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>
<th>DEGREES OF FREEDOM &amp; F/T/Z/R/ETC VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIGURE NUMBER</td>
<td>WHICH TEST</td>
<td>SECTION &amp; PARAGRAPH #</td>
<td>EXACT VALUE</td>
<td>DEFINED?</td>
</tr>
<tr>
<td>1a</td>
<td>one-way ANOVA</td>
<td>Methods para 8</td>
<td>9, 9, 10, 15 mice from at least 3 litters/group</td>
<td></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></td>
</tr>
</tbody>
</table>
### Representative figures

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

   If so, what figure(s)?

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 #)?

---

Fig. 1a, inset panels (i) and (ii): point-spread function measurements; Fig. 3b inset panels: optical field response profiles.

Fig 1a, inset panels (i) and (ii): the optical point spread function is a physical measurement and it would be unusual to expect variability under normal experimental conditions. Fig. 3b inset panels: yes (Figure 3 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.  
   
   No calculation was performed to determine sample size a priori. Sample sizes were chosen to be similar to previous studies characterizing optical stimulation (tens of stimulated cells) and analyzing in vivo fluorescence recordings (hundreds).

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?  
   b. Do the data meet the assumptions of the specific statistical test you chose (e.g. normality for a parametric test)?  
   c. Is there any estimate of variance within each group of data?  
   Is the variance similar between groups that are being statistically compared?  
   d. Are tests specified as one- or two-sided?  
   e. Are there adjustments for multiple comparisons?  

   Statistical significance values determined by bootstrap resampling and validated by permutation testing (exchangeability under label randomization; see Methods p.34-35)

   Yes (see Methods p.34-35)

   Bootstrap resampling is a non-parametric test. Permutation testing was used to verify the assumption of exchangeability (see Methods p.34-35)

   No

   Two-sided

   No

   Yes (Methods section). Periods of 1) non-running in behavior experiments, 2) detector saturation during visible-light stimulation, and 3) significant transients (in resting fluorescence measurements) were excluded from analysis as described.

   All animal subjects in behavior experiments were part of a single group. For each subject, labels on trials (stimulus or control) within a behavior session were randomized during analysis of statistical significance (see Methods p.34-35)

   N/A

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

   Yes (Methods section). Periods of 1) non-running in behavior experiments, 2) detector saturation during visible-light stimulation, and 3) significant transients (in resting fluorescence measurements) were excluded from analysis as described.

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 #)?

   All animal subjects in behavior experiments were part of a single group. For each subject, labels on trials (stimulus or control) within a behavior session were randomized during analysis of statistical significance (see Methods p.34-35)

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 #)?

   N/A
6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included?  
   Where (section, paragraph #)?  
   Yes (reference: Methods section, subsection “Mouse surgical procedures and behavior training”)

7. Is the species of the animals used reported?  
   Where (section, paragraph #)?  
   Yes (reference: Methods section, subsection “Mouse surgical procedures and behavior training” and “Data Analysis”)

8. Is the strain of the animals (including background strains of KO/transgenic animals used) reported?  
   Where (section, paragraph #)?  
   Yes (reference: Methods section, subsections “Mouse surgical procedures and behavior training” and “Data Analysis”)

9. Is the sex of the animals/subjects used reported?  
   Where (section, paragraph #)?  
   Yes (reference: Methods section, subsections “Mouse surgical procedures and behavior training” and “Data Analysis”)

10. Is the age of the animals/subjects reported?  
    Where (section, paragraph #)?  
    Yes (reference: Methods section, subsections “Mouse surgical procedures and behavior training” and “Data Analysis”)

11. For animals housed in a vivarium, is the light/dark cycle reported?  
    Where (section, paragraph #)?  
    Yes (reference: Methods section, subsections “Mouse surgical procedures and behavior training” and “Data Analysis”)

12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported?  
    Where (section, paragraph #)?  
    Yes (reference: Methods section, subsections “Mouse surgical procedures and behavior training” and “Data Analysis”)

13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)?  
    Where (section, paragraph #)?  
    Yes (reference: Methods section, subsections “Mouse surgical procedures and behavior training” and “Data Analysis”)

14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported?  
    Where (section, paragraph #)?  
    The mice used in these experiments were not used in any other studies. The surgery and behavior training timeline is reported in Methods.

   a. If multiple behavioral tests were conducted in the same group of animals, is this reported?  
      Where (section, paragraph #)?  
      N/A

15. If any animals/subjects were excluded from analysis, is this reported?  
    Where (section, paragraph #)?  
    No animals were excluded.

   a. How were the criteria for exclusion defined?  
      Where is this described (section, paragraph #)?  
      N/A
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 #)? N/A
   b. Where were the validation data reported (citation, supplementary information, Antibodypedia)? Where does this appear (section, paragraph #)? N/A

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

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 scripts written in Matlab were used for data analysis, and custom software written in Labview was used for instrument control.
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.

No. Our software employed standard methods in the field for data analysis and instrument control.

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?  N/A
   Where (section, paragraph #)?

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?  N/A
   If not, state area of acquisition.
   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?  N/A
   a. If not, is this labeled as uncorrected?  N/A

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