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.
- 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 page 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.

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<td>EXACT VALUE</td>
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<td>REPORTED?</td>
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<td>&gt; 400 neurons/ mouse from 3 mice</td>
<td>&gt; 400 neurons/ mouse from 3 mice</td>
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### Representative figures

1. Are any representative images shown (including Western blots and immunohistochemistry/staining) in the paper?  
   If so, what figure(s)?
   
   Yes we have representative images showed from figure 1 to figure 7.

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, on what page(s) is this reported?
   
   The experiment for each representative image was successfully repeated for at least three times. We did not have a statement for each image because we have >50 images in the manuscript, but we made a statement at the method part on page 33.

### Statistics and general methods

1. Is there a justification of the sample size?  
   If so, how was it justified?  
   On what page(s)?
   
   No sample size calculation was performed.  
   For staining assays, we counted more than 300 neurons/mouse from 3 mice, according to previously published papers doing the same quantification (e.g. Guzowski et al., 1999, Nature Neuroscience, reference #37 in manuscript; Lin et al., 2011, Nature, reference #56 in manuscript).  
   For place preference assay, we used at least 13 mice each group; for place avoidance assay, 5 mice each group. These numbers are commonly seen in published literature using the same behavioral tests.

2. Are statistical tests justified as appropriate for every figure?  
   On what page(s)?
   
   For Figure 1d and 1e, we used Mann-Whitney U tests based on the experimental design (2 independent samples) and the data’s violation of t-test normality assumption.  
   For Figure 4f, 5c, 5d and 7e, paired t-tests was used according to studies doing the same quantification (Guzowski et al., 1999; Lin et al., 2011).

   a. If there is a section summarizing the statistical methods in the methods, is the statistical test for each experiment clearly defined?
      
      Yes. The "statistical analysis" section (pg. 33) in manuscript summarized the statistical methods used, which are clearly defined.

   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?
      
      For Figure 1d and Figure 1e, a nonparametric test, Whitney-Mann U test was used, which is equivalent to independent sample t tests but does not assume normality in data.  
      For Figure 4f, 5b, 5d and 7e, we used paired t-tests according to Guzowski et al., 1999 and Lin et al., 2011.
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?

For both Figure 1d and Figure 1e, since the nonparametric tests were used, there is no concern of homogeneity of variance. For other figures, Levene's test for homogeneity of variance suggested unequal variances among groups (all p<0.05).

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

Two-sided.

e. Are there adjustments for multiple comparisons?

Yes, the Benjamini-Hochberg procedure controlling the false discovery rate was performed to adjust p-values.

3. Are criteria for excluding data points reported?
   Was this criterion established prior to data collection?
   On what page(s) is this described?

No data points were excluded.

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.
   On what page(s) does this appear?

No randomization was used.

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.
   On what page(s)?

Single blinding was done in that the experimenters were not aware of the treatment group while counting cell numbers in slices. On page 30

6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included?
   On what page(s)?

Yes, on page 26

7. Is the species of the animals used reported?
   On what page(s)?

Yes, on page 26

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

Yes, on page 26

9. Is the sex of the animals/subjects used reported?
   On what page(s)?

Yes, on page 26

10. Is the age of the animals/subjects reported?
    On what page(s)?

Yes, on page 26

11. For animals housed in a vivarium, is the light/dark cycle reported?
    On what page(s)?

Yes, on page 26
12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported?
   Yes, on page26

13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)?
   Yes, on page26

14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported?
   Yes, on page26

   a. If multiple behavioral tests were conducted in the same group of animals, is this reported?
      No multiple behavioral tests were conducted in the same group of animals.

15. If any animals/subjects were excluded from analysis, is this reported?
   No.

   a. How were the criteria for exclusion defined?
      Where is this described?

   b. Specify reasons for any discrepancy between the number of animals at the beginning and end of the study.
      Where is this described?

▶ Reagents

1. Have antibodies been validated for use in the system under study (assay and species)?
   Yes, we tested all the antibodies in control and experimental group mice.

   a. Is antibody catalog number given?
      Yes, on page 29-30.

   b. Where were the validation data reported (citation, supplementary information, Antibodypedia)?
      Not reported.

2. If cell lines were used to reflect the properties of a particular tissue or disease state, is their source identified?
   No cell lines were used
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.

1. Are accession codes for deposit dates provided?

   On what page(s)?

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.

2. Is computer source code/software provided with the paper or deposited in a public repository? Indicate in what form this is provided or how it can be obtained.

Human subjects

1. Which IRB approved the protocol?

   Where is this stated?

2. Is demographic information on all subjects provided?

   On what page(s)?

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

   On what page(s)?

4. Are the inclusion and exclusion criteria (if any) clearly specified?

   On what page(s)?
5. How well were the groups matched?
   Where is this information described?

6. Is a statement confirming that informed consent was obtained from all subjects included?
   On what page(s)?

7. For publication of patient photos, is a statement confirming that consent to publish was obtained included?
   On what page(s)?

▶ 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?
      On what page(s)?

2. Is the number of blocks, trials or experimental units per session and/or subjects specified?
   On what page(s)?

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?

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? On what page(s)?

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? On what page(s)?

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