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Manuscript Type: Article

Main Figures: Eight

Supplementary Figures: Seven

Supplementary Tables: One

Supplementary Videos: None

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 | |

| FIGURE NUMBER | TEST USED | | n | | | DESCRIPTIVE STATS (AVERAGE, VARIANCE) | | P VALUE | | DEGREES OF FREEDOM & F/t/z/R/ETC VALUE | | |
|---------------|-------------|-----------------------|--|--|---|---|-----------------------------|-----------------|-----------------------|--|-----------------------|----|
| | WHICH TEST? | SECTION & PARAGRAPH # | EXACT VALUE | DEFINED? | SECTION & PARAGRAPH # | REPORTED? | SECTION & PARAGRAPH # | EXACT VALUE | SECTION & PARAGRAPH # | VALUE | SECTION & PARAGRAPH # | |
| + - | 1B | NA | NA | 7 neurons | neurons cultured from 5 pups | results, para 2; Fig. 1B legend | error bars are mean +/- SEM | methods, para 8 | NA | NA | NA | NA |
| + - | 2C | Unpaired t-test | results, para 5; Fig. 2C, legend | 11 neurons tested for each group | neurons cultured from 5-6 pups | NA | error bars are mean +/- SEM | methods, para 8 | 0.0002 | Fig. 2 legend | NA | NA |
| + - | 3A, 3B | NA | results, para 6; Fig. 3B, legend | 283 spines from 10-12 neurons | neurons cultured from 5-6 pups, 2 separate cultures | discussion, para 2; Fig. 3B legend | NA | methods, para 8 | NA | NA | NA | NA |
| + - | 3C, 3D | One-way-ANOVA | Results, para 6; Fig. 3D, legend | 20-24 dendrites from 12-18 neurons for each group | neurons cultured from 5-6 pups, 2 separate cultures | discussion, para 2; Fig. 3C and 3D legend | error bars are mean +/- SEM | methods, para 8 | 0.0001 | Fig. 3C and 3D legend | NA | NA |
| + - | 3E | One-way-ANOVA | Results, para 6; Fig. 3E, legend | 18-25 dendrites from 10-14 neurons for each group | neurons cultured from 5-6 pups, 2 separate cultures | Fig. 3E legend | error bars are mean +/- SEM | methods, para 8 | 0.0148 | Fig. 3E legend | NA | NA |
| + - | 4C | unpaired t-test | Results, para 7; Fig. 4C, legend | 20-25 axons from 20-25 neurons for each group | neurons cultured from 5-6 pups, 2 separate cultures | Fig. 4C legend | error bars are mean +/- SEM | methods, para 8 | 0.0001 | Fig. 4C legend | NA | NA |
| + - | 4E | NA | Results, para 7; discussion, para 5; Fig. 4E, legend | 257 boutons from 15 neurons | neurons cultured from 5-6 pups, 2 separate cultures | Fig. 4E legend | error bars are mean +/- SEM | methods, para 8 | NA | NA | NA | NA |
| + - | 4H | unpaired t-test | Results, para 7; discussion, para 5; Fig. 4H, legend | 370-395 boutons from 20 neurons | neurons cultured from 5-6 pups, 2 separate cultures | Fig. 4H legend | error bars are mean +/- SEM | methods, para 8 | 0.0001 | Fig. 4H legend | NA | NA |
| + - | 5A | NA | Results, para 8; Fig. 5A, legend | 360 APP and 300 BACE-1 vesicles from 20-22 neurons | neurons cultured from 5-6 pups, 2 separate cultures | Fig. 5A legend | error bars are mean +/- SEM | methods, para 8 | NA | NA | NA | NA |

| | | | | | | | | | | | | |
|--------|--------|-----------------|--------------------------------------|--|---|-------------------|-----------------------------|-----------------|--|-------------------|----|----|
| + - | 5B | NA | Results , para 8; Fig 5B, legend | For APP/ NPYss: 320 APP and 390 NPYss vesicles; for BACE-1/ NPYss: 410 BACE-1 and 500 NPYss vesicles from 18-20 neurons for each set | neurons cultured from 5-6 pups, 2 separate cultures | Fig. 5B legend | error bars are mean +/- SEM | methods, para 8 | NA | NA | NA | NA |
| + - | 5C | NA | Results , para 8; Fig 5C, legend | 350 APP and 430 BACE-1 vesicles from 20-25 neurons | neurons cultured from 5-6 pups, 2 separate cultures | Fig. 5C legend | error bars are mean +/- SEM | methods, para 8 | NA | NA | NA | NA |
| + - | 6B | unpaired t-test | Results , para 9; Fig 6B, legend | 200 vesicles for DIV7 and 160 vesicles for DIV14 from 12-14 axons | neurons cultured from 5-6 pups, 2 separate cultures | Fig. 6B legend | error bars are mean +/- SEM | methods, para 8 | $p = 0.7488$ (between the mobile groups) $p = 0.6634$ (between the stationary groups) | results, para 9 | NA | NA |
| + - | 6C | unpaired t-test | Results , para 9; Fig 6C, legend | 200 vesicles for DIV7 and 170 vesicles for DIV14 from 12-14 axons | neurons cultured from 5-6 pups, 2 separate cultures | Fig. 6C legend | error bars are mean +/- SEM | methods, para 8 | $p = 0.0002$ (between the mobile groups) $p = 0.0002$ (between the stationary groups) | results, para 9 | NA | NA |
| + - | 7C | NA | NA | 5-7 neurons in each group | neurons cultured from 5-6 pups | Fig. 7C legend | NA | methods, para 8 | NA | NA | NA | NA |
| + - | 7D | NA | NA | 128 particles from 12 neurons | neurons cultured from 5-6 pups, 2 separate cultures | Fig. 7D legend | error bars are mean +/- SEM | methods, para 8 | 0.0001 | Fig. 7D legend | NA | NA |
| + - | 7E, 7F | One-way ANOVA | Results , para 11; Fig 7F legend | 18-22 dendrites from 15-18 neurons for each group | neurons cultured from 5-6 pups, 2 separate cultures | Fig. 7F legend | error bars are mean +/- SEM | methods, para 8 | 0.0001 | Fig. 7F legend | NA | NA |
| + - | 8B, 8C | unpaired t-test | Results , para 12; Fig 8B-8C, legend | 22-24 dendrites from 14-18 neurons in each group | neurons cultured from 5-6 pups, 3 separate cultures | Fig. 8B-8C legend | error bars are mean +/- SEM | methods, para 8 | 0.0001 (for panel D, left); 0.0042 (for panel D, right) | Fig. 8B-8C legend | NA | NA |

| | | | | | | | | | | | | |
|--------|----------------|-----------------|----------------------------------|--|---|-----------------------|-----------------------------|-----------------|---|------------------|----|----|
| + - | 8D | unpaired t-test | Results, para 12; Fig 8D, legend | data from 4 independent experiments | cells from 4 separate cultures | Fig. 8D legend | error bars are mean +/- SEM | methods, para 8 | 0.0272 | Fig. 8D legend | NA | NA |
| + - | Suppl. Fig. 2B | unpaired t-test | Suppl. Fig 2B, legend | 15-20 dendrites from 15-20 neurons | neurons cultured from 5-6 pups, 3 separate cultures | Suppl. Fig 2B, legend | error bars are mean +/- SEM | methods, para 8 | ns | results, para 4 | NA | NA |
| + - | Suppl. Fig. 2C | unpaired t-test | Suppl. Fig 2B, legend | 12-20 neurons | neurons cultured from 5-6 pups, 2 separate cultures | Suppl. Fig 2B, legend | error bars are mean +/- SEM | methods, para 8 | ns | results, para 6 | NA | NA |
| + - | Suppl. Fig. 2D | unpaired t-test | Suppl. Fig 2D, legend | 15-20 dendrites from 15-20 neurons for each group | neurons cultured from 5-6 pups, 2 separate cultures | Suppl. Fig 2D, legend | error bars are mean +/- SEM | methods, para 8 | $p=0.1729$ (Alexa-Tf vs Rab5) $p=0.0107$ (Alexa-Tf vs Lamp1) $p=0.0001$ (Alexa-Tf vs NPYss) | results, para 6 | NA | NA |
| + - | Suppl. Fig. 3A | NA | Suppl. Fig 3A, legend | for APP/Rab5: 254 APP vesicles and 121 Rab5 vesicles; for BACE-1/Rab5: 461 BACE-1 vesicles and 163 Rab5 vesicles from 20-22 neurons | neurons cultured from 5-6 pups, 2 separate cultures | Suppl. Fig 3A, legend | error bars are mean +/- SEM | methods, para 8 | NA | NA | NA | NA |
| + - | Suppl. Fig. 3B | NA | Suppl. Fig 3B, legend | for APP/Rab11: 174 APP vesicles and 132 Rab11 vesicles; for BACE-1/Rab11: 201 BACE-1 vesicles and 58 Rab11 vesicles from 14-16 neurons | neurons cultured from 5-6 pups, 2 separate cultures | Suppl. Fig 3B, legend | error bars are mean +/- SEM | methods, para 8 | NA | NA | NA | NA |
| + - | Suppl. Fig. 4B | NA | Suppl. Fig 4B, legend | 10-18 neurons tested for each group | neurons cultured from 5-6 pups, 2 separate cultures | Suppl. Fig 4B, legend | error bars are mean +/- SEM | methods, para 8 | NA | NA | NA | NA |
| + - | Suppl. Fig. 5B | unpaired t-test | Suppl. Fig 5B, legend | 26-28 neurons tested for each group | neurons cultured from 5-6 pups, 3 separate cultures | Suppl. Fig 5B, legend | error bars are mean +/- SEM | methods, para 8 | ns | results, para 12 | NA | NA |

| | | | | | | | | | | | | |
|---|----------------|-----------------|-----------------------|----------------------------------|---|-----------------------|-----------------------------|-----------------|----|------------------|----|----|
| + | Suppl. Fig. 5C | unpaired t-test | Suppl. Fig 5C, legend | 450 particles from 28-36 neurons | neurons cultured from 5-6 pups, 3 separate cultures | Suppl. Fig 5C, legend | error bars are mean +/- SEM | methods, para 8 | ns | results, para 12 | NA | NA |
|---|----------------|-----------------|-----------------------|----------------------------------|---|-----------------------|-----------------------------|-----------------|----|------------------|----|----|

► Representative figures

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

If so, what figure(s)?

Western blots in Figs. 1D, E; fig. 8E and supplementary fig 6B. Live imaging, figs. 1B; fig. 4E, G; fig. 5A, B, C; fig. 6B, C, D; fig 7D; suppl. figs. 3A, B and 5C. Immunofluorescence in fig. 1C; fig. 2B; fig. 3A, C, E; fig. 4B, C; fig. 7B, C, E; fig. 8B, C; suppl fig. 1A; 2B, 2D, 4A, 5B, 6A and 7.

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 #)?

For all the representative WBs, immunofluorescence/live images, it has been clearly stated in either the accompanying legend or the methods (or both).

► 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 determined as per conventional cell biology parameters that allow adequate sampling.

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

Where (section, paragraph #)?

Yes

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

Described in the method section

- b. Do the data meet the assumptions of the specific statistical test you chose (e.g. normality for a parametric test)?

NA

Where is this described (section, paragraph #)?

- c. Is there any estimate of variance within each group of data?

NA

Is the variance similar between groups that are being statistically compared?

Where is this described (section, paragraph #)?

- d. Are tests specified as one- or two-sided?

- e. Are there adjustments for multiple comparisons?

3. Are criteria for excluding data points reported?
Was this criterion established prior to data collection?
Where is this described (section, paragraph #)?
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 #)?
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 #)?
6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included?
Where (section, paragraph #)?
7. Is the species of the animals used reported?
Where (section, paragraph #)?
8. Is the strain of the animals (including background strains of KO/transgenic animals used) reported?
Where (section, paragraph #)?
9. Is the sex of the animals/subjects used reported?
Where (section, paragraph #)?
10. Is the age of the animals/subjects reported?
Where (section, paragraph #)?
11. For animals housed in a vivarium, is the light/dark cycle reported?
Where (section, paragraph #)?
12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported?
Where (section, paragraph #)?
13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)?
Where (section, paragraph #)?
- NA
- NA
- NA
- NA
- Method section, para 3
- Method section, para 3
- Mixed population of male and female pups
- Method section, para 3
- NA
- NA
- NA

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

NA

Where (section, paragraph #)?

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

NA

Where (section, paragraph #)?

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

NA

Where (section, paragraph #)?

a. How were the criteria for exclusion defined?

NA

Where is this described (section, paragraph #)?

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

NA

Where is this described (section, paragraph #)?

▶ Reagents

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

All the antibodies were purchased from commercial sources

a. Is antibody catalog number given?

Yes, method section, para 2.

Where does this appear (section, paragraph #)?

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

NA

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?

Method section, para 3

Where (section, paragraph #)?

a. Were they recently authenticated?

NA

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

▶ Data deposition

Data deposition in a public repository is mandatory for:

- Protein, DNA and RNA sequences
- Macromolecular structures
- Crystallographic data for small molecules
- 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.

- Are accession codes for deposit dates provided?

Where (section, paragraph #)?

NA

▶ 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.

- Identify all custom software or scripts that were required to conduct the study and where in the procedures each was used.

A previously-published algorithm was used to determine colocalization, used in figs. 2, 3, 4, 7 and Supp. fig. 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.

Ok

▶ Human subjects

- Which IRB approved the protocol?

Where is this stated (section, paragraph #)?

NA

- Is demographic information on all subjects provided?

Where (section, paragraph #)?

NA

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

Where (section, paragraph #)?

NA

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

Where (section, paragraph #)?

NA

5. How well were the groups matched?

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

NA

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

Where (section, paragraph #)?

NA

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

Where (section, paragraph #)?

NA

► 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?

NA

a. If yes, is the number rejected and reasons for rejection described?

Where (section, paragraph #)?

NA

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

Where (section, paragraph #)?

NA

3. Is the length of each trial and interval between trials specified?

NA

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.

NA

5. Is the task design clearly described?

Where (section, paragraph #)?

NA

6. How was behavioral performance measured?

NA

7. Is an ANOVA or factorial design being used?

NA

8. For data acquisition, is a whole brain scan used?

If not, state area of acquisition.

NA

a. How was this region determined?

NA

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?

NA

a. If so, is the rationale clearly described?

NA

b. How were the ROI's defined (functional vs anatomical localization)?

NA

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

NA

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

NA

► Additional comments

Additional Comments

None