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, and it is misleading not to state this clearly.

<table>
<thead>
<tr>
<th>FIGURE NUMBER</th>
<th>WHICH TEST?</th>
<th>PAGE</th>
<th>n DEFINED?</th>
<th>PAGE</th>
<th>REPORTED?</th>
<th>PAGE</th>
<th>EXACT VALUE</th>
<th>PAGE</th>
<th>VALUE</th>
<th>DEGREES OF FREEDOM</th>
<th>F/T/Z/R/ETC VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>one-way ANOVA</td>
<td>4</td>
<td>9, 9, 10, 15</td>
<td>mice from at least 3 litters/group</td>
<td>4</td>
<td>error bars are mean +/- SEM</td>
<td>4</td>
<td>p = 0.044</td>
<td>4</td>
<td>F(3, 36) = 2.97</td>
<td>4</td>
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<tr>
<td>1b cocaine cue</td>
<td>One-Way ANOVA</td>
<td>7</td>
<td>21</td>
<td>average of 5 slices per rat from 21 rats</td>
<td>7</td>
<td>error bars are mean +SEM</td>
<td>7</td>
<td>p &lt; .0001</td>
<td>7</td>
<td>F(3,17) = 17.80</td>
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<tr>
<td>1b nicotine</td>
<td>One-Way ANOVA</td>
<td>7</td>
<td>16</td>
<td>average of 5 slices per rat from 16 rats</td>
<td>7</td>
<td>error bars are mean +SEM</td>
<td>7</td>
<td>p = .0001</td>
<td>7</td>
<td>F(2,13) = 19.70</td>
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<tr>
<td>1b heroin</td>
<td>One-Way ANOVA</td>
<td>7</td>
<td>14</td>
<td>average of 5 slices per rat from 14 rats</td>
<td>7</td>
<td>error bars are mean +SEM</td>
<td>7</td>
<td>p &lt; .0001</td>
<td>7</td>
<td>t(3) = 3.722</td>
<td>7</td>
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<tr>
<td>FIGURE NUMBER</td>
<td>WHICH TEST?</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>
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<tr>
<td>1b</td>
<td>One-Way ANOVA</td>
<td>7</td>
<td>average of 5 slices per rat from 18 rats</td>
<td>7 p &lt; .0001</td>
<td>7 F(3, 14) = 23.42</td>
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<tr>
<td>2a, MMP2i</td>
<td>paired t-test vehicle vs MMP-2i</td>
<td>7</td>
<td>average of 5 opposite hemispheres were analyzed per animal from 4 animals</td>
<td>7 p = 0.034</td>
<td>7 t(3) = 3.72</td>
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<tr>
<td>2a, MMP9i</td>
<td>paired t-test vehicle vs MMP-9i</td>
<td>7</td>
<td>average of 5 opposite hemispheres were analyzed per animal from 4 animals</td>
<td>7 p = 0.040</td>
<td>7 t(3) = 3.47</td>
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<tr>
<td>2a, MMP2i</td>
<td>paired t-test vehicle vs MMP-2i</td>
<td>7</td>
<td>average of 5 opposite hemispheres were analyzed per animal from 4 animals</td>
<td>16 p = .037</td>
<td>16 t(3) = 3.77</td>
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<tr>
<td>1h</td>
<td>One-Way ANOVA</td>
<td>7</td>
<td>samples from 25 animals</td>
<td>7 p = .0412</td>
<td>7 F(2,20) = 3.756</td>
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<tr>
<td>1h</td>
<td>One-Way ANOVA</td>
<td>7</td>
<td>samples from 22 animals</td>
<td>7 p = .0009</td>
<td>7 F(2,19) = 10.35</td>
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<tr>
<td>1h</td>
<td>One-Way ANOVA</td>
<td>7</td>
<td>samples from 22 animals</td>
<td>7 p = .0287</td>
<td>7 F(2,19) = 4.306</td>
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<tr>
<td>2b</td>
<td>One-Way ANOVA</td>
<td>7</td>
<td>cells recorded</td>
<td>7 p &lt; .0001</td>
<td>7 F(6,57) = 13.08</td>
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<tr>
<td>2c</td>
<td>One-Way ANOVA</td>
<td>8</td>
<td>rats</td>
<td>8 p &lt; .0001</td>
<td>8 F(8,27) = 11.68</td>
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<tr>
<td>2d</td>
<td>One-Way ANOVA</td>
<td>8</td>
<td>rats</td>
<td>8 p &lt; .0001</td>
<td>8 F(8,27) = 13.47</td>
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<td>2e</td>
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<td>8</td>
<td>rats</td>
<td>8 p &lt; .0001</td>
<td>8 F(7,172) = 8.02</td>
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<td>2f</td>
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<td>8</td>
<td>rats</td>
<td>8 p &lt; .0001</td>
<td>8 F(4,57) = 11.28</td>
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<tr>
<td>2g</td>
<td>Kruskal-Wallis</td>
<td>8</td>
<td>rats</td>
<td>8 p = .148</td>
<td>8 H(4,30) = 10.61</td>
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<td>s3</td>
<td>Unpaired t-test (dorsal striatum)</td>
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<td>average of 5 slices per rat from 8 rats</td>
<td>s3 p = .2312</td>
<td>s3 t(4) = 1.411</td>
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<td>s3</td>
<td>Unpaired t-test (NAshell)</td>
<td>s3</td>
<td>average of 5 slices from 8 rats</td>
<td>Fig S4 p = .8431</td>
<td>s3 t(4) = 0.2112</td>
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<td>s4</td>
<td>Students’ t-test</td>
<td>s4</td>
<td>rats</td>
<td>s4 p = .5415</td>
<td>s4 t(10) = 0.6321</td>
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<tr>
<td>s4</td>
<td>student’s t-test</td>
<td>s4</td>
<td>rats</td>
<td>s4 p = .3723</td>
<td>s4 t(10) = 0.934</td>
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<tr>
<td>s4</td>
<td>student’s t-test</td>
<td>s4</td>
<td>rats</td>
<td>s4 p = .4346</td>
<td>s4 t(10) = 0.814</td>
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<td>s7a</td>
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<td>s8</td>
<td>cells recorded</td>
<td>s8 p = .0552</td>
<td>s8 F(2,19) = 3.410</td>
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<tr>
<td>s7b</td>
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<td>s8</td>
<td>rats</td>
<td>s8 p = .402</td>
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<td>s7c</td>
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<td>s8</td>
<td>rats</td>
<td>s8 p = .8854</td>
<td>s8 F(2,9) = 0.1234</td>
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<tr>
<td>s5c</td>
<td>One-Way ANOVA</td>
<td>s5</td>
<td>cells recorded</td>
<td>s5 p = .2402</td>
<td>s5 F(8,67) = 1.338</td>
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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, Figure 1c-g and Supplemental figures S4, S5a, S6a-c

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?
   - Yes, the N is indicated in each data figure corresponding to the representative data. Western blots were each repeated at least 2x. PCR was run once. This is stated in supplemental methods pg 4.

### Statistics and general methods

1. Is there a justification of the sample size?  
   If so, how was it justified?  
   On what page(s)?
   - Yes, on supplemental methods page 8. Sample size was determined by power analysis by G*Power and from analysis of power in similar experiments previously conducted in the lab.

2. Are statistical tests justified as appropriate for every figure?  
   On what page(s)?
   - Yes, online methods page 7.

   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?
   - Yes, appropriate tests were used depending on assumptions about the data. This is described in the online methods, page 7.

   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?
   - The estimate of variance within each group is represented by error bars on each graph (error bars are + SEM or +/- SEM

   d. Are tests specified as one- or two-sided?
   - Yes.
e. Are there adjustments for multiple comparisons?

Yes.

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

Yes they are reported, and yes the criterion was established prior to data collection. Described on pages 3-6 of online methods (each technique has description of exclusion parameters in its subsection).

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?

For experiments containing yoked-saline animals, rats were randomly assigned to the cocaine or saline conditions, stated on supplemental methods pg 2.

For experiments which compared extinguished to reinstated animals, groups were counterbalanced by self-administration responding to control for amount of drug intake. This is stated in supplemental methods pg 2.

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, is a statement to this effect included?
   On what page(s)?

Yes
Supplemental Methods pg 3 for zymography
Supplemental Methods pg 6 for spine morphology
Supplemental Methods pg 8 for electrophysiology

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

Yes. Supplemental Methods Page 1.

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

Yes. Supplemental Methods page 1.

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

Yes. Supplemental Methods page 1.

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

Yes. Supplemental Methods page 1.

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

Yes. Supplemental Methods page 1

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

Yes. Supplemental Methods page 1.

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

Yes. Supplemental Methods page 1.
13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)?
   On what page(s)?
   Yes. Supplemental Methods page 1.

14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported?
   On what page(s)?
   All animals were trained to self-administer drug or saline and underwent surgery, reported on page 1 of Supplemental Methods.
   a. If multiple behavioral tests were conducted in the same group of animals, is this reported?
      On what page(s)?
      Yes. For behavioral studies, rats were given more than one microinjection using a randomized repeated measures design. Described on page 8 in figure legend 2, and in supplemental methods page 2.

15. If any animals/subjects were excluded from analysis, is this reported?
   On what page(s)?
   The behavioral exclusion criteria are described on supplemental methods, page 2, and include pressing >10 infusions of drug for 3 days, and extinguishing to <25 active lever presses.
   a. How were the criteria for exclusion defined?
      Where is this described?
      Unable to visualize the site of microinjection in the same frame as the anterior commissure, while using a 10x magnification objective, described on supplemental methods page 3 and figure legend 1.
   b. Specify reasons for any discrepancy between the number of animals at the beginning and end of the study.
      Where is this described?
      Animals which were excluded because they did not acquire self-administration, or for problems with microinjection cannulae were not reported. Supplemental methods page 2, supplemental figure legend S8, pg 9.

Reagents

1. Have antibodies been validated for use in the system under study (assay and species)?
   Yes.
   a. Is antibody catalog number given?
      On what page(s) does this appear?
      Yes, on pg 4 supplemental methods.
   b. Where were the validation data reported (citation, supplementary information, Antibodypedia)?
      On what page(s) does this appear?
      Validation data is on manufacturer product pages. This was not referenced in the supplemental methods of this manuscript.

2. If cell lines were used to reflect the properties of a particular tissue or disease state, is their source identified?
   On what page(s)?
   a. Were they recently authenticated?
      On what page(s) is this information reported?
### 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

1. Is there any custom algorithm/software that is integral to the study that has not been previously reported?

   If so, is this algorithm/software provided in a usable and readable form for the referees?

   Indicate in what form this is provided.

### 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 design used?
   If so, is length of blocks specified?

5. Is an event-related design being used?
   If so, how was the design optimized?

6. Is the task design clearly described?
   Where?

7. How was behavioral performance measured?

8. Are any planned comparisons being used?
   a. Are they clearly described?
   b. Is an ANOVA used?

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

10. Is the field strength (in Tesla) of the MRI system stated?
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<tr>
<td>a.</td>
<td>Is the pulse sequence type (gradient/spin echo, EPI/spiral) stated?</td>
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<td>11.</td>
<td>Is the software used for data processing and pre-processing clearly stated?</td>
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<td>12.</td>
<td>For any anatomical imaging, is the coordinate space defined?</td>
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<td>13.</td>
<td>How was the brain image template space, name, modality and resolution determined?</td>
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<td>14.</td>
<td>How were anatomical locations determined?</td>
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<td>15.</td>
<td>Is the statistical model and estimation method clearly described?</td>
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<td>16.</td>
<td>Were any additional regressors (behavioral covariates, motion etc) used?</td>
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<td>17.</td>
<td>Is the contrast construction clearly defined?</td>
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<td>18.</td>
<td>Is a mixed/random effects or fixed inference used?</td>
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<tr>
<td>a.</td>
<td>If fixed effects inference used, is this justified?</td>
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<td>19.</td>
<td>Were repeated measures used (multiple measurements per subject)?</td>
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<td>a.</td>
<td>If so, are the method to account for within subject correlation and the assumptions made about variance clearly stated?</td>
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<td>20.</td>
<td>If the threshold used for inference and visualization in figures varies, is this clearly stated?</td>
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<td>21.</td>
<td>Are statistical inferences corrected for multiple comparisons?</td>
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<td>a.</td>
<td>If not, is this labeled as uncorrected?</td>
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<td>22.</td>
<td>Are the results based on an ROI (region of interest) analysis?</td>
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<td>a.</td>
<td>If so, is the rationale clearly described?</td>
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<td>b.</td>
<td>How were the ROI’s defined (functional vs anatomical localization)?</td>
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<td>23.</td>
<td>Is there correction for multiple comparisons within each voxel?</td>
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<td>24.</td>
<td>For cluster-wise significance, is the cluster-defining threshold and the corrected significance level defined?</td>
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