# 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? | REPORTED? | SECTION & PARAGRAPH # | EXACT VALUE | SECTION & PARAGRAPH # | VALUE | SECTION & PARAGRAPH # |
| 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 |
| 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 |
| 1C | 1-way ANOVA | Fig 1 | 6,6,6,6 mice | Fig 1 | mean ± s.e.m | Fig 1 | p< 0.0001 | Fig 1 | F(3,20) = 12.4 | Fig 1 |
| FIGURE NUMBER | WHICH TEST? | SECTION & PARAGRAPH # | EXACT VALUE | DEFINED? | n | SECTION & PARAGRAPH # | REPORTED? | SECTION & PARAGRAPH # | EXACT VALUE | P VALUE | SECTION & PARAGRAPH # | DEGREES OF FREEDOM & F/T/Z/R/ETC VALUE |
|--------------|------------|------------------------|-------------|----------|---|------------------------|-----------|------------------------|-------------|---------|------------------------|----------------------------------|
| 1E           | 1-way ANOVA | Fig 1                  | 6,6,6,6     | mice     | Fig 1 | mean ± s.e.m           | Fig 1     | p = 0.0004             | F(3,20) = 9.4 | Fig 1   |
| 1G:D 2       | 1-way ANOVA | Fig 1                  | 9,9,8,8     | mice     | Fig 1 | mean ± s.e.m           | Fig 1     | p<0.0001               | F(3,30) = 16.2 | Fig 1   |
| 1G:D 3       | 1-way ANOVA | Fig 1                  | 9,9,8,8     | mice     | Fig 1 | mean ± s.e.m           | Fig 1     | p<0.0001               | F(3,30) = 19.2 | Fig 1   |
| 1F           | 2-way RM-ANOVA | Fig 1               | 9,9,8,8     | mice     | Fig 1 | mean ± s.e.m           | Fig 1     | p<0.0001               | F(3,30) = 2.8 | Fig 1   |
| 2D           | 1-way ANOVA | Fig 2                  | 6,6,6,6,6,6 | mice     | Fig 2 | mean ± s.e.m           | Fig 2     | p<0.0001               | F(6,36) = 11.0 | Fig 2   |
| 3B           | 2-way RM-ANOVA | Fig 3               | 9,9,9       | # of experiments | Fig 3 | mean ± s.e.m           | Fig 3     | p = 0.0155              | F(4,48) = 3.4 | Fig 3   |
| 3C           | 1-way ANOVA | Fig 3                  | 9,9,9       | # of experiments | Fig 3 | mean ± s.e.m           | Fig 3     | p = 0.0067              | F(2,24) = 6.2 | Fig 3   |
| S1A          | 2-way RM-ANOVA | Fig S1A              | 6,6         | mice     | Fig S1A | mean ± s.e.m       | Fig S1A   | p = 0.0056              | F(2,10) = 12.4 | Fig S1A |
| S1D          | 1-way ANOVA | Fig S1D                | 6,6,5,6     | mice     | Fig S1D | mean ± s.e.m           | Fig S1D   | p = 0.0029              | F(3,20) = 6.69 | Fig S1D |
| S1F          | 2-way RM-ANOVA | S1F                 | 12,12       | mice     | S1F   | mean ± s.e.m           | S1F       | p = 0.0015              | S1G [stim x time] (2,24) = 7.5 | S1F   |
| S1G          | 2-way RM-ANOVA | S1G                 | 6,6,6,6     | mice     | S1G   | mean ± s.e.m           | S1G       | p = 0.33                | S1G [stim x time] (3,30) = 1.2 | S1G   |
| S1K          | t-test (AMPA) | S1K                 | 6,12        | mice     | S1K   | mean ± s.e.m           | S1K       | p = 0.0003              | t(16) = 4.7 | S1K   |
| S1K          | t-test (NMDA) | S1K                 | 6,6         | mice     | S1K   | mean ± s.e.m           | S1K       | p = 0.0063              | t(10) = 3.4 | S1K   |
| S1K          | t-test (SP)  | S1K                  | 6,5         | mice     | ST1   | mean ± s.e.m           | ST1       | p = 0.84                | t(9) = 0.2  | S1K   |
| ST1          | 1-way RM-ANOVA (Veh + Aniso) | ST1        | 8          | mice     | ST1   | mean ± s.e.m           | ST1       | p<0.0001               | F(8,56) = 22.4 | ST1   |
| ST1          | 1-way RM-ANOVA (Veh + Veh)  | ST1        | 8          | mice     | ST1   | mean ± s.e.m           | ST1       | p<0.0001               | F(8,56) = 22.6 | ST1   |
| ST1          | 1-way RM-ANOVA (Cap + Aniso) | ST1       | 9          | mice     | ST1   | mean ± s.e.m           | ST1       | p<0.0001               | F(8,64) = 12.3 | ST1   |
| ST1          | 1-way RM-ANOVA (Cap + Veh)  | ST1       | 9          | mice     | ST1   | mean ± s.e.m           | ST1       | p<0.0001               | F(8,64) = 32.8 | ST1   |

### Representative figures

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

   If so, what figure(s)?

   Representative electrophysiology traces are shown in figure 3A.
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 averaged data from the complete data set with analysis of complete data are also shown in figures 3. This information is in the 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.

   No sample size calculation was performed. Samples sizes used reflect a balance between commonly used sample sizes in the field and a desire to reduce the use of animals in pain experiments. This information is reported in the Methods in the “Statistics” section.

2. Are statistical tests justified as appropriate for every figure?
   Where (section, paragraph #)?

   The statistical tests used are appropriate for the comparisons made.

   a. If there is a section summarizing the statistical methods in the methods, is the statistical test for each experiment clearly defined?

   This information is reported in the Methods in the “Statistics” section.

   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 #)?

   A D’Agostino-Pearson omnibus normality test was conducted on groups with n > 6, otherwise the Kolmogorov-Smirnov normality test was used. This information is reported in the Methods in the “Statistics” 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 #)?

   No estimate of variance was conducted.

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

   All tests were two-sided. This information is reported in the Methods in the "Statistics" section.

   e. Are there adjustments for multiple comparisons?

   Yes. Multiple comparisons within groups were done via post-hoc tests. This information is reported in the Methods in the "Statistics" section.

3. Are criteria for excluding data points reported?
   Was this criterion established prior to data collection?
   Where is this described (section, paragraph #)?

   The exclusion criterion for exclusion was established prior to experiments. In behavioural experiments, mice were excluded if they did not exhibit a reduction in withdrawal threshold greater than 10% after sensitization. One mouse was excluded from the study (Substance P + Anisomycin group) based on this criterion.

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 randomly assigned to experimental groups in behavioral experiments using a counter-balanced approach. A statement of randomization is included in the Methods, paragraph 1.
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 #)?

5. The experimenter was blinded to experimental groups during testing in behavioral experiments. A statement of blinding is provided in the Methods on page 5.

6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included?

Where (section, paragraph #)?

6. All behavioral experiments were conducted in accordance with the guidelines established by the Canadian Council for Animal Care as indicated in the Methods on page 5, paragraph 1.

7. Is the species of the animals used reported?

Where (section, paragraph #)?

7. Mice were used in all experiments as indicated in the Methods, paragraph 1.

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

Where (section, paragraph #)?

8. Male C57BL/6 mice were used in most experiments. Male Nav1.8-ChR2 mice were used in one experiment and the background is indicated in the Methods, paragraph 1.

9. Is the sex of the animals/subjects used reported?

Where (section, paragraph #)?

9. Male mice were used and the sex is reported in the Methods, paragraph 1.

10. Is the age of the animals/subjects reported?

Where (section, paragraph #)?

10. Yes, the age of the animals is reported in the Methods, paragraph 1.

11. For animals housed in a vivarium, is the light/dark cycle reported?

Where (section, paragraph #)?

11. Animals were kept on a 12:12-hour light/dark cycle, with food and water provided ad libitum. This is reported in the Methods, paragraph 1.

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

Where (section, paragraph #)?

12. Animals were maintained in groups of 1 to 4 mice per cage. This is reported in the Methods, paragraph 1.

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

Where (section, paragraph #)?

13. Behavioural experiments were started prior to 10:00. his is reported in the Methods, paragraph 1.

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

Where (section, paragraph #)?

14. Animals were not re-used in behavioral experiments and naive mice were used in electrophysiological experiments. This is reported in the Methods, paragraph 1.

a. If multiple behavioral tests were conducted in the same group of animals, is this reported?

Where (section, paragraph #)?

a. N/A

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

Where (section, paragraph #)?

15. As described in question 3, one mouse was excluded from the study (Substance P + Anisomycin group).
a. How were the criteria for exclusion defined?
   Where is this described (section, paragraph #)?

   The exclusion criterion for exclusion was established prior to experiments. In behavioural experiments, mice were excluded if they did not exhibit a reduction in withdrawal threshold greater than 10% after sensitization. This is reported in the Methods, paragraph 1.

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)?
   N/A

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

   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.

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.
   - N/A

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.
   - N/A

### 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 #)?

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?  
   - N/A
   - 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

N/A