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>FIGURE NUMBER</th>
<th>WHICH TEST?</th>
<th>SECTION &amp; PARAGRAPH #</th>
<th>n</th>
<th>DESCRIPTIVE STATS (AVERAGE, VARIANCE)</th>
<th>P VALUE</th>
<th>DEGREES OF FREEDOM &amp; F/T/Z/R/ETC VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>one-way ANOVA</td>
<td>Fig. legend</td>
<td>9, 9, 10, 15 mice from at least 3 litters/group</td>
<td>Methods para 8</td>
<td>error bars are mean +/- SEM</td>
<td>Fig. legend</td>
</tr>
<tr>
<td>results para 6</td>
<td>unpaired t-test</td>
<td>Results para 6</td>
<td>15 slices from 10 mice</td>
<td>Results para 6</td>
<td>error bars are mean +/- SEM</td>
<td>Results para 6</td>
</tr>
<tr>
<td>FIGURE NUMBER</td>
<td>TEST USED</td>
<td>n</td>
<td>DESCRIPTIVE STATS (AVERAGE, VARIANCE)</td>
<td>P VALUE</td>
<td>DEGREES OF FREEDOM &amp; F/T/Z/R/ETC VALUE</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>-----------</td>
<td>---</td>
<td>--------------------------------------</td>
<td>---------</td>
<td>--------------------------------------</td>
<td></td>
</tr>
<tr>
<td>2c, left panel</td>
<td>two-tailed unpaired t test</td>
<td>3</td>
<td>mice (2 slices averaged per mouse)</td>
<td>Fig. legend mean +/- SEM</td>
<td>Fig. legend 0.54</td>
<td>t(4)=0.6666</td>
</tr>
<tr>
<td>2c, right panel</td>
<td>two-tailed unpaired t test</td>
<td>4</td>
<td>mice (2 slices averaged per mouse)</td>
<td>Fig. legend mean +/- SEM</td>
<td>Fig. legend 0.13</td>
<td>t(6)=1.748</td>
</tr>
<tr>
<td>2d, left panel</td>
<td>two-tailed unpaired t test</td>
<td>5</td>
<td>mice (2 slices averaged per mouse)</td>
<td>Fig. legend mean +/- SEM</td>
<td>Fig. legend 0.0027</td>
<td>t(8)=4.276</td>
</tr>
<tr>
<td>2d, right panel</td>
<td>two-tailed unpaired t test</td>
<td>4</td>
<td>mice (2 slices averaged per mouse)</td>
<td>Fig. legend mean +/- SEM</td>
<td>Fig. legend 0.031</td>
<td>t(6)=2.798</td>
</tr>
<tr>
<td>results, para 6</td>
<td>two tailed unpaired t test</td>
<td>3</td>
<td>mice (2 slices averaged per mouse)</td>
<td>Results para 6 mean +/- SEM</td>
<td>Results para 6 0.59</td>
<td>t(4)=0.5930</td>
</tr>
<tr>
<td>results, para 6</td>
<td>two tailed unpaired t test</td>
<td>4</td>
<td>mice (2 slices averaged per mouse)</td>
<td>Results para 6 mean +/- SEM</td>
<td>Results para 6 0.60</td>
<td>t(6)=0.5520</td>
</tr>
<tr>
<td>results, para 7</td>
<td>two tailed unpaired t test</td>
<td>4</td>
<td>mice (2 slices averaged per mouse)</td>
<td>Results para 7 mean +/- SEM</td>
<td>Results para 7 P&lt;0.0001</td>
<td>Results para 7 t(6)=10.01</td>
</tr>
<tr>
<td>results, para 7</td>
<td>two tailed unpaired t test</td>
<td>5</td>
<td>mice (2 slices averaged per mouse)</td>
<td>Results para 7 mean +/- SEM</td>
<td>Results para 7 P&lt;0.0001</td>
<td>Results para 7 t(8)=21.47</td>
</tr>
<tr>
<td>3b</td>
<td>two way ANOVA with Bonferroni post test</td>
<td>5</td>
<td>mice (1 or occasionally 2 slices averaged per mouse)</td>
<td>Fig. legend mean +/- SEM</td>
<td>Fig. legend P&lt;0.0001 for time, genotype and interaction</td>
<td>F(21,171)=14.47 (interaction)</td>
</tr>
<tr>
<td>4d</td>
<td>one way ANOVA</td>
<td>11, 6, 15</td>
<td>mice (1 or occasionally 2 slices averaged per mouse per condition)</td>
<td>Fig. legend mean +/- SEM</td>
<td>Fig. legend P&lt;0.0001</td>
<td>F(2,29)=23.01</td>
</tr>
<tr>
<td>5a</td>
<td>one way ANOVA</td>
<td>7, 8, 11, 12</td>
<td>mice (1 or occasionally 2 slices averaged per mouse per condition)</td>
<td>Fig. legend mean +/- SEM</td>
<td>Fig. legend 0.1039</td>
<td>F(3,34)=2.218</td>
</tr>
</tbody>
</table>
### Representative figures

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

   Yes. Representative images are shown in Fig. 1c-e, Fig. 2a-b, Fig. 3a, c, Fig. 4a, Fig. 6, Fig. 7a-e and Supplementary 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 #)?

Representative images represent experiments that have been repeated and quantified and that information is provided:

- Fig. 1c - Results, paragraph 2
- Fig. 1d - Results, paragraph 4
- Fig. 1e - Results, paragraph 5
- Fig. 2a - Results, paragraph 6 and Fig. Legend
- Fig. 2b - Results, paragraph 7 and Fig. Legend
- Fig. 3a, c - Fig. Legend
- Fig. 4a - Fig. Legend
- Fig. 6 - Results, paragraph 17
- Fig. 7a-d - Results, paragraph 18
- Fig. 7e - Results, paragraph 19 and Fig. Legend
- Suppl. Fig. 1a - Suppl. Fig. legend

\section*{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.

Sample size was chosen based on studies using related methods and is similar to what is generally employed in the field. This is described in the "Statistics" subheading of the Methods section.

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?

A summary of the statistical methods is presented in the "Statistics" subheading of the Methods. The statistical test used in each experiment is clearly defined in the corresponding figure legend.

- 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 #)?

Whenever possible, data was confirmed as normally distributed by the Kolmogorov-Smirnov test. In other cases (e.g. when sample size didn't allow it), data distribution was assumed to be normal but this was not formally tested. This is described in the "Statistics" subheading of the Methods.

- 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, all groups of data being compared are presented as mean +/- SEM. Equal variance of data groups being compared by two tailed t test was confirmed using F test or assumed without formal testing for ANOVA comparisons. This is described in the "Statistics" subheading of the Methods.

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

Yes, two-tailed t tests were always performed, as described in Fig. legends.
<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>e. Are there adjustments for multiple comparisons?</td>
<td>Adjustments for multiple comparisons were done automatically by the GraphPad Prism software used to perform the statistical analysis (Bonferroni or Newman-Keuls multiple comparison tests were performed).</td>
</tr>
<tr>
<td>3. Are criteria for excluding data points reported?</td>
<td>For the data reported in Fig. 4, extreme outliers and data not properly fitted with a monoexponential were excluded from analysis, as described in the Methods at the end of paragraph 18.</td>
</tr>
<tr>
<td>Was this criterion established prior to data collection?</td>
<td></td>
</tr>
<tr>
<td>Where is this described (section, paragraph #)?</td>
<td>N/A; We state that no randomization was used in the &quot;Statistics&quot; subheading of the Methods.</td>
</tr>
<tr>
<td>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 #)?</td>
<td>No blinding was done. This is stated in the &quot;Statistics&quot; subheading of the Methods.</td>
</tr>
<tr>
<td>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 #)?</td>
<td>A compliance statement is included in Methods, paragraph 6.</td>
</tr>
<tr>
<td>6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included? Where (section, paragraph #)?</td>
<td>The species used (mouse) is reported in Methods, paragraph 6 and several times throughout the manuscript (e.g. Results, paragraph 2).</td>
</tr>
<tr>
<td>7. Is the species of the animals used reported?</td>
<td>All mouse strains are reported in Methods, paragraph 6.</td>
</tr>
<tr>
<td>Where (section, paragraph #)?</td>
<td>The sex of the animals used is reported in Methods, paragraph 7.</td>
</tr>
<tr>
<td>8. Is the strain of the animals (including background strains of KO/transgenic animals used) reported? Where (section, paragraph #)?</td>
<td>The age of the mice used is reported in Methods, paragraph 7.</td>
</tr>
<tr>
<td>9. Is the sex of the animals/subjects used reported?</td>
<td>The light/dark cycle is reported in Methods, paragraph 6.</td>
</tr>
<tr>
<td>Where (section, paragraph #)?</td>
<td>Housing groups are reported in Methods, paragraph 6.</td>
</tr>
<tr>
<td>10. Is the age of the animals/subjects reported?</td>
<td></td>
</tr>
<tr>
<td>Where (section, paragraph #)?</td>
<td></td>
</tr>
<tr>
<td>11. For animals housed in a vivarium, is the light/dark cycle reported?</td>
<td></td>
</tr>
<tr>
<td>Where (section, paragraph #)?</td>
<td></td>
</tr>
<tr>
<td>12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported? Where (section, paragraph #)?</td>
<td></td>
</tr>
<tr>
<td>Q</td>
<td>A</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)? Where (section, paragraph #)?</td>
<td>N/A</td>
</tr>
<tr>
<td>14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported? Where (section, paragraph #)?</td>
<td>N/A</td>
</tr>
<tr>
<td>a. If multiple behavioral tests were conducted in the same group of animals, is this reported? Where (section, paragraph #)?</td>
<td>N/A</td>
</tr>
<tr>
<td>15. If any animals/subjects were excluded from analysis, is this reported? Where (section, paragraph #)?</td>
<td>N/A</td>
</tr>
<tr>
<td>a. How were the criteria for exclusion defined? Where is this described (section, paragraph #)?</td>
<td>N/A</td>
</tr>
<tr>
<td>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 #)?</td>
<td>N/A</td>
</tr>
</tbody>
</table>

### Reagents

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

   - Primary antibodies have been validated by the manufacturers and are widely used in the field. The mouse monoclonal anti-synaptophysin antibody (clone SY38) is no longer available from DAKO but can be purchased from Millipore, where validation information is available. Further validation of the rabbit anti-GFP and mouse anti-TH antibodies is provided by our study since immunoreactivity is confined to expected brain regions as determined by immunohistochemistry (Fig. 6a-d and Results, paragraph 16).

   - HEK cells, stably transfected with VMAT2, were used for the results reported in paragraph 1 of the Results section (see also Methods, paragraph 5). Although HEK cells are reported to be cross-contaminated (ICLA), a cell clone stably expressing VMAT2 was isolated and grown based on survival in MPP+ containing media (Adam et al., 2008). Therefore, cross-contamination is not an issue in this case.
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.

| N/A |

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 #)?

This information is included in Methods, paragraph 5.

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?

| 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.

An in-house written macro for ImageJ was used to correct for shifts in the z dimension for the KCl-induced destaining experiments in Fig. 3 a-b. An in-house written Matlab script for the analysis of FFN200 fluorescent puncta destaining was used in the experiments described in Fig. 4, Fig. 5 and in Figure 7 f-h.

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.

The following statement was included in the Methods: "An ImageJ macro to correct for shifts in the z dimension and the Matlab script for fluorescent puncta destaining analysis will be available from our laboratory’s website at www.sulzerlab.org."

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

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

3. Is the length of each trial and interval between trials specified?
   N/A

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

6. How was behavioral performance measured?
   N/A

7. Is an ANOVA or factorial design being used?
   N/A

8. For data acquisition, is a whole brain scan used?
   If not, state area of acquisition.
   N/A
   a. How was this region determined?
   N/A

9. Is the field strength (in Tesla) of the MRI system stated?
   N/A
   a. Is the pulse sequence type (gradient/spin echo, EPI/spiral) stated?
   N/A
   b. Are the field-of-view, matrix size, slice thickness, and TE/TR/flip angle clearly stated?
   N/A

10. Are the software and specific parameters (model/functions, smoothing kernel size if applicable, etc.) used for data processing and pre-processing clearly stated?
    N/A

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 #)?
    N/A

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 #)?
    N/A

13. How were anatomical locations determined, e.g., via an automated labeling algorithm (AAL), standardized coordinate database (Talairach daemon), probabilistic atlases, etc.?
    N/A

14. Were any additional regressors (behavioral covariates, motion etc) used?
    N/A

15. Is the contrast construction clearly defined?
    N/A

16. Is a mixed/random effects or fixed inference used?
    N/A
    a. If fixed effects inference used, is this justified?
    N/A

17. Were repeated measures used (multiple measurements per subject)?
    N/A
a. If so, are the method to account for within subject correlation and the assumptions made about variance clearly stated?

N/A

18. If the threshold used for inference and visualization in figures varies, is this clearly stated?

N/A

19. Are statistical inferences corrected for multiple comparisons?

a. If not, is this labeled as uncorrected?

N/A

20. Are the results based on an ROI (region of interest) analysis?

a. If so, is the rationale clearly described?

N/A

b. How were the ROI’s defined (functional vs anatomical localization)?

N/A

21. Is there correction for multiple comparisons within each voxel?

N/A

22. For cluster-wise significance, is the cluster-defining threshold and the corrected significance level defined?

N/A

Additional comments

Additional Comments

None.