nature neuroscience

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Manuscript Number:	NN-A50525B	# Supplementary Figures:	11
Manuscript Type:	Article	# Supplementary Tables:	2
		# Supplementary Videos:	9

Reporting Checklist for Nature Neuroscience

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. For more information, please read Reporting Life Sciences Research.

Please note that in the event of publication, it is mandatory that authors include all relevant methodological and statistical information in the manuscript.

Statistics reporting, by figure

- Please specify the following information for each panel reporting quantitative data, and where each item is reported (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 #
+ -	1d	unpaired Student's t test	Results & legend	109, 9 857, 232 3500, 1667	Yes (# fluctuations of each type from 5 mice)	In Fig 1	Yes	In Fig 1	0.0418 <0.00001 0.19532	In Fig 1		
+ -	1e	unpaired Student's t test	Results & legend	109, 9 857, 232 3500, 1667	Yes (# fluctuations of each type from 5 mice)	In Fig 1	Yes	In Fig 1	0.00115 <0.00001 <0.00001	In Fig 1		
+ -	1f	unpaired Student's t test	Results & legend	109, 9 857, 232 3500, 1667	Yes (# fluctuations of each type from 5 mice)	In Fig 1	Yes	In Fig 1	0.06109 0.0001 <0.00001	In Fig 1		
+ -	2c	unpaired Student's t test	Results & legend	20, 21	Yes (# cells from 5 mice)	In Fig 2	Yes	In Fig 2	0.63	In Fig 2		
+ -	2d (1st graph)	unpaired Student's t test	Results & legend	291, 371 3306, 1498	Yes (frequency of pooled fluctuations in proceses from 5 mice)	In Fig 2	Yes	In Fig 2	0.0001	In Fig 2		
+ -	2d (2nd graph)	unpaired Student's t test	Results & legend	291, 371 3306, 1498	Yes (amplitude of fluctuations in processes from 5 mice)	In Fig 2	Yes	In Fig 2	<0.00001	In Fig 2		
+ -	2d (3rd graph)	unpaired Student's t test	Results & legend	291, 371 3306, 1498	Yes (half width of pooled fluctuations in processes from 5 mice)	In Fig 2	Yes	In Fig 2	< 0.00001	In Fig 2		
+ -	2d (4th graph)	unpaired Student's t test	Results & legend	291, 371 3306, 1498	Yes (area of pooled fluctuations in processes from 5 mice)	In Fig 2	Yes	In Fig 2	<0.00001	In Fig 2		
+ -	3a	paired Student's t test	Results & legend	basal intensity from 11 cells, 4 mice	Yes	In Fig 3	Yes	In Fig 3	<0.00001	In Fig 3		
+ -	3b	paired Student's t test	Results & legend	somatic fluctation ferquency from 11 cells, 4 mcie	Yes	In Fig 3	Yes	In Fig 3	>0.05	In Fig 3		
+ -	3c	paired Student's t test	Results & legend	wave frequency from 11 cells, 4 mcie	Yes	In Fig 3	Yes	In Fig 3	<0.00001	In Fig 3		
+ -	3d	paired Student's t test	Results & legend	microdomain ferquency 11 cells, 4 mcie	Yes	In Fig 3	Yes	In Fig 3	<0.00001	In Fig 3		
+ -	4b	paired Student's t test	Results & legend	18 cells from 5 mice	Yes (# cells from 5 mice)	In Fig 4	Yes	In Fig 3	0.00487	In Fig 4		

+ -	4c	paired Student's t test	Results & legend	23 cells from 5 mice	Yes (# cells from 5 mice)	In Fig 4	Yes	In Fig 3	0.26252	In Fig 4		
+ -	4e	paired Student's t test	Results & legend	8 cells from 5 mice	Yes (# cells from 5 mice)	In Fig 4	Yes	In Fig 3	0.0094	In Fig 4		
+ -	4f	paired Student's t test	Results & legend	9 cells from 5 mice	Yes (# cells from 5 mice)	In Fig 4	Yes	In Fig 3	0.0037	In Fig 4		
+ -	5d	Mann-Whitney test	Results & legend	41,5 238, 83 453, 496	Yes (frequency of fluctuations of each type from 5 mice)	In Fig 5	Yes	In Fig 5	0.0197 0.07 0.037	In Fig 5		
+ -	5e	Mann-Whitney test	Results & legend	41,5 238, 83 453, 496	Yes (amplitude of fluctuations of each type from 5 mice)	In Fig 5	Yes	In Fig 5	0.0059 0.97 0.0004	In Fig 5		
+ -	5f	Mann-Whitney test	Results & legend	41,5 238, 83 453, 496	Yes (half width of fluctuations of each type from 5 mice)	In Fig 4	Yes	In Fig 5	0.697 0.0001 0.468	In Fig 5		
+ -	6b (1st graph)	Mann-Whitney test	Results & legend	115, 75 signals from 4 mice	Yes (frequency of fluctuations in endfeet)	In Fig 6	Yes	In Fig 6	0.0357	In Fig 6		
+ -	6b (2nd graph)	Mann-Whitney test	Results & legend	115, 75 signals from 4 mice	Yes (amplitude of fluctuations in endfeet)	In Fig 6	Yes	In Fig 6	<0.00001	in Fig 6		
+ -	6b (3rd graph)	unpaired Student's t test	Results & legend	115, 75 signals from 4 mice	Yes (half width of fluctuations in endfeet)	In Fig 6	Yes	In Fig 6	0.7217	in Fig 6		

Representative figures

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

   If so, what figure(s)?

Fig 1b,c
Fig 2a
Fig 5b,c
Fig 6a
Fig 7a
Fig 8a.

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. The number of times the experiments were repeated and the data for repeatability are all shown in the main text and figures for the paper.

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.

No we did not include a justification for the sample size and were unaware this was required.

We chose the sample size based on the collective experiences of the lead PIs, who have been running labs for 20 years (between them).

2. Are statistical tests justified as appropriate for every figure? Where (section, paragraph #)?	Yes. This is described in detail in the Data Analysis Section.
a. If there is a section summarizing the statistical methods in the methods, is the statistical test for each experiment clearly defined?	Yes.
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. This is described in detail in the Data Analysis Section.
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 #)?	If we understand this question properly, then the answer is No. The way we analyzed the data is described in detail in the Data Analysis Section.
d. Are tests specified as one- or two-sided?	Yes
e. Are there adjustments for multiple comparisons?	No. If we understand this question properly, then we did not make multiple comparisons.
3. Are criteria for excluding data points reported? Was this criterion established prior to data collection? Where is this described (section, paragraph #)?	We did not exclude data points.
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 don't understand this. It does not seem to apply.
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 #)?	No blinding was done.
6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included? Where (section, paragraph #)?	Yes. In the methods.
7. Is the species of the animals used reported? Where (section, paragraph #)?	Yes. In the methods.

8. Is the strain of the animals (including background strains of KO/ transgenic animals used) reported? Where (section, paragraph #)?	Yes. In the methods and throughout the paper.
9. Is the sex of the animals/subjects used reported? Where (section, paragraph #)?	We used either sex. This is stated in the methods
10. Is the age of the animals/subjects reported? Where (section, paragraph #)?	Yes. In the methods and throughout the paper.
11. For animals housed in a vivarium, is the light/dark cycle reported? Where (section, paragraph #)?	Yes. Methods
12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported? Where (section, paragraph #)?	Yes. Methods
13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)? Where (section, paragraph #)?	Yes. Methods
14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported? Where (section, paragraph #)?	Yes. This is reported in the relevant sections of the results. The mice that underwent behavioral evaluations had undergone surgery to deliver AAVs.
a. If multiple behavioral tests were conducted in the same group of animals, is this reported? Where (section, paragraph #)?	Yes this is reported and the data are presented in Fig 8 and the associated text.
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)?

   N/A

   a. Is antibody catalog number given?

      Where does this appear (section, paragraph #)?

      N/A

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

      Where does this appear (section, paragraph #)?

      N/A

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.

   GECIquant software was written specifically for these experiments and was used throughout the study. The script and a user manual are submitted as Supporting information.

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.	Yes it is provided as Supplementary information as the coding script. An executable file for its use with Fiji can be obtained from the corresponding author. We are also happy to provide this via the journal's web site if this is possible.

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	Comment 1: In the large table at the start of this file, we have listed precise p values where possible. In some cases where the p value was less than 0.00001, we simply stated "p < 0.00001". This is because the program we use for analysis (Graphpad Instat) does not give finite values for such low p values. It simply states that p is less than 0.00001, which is what we stated in the table up front. Thank you and bests, Bal Khakh (on behalf of all authors)