th>
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</table>
Representative figures

1. Are any representative images shown (including Western blots and immunohistochemistry/staining) in the paper?
   If so, what figure(s)?
   
   Yes, IHC and electrophysiology traces
   Figures 1b,c,f,g; 2a,c,e; 3a,d
   Supp Figures 1a,d,g,h,i,j; 3a,b; 6a,b; 9a,b

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, all experiments were done w multiple mice per group.
   All data collected was collapsed to an average per animal or experimental condition.
   N per group is listed in the figure legends
   For spine density experiments, ~10 dendrites/animal~1,000 spines per group were analyzed. This is listed in the methods under Imaging and Analysis on pg 20

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 justification of sample size is included in the manuscript. However, sample size was based on previously published work (Krishnan et al., Cell, 2007; Christoffel et al., J. Neuro., 2011; Christoffel et al., Neuropsychopharmac., 2012) and animal protocol (approved by IACUC). This is stated in the methods section. A sample size of 5-15 is appropriate to see a significant difference both in behavior tests, 5-10 cells for electrophysiology, 4-6 for dendritic spine density and VGLUT puncta, and 3 for c-Fos mapping experiments without unnecessary sacrifice.

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?
      
      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 in methods under statistics
   
   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, F tests for variance were carried out for all analysis, where appropriate a Bartlett’s test for equal variances was performed for the one-way ANOVAs. It is stated in the methods statistics section that the data has equal variance.
   
   d. Are tests specified as one- or two-sided?
   
   Yes in methods statistics section
   
   e. Are there adjustments for multiple comparisons?
   
   N/A
3. Are criteria for excluding data points reported?
   Was this criterion established prior to data collection?
   Where is this described (section, paragraph #)?
   No, we did not have data points to exclude.

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 #)?
   Animals were randomized by cages before surgeries. For example, if there were 30 animals in an experiment, with 5 animals per cage, animals were randomly assigned to be control or manipulation groups. Additionally, the order of the animals were randomized prior to behavioral tests.
   This is mentioned in the text under animals section in 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 #)?
   This is mentioned in the imaging analysis and in vitro electrophysiology section of the methods. All behavioral data collected was automated through the use of video tracking software.

6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included?
   Where (section, paragraph #)?
   Yes, methods animals

7. Is the species of the animals used reported?
   Where (section, paragraph #)?
   Yes, methods animals

8. Is the strain of the animals (including background strains of KO/transgenic animals used) reported?
   Where (section, paragraph #)?
   Yes, methods animals

9. Is the sex of the animals/subjects used reported?
   Where (section, paragraph #)?
   Yes, methods animals

10. Is the age of the animals/subjects reported?
    Where (section, paragraph #)?
    Yes, methods animals

11. For animals housed in a vivarium, is the light/dark cycle reported?
    Where (section, paragraph #)?
    Yes, pg 13

12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported?
    Where (section, paragraph #)?
    No, animals were housed 5 per cage until performance of surgery. Animals were then singly housed till the end of the experiment

13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)?
    Where (section, paragraph #)?
    Yes, methods animals
14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported?  
   Where (section, paragraph #)?
   This did not occur

   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 #)?
   Animals were excluded for the following reasons.
   1. missed viral injection
   2. missed cannula placement
   3. no/low viral expression
   This is listed under perfusion and tissue processing, of the methods

   a. How were the criteria for exclusion defined?  
      Where is this described (section, paragraph #)?
      See above

   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 #)?
      For spine morphology analysis we processed tissue from only a subset of the total animals run through social defeat behavior. This was due to the labor intensive nature of this analysis and our previous results which suggested that we only need between 4-7 animals for appropriate power (Christoffel et al., J Neurosci, 2011; Golden et al., Nat Med., 2013).

### 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 under immunohistochemistry in methods

   b. Where were the validation data reported (citation, supplementary information, Antibodypedia)?  
      Where does this appear (section, paragraph #)?
      Antibodies used for protein quantification were mentioned on page the immunohistochemistry section in the methods. These were chosen based on previous published work although references were not give in the methods.

2. If cell lines were used to reflect the properties of a particular tissue or disease state, is their source identified?  
   Where (section, paragraph #)?
   NA

   a. Were they recently authenticated?  
      Where is this information reported (section, paragraph #)?
      NA
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.

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

1. Are accession codes for deposit dates provided?
   Where (section, paragraph #)?
   NA

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.
   NA

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.
   NA

Human subjects

1. Which IRB approved the protocol?
   Where is this stated (section, paragraph #)?
   NA

2. Is demographic information on all subjects provided?
   Where (section, paragraph #)?
   NA

3. Is the number of human subjects, their age and sex clearly defined?
   Where (section, paragraph #)?
   NA

4. Are the inclusion and exclusion criteria (if any) clearly specified?
   Where (section, paragraph #)?
   NA
5. How well were the groups matched?
   Where is this information described (section, paragraph #)?
   NA

6. Is a statement included confirming that informed consent was obtained from all subjects?
   Where (section, paragraph #)?
   NA

7. For publication of patient photos, is a statement included confirming that consent to publish was obtained?
   Where (section, paragraph #)?
   NA

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?
   NA

   a. If yes, is the number rejected and reasons for rejection described?
      Where (section, paragraph #)?
      NA

2. Is the number of blocks, trials or experimental units per session and/or subjects specified?
   Where (section, paragraph #)?
   NA

3. Is the length of each trial and interval between trials specified?
   NA

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.
   NA

5. Is the task design clearly described?
   Where (section, paragraph #)?
   NA

6. How was behavioral performance measured?
   NA

7. Is an ANOVA or factorial design being used?
   NA

8. For data acquisition, is a whole brain scan used?
   If not, state area of acquisition.

   a. How was this region determined?
      NA
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