# 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.

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<th>DESCRIPTIVE STATS (AVERAGE, VARIANCE)</th>
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<th>DEGREES OF FREEDOM &amp; F/T/Z/R/ETC VALUE</th>
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Nature Neuroscience: doi:10.1038/nn.4392
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**3e** One-way ANOVA Figure legend n=15 n=trials Within subject Mice from multiple litters Figure legend mean±SEM Figure legend p=0.0002 Figure legend F(14,28)=1.88 post-hoc: Appro. v Base, ****p=0.0026; Consum. v Base, ***p=0.001

**3g** One-way ANOVA Figure legend n=6 n=mice Within subject Mice from multiple litters Figure legend mean±SEM Figure legend p=0.0001 Figure legend F(5,15)=1.29 Fasted - Obj v chow, **p=0.004; Fed - Obj v Chow, *p=0.027; Fasted chow v Fed chow, *p=0.019

**3h** Paired t-test Figure legend n=7 n=mice Within subject Mice from multiple litters Figure legend mean±SEM Figure legend p=0.008 Figure legend t(6)=3.88

**3j** One-way ANOVA Figure legend n=6 n=mice Within subject Mice from multiple litters Figure legend mean±SEM Figure legend p=0.0003 Figure legend F(4,12)=24.12 post-hoc, Fasted – Obj v Chow, *p=0.015; Fed – Obj v Chow, *p=0.018

**3k** Paired t-test Figure legend n=6 n=mice Within subject Mice from multiple litters Figure legend mean±SEM Figure legend p=0.006 Figure legend t(5)=4.57

**4d** One-way ANOVA Figure legend n=6 n=mice Within subject Mice from multiple litters Figure legend mean±SEM Figure legend p=0.0001 Figure legend F(5,15)=38.08 post-hoc, Fasted – Obj v Chow, p=0.0005; Fed – Obj v Chow, p=0.136; Fasted chow v Fed chow, **p=0.012

**4e** One-way ANOVA Figure legend n=36 n=trials Within subject Mice from multiple litters Figure legend mean±SEM Figure legend p<0.0001 Figure legend F(35,70)=35.50; post-hoc: Appro. v Base, ****p=0.0003; Consum. v Base, ****p<0.0001; Appro. v. Consum., ***p=0.0002

**4h** Paired t-test Figure legend n=6 n=mice Within subject Mice from multiple litters Figure legend mean±SEM Figure legend p=0.015 Figure legend t(5)=3.6

**4i** Paired t-test Figure legend n=6 n=mice Within subject Mice from multiple litters Figure legend mean±SEM Figure legend p=0.026 Figure legend t(5)-3.13

**5a** N/A Figure legend n=21 n=AgRP neurons 4 mice Figure N/A N/A N/A N/A N/A N/A

**5b** N/A Figure legend n=12 n=POMC neurons 4 mice Figure N/A N/A N/A N/A N/A N/A
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**Figure legend**

- **Repeated measures Two-way ANOVA**
- **n=mice**
- **Within subject. Mice from multiple litters**
- **mean±SEM**
- **Treatment**
- **Time**
- **Interaction**
- **post-hoc:**
  - 1 h, p=0.062; 2 h, ***p=0.0006; 3 h, ****p=0.0001
  - 1 h, ***p=0.0002; 2 h, ****p=0.0001; 3 h, ****p=0.0001
  - 1 h, ***p=0.0000; Fed – Obj v Chow, ****p<0.0001; Fasted chow v Fed Chow, *p=0.028
  - F(43,86)=3.12
  - post-hoc: Appro. v Base, ****p<0.0001; Consum. v Base, ****p<0.0001; Appro. v. Consum., p=0.304
  - F(3,18)=13.43
  - post-hoc: Fasted – Obj v Chow, ****p<0.0001; Fed – Obj v Chow, p=0.98; Fasted chow v Fed Chow, *p=0.028
  - F(3,18)=18.03
  - post-hoc: Fasted – Obj v Choc, ***p=0.001; Fed – Obj v Choc, **p=0.001; Fasted choc v Fed choc, p=0.99
  - t(5)=3.56
  - t(5)=5.09
  - t(5)=5.9
| S1a | N/A | N/A | n=10 | n=AgRP neurons 4 mice | Figure and methods | N/A | N/A | N/A | N/A | N/A | N/A |
| S1b | N/A | N/A | n=13 | n=AgRP neurons 5 mice | Figure and methods | N/A | N/A | N/A | N/A | N/A | N/A |
| S1c | N/A | N/A | n=20 | n=POMC neurons 2 mice | Figure and methods | N/A | N/A | N/A | N/A | N/A | N/A |
| S1d | N/A | N/A | n=11 | n=non-AgRP neurons 4 mice | Figure and methods | N/A | N/A | N/A | N/A | N/A | N/A |
| S1e | N/A | N/A | n=13 | n=AgRP neurons 5 mice | Figure and methods | N/A | N/A | N/A | N/A | N/A | N/A |
| S1f | N/A | N/A | n=20 | n=POMC neurons 2 mice | Figure and methods | N/A | N/A | N/A | N/A | N/A | N/A |
| S1g | N/A | N/A | n=13 | n=POMC neurons 4 mice | Figure and methods | N/A | N/A | N/A | N/A | N/A | N/A |
| S1h | N/A | N/A | n=21 | n=POMC neurons 2 mice | Figure and methods | N/A | N/A | N/A | N/A | N/A | N/A |
| S1i | N/A | N/A | n=21 | n=POMC neurons 2 mouse | Figure and methods | N/A | N/A | N/A | N/A | N/A | N/A |
| S1j | N/A | N/A | n=15 | n=AgRP neurons 3 mice | Figure and methods | N/A | N/A | N/A | N/A | N/A | N/A |

**S4**

- S4a. Two-way ANOVA. Figure legend. n=4. n=mice. Within subject. Mice from multiple litters. Figure legend. mean±SEM. Figure legend. Treatment p=0.642. Time p=0.0001. Interaction p=0.075. Figure legend. Treatment F (1, 12) = 0.65. Time F (3, 12) = 84.95. Interaction F (3, 12) = 2.96. Figure legend.

- S4b. Repeated measures one-way ANOVA. Figure legend. n=6. n=mice. Within subject. Mice from multiple litters. Figure legend. mean±SEM. Figure legend. p=0.003. Figure legend. ANOVA, F(5,10)=15.52, p=0.011. post-hoc compared to ‘OFF’ : ON (Before), *p=0.02; ON (After), *p=0.03. Figure legend.

- S4c. Paired t-test. Figure legend. n=4. n=mice. Within subject. Mice from multiple litters. Figure legend. mean±SEM. Figure legend. p=0.44. Figure legend. t(3)=0.88. Figure legend.

- S4d. Paired t-test. Figure legend. n=6. n=mice. Within subject. Mice from multiple litters. Figure legend. mean±SEM. Figure legend. p=0.60. Figure legend. t(5)=0.59. Figure legend.

- S4e. Paired t-test. Figure legend. n=6. n=mice. Within subject. Mice from multiple litters. Figure legend. mean±SEM. Figure legend. p=0.97. Figure legend. t(5)=0.03. Figure legend.

- S4f. Paired t-test. Figure legend. n=6. n=mice. Within subject. Mice from multiple litters. Figure legend. mean±SEM. Figure legend. p=0.85. Figure legend. t(5)=0.19. Figure legend.

- S4g. Paired t-test. Figure legend. n=6. n=mice. Within subject. Mice from multiple litters. Figure legend. mean±SEM. Figure legend. p=0.74. Figure legend. t(5)=0.74. Figure legend.
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<td>Figure legend</td>
<td>p&lt;0.0001</td>
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Representative figures

1. Are any representative images shown (including Western blots and immunohistochemistry/staining) in the paper? If so, what figure(s)?
   - Figure 3a, h; Figure S1a-c; Figure S2; Figure S3; Figure S4a; Fig S5b

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 #)?
   - Yes
   - Relevant figure legend

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.
   - Power analyses were calculated to estimate sample size using statistical conventions for 80% power, assuming a standard deviation of change of 1.0, a difference between the means of 1.5-fold and alpha level of 0.05. For a two-tailed paired analysis a sample size of 6 was indicated.

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?
     - Yes
   - b. Do the data meet the assumptions of the specific statistical test you chose (e.g. normality for a parametric test)?
     - Yes
     - Data was normally distributed as determined by Shapiro-Wilk normality test.
   - 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.
     - Material and methods - Statistical analysis
   - 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?
   - Where is this described (section, paragraph #)?
   - Exclusion criteria for experimental animals were a) sickness or death during the testing period or b) if histological validation of the injection site demonstrated an absence of reporter gene expression. These criteria were established prior to data collection.
   - Material and methods - Statistical analysis
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 #)?

Due to the within subject design of most studies no randomization was employed.
Material and methods - Statistical analysis

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 #)?

Experimenters were not blinded to treatment (saline vs. CNO or Laser ON vs Laser OFF) but the requirement for post-hoc validation of injection accuracy and viral transgene expression ensures blinding with regard to genotype.
Material and methods - Feeding studies

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

Yes.
Material and methods - Animals

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

Yes.
Material and methods - Animals

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

Yes.
Material and methods - Animals

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

Yes.
Material and methods - Animals

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

Yes.
Material and methods - Animals

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

Yes.
Material and methods - Animals

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

Yes.
Material and methods - Food intake studies

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

Yes.
Throughout manuscript.

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

Yes.
Material and methods - Viral injections
Material and methods - Food intake studies
a. If multiple behavioral tests were conducted in the same group of animals, is this reported?
   Where (section, paragraph #)?
   Yes
   Material and methods - Feeding studies
   Material and methods - In vivo photometry

15. If any animals/subjects were excluded from analysis, is this reported?
   Where (section, paragraph #)?
   No. Exclusion of mice is detailed above (Box 3). n-numbers reported reflect final numbers after exclusion.
   a. How were the criteria for exclusion defined?
       Where is this described (section, paragraph #)?
       See Box 3
   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-numbers reported reflect final numbers after exclusion.

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 #)?
       Yes
       Material and methods - Immunohistochemistry
   b. Where were the validation data reported (citation, supplementary information, Antibodypedia)?
       Where does this appear (section, paragraph #)?
       All antibodies are validated and represented on the Journal of Comparative Neurology Antibody Database v14.2

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 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.

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

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. 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.
   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 #)?
   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?  
   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**