nature neuroscience

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Manuscript Number:	NN-A49746B	# Supplementary Figures:	7
Manuscript Type:	Article	# Supplementary Tables:	0
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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
+ -												

		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 #
+ -	1b	unpaired two-way t-test	Fig legend	37, 39 animals and 406, 296 sheaths	# animals. The total # of sheaths analyzed for each group is also provided.	Fig legend	error bars are mean +/- SEM	Fig legend	P = 0.0005	Fig legend	df = 74 , t = 3.652	not reported
+ -	1c	unpaired two-way t-test	Fig legend	37, 39 animals and 406, 296 sheaths	# animals. The total # of sheaths analyzed for each group is also provided.	Fig legend	error bars are mean +/- SEM	Fig legend	P = 0.3426	Fig legend	df = 74 , t=0.9552	not reported
+ -	1d	unpaired two-way t-test	Fig legend	16, 12, 16, 12 animals; 184, 108, 222, 188 sheaths	# animals. The total # of sheaths analyzed within these groups is also provided.	Fig legend	error bars are mean +/- SEM	Fig legend	left green comparison P = 0.7468, right grey comparison P = 0.1540	Fig legend	left green comparison df = 26 , t = 0.3263, right grey comparison df = 26, t = 1.468	not reported
+ -	2c	unpaired two-way t-test	Fig legend	30, 28	# animals	Fig legend	error bars are mean +/- SEM	Fig legend	P = 0.2147	Fig legend	df = 56, t = 1.255	not reported
+ -	2d	unpaired two-way t-test	Fig legend	30, 28	# animals	Fig legend	error bars are mean +/- SEM	Fig legend	P = 0.2173	Fig legend	df = 56, t = 1.248	not reported
+ -	2e	unpaired two-way t-test	Fig legend	30, 28	# animals	Fig legend	error bars are mean +/- SEM	Fig legend	For left comparison P = 0.0011, for right comparison P = 0.0342	Fig legend	df = 45, t =3.447 (left) and 2.171 (right)	not reported
+ -	3c	Mann-Whitney Test	Fig legend	38, 19, 28, 38	total number of axons analyzed, derived from 17, 17, 22, and 29 animals	Fig legend, also directed to Fig 3b	error bars are mean +/- SEM	Fig legend	For upper * P = 0.0096, for lower * P = 0.0294, for ns P = 0.7157	Fig legend	For all, df= n/a. For upper * U = 501, For lower* U = 242.5, For ns U = 252	not reported
+ -	3d	Mann-Whitney Test	Fig legend	axons derived from 17, 17, 22, and 29 animals	# animals	Fig legend, also directed to Fig 3b	error bars are mean +/- SEM	Fig legend	For upper * P = 0.0002, for lower * P < 0.0001	Fig legend	For upper * df = n/a; Mann-Whitney U = 1, For lower* df = n/a; Mann-Whitney U =87	not reported
+ -	4c	unpaired two-way t-test	Fig legend	12/7 (272 and 31 vesicles)	Total numbers of animals analyzed. The total number of vesicles analyzed is also given.	Figure legend	error bars are mean +/- SEM	Fig legend	P = 0.0009	Fig legend	df = 17 , t = 4.028	not reported
+ -	5b	Mann-Whitney Test	Fig legend	12/8 and 66/57	# animals. The total # of sheaths analyzed within these groups is also provided.	Fig legend	error bars are mean +/- SEM	Fig legend	P = 0.5624	Fig legend	df = n/a; Mann-Whitney U =35	not reported
+ -	5c	Mann-Whitney Test	Fig legend	12/8 and 50/41	# animals. The total # of sheaths analyzed within these groups is also provided.	Fig legend	error bars are mean +/- SEM	Fig legend	P = 0.0411	Fig legend	df = n/a; Mann-Whitney U = 770	not reported

+ -	6b	Mann-Whitney Test	Fig legend	7, 9 animals and 99, 129 sheaths	# animals. The total # of sheaths analyzed within these groups is also provided.	Fig legend	error bars are mean +/- SEM	Fig legend	P = 0.0071	Fig legend	df = n/a; U = 7	not reported
+ -	6c	Mann-Whitney Test	Fig legend	7, 9 animals and 78, 125 sheaths	# animals. The total # of sheaths analyzed within these groups is also provided.	Fig legend	error bars are mean +/- SEM	Fig legend	For upper ns P = 0.3737, for lower ns P = 0.6105 , for ** P = 0.0034, for *** P = 0.0002	Fig legend	For all, df= n/a. For upper ns U = 42.5, For lower NS U = 3644, For ** U = 67.5 , For *** U = 481.5	not reported
+ -	S2B	unpaired two-way t-test	Figure legend	13, 13	# animals. The value for each animal represents the average cell count from 10 independent transverse sections on the same animal	Figure legend	error bars are mean +/- SEM	Figure legend	p = 0.3503	Fig legend	df = 23 , t =0.9533	not reported
+ -	S2D	unpaired two-way t-test	Figure legend	21, 21	animals	Figure legend	error bars are mean +/- SEM	Figure legend	P = 0.7210	Figure legend	df = 40, t = 0.3596	not reported
+ -	S3B	unpaired two-way t-test	Fig legend	12, 16	# axons. The number of animals is indicated as 12, 15.	Fig legend	error bars are mean +/- SEM	Fig legend	P = 0.9383	Fig legend	df = 25, t = 0.07822	not reported
+ -	S5B	unpaired two-way t-test	Fig legend	13, 13	animals	Fig legend	error bars are mean +/- SEM	Fig legend	P = 0.0043	Fig legend	df = 24, t = 3.156	not reported
+ -	S5C	unpaired two-way t-test	Fig legend	13, 13	animals	Fig legend	error bars are mean +/- SEM	Fig legend	P = 0.2023	Fig legend	df = 24, t = 1.311	not reported
+ -	S5D	unpaired two-way t-test	Fig legend	13, 9, 7, 6, 16, and 13	animals	Fig legend	error bars are mean +/- SEM	Fig legend	P < 0.0001 (left comparison), P < 0.0001 (middle comparison), and P = 0.0011 (right comparison)	Fig legend	For left comparison df = 20 and t = 5.492; for middle comparison df = 11 and t = 6.197; for right comparison df = 27 and t = 3.655	not reported
+ -	S6B	Kolmogorov-Smirnov test	Fig legend	3, 3 animals and 204, 185 axons	animals and axons	Figure legend and methods	size diameter distributions in 0.05 µm bins	Figure and fig legend	P = 0.41	Fig legend	KD-D value = 0.133	not reported
+ -	S6C	unpaired two-way t-test	Fig legend	3, 3 animals and 204,185 axons	animals and axons	Figure legend and methods	error bars are mean +/- SEM	Fig legend	P = 0.98	Fig legend	df = 4 , t = 0.02977	not reported
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Representative figures

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

   If so, what figure(s)?

Yes. All the images are representative.

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 #)?	All representative images except for Supplementary Figures 1 and 2 were used for measurements included in quantitative and statistically tested data.

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.	We have not performed a sample size calculation. The sample numbers used in our experiments are well within the range of studies in our field. This is stated in the final paragraph of the methods section.
2. Are statistical tests justified as appropriate for every figure? Where (section, paragraph #)?	We used the D'Agonstino and Pearson omnibus normality test to determine whether data follow a normal distribution. When normal, we used an unpaired two-tailed t-test. When not normal, we used the Mann-Whitney test.
a. If there is a section summarizing the statistical methods in the methods, is the statistical test for each experiment clearly defined?	Use of the D'Agonstino and Pearson omnibus and t-test/Mann-Whitney test is indicated in the Methods. Each individual figure legend indicates which test was used.
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. Methods section final paragraph
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 #)?	We did not systematically assess the variance within groups.
d. Are tests specified as one- or two-sided?	Two-sided
e. Are there adjustments for multiple comparisons?	Our data did not require use of multiple comparison tests
3. Are criteria for excluding data points reported? Was this criterion established prior to data collection? Where is this described (section, paragraph #)?	No data points were excluded
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 #)?	We used random file name extensions for electron microscopy image acquisition. Randomization was not used in other experiments. Methods paragraph 9

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 #)?	The blinded investigator did not know the group each randomized electron micrograph belonged to. Randomization was not used in other experiments. Methods paragraph 9
6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included? Where (section, paragraph #)?	Experiments complied with the University of Colorado IACUC committee - see Methods paragraph 1
7. Is the species of the animals used reported? Where (section, paragraph #)?	Danio rerio, methods paragraph 1 indicates zebrafish
8. Is the strain of the animals (including background strains of KO/ transgenic animals used) reported? Where (section, paragraph #)?	We use a mix of Tuebingen, AB and TAB as background strains in our transgenics. This has helped to reduce phenotypes that result from in-crossing individual strains, which most likely reflects recessive background mutations. This is not discussed.
9. Is the sex of the animals/subjects used reported? Where (section, paragraph #)?	Sex has not been biologically determined at the larval stage when experiments were performed
10. Is the age of the animals/subjects reported? Where (section, paragraph #)?	Yes, the age post-fertilization is always reported in figure legends and methods sections. This is very important in developmental studies.
11. For animals housed in a vivarium, is the light/dark cycle reported? Where (section, paragraph #)?	This is not reported and irrelevant for embryos & larvae. Adult zebrafish are maintained in a zebrafish "vivarium". Zebrafish researchers consistently use 14:10 light:dark cycle.
12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported? Where (section, paragraph #)?	Zebrafish are maintained in a zebrafish "vivarium" in tanks typically housing 2-40 adults. This is irrelevant for embryos & larvae.
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 #)?	N/A
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 #)?

    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)?

   We used an antibody to myelin basic protein. This antibody produces a signal by immunocytochemistry and western blot that is perfectly consistent with the expected staining pattern and protein band size. We used an additional antibody to sox10.

   a. Is antibody catalog number given?

      Where does this appear (section, paragraph #)?

      See Methods paragraph 3

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

      Where does this appear (section, paragraph #)?

      These antibodies have been used in multiple previously published studies from our lab, and others. These papers are cited in Methods paragraph 3

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

   Where (section, paragraph #)?

   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.

We encourage publication of Data Descriptors (see Scientific Data) to maximize data reuse.

1. Are accession codes for deposit dates provided?

   Where (section, paragraph #)?

   N/A

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.

N/A

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
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? Where (section, paragraph #)?	N/A
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? If not, state area of acquisition.	N/A
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