# 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 (AVERAGE, VARIANCE)</th>
<th>P VALUE</th>
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<tr>
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<td>Fig legend</td>
<td>9, 9, 10, 15 mice from at least 3 litters/group</td>
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Nature Neuroscience: doi:10.1038/nn.4335
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<tr>
<th>FIGURE NUMBER</th>
<th>WHICH TEST?</th>
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<th>P VALUE</th>
<th>DEGREES OF FREEDOM &amp; F/1/2/R/ETC VALUE</th>
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<tr>
<td>5d</td>
<td>two tailed t-test</td>
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<td>Fig. legend 6 (p.17)</td>
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<td>Fig. legend 6 (p.17)</td>
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<td>NAcc-projecting TH+ neurons</td>
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<td>Fig. legend 7 (p.18)</td>
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<td><strong>8c</strong></td>
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<td>Fig. legend 8 (p. 18-19)</td>
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<td>n=6 rats, across n=3 mediolateral levels</td>
<td>Online Meth para #21, p.12</td>
<td>Mean +/- SEM</td>
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<tr>
<td>S 4b</td>
<td>Wilcoxon signed-rank test</td>
<td>Online Meth para #21, p.12</td>
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<td>n=6 rats, across n=4 rostrocaudal levels and n=3 mediolateral levels</td>
<td>Suppl. Fig. legend 4 (p.2)</td>
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<td>S8e &amp; Results para #4 (p.6)</td>
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<td>Suppl. Fig. legend 8 (p. 4) &amp; Results para #4 (p.6)</td>
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<td>Mean +/- SEM</td>
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<td>Suppl. Fig. legend 11 (p. 5)</td>
<td>PPN n=8 LDT n=8 WT n=8 Stim n=13</td>
<td>Number of animals per stimulation and trials</td>
<td>Suppl. Fig. legend 11 (p. 5)</td>
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<td>S11B</td>
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<td>Suppl. Fig. legend 11 (p. 5)</td>
<td>PPN n=8 LDT n=6 WT n=8</td>
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<td>Fig. legend 3 (p.15)</td>
<td>Magnitude of the responses</td>
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<td>Results para#5 (p. 7)</td>
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<td>TH+ neurons that showed bursting activity after laser stimulation</td>
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<td>Results Fig. legend 6 (p.17)</td>
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<td>Fig. legend 6 (p.17)</td>
<td>TH+ n=60 TH- n=36</td>
<td>Action potential duration for tracer-positive vs tracer-negative neurons</td>
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<tr>
<td>Results Fig. legend 6 (p.17)</td>
<td>Mann-Whitney Rank Sum Test</td>
<td>Fig. legend 6 (p.17)</td>
<td>TH+ n=60 TH- n=36</td>
<td>Basal firing rate for tracer-positive vs tracer-negative neurons</td>
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<td>3a &amp; 3b</td>
<td>Cluster based permutation test</td>
<td>Fig. legend 3 (p.15)</td>
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<td>Activation of DA neurons</td>
<td>Fig. legend 3 (p.15)</td>
<td>Magnitude of response across time</td>
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<td>6d</td>
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<td>Fig. legend 6 (p.17)</td>
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<td>basal firing rate of mesolimbic neurons</td>
<td>Fig. legend 6 (p.17)</td>
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<tr>
<td>6d</td>
<td>Mann-Whitney Rank Sum Test</td>
<td>Fig. legend 6 (p.17)</td>
<td>TH+ n=43 TH- n=30</td>
<td>duration of action potential</td>
<td>Fig. legend 6 (p.17)</td>
<td>-</td>
</tr>
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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)?
   1b, 1f, 2b, 2f, 2g, 4b, 6c, 6d, 8a, S1b, S2, S3, S6b, S7a, S7d, S8a, S8f, S9, S10a, S10b: immunohistochemistry
   1d, 1e, 2d, 2e: electron microscopy
   S1c: retrogradely-labeled cells distribution
   S4a: axon distribution and mapping

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 #)?
   1b, 2b, S3a: Results paragraph #1 (p. 3) & Figs. 1c and 2c
   1f, 1g, 2f, 2g: Results paragraph #2 (p. 5)
   4b: Results paragraph #4 (p. 7)
   6c, 6d: Results paragraph #6 (p. 8)
   8: online methods paragraph #12 (p.13) & Results paragraph# 7 (p.9)
   S1b, S1c: Quantified in figs. S1d and S1e
   S2: Suppl. table 2
   S3: online methods paragraph #23 (p.13)
   S7a, S7d: Results paragraph #9 (p. 6)
   S8: online methods paragraph #10 (p.6)
   S11: online methods paragraph #29 (p.18) & Results paragraph 8 (p.9)
   1d, 1e, 2d, 2e: Results paragraph #1 (p.3) & Online methods paragraph #18 (p.10)

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 decided on the basis of our previous experience in the field and was not pre-determined by a sample size calculation. 
   The sample size is justified by the high rate of exclusion due to the difficulty of the combined methodological approaches (site of injection, labeling of the neurons, location of the neurons, etc).

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?
   Sections "Analysis of connectivity" (p.12), "Electrophysiological data analysis" (p.14), "In vitro recordings" p.15, "Behavior data analysis" (p. 18) in Online Methods.
   Yes, data was tested for normality. If the data passed the normality test, parametric statistics were used. If failed, non-parametric test were chosen (Online Methods, p.15).

   b. Do the data meet the assumptions of the specific statistical test you chose (e.g. normality for a parametric test)?
   The statistical tests used in this study are of standard use and described in the appropriate sections (see below).

   c. Is there any estimate of variance within each group of data?
   The data is reported as mean and standard error of the mean. In most cases when we show group data, we also show individual cases to illustrate variability within groups (Figs. 1h, 1i, 2h, 2i, 4d, 4e, 5e, 6e, 6g, 7a, 7b, S7).
   Data for z-score (Fig. 3, 6F, 8b, 8c, S11a, S11b) are represented as mean and confidence interval.
d. Are tests specified as one- or two-sided?  
Yes, all tests were two-sided with only exceptions which have been specified.

e. Are there adjustments for multiple comparisons?  
Yes, for the 2-way ANOVA (Fig. 4).

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.

4. Are criteria for excluding data points reported?  
Neurons were excluded from the analysis if (1) their location was out of the VTA, (2) their location did not match the stereotaxic coordinates during the recording, (3) their neurochemical identity could not be verified or (4) the virus infection in the brainstem was not successful. Outliers for the linear regressions in Fig. 7 were detected by calculating the Studentized Deleted Residuals and removed from the analysis (Online Methods, p.13).

5. Define the method of randomization used to assign subjects (or samples) to the experimental groups and to collect and process data.  
No randomization was used for electrophysiology or anatomy. For behavior: operant boxes, testing order, drugs order were randomized using unbiased simple randomization.

6. Is a statement of the extent to which investigator knew the group allocation during the experiment and in assessing outcome included?  
No blinding for experimental groups, although neurons were sampled without distinction of their firing properties or stereotaxic coordinates within the VTA, and the neurochemical identity was revealed post hoc. No blinding was done for behavior experiments.

7. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included?  
Online methods, paragraph#1 (p.1): "All procedures were performed in accordance with the Society for Neuroscience policy on the use of animals in neuroscience and the Animals (Scientific Procedures) Act, 1986 (UK) and EU Directive 2010/63/EU, under the authority of Project License 30-2639 approved by the Home Office and the local ethical committee of the University of Oxford."

8. Is the species of the animals used reported?  
Online methods, paragraph#1 (p.1): "Male adult (250-450g) Long Evans (LE) wild-type and ChAT::Cre+ rats were used for all experiments."

9. Is the strain of the animals (including background strains of KO/transgenic animals used) reported?  
Online methods, paragraph#1 (p.1): "Male adult (250-450g) Long Evans (LE) wild-type and ChAT::Cre+ rats were used for all experiments."

10. Is the sex of the animals/subjects used reported?  
Online methods, paragraph#1 (p.1): "Male adult (250-450g) ..."
11. Is the age of the animals/subjects reported?
Where (section, paragraph #)?

Instead of age, weight was reported. Online methods, paragraph#1 (p.1): "Male adult (250-450g) ..."

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

Online methods, paragraph#1 (p.1): "Rats were maintained on a 12 h light cycle (lights on 07:00) and had ad libitum access to water and food."

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

For behavior experiments "house-caged in pairs" paragraph #13 (p.16).

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

Online methods, paragraph #28 (p.16) "All behavioral experiments were performed during the active (dark) phase"

15. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported?
Where (section, paragraph #)?

Online methods, paragraph #28 (p.16)

16. If any animals/subjects were excluded from analysis, is this reported?
Where (section, paragraph #)?

Online methods, paragraph#17 (p.9): "If tracers were off target, the recorded and juxtacellularly labeled neurons were still included in the analysis but the retrograde labeling was not taken into consideration. If the transduction was weak or overlapped between the two cholinergic nuclei, the neurons were excluded."

Reagents

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

No, but we used conventional (commercially-available) antibodies that have been used extensively in the system under study in a series of studies by ourselves and others.

a. Is antibody catalog number given?
Where does this appear (section, paragraph #)?

Online methods, paragraphs#12-17, "Immunohistochemistry and Image Processing" (p.7-9).
b. Where were the validation data reported (citation, supplementary information, Antibodypedia)? Where does this appear (section, paragraph #)?

No validation reported.

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 #)?

   N/A

   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 #)?

   N/A
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:

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.

We encourage publication of Data Descriptors (see Scientific Data) to maximize data reuse.

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

Methods:
"Data availability
The data that support the findings of this study and the custom Matlab code are available from corresponding author upon request."

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.

Custom code was implemented to calculate the average response of the neurons to the laser stimulation and to assess its significance.

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.

Custom made routines will be available upon request (see above under Data availability).

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
<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>5. Is the task design clearly described?</td>
<td>N/A</td>
</tr>
<tr>
<td>Where (section, paragraph #)?</td>
<td></td>
</tr>
<tr>
<td>6. How was behavioral performance measured?</td>
<td>N/A</td>
</tr>
<tr>
<td>7. Is an ANOVA or factorial design being used?</td>
<td>N/A</td>
</tr>
<tr>
<td>8. For data acquisition, is a whole brain scan used?</td>
<td>N/A</td>
</tr>
<tr>
<td>If not, state area of acquisition.</td>
<td></td>
</tr>
<tr>
<td>a. How was this region determined?</td>
<td>N/A</td>
</tr>
<tr>
<td>9. Is the field strength (in Tesla) of the MRI system stated?</td>
<td>N/A</td>
</tr>
<tr>
<td>a. Is the pulse sequence type (gradient/spin echo, EPI/spiral) stated?</td>
<td>N/A</td>
</tr>
<tr>
<td>b. Are the field-of-view, matrix size, slice thickness, and TE/TR/flip angle clearly stated?</td>
<td>N/A</td>
</tr>
<tr>
<td>10. Are the software and specific parameters (model/functions, smoothing kernel size if applicable, etc.) used for data processing and pre-processing clearly stated?</td>
<td>N/A</td>
</tr>
<tr>
<td>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 #)?</td>
<td>N/A</td>
</tr>
<tr>
<td>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 #)?</td>
<td>N/A</td>
</tr>
<tr>
<td>13. How were anatomical locations determined, e.g., via an automated labeling algorithm (AAL), standardized coordinate database (Talairach daemon), probabilistic atlases, etc.?</td>
<td>N/A</td>
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<tr>
<td>14. Were any additional regressors (behavioral covariates, motion etc) used?</td>
<td>N/A</td>
</tr>
<tr>
<td>15. Is the contrast construction clearly defined?</td>
<td>N/A</td>
</tr>
<tr>
<td>16. Is a mixed/random effects or fixed inference used?</td>
<td>N/A</td>
</tr>
<tr>
<td>a. If fixed effects inference used, is this justified?</td>
<td>N/A</td>
</tr>
<tr>
<td>17. Were repeated measures used (multiple measurements per subject)?</td>
<td>N/A</td>
</tr>
</tbody>
</table>
### Additional comments

Additional Comments

<table>
<thead>
<tr>
<th>Question</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. If so, are the method to account for within subject correlation and the assumptions made about variance clearly stated?</td>
<td>N/A</td>
</tr>
<tr>
<td>18. If the threshold used for inference and visualization in figures varies, is this clearly stated?</td>
<td>N/A</td>
</tr>
<tr>
<td>19. Are statistical inferences corrected for multiple comparisons?</td>
<td>N/A</td>
</tr>
<tr>
<td>a. If not, is this labeled as uncorrected?</td>
<td>N/A</td>
</tr>
<tr>
<td>20. Are the results based on an ROI (region of interest) analysis?</td>
<td>N/A</td>
</tr>
<tr>
<td>a. If so, is the rationale clearly described?</td>
<td>N/A</td>
</tr>
<tr>
<td>b. How were the ROI’s defined (functional vs anatomical localization)?</td>
<td>N/A</td>
</tr>
<tr>
<td>21. Is there correction for multiple comparisons within each voxel?</td>
<td>N/A</td>
</tr>
<tr>
<td>22. For cluster-wise significance, is the cluster-defining threshold and the corrected significance level defined?</td>
<td>N/A</td>
</tr>
</tbody>
</table>