nature neuroscience

| Corresponding Author: | István Katona | # Main Figures: | 6 |
|-----------------------|---------------|--------------------------|----|
| Manuscript Number: | NN-A48195A | # Supplementary Figures: | 10 |
| Manuscript Type: | Article | # Supplementary Tables: | 1 |
| | | # Supplementary Videos: | 0 |

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.

| | | TEST USED | | n | | | DESCRIPTIVE STATS (AVERAGE, VARIANCE) | | P VALUE | | DEGREES OF FREEDOM & F/t/z/R/ETC VALUE | |
|---------|--------------------|---|--------------------------|-----------------|---------------------------------------|--------------------------|--|--------------------------|-------------|--------------------------|--|--------------------------|
| | FIGURE NUMBER | WHICH TEST? | SECTION & PARAGRAPH # | EXACT VALUE | DEFINED? | SECTION & PARAGRAPH # | REPORTED? | SECTION & PARAGRAPH # | EXACT VALUE | SECTION & PARAGRAPH # | VALUE | SECTION & PARAGRAPH # |
| example | 1a | one-way ANOVA | Fig. legend | 9, 9, 10, 15 | mice from at least 3 litters/group | Methods para 8 | error bars are mean +/- SEM | Fig. legend | p = 0.044 | Fig. legend | F(3, 36) = 2.97 | Fig. legend |
| example | results, para 6 | unpaired t- test | Results para 6 | 15 | slices from 10 mice | Results para 6 | error bars are mean +/- SEM | Results para 6 | p = 0.0006 | Results para 6 | t(28) = 2.808 | Results para 6 |
| + - | S1d | Spearman's rank order correlation | Fig. legend | 46 | cells from 4 dishes | figure | scatterplot | figure | p<0.000001 | figure | R=0.92 | figure |

| ſ | | TEST USED | | D n | | DESCRIPTIVE S (AVERAGE, VARI | | P VALU | JE | DEGREES OF FREEDOM & F/t/z/R/ETC VALUE | | |
|--------|------------------|--|--------------------------|----------------|---|---------------------------------|------------|--------------------------|-------------|--|--------------------------|--------------------------|
| | FIGURE NUMBER | WHICH TEST? | SECTION & PARAGRAPH # | EXACT VALUE | DEFINED? | SECTION & PARAGRAPH # | REPORTED? | SECTION & PARAGRAPH # | EXACT VALUE | SECTION & PARAGRAPH # | VALUE | SECTION & PARAGRAPH # |
| + - | 1i | N/A | N/A | 208,200 | terminals from 3 and 2 mice | Fig. legend | histogram | figure | N/A | N/A | N/A | N/A |
| + - | 2c | N/A | N/A | 2168 | terminals from 1 cell | figure | histogram | figure | N/A | N/A | N/A | N/A |
| + - | 2i | N/A | N/A | 1934 | terminals from 1 cell | figure | histogram | figure | N/A | N/A | N/A | N/A |
| + - | s2 | N/A | N/A | 1341 | localization points from 160 groups | Fig. legend | histogram | figure | N/A | N/A | N/A | N/A |
| + - | S3a | unpaired two-sided t- test | Fig. legend | 14, 18 | neurons form 11 and 12 mice | Fig. legend | median±IQR | Fig. legend | p=0.1903 | Fig. legend | t(30)=-1.3401 | Fig. legend |
| + - | S3b | unpaired two-sided t- test | Fig. legend | 14,18 | neurons form 11 and 12 mice | Fig. legend | median±IQR | Fig. legend | p=0.1691 | Fig. legend | t(30)=-1.4089 | Fig. legend |
| + - | S3c | Mann- Whitney U Test | Fig. legend | 14,18 | neurons form 11 and 12 mice | Fig. legend | median±IQR | Fig. legend | p=0.2351 | Fig. legend | U=94.5 | Fig. legend |
| + - | S3d | Mann- Whitney U Test | Fig. legend | 14,17 | neurons form 11 and 12 mice | Fig. legend | median±IQR | Fig. legend | p=0.2455 | Fig. legend | U=89 | Fig. legend |
| + - | S3e | unpaired two-sided t- test | Fig. legend | 14,18 | neurons form 11 and 12 mice | Fig. legend | median±IQR | Fig. legend | p=0.1515 | Fig. legend | t(30)=-1.4717 | Fig. legend |
| + - | S3f | Mann- Whitney U Test | Fig. legend | 14,18 | neurons form 11 and 12 mice | Fig. legend | median±IQR | Fig. legend | p=0.0024 | Fig. legend | U=48.5 | Fig. legend |
| + - | S3g | unpaired two-sided t- test | Fig. legend | 14,18 | neurons form 11 and 12 mice | Fig. legend | median±IQR | Fig. legend | p=0.0373 | Fig. legend | t(30)=-2.1783 | Fig. legend |
| + - | S3h | unpaired two-sided t- test | Fig. legend | 14,18 | neurons form 11 and 12 mice | Fig. legend | median±IQR | Fig. legend | p=0.0133 | Fig. legend | t(30)=-2.6313 | Fig. legend |
| + - | S4a | Kolmogorov -Smirnov two-sample test | Fig. legend | 150,279 | neurons form 11 and 12 mice | Fig. legend | histogram | figure | p>0.05 | Fig. legend | Max. Difference=0.128 | not shown |
| + - | S4b | Kolmogorov -Smirnov two-sample test | Fig. legend | 279,302 | boutons from 6 and 6 neurons from 6 and 5 mice | Fig. legend | histogram | figure | p>0.1 | Fig. legend | Max. Difference=0.147 | not shown |
| + - | S4c | Mann- Whitney U Test | Fig. legend | 265,233 | boutons from 6 slices from 6 mice | Fig. legend | median±IQR | Fig. legend | p=0.3060 | Fig. legend | U=29232 | Fig. legend |
| + | S4d | Mann- Whitney U Test | Fig. legend | 202,202 | boutons from 6 sclices from 6 mice | Fig. legend | median±IQR | Fig. legend | p=0.1965 | Fig. legend | U=4563.5 | Fig. legend |
| + - | S5a | Mann- Whitney U Test | Fig. legend | 6,6 | mean values by cell from 279 and 334 boutons from 6 and 3 mice | Fig. legend | median±IQR | Fig. legend | p=0.82 | Fig. legend | U=16 | Fig. legend |
| + - | S5b | Mann- Whitney U Test | Fig. legend | 6,6 | mean values by cell from 279 and 334 boutons from 6 and 3 mice | Fig. legend | median±IQR | Fig. legend | p=0.70 | Fig. legend | U=15 | Fig. legend |

| + | S5c | Mann- Whitney U Test | Fig. legend | 6,6 | mean values by cell from 279 and 334 boutons from 6 and 3 mice | Fig. legend | median±lQR | Fig. legend | p=0.94 | Fig. legend | U=17 | Fig. legend |
|--------|-----|---|----------------|-----|---|----------------|-------------|----------------|------------|----------------|-------------|----------------|
| + | За | Mann- Whitney U Test | Fig. legend | 6,6 | mean values by cell from 279 and 334 boutons from 6 and 3 mice | Fig. legend | median±IQR | Fig. legend | p=0.0043 | Fig. legend | U=1 | Fig. legend |
| + | 3b | Mann- Whitney U Test | Fig. legend | 6,6 | mean values by cell from 279 and 334 boutons from 6 and 3 mice | Fig. legend | median±IQR | Fig. legend | p=0.0022 | Fig. legend | U=0 | Fig. legend |
| + | Зc | Mann- Whitney U Test | Fig. legend | 6,6 | mean values by cell from 279 and 334 boutons from 6 and 3 mice | Fig. legend | median±IQR | Fig. legend | p=0.0152 | Fig. legend | U=3 | Fig. legend |
| + | 3d | Spearman's rank order correlation | Fig. legend | 279 | boutons from 6 perisomatic cells from 6 mice | Fig. legend | scatterplot | figure | p<0.001 | Fig. legend | R=0.73 | figure |
| + - | 3d | Spearman's rank order correlation | Fig. legend | 334 | boutons from 6 dendritic cells from 3 mice | Fig. legend | scatterplot | figure | p<0.001 | Fig. legend | R=0.61 | figure |
| + - | 3e | Mann- Whitney U Test | Fig. legend | 6,6 | mean values by cell from 279 and 334 boutons from 6 and 3 mice | Fig. legend | median±IQR | Fig. legend | p=0.6991 | Fig. legend | U=15 | Fig. legend |
| + | 3f | Mann- Whitney U Test | Fig. legend | 6,6 | mean values by cell from 279 and 334 boutons from 6 and 3 mice | Fig. legend | median±IQR | Fig. legend | p=0.3095 | Fig. legend | U=11 | Fig. legend |
| + | 3m | Mann- Whitney U Test | Fig. legend | 5,5 | mean values by cell from 185 and 226 boutons from 3 and 3 mice | Fig. legend | median±IQR | Fig. legend | p=0.007937 | Fig. legend | U=0 | Fig. legend |
| + | S6a | Mann- Whitney U Test | Fig. legend | 6,6 | mean values by cell from 280 and 338 boutons from 6 and 3 mice | figure | median±IQR | Fig. legend | p=0.59 | Fig. legend | U=14 | Fig. legend |
| + | S6b | Spearman's rank order correlation | Fig. legend | 20 | cells from 4 dishes | figure | scatterplot | figure | p<0.0001 | figure | R=0.88 | figure |
| + - | S6d | Mann- Whitney U Test | Fig. legend | 6,6 | mean values by cell from 279 and 334 boutons from 6 and 3 mice | Fig. legend | median±IQR | Fig. legend | p=0.01515 | Fig. legend | U=3 | Fig. legend |
| + | S6e | Mann- Whitney U Test | Fig. legend | 6,6 | mean values by cell from 279 and 334 boutons from 6 and 3 mice | Fig. legend | median±IQR | Fig. legend | p=0.0411 | Fig. legend | U=5 | Fig. legend |
| + | S7e | paired two- sided t-test | Fig. legend | 3 | 3 mice, 100 boutons sampled from both layers from each animal | Fig. legend | median±IQR | Fig. legend | p=0.0246 | Fig. legend | t(2)=6.2551 | Fig. legend |
| + | S7f | paired two- sided t-test | Fig. legend | 3 | 3 mice, 100 boutons sampled from both layers from each animal | Fig. legend | median±IQR | Fig. legend | p=0.0334 | Fig. legend | t(2)=5.3319 | Fig. legend |
| + | S7g | paired two- sided t-test | Fig. legend | 3 | 3 mice, 100 boutons sampled from both layers from each animal | Fig. legend | median±IQR | Fig. legend | p=0.0360 | Fig. legend | t(2)=5.1301 | Fig. legend |
| + - | S7h | paired two- sided t-test | Fig. legend | 3 | 3 mice, 100 boutons sampled from both layers from each animal | Fig. legend | median±lQR | Fig. legend | p=0.0641 | Fig. legend | t(2)=3.7569 | Fig. legend |

| + | resul ts, p11 | Mann- Whitney U Test | results, p11 | 452,452 | boutons from 15 neurons from 12 mice | results, p11 | not shown | N/A | p=0.5522 | results, p11 | U=99818 | not shown |
|--------|---------------------|--|-----------------|---------|---|-----------------|-------------|----------------|------------|-----------------|--------------------------------|----------------|
| + - | 40 | Mann- Whitney U Test | Fig. legend | 311,141 | boutons from 10 and 5 neurons from 9 and 3 mice | Fig. legend | median±IQR | Fig. legend | p=0.6947 | Fig. legend | U=21420 | Fig. legend |
| + - | 40 | Mann- Whitney U Test | Fig. legend | 33,22 | boutons from 10 and 5 neurons from 9 and 3 mice | Fig. legend | median±IQR | Fig. legend | p=0.3261 | Fig. legend | U=305 | Fig. legend |
| + - | 4p | Mann- Whitney U Test | Figure | 33,26 | boutons from 10 neurons from 9 mice | Fig. legend | median±IQR | Fig. legend | p=0.6439 | Figure | U=398 | Figure |
| + - | 4p | Mann- Whitney U Test | Figure | 22, 16 | boutons from 5 neurons from 3 mice | Fig. legend | median±IQR | Fig. legend | p=0.2842 | Figure | U=139 | Figure |
| + - | 4p | Mann- Whitney U Test | Figure | 33,22 | boutons from 10 and 5 neurons from 9 and 3 mice | Fig. legend | median±IQR | Fig. legend | p=0.8581 | Figure | U=352 | Figure |
| + | 4p | Mann- Whitney U Test | Figure | 26,16 | boutons from 10 and 5 neurons from 9 and 3 mice | Fig. legend | median±IQR | Fig. legend | p=0.3898 | Figure | U=174 | Figure |
| + | s9d | unpaired two-sided t- test | Fig. legend | 50,50 | simulations of distribution | Fig. legend | median±IQR | Fig. legend | see figure | Figure | not shown | not shown |
| + - | s10g | Kolmogorov -Smirnov two-sample test | Fig. legend | 129,141 | 1 and 5 neurons from 1 and 3 mice | Fig. legend | histogram | figure | p>0.1 | Fig. legend | Max. Difference=-0.08 89 | not shown |
| + | s10h | Kolmogorov -Smirnov two-sample test | Fig. legend | 129,141 | 1 and 5 neurons from 1 and 3 mice | Fig. legend | histogram | figure | p>0.1 | Fig. legend | Max. Difference=-0.05 98 | not shown |
| + | s10i | Spearman's rank order correlation | Fig. legend | 129 | boutons from 1 neuron | Fig. legend | scatterplot | figure | p<0.001 | Fig. legend | R=0.68 | figure |
| + - | 5g | Mann- Whitney U Test | Fig. legend | 10,12 | mean values by cell from 349 and 374 boutons from 6 and 7 mice | Fig. legend | median±IQR | Fig. legend | p=0.6743 | Figure | U=53 | Figure |
| + - | 5h | Kolmogorov -Smirnov two-sample test | Fig. legend | 349,374 | boutons from 10 and 12 neurons from 6 and 7 mice | Fig. legend | histogram | figure | p>0.1 | Figure | Max. Difference=0.040 1 | not shown |
| + - | 5h | Kolmogorov -Smirnov two-sample test | Fig. legend | 279,334 | boutons from 6 and 6 neurons from 6 and 3 mice | Fig. legend | histogram | figure | p<0.001 | Figure | Max. Difference=-0.20 61 | not shown |
| + | 5i | Mann- Whitney U Test | Fig. legend | 10,12 | mean values by cell from 349 and 374 boutons from 6 and 7 mice | Fig. legend | median±IQR | figure | p=0.00896 | Figure | U=21 | Figure |
| + | 5j | Mann- Whitney U Test | Fig. legend | 10,12 | mean values by cell from 349 and 374 boutons from 6 and 7 mice | Fig. legend | median±IQR | figure | p=0.0249 | Figure | U=26 | Figure |
| + | 5k | Kolmogorov -Smirnov two-sample test | Fig. legend | 80,80 | boutons selected at random | Fig. legend | median±IQR | figure | p<0.001 | Figure | Max. Difference=0.312 5 | not shown |
| + | 51 | Kolmogorov -Smirnov two-sample test | Fig. legend | 192,192 | boutons selected at random | Fig. legend | histogram | figure | p>0.1 | Figure | Max. Difference=0.020 8 | not shown |

| + - | 5m | Kolmogorov -Smirnov two-sample test | Fig. legend | 192,192 | boutons selected at random | Fig. legend | histogram | figure | p<0.025 | Figure | Max. Difference=-0.16 | not shown |
|--------|----|--|----------------|---------|--|----------------|------------|--------|-----------|--------|--------------------------------|--------------|
| + - | 5m | Kolmogorov -Smirnov two-sample test | Fig. legend | 192,192 | boutons selected at random | Fig. legend | histogram | figure | p>0.1 | Figure | Max. Difference=-0.09 | not shown |
| + - | 5n | Kolmogorov -Smirnov two-sample test | Fig. legend | 197,197 | boutons selected at random | Fig. legend | histogram | figure | p>0.1 | Figure | Max. Difference=0.06 | not shown |
| + - | 50 | Kolmogorov -Smirnov two-sample test | Fig. legend | 200,200 | boutons selected at random | Fig. legend | histogram | figure | p<0.001 | Figure | Max. Difference=-0.21 5 | not shown |
| + - | 5p | Kolmogorov -Smirnov two-sample test | Fig. legend | 80,80 | boutons selected at random | Fig. legend | median±IQR | figure | p>0.1 | Figure | Max. Difference=-0.13 75 | not shown |
| + - | 6g | Mann- Whitney U Test | Fig. legend | 185,117 | boutons from 4 and 2 neurons from 3 and 2 mice | Fig. legend | median±IQR | figure | <0.0001 | Figure | U=1813 | not shown |
| + - | 6g | Mann- Whitney U Test | Fig. legend | 283,113 | boutons from 5 and 3 neurons from 3 and 3 animals | Fig. legend | median±IQR | figure | <0.0001 | Figure | U=7096 | not shown |
| + - | 6g | Mann- Whitney U Test | Fig. legend | 113,92 | boutons from 3 and 3 neurons from 2 and 3 animals | Fig. legend | median±IQR | figure | p=0.7977 | Figure | U=4854 | not shown |
| + - | 6g | Mann- Whitney U Test | Fig. legend | 129,153 | boutons from 4 and 4 cells from 2 and 3 animals | Fig. legend | median±IQR | figure | p<0.0001 | Figure | U=7184 | not shown |
| + - | 6h | unpaired two-sided t- test | Fig. legend | 185,117 | boutons from 4 and 2 neurons from 3 and 2 mice | Fig. legend | median±IQR | figure | p=0.00059 | Figure | t(300)=3.474 | not shown |
| + - | 6h | unpaired two-sided t- test | Fig. legend | 238,113 | boutons from 5 and 3 neurons from 3 and 3 animals | Fig. legend | median±IQR | figure | p=0.483 | figure | t(349)=0.702 | not shown |
| + - | 6h | unpaired two-sided t- test | Fig. legend | 113,92 | boutons from 3 and 3 neurons from 2 and 3 animals | Fig. legend | median±IQR | figure | p=0.574 | figure | t(203)=-0.563 | not shown |
| + - | 6h | unpaired two-sided t- test | Fig. legend | 129,153 | boutons from 4 and 4 cells from 2 and 3 animals | Fig. legend | median±IQR | figure | p=0.331 | figure | t(280)=-0.974 | not shown |

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 #)?

Representative images are shown: Figures 1, 2, 3, 4, 5, 6 Supplementary figures 1, 7, 10.

All shown differences are quantified and reported in the paper with sample sizes. For figures showing representative images for illustrative purposes(Figures 2, 3, 4, Supplementary Figure 10), the nuber of repeated experiments is stated in the figure legends.

Statistics and general methods

| 1. | Is there a | a justification of the sample size? | Post-hoc power analysis reported in Methods - Statistical analysis |
|----|------------|--|--|
| | If so, how | v was it justified? | and figure preparation p1. |
| | Where (s | section, paragraph #)? | |
| | | o sample size calculation was performed, authors should hy the sample size is adequate to measure their effect size. | |
| 2. | | stical tests justified as appropriate for every figure? | Statement in Methods: Statistical analysis and figure preparation p1. |
| | Where (s | 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. Statistical analysis and figure preparation p1. At each reported experiment, the appled statistical probe is defined. |
| | 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 (section, paragraph #)? | Yes. Statement in Methods: Statistical analysis and figure preparation p1. |
| | C. | Is there any estimate of variance within each group of data? | Out of the 66 statistical comparisons reported in the paper, 13 |
| | | Is the variance similar between groups that are being statistically compared? | were parametric tests (Student's t-test). Because the t-test is highly robust to unequal variances and the sample sizes were similar in the experimental groups compared, therefore equal variances were |
| | | Where is this described (section, paragraph #)? | not assessed. |
| | d. | Are tests specified as one- or two-sided? | Yes (all tests in the study are two-sided). |
| | e. | Are there adjustments for multiple comparisons? | Multiple comparisons were not performed. |
| 3. | Are crite | ria for excluding data points reported? | Criteria were reported in every case where exclusions were made. |
| | Was this | criterion established prior to data collection? | fig. s8 Methods: Development and analysis of in vitro and in vivo recorded |
| | Where is | this described (section, paragraph #)? | cells p2 |
| 4. | | ne method of randomization used to assign subjects (or to the experimental groups and to collect and process data. | Mice were randomly assigned to vehicle or THC treatment. Methods: Chronic drug treatment, p1. |
| | If no ran | domization was used, state so. | |
| | Where d | oes this appear (section, paragraph #)? | |
| 5. | | ement of the extent to which investigator knew the group n during the experiment and in assessing outcome included? | Methods: Chronic drug treatment, p1. |
| | If no blin | ding was done, state so. | |

Where (section, paragraph #)?

| 6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included? Where (section, paragraph #)? | Yes. Statements in: Methods: Perfusion and preparation of tissue sections, p1 Methods: In vivo recording, p1 Methods: Preparation of the rabbit anti-CB1 antibody, p1 Methods: Chronic drug treatment, p1. |
|---|--|
| 7. Is the species of the animals used reported? Where (section, paragraph #)? | Yes. Methods: Perfusion and preparation of tissue sections, p1 Methods: In vivo recording, p1 Methods: Preparation of the rabbit anti-CB1 antibody, p1 Methods: Chronic drug treatment, p1. |
| Is the strain of the animals (including background strains of KO/ transgenic animals used) reported? Where (section, paragraph #)? | Yes. Methods: Perfusion and preparation of tissue sections, p1 Methods: In vivo recording, p1 Methods: Preparation of the rabbit anti-CB1 antibody, p1 Methods: Chronic drug treatment, p1. |
| 9. Is the sex of the animals/subjects used reported? Where (section, paragraph #)? | Yes. Methods: Perfusion and preparation of tissue sections, p1 Methods: Preparation of the rabbit anti-CB1 antibody, p1 Methods: In vivo recording, p1 Methods: Chronic drug treatment, p1. |
| 10. Is the age of the animals/subjects reported? Where (section, paragraph #)? | Yes. Methods: Perfusion and preparation of tissue sections, p1 Methods: In vivo recording, p1 Methods: Preparation of the rabbit anti-CB1 antibody, p1 Methods: Chronic drug treatment, p1. |
| 11. For animals housed in a vivarium, is the light/dark cycle reported? Where (section, paragraph #)? | Yes. Methods: Chronic drug treatment, p1. |
| 12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported? Where (section, paragraph #)? | Yes. Methods: Chronic drug treatment, p1. |
| 13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)? Where (section, paragraph #)? | N/A |
| 14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported? Where (section, paragraph #)? | Yes. Methods: Chronic drug treatment, p1. |
| 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? No animals were excluded.

Where (section, paragraph #)?

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a. How were the criteria for exclusion defined?

N/A

- Where is this described (section, paragraph #)?
- b. Specify reasons for any discrepancy between the number of N/A animals at the beginning and end of the study.

Where is this described (section, paragraph #)?

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 #)?

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

Where does this appear (section, paragraph #)?

2. If cell lines were used to reflect the properties of a particular tissue or disease state, is their source identified?

Where (section, paragraph #)?

a. Were they recently authenticated?

Where is this information reported (section, paragraph #)?

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.

N/A

1. Are accession codes for deposit dates provided?

Where (section, paragraph #)?

All antibodies have been validated for immunohistochemistry in mice.

Yes. Methods: Immunostaining for STORM imaging, p1.

Validation data are cited in: Methods: Immunostaining for STORM imaging, p1.

N/A

N/A

March 2014

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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. | Algorithms used throughout the analysis are described in: Methods: Combined confocal/STORM image processing, p1-4 Methods: Quantitative analysis of combined confocal/STORM images of axon terminals, p1-5. |
|----|---|--|
| 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. | Scripts used in the study will be freely provided to any member of the scientific community upon request after the study is published We are also developing a standalone software to release. |

Human subjects

| 1. | Which IRB approved the protocol? | N/A |
|----|--|-----|
| | Where is this stated (section, paragraph #)? | |
| 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 |

nature neuroscience | reporting checklist

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?

Where (section, paragraph #)?

2. Is the number of blocks, trials or experimental units per session and/ or subjects specified?

Where (section, paragraph #)?

- 3. Is the length of each trial and interval between trials specified?
- 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.
- 5. Is the task design clearly described?

Where (section, paragraph #)?

- 6. How was behavioral performance measured?
- 7. Is an ANOVA or factorial design being used?
- 8. For data acquisition, is a whole brain scan used?

If not, state area of acquisition.

- a. How was this region determined?
- 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?

| e | N/A |
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| | 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 #)?
- 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?

| N/A | |
|-----|--|
| N/A | |

Additional comments

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