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>
<th>TEST USED</th>
<th>n</th>
<th>DESCRIPTIVE STATS (AVG, VARIANCE)</th>
<th>P VALUE</th>
<th>DEGREES OF FREEDOM &amp; F/T/Z/R/etc VALUE</th>
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</thead>
<tbody>
<tr>
<td>FIGURE NUMBER</td>
<td>WHICH TEST?</td>
<td>SECTION &amp; PARAGRAPH #</td>
<td>EXACT VALUE</td>
<td>DEFINED?</td>
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<tr>
<td>1a</td>
<td>one-way ANOVA</td>
<td>Fig. legend</td>
<td>9, 9, 10, 15</td>
<td>mice from at least 3 litters/group</td>
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<td>results para 6</td>
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<td>Results para 6</td>
<td>15</td>
<td>slices from 10 mice</td>
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Nature Neuroscience: doi:10.1038/nn.4339
<table>
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<tr>
<th>Figure Number</th>
<th>WHICH TEST?</th>
<th>TEST USED</th>
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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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<tbody>
<tr>
<td>Suppl Fig 2 d Vm</td>
<td>Unpaired t-tests</td>
<td>Suppl Fig 2 d Vm</td>
<td>102,72 neurons from 78 mice</td>
<td>Suppl Fig 2 d Vm; mean +/- SD</td>
<td>p = 3.0173e-07</td>
<td>Suppl Fig 2 d Vm; t(172) = 5.3323</td>
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<tr>
<td>Suppl Fig 2 d Cm</td>
<td>Unpaired t-tests</td>
<td>Suppl Fig 2 d Cm</td>
<td>79,55 neurons from 60 mice</td>
<td>Suppl Fig 2 d Cm; mean +/- SD</td>
<td>p = 1.2359e-26</td>
<td>Suppl Fig 2 d Cm; t(132) = 13.5010</td>
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<td>Unpaired t-tests</td>
<td>Suppl Fig 2 d Rm</td>
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<td>Suppl Fig 2 d Rm; mean +/- SD</td>
<td>p = 0.1312</td>
<td>Not reported; t(132) = 1.5189</td>
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<tr>
<td>Tabl e 1 L4 vs L23</td>
<td>z-score</td>
<td>Table 1 L4 vs L23</td>
<td>39/82, 14/58 Connected/Tested from 93 mice</td>
<td>Table 1 L4 vs L23; Percentage</td>
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<td>Table 1 L4 vs L23; z = 2.8147</td>
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<td>Tabl e 1 L4 to L23</td>
<td>z-score</td>
<td>Table 1 L4 to L23</td>
<td>39/82, 16/110 Connected/Tested from 117 mice</td>
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<td>Table 1 L4 to L23; z = 5.0053</td>
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<td>Tabl e 1 L23 vs L4 to L23</td>
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<td>results, para 3; mean +/- SD</td>
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<td>results, para 7</td>
<td>1727 Locations from 29 pairs from 26 mice</td>
<td>4b, Fig. legend association between variables</td>
<td>p = 0</td>
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<td>Cochran–Armitage test</td>
<td>results, para 7</td>
<td>2312 Locations from 40 pairs from 34 mice</td>
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<td>Cochran–Armitage test</td>
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<td>Wilcoxon rank sum test</td>
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<td>17,12 pairs from 26 mice</td>
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<td>Wilcoxon rank sum test</td>
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<td>18,21 pairs from 34 mice</td>
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<td>Suppl Fig 7a</td>
<td>18,21 pairs from 34 mice</td>
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<td>Suppl Fig 7b</td>
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<td>Suppl Fig 7b</td>
<td>17,12 pairs from 26 mice</td>
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<td>Suppl Fig 7b; z = -0.4207 ranksum:170</td>
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<tr>
<th>Suppl Fig 7c (left)</th>
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<td>Suppl Fig 7c (right)</td>
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<td>all data points and mean</td>
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<td>all data points and mean</td>
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<td>Suppl Fig 7h</td>
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<td>all data points and mean</td>
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<td>Suppl Fig 7i (inset)</td>
<td>Wilcoxon rank sum test</td>
<td>Suppl Fig 7i (inset)</td>
<td>11,8 pairs from 26 mice</td>
<td>all data points and mean</td>
<td>Suppl Fig 7i (inset)</td>
<td>p=0.0187</td>
<td>Suppl Fig 7i (inset)</td>
<td>ranksum=82.5</td>
<td>not reported</td>
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</tbody>
</table>

| Suppl Fig 7j | Linear regression model fit | Suppl Fig 7j | 39 pairs from 34 mice | all data points and linear fit | Suppl Fig 7j | p=0.571 | Suppl Fig 7j | Error degrees of freedom: 37 Root Mean Squared Error: 0.119 R-squared: 0.00874, Adjusted R-Squared: -0.018 F-statistic vs. constant model: 0.326 |
| Suppl Fig 7k | Linear regression model fit | Suppl Fig 7k | 29 pairs from 26 mice | all data points and linear fit | Suppl Fig 7k | p=0.909 | Suppl Fig 7k | Error degrees of freedom: 27 Root Mean Squared Error: 0.0619 R-squared: 0.000497, Adjusted R-Squared: -0.0365 F-statistic vs. constant model: 0.0134 |
### 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

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 #)?

   The quantification on the right panel of Fig 1a is the mean of 3 animals (reported in the figure caption)
### 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.
   - **We did not do statistical tests to predetermine sample size. Our sample size is similar to other reported studies. This is stated in the methods section, Statistical analyses.**

2. Are statistical tests justified as appropriate for every figure?
   - Where (section, paragraph #)?
     - **Statistical tests are stated in results and figure legends where appropriate.**
     - a. If there is a section summarizing the statistical methods in the methods, is the statistical test for each experiment clearly defined?
     - Statistical tests are defined in each section according to when they are used.
     - b. Do the data meet the assumptions of the specific statistical test you chose (e.g. normality for a parametric test)?
     - Most comparison were done using non-parametric tests. Data distribution was assumed to be normal for samples of big size but this was not formally tested. See Methods, Statistical analyses.
     - c. Is there any estimate of variance within each group of data?
     - For each data group all data points and mean are shown in figure panels. For data in the text we report standard deviations. See Methods, Statistical analyses.
     - d. Are tests specified as one- or two-sided?
     - All tests are two-sided
     - e. Are there adjustments for multiple comparisons?
     - We made no multiple comparisons

3. To promote transparency, *Nature Neuroscience* has stopped allowing bar graphs to report statistics in the papers it publishes. If you have bar graphs in your paper, please make sure to switch them to dot-plots (with central and dispersion statistics displayed) or to box-and-whisker plots to show data distributions.
   - No bar graphs were used

4. Are criteria for excluding data points reported?
   - Was this criterion established prior to data collection?
   - Where is this described (section, paragraph #)?
   - No data points were excluded. Full data is shown in Supplementary Fig. 5. See Methods, Data analysis, paragraph 4.

5. 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 #)?
   - No randomization was used. Pairs of neurons were classified according to their local connectivity. This is described in Methods, Data analysis, paragraph 1.
6. 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 #)?

   In general the investigator was blind to the local connectivity of the recorded pairs during the experiments. For some L4→L2/3 recordings connectivity analyses were done during the experiments, before photostimulation, in order to balance data groups and increase data collection from the relatively rare connected L4→L2/3 pairs. See Methods, Electrophysiology and photostimulation.

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

   Yes. Methods, paragraph 1 (Animals surgeries)

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

   Yes. Methods, paragraph 1 (Animals surgeries)

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

   Yes. Methods, paragraph 1 (Animals surgeries)

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

    Yes. Methods, paragraph 1 (Animals surgeries)

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

    Yes. Methods, paragraph 1 (Animals surgeries)

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

    Yes. Methods, paragraph 1 (Animals surgeries)

13. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported?
    Where (section, paragraph #)?

    Yes. Methods, paragraph 1 (Animals surgeries)

14. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)?
    Where (section, paragraph #)?

    N/A

15. 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 #)?

      N/A
16. If any animals/subjects were excluded from analysis, is this reported? N/A

   Where (section, paragraph #)?

   a. How were the criteria for exclusion defined? N/A

      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. N/A

      Where is this described (section, paragraph #)?

Reagents

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

   a. Is antibody catalog number given? N/A

      Where does this appear (section, paragraph #)?

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

      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? N/A

      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 availability

Provide a Data availability statement in the Methods section under "Data availability", which should include, where applicable:

- Accession codes for deposited data
- Other unique identifiers (such as DOIs and hyperlinks for any other datasets)
- At a minimum, a statement confirming that all relevant data are available from the authors
- Formal citations of datasets that are assigned DOIs
- A statement regarding data available in the manuscript as source data
- A statement regarding data available with restrictions

See our data availability and data citations policy page for more information.

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.

Where is the Data Availability statement provided (section, paragraph #)?

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. 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.

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 #)?

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**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