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Main Figures: 6

Supplementary Figures: 6

Supplementary Tables: 0

Supplementary Videos: 2

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.

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 #
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

		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 #	
+ -	1e	One-Way-ANOVA followed by Bonferroni post-hoc test	Fig. legend	14, 13, 21	eye primordia from 3 biological replicates	Figure; Methods last para	error bars are mean +/- SEM	Meth ods last para	1.257E-8 (2.785E-7; 1.000; 5.010E-8)	Fig. legend	F(2, 45)=28.003 (t(47)= 6.354, 0.082, -6.855)	N/A
+ -	1g	Mann-Whitney-Test	Fig. legend	60, 60	axons from 3 experiments	Figure, Methods: In vitro time-lapse expts.	boxes showing the 25th, 50th, and 75th percentiles, whiskers the spread of the data, O the mean score for a group	Meth ods last para	9.316E-6	Fig. legend	Z=4.432	N/A
+ -	1h	two-tailed t-test	Fig. legend	60, 60	axons from 3 experiments	Figure, Methods: In vitro time-lapse expts.	error bars are mean +/- SEM	Meth ods last para	0.00867	Fig. legend	t(118)=2.669	N/A
+ -	1i	Mann-Whitney-Test	Fig. legend	60, 60	axons from 3 experiments	Figure, Methods: In vitro time-lapse expts.	boxes showing the 25th, 50th, and 75th percentiles, whiskers the spread of the data, O the mean score for a group	Meth ods last para	1.097E-6	Fig. legend	Z=4.873	N/A
+ -	2f	Mann-Whitney-Test	Fig. legend	588, 454	measurement points from 9 brains from 3 experiments	Figure, Methods last para	boxes showing the 25th, 50th, and 75th percentiles, whiskers the spread of the data, O the mean score for a group	Meth ods last para	2.256E-9	Fig. legend	Z=-5.978	N/A
+ -	2g	Mann-Whitney-Test	Fig. legend	215, 225	measurement points from 7 brains from 3 experiments	Figure, Methods last para	boxes showing the 25th, 50th, and 75th percentiles, whiskers the spread of the data, O the mean score for a group	Meth ods last para	0.0033	Fig. legend	Z=2.933	N/A
+ -	2h	Mann-Whitney-Test	Fig. legend	329, 403	measurement points from 7 brains from 3 experiments	Figure, Methods last para	boxes showing the 25th, 50th, and 75th percentiles, whiskers the spread of the data, O the mean score for a group	Meth ods last para	2.973E-5	Fig. legend	Z=4.163	N/A
+ -	2i	paired two-tailed t-test	Fig. legend	10	brains from 3 experiments	Figure, Methods last para	error bars are mean +/- SEM	Meth ods last para	3.96E-06	Fig. legend	t(9)=9.879	N/A
+ -	3c	one-sample Wilcoxon Signed Rank Test	Fig. legend	51	measurement points from 7 brains from 3 experiments	Figure, Methods last para	boxes showing the 25th, 50th, and 75th percentiles, whiskers the spread of the data, O the mean score for a group	Meth ods last para	1.441E-4	Fig. legend	Z=3.801	N/A

+ -	3e	one-sample Wilcoxon Signed Rank Test	Fig. legend	30	turning essays from 11 experiments	Figure, Methods last para	boxes showing the 25th, 50th, and 75th percentiles, whiskers the spread of the data, O the mean score for a group	Meth ods last para	0.0549	Fig. legend	Z=-1.913	N/A
+ -	3g	one-sample Wilcoxon Signed Rank Test	Fig. legend	14	explants from 3 experiments	Figure, Methods last para and In vitro turning angle analysis	boxes showing the 25th, 50th, and 75th percentiles, whiskers the spread of the data, O the mean score for a group	Meth ods last para	0.0264	Fig. legend	Z=2.197	N/A
+ -	4b	Mann-Whitney-Test	Fig. legend	1346 and 1681	measurement points from 7 and 9 brains from 3 experiments each	Figure, Methods last para	boxes showing the 25th, 50th, and 75th percentiles, whiskers the spread of the data, O the mean score for a group	Meth ods last para	6.617E-10	Fig. legend	Z=6.175	N/A
+ -	4c	Mann-Whitney-Test	Fig. legend	66 and 105	measurement points from 7 and 9 brains from 3 experiments each	Figure, Methods last para	boxes showing the 25th, 50th, and 75th percentiles, whiskers the spread of the data, O the mean score for a group	Meth ods last para	8.571E-11	Fig. legend	Z=6.490	N/A
+ -	4e	two-tailed t-test	Fig. legend	24, 16	optic tracts from 4 experiments	Figure, Methods last para	error bars are mean +/- SEM	Meth ods last para	3.116E-6	Fig. legend	t(38)=5.462	N/A
+ -	5i	two-tailed t-test	Fig. legend	24, 19, 35, 27	optic tracts from 3 and 4 experiments	Figure, Methods last para	error bars are mean +/- SEM	Meth ods last para	0.00243 0.00476 0.782 0.0976	Fig. legend	t(41)=3.231 t(60)=2.932 t(57)=0.278 t(44)=1.692	N/A
+ -	S1d	two-tailed t-test	Fig. legend	14, 14	eye primordia from 3 biological replicates	Figure, Methods last para	error bars are mean +/- SEM	Meth ods last para	1.815E-06	Fig. legend	t(26)=-6.118	N/A
+ -	S1g	two-tailed t-test	Fig. legend	36, 47	ROIs from 3 experiments	Figure, Methods last para	error bars are mean +/- SEM	Meth ods last para	0.026	Fig. legend	t(81)=2.274	N/A
+ -	S3c	Mann-Whitney-Test	Fig. legend	72, 72	randomly selected areas of 3 substrates	Figure caption, Methods: Substrate coating measurements	boxes showing the 25th, 50th, and 75th percentiles, whiskers the spread of the data, O the mean score for a group	Meth ods last para	0.430	Fig. legend	Z=0.789	N/A
+ -	S4b	one-sample Wilcoxon Signed Rank Test; Mann-Whitney-Test	Fig. legend	89; 51, 89	measurement points from 9; 7 and 9 brains from 3 experiments each	Figure, Methods last para	boxes showing the 25th, 50th, and 75th percentiles, whiskers the spread of the data, O the mean score for a group	Meth ods last para	5.627E-4; 0.354	Fig. legend	Z=3.449; Z=0.927	N/A
+ -	S5h	Kruskal-Wallis ANOVA	Fig. legend	13, 13, 13	measurements from 4 brains	Figure legend, Methods last para	boxes showing the 25th, 50th, and 75th percentiles, whiskers the spread of the data, O the mean score for a group	Meth ods last para	6.83E-3	Fig. legend	Chi-Square=9.97396	N/A

+ -	S6d	two-tailed t-test	Fig. legend	16, 15	brains from 3 experiments	Figure legend, Methods last para	error bars are mean +/- SEM	Meth ods last para	5.21E-4	Fig. legend	t(29)=3.902	N/A
+ -	S6e Fig. legend	one-sample t-test	Fig. legend	4	Western Blots from 2 lysates	Figure legend, Methods last para	mean +/- SEM	Meth ods last para	6.98E-3	Fig. legend	t(3)=6.634	N/A

► Representative figures

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

If so, what figure(s)?

Yes. Figs. 1a-c,f,j, 2c-e, 3d,f, 4a,d,g,h, 5a-h, S1a,b, S2, S3b, S5a-g, S6a,b,e

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. Reported in the last paragraph of the Methods as well as in the figure legends.

► 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.

Yes. Sample sizes were chosen using a Power & Sample Size Calculator, as mentioned in the last paragraph of the Methods.

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

Where (section, paragraph #)?

Yes. Reported in the last paragraph of the Methods.

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

Yes. Reported in the last paragraph of the Methods.

- 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. Reported in the last paragraph of the Methods.

- 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. Reported in the last paragraph of the Methods.

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

Yes.

- e. Are there adjustments for multiple comparisons?

Yes.

- | | |
|--|---|
| <p>3. To promote transparency, <i>Nature Neuroscience</i> has stopped allowing bar graphs to report statistics in the papers it publishes. If you have bar graphs in your paper, please make sure to switch them to dot-plots (with central and dispersion statistics displayed) or to box-and-whisker plots to show data distributions.</p> | <p>OK.</p> |
| <p>4. Are criteria for excluding data points reported?
Was this criterion established prior to data collection?
Where is this described (section, paragraph #)?</p> | <p>No data points were excluded, as mentioned in the last paragraph of the online Methods.</p> |
| <p>5. 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 #)?</p> | <p>The order of data collection was randomized, as reported in the last paragraph of the Methods.</p> |
| <p>6. 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 #)?</p> | <p>No blinding was done, as reported in the last paragraph of the Methods.</p> |
| <p>7. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included?
Where (section, paragraph #)?</p> | <p>Yes, in the Methods, section Animal model.</p> |
| <p>8. Is the species of the animals used reported?
Where (section, paragraph #)?</p> | <p>Yes, in the first line of the Results section and in the Methods, section Animal model.</p> |
| <p>9. Is the strain of the animals (including background strains of KO/transgenic animals used) reported?
Where (section, paragraph #)?</p> | <p>N/A</p> |
| <p>10. Is the sex of the animals/subjects used reported?
Where (section, paragraph #)?</p> | <p>Yes, in the Animal model paragraph in the methods.</p> |
| <p>11. Is the age of the animals/subjects reported?
Where (section, paragraph #)?</p> | <p>Yes, in the main text, paragraph 2 under the 'Stiffness gradients in developing brain tissue' heading.</p> |
| <p>12. For animals housed in a vivarium, is the light/dark cycle reported?
Where (section, paragraph #)?</p> | <p>N/A</p> |
| <p>13. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported?
Where (section, paragraph #)?</p> | <p>N/A</p> |

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

Where (section, paragraph #)?

N/A

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

Where (section, paragraph #)?

N/A

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

Where (section, paragraph #)?

N/A

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

Where (section, paragraph #)?

N/A

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)?

No.

a. Is antibody catalog number given?

Where does this appear (section, paragraph #)?

Yes, in the Methods.

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

Where does this appear (section, paragraph #)?

N/A

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 #)?

No.

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 availability

Provide a Data availability statement in the Methods section under "Data availability", which should include, where applicable:

- Accession codes for deposited data
- Other unique identifiers (such as DOIs and hyperlinks for any other datasets)
- At a minimum, a statement confirming that all relevant data are available from the authors
- Formal citations of datasets that are assigned DOIs
- A statement regarding data available in the manuscript as source data
- A statement regarding data available with restrictions

See our [data availability and data citations policy page](#) for more information.

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.

Where is the Data Availability statement provided (section, paragraph #)?

Done at the end of the Methods.

► 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.

Image analysis was done using custom-written MatLab codes as described in the Methods; AFM stiffness mapping was automated using a custom-written script as described.

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

<p>2. Is the number of blocks, trials or experimental units per session and/or subjects specified?</p> <p>Where (section, paragraph #)?</p>	N/A
<p>3. Is the length of each trial and interval between trials specified?</p>	N/A
<p>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.</p>	N/A
<p>5. Is the task design clearly described?</p> <p>Where (section, paragraph #)?</p>	N/A
<p>6. How was behavioral performance measured?</p>	N/A
<p>7. Is an ANOVA or factorial design being used?</p>	N/A
<p>8. For data acquisition, is a whole brain scan used?</p> <p>If not, state area of acquisition.</p>	N/A
<p>a. How was this region determined?</p>	N/A
<p>9. Is the field strength (in Tesla) of the MRI system stated?</p>	N/A
<p>a. Is the pulse sequence type (gradient/spin echo, EPI/spiral) stated?</p>	N/A
<p>b. Are the field-of-view, matrix size, slice thickness, and TE/TR/flip angle clearly stated?</p>	N/A
<p>10. Are the software and specific parameters (model/functions, smoothing kernel size if applicable, etc.) used for data processing and pre-processing clearly stated?</p>	N/A
<p>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 #)?</p>	N/A
<p>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 #)?</p>	N/A
<p>13. How were anatomical locations determined, e.g., via an automated labeling algorithm (AAL), standardized coordinate database (Talairach daemon), probabilistic atlases, etc.?</p>	N/A

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