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# Main Figures: 7

# Supplementary Figures: 14

# Supplementary Tables: 1

# Supplementary Videos: 9

# 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.

| | | TEST USED | | n | | | DESCRIPTIVE STATS (AVERAGE, VARIANCE) | | P VALUE | | DEGREES OF FREEDOM & F/t/z/R/ETC VALUE | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | FIGURE NUMBER | WHICH TEST? | PAGE | EXACT VALUE | DEFINED? | PAGE | REPORTED? | PAGE | EXACT VALUE | PAGE | VALUE | PAGE |
| example | 1a | one-way ANOVA | 4 | 9, 9, 10, 15 | mice from at least 3 litters/group | 4 | error bars are mean +/- SEM | 4 | p = 0.044 | 4 | F(3, 36) = 2.97 | 4 |
| example | results, pg 6 | unpaired t-test | 6 | 15 | slices from 10 mice | 6 | error bars are mean +/- SEM | 6 | p = 0.0006 | 6 | t(28) = 2.808 | 6 |
| + - | result, page 7 | unpaired t-test | 7 | 4 | thalamic structures | 6 | mean +/- SD | 6 | p = 0.35 | 6 | df = 6 | 6 |

## Representative figures

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

   If so, what figure(s)?

Figure 1b-c
Figure 5b
Figure 7a-b
Supplementary Figure 1a, b, c, f and g
Supplementary Figure 2b and c
Supplementary Figure 3a-b
Supplementary Figure 8a
Supplementary Figure 14a

2. For each representative image, is there a clear statement of how many time s this experiment was successfully repeated and a discussion of any limitations in repeatability?

   If so, on what page(s) is this reported?

Yes:
Figure 1, 2, 7, Supplementary Figure 2, Supplementary Figure 8 and Supplementary Figure 14: Page 5
Figure 6: Page 11and labeled in the figure
Supplementary Figure 1: page 21 and 38

## Statistics and general methods

1. Is there a justification of the sample size?

   If so, how was it justified?

   On what page(s)?

   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 justified post hoc based on the feedback of how well our injections cover the thalamus, shown in Supplementary Figure 3 and Supplementary Figure 7. We achieved 93.4% of thalamus coverage with 75 mice and 254 injections (Page 5 and 7).

2. Are statistical tests justified as appropriate for every figure?

   On what page(s)?

No. There is only one statistical test in our manuscript (page 7). It is for comparing the similarity between the aligned thalamus and published thalamic nuclei/structures. Because there are only 4 structures could be faithfully traced from the experimental brains, it is difficult to determine whether t test is appropriate. However, an independent non-parametric test (Wilcoxon rank sum test) gave nearly identical p value.

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

Yes, page 7

   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?

Because the limited n due to technical limitation mentioned above, it was not possible to determine whether the assumptions are met. However, an independent non-parametric test (Wilcoxon rank sum test) gave nearly identical p value.

   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?

Yes. This is given by the standard deviation described in page 6. The values are similar across the two groups.

| | |
|---|---|
| d. Are tests specified as one- or two-sided? | it is two sided. |
| e. Are there adjustments for multiple comparisons? | N/A |
| 3. Are criteria for excluding data points reported?<br>Was this criterion established prior to data collection?<br>On what page(s) is this described? | No data were excluded except for obvious reasons: injections went outside of the thalamus, the injection tract infected a significant number of cortical neurons on the injection track, or the tissue became damaged during processing steps. This is not currently in the text |
| 4. Define the method of randomization used to assign subjects (or samples) to the experimental groups and to collect and process data.<br>If no randomization was used, state so.<br>On what page(s) does this appear? | No randomization was used. |
| 5. Is a statement of the extent to which investigator knew the group allocation during the experiment and in assessing outcome included?<br>If no blinding was done, is a statement to this effect included?<br>On what page(s)? | The scoring of frontal projections were done without the knowledge of the injection location in the thalamus. This statement is included in revision. |
| 6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included?<br>On what page(s)? | Yes, Page 19 |
| 7. Is the species of the animals used reported?<br>On what page(s)? | Yes, Page 4 |
| 8. Is the strain of the animals (including background strains of KO/ transgenic animals used) reported?<br>On what page(s)? | Yes, Page 18 |
| 9. Is the sex of the animals/subjects used reported?<br>On what page(s)? | Yes, Page 19 |
| 10. Is the age of the animals/subjects reported?<br>On what page(s)? | Yes, Page 19, 28 and 29 |
| 11. For animals housed in a vivarium, is the light/dark cycle reported?<br>On what page(s)? | No |
| 12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported?<br>On what page(s)? | No |

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

    On what page(s)?

N/A

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

    On what page(s)?

No, there were no prior treatments/surgeries performed on any animals in these experiments.

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

       On what page(s)?

N/A

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

    On what page(s)?

None were excluded from analysis except for those with obvious experimental failures (see #3).

    a. How were the criteria for exclusion defined?

       Where is this described?

    b. Specify reasons for any discrepancy between the number of animals at the beginning and end of the study.

       Where is this described?

## Reagents

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

N/A

    a. Is antibody catalog number given?

       On what page(s) does this appear?

N/A

    b. Where were the validation data reported (citation, supplementary information, Antibodypedia)?

       On what page(s) does this appear?

N/A

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)?

N/A

    a. Were they recently authenticated?

       On what page(s) is this information reported?

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?

   On what page(s)?

N/A

## 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.

Yes. The adjustment, alignment and normalization, cluster analysis, vM1 fluorescence analysis are are all custom software written in MATLAB (MathWorks). All codes are available to referees upon request and all algorithms are described in details in Methods on Pages 21-28.

## Human subjects

1. Which IRB approved the protocol?

   Where is this stated?

N/A

2. Is demographic information on all subjects provided?

   On what page(s)?

N/A

3. Is the number of human subjects, their age and sex clearly defined?

   On what page(s)?

N/A

4. Are the inclusion and exclusion criteria (if any) clearly specified?

   On what page(s)?

N/A

5. How well were the groups matched?

   Where is this information described?

N/A

6. Is a statement confirming that informed consent was obtained from all subjects included?

   On what page(s)?

N/A

7. For publication of patient photos, is a statement confirming that consent to publish was obtained included?

   On what page(s)?

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?

   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?

a. Is the pulse sequence type (gradient/spin echo, EPI/spiral) stated?

11. Is the software used for data processing and pre-processing clearly stated?

12. For any anatomical imaging, is the coordinate space defined?

13. How was the brain image template space, name, modality and resolution determined?

14. How were anatomical locations determined?

15. Is the statistical model and estimation method clearly described?

16. Were any additional regressors (behavioral covariates, motion etc) used?

17. Is the contrast construction clearly defined?

18. Is a mixed/random effects or fixed inference used?

a. If fixed effects inference used, is this justified?

19. 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?

20. If the threshold used for inference and visualization in figures varies, is this clearly stated?

21. Are statistical inferences corrected for multiple comparisons?

a. If not, is this labeled as uncorrected?

22. 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)?

23. Is there correction for multiple comparisons within each voxel?

24. For cluster-wise significance, is the cluster-defining threshold and the corrected significance level defined?

## Additional comments

Additional Comments