nature neuroscience

Corresponding Author:	Viviana Gradinaru	# Main Figures:	5
Manuscript Number:	NN-T58480A	# Supplementary Figures:	8
Manuscript Type:	Technical Report	# Supplementary Tables:	3
		# Supplementary Videos:	1

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 #
+ -	Fig. 2D	unpaired t- test		4	yes, 4 mice per group	Line 385 and Lines 821-823	Error bars are mean +/- SEM shown in main 2d and Supplemental 1a-c show raw data plotted as a cumulative a distribution	Suppl ement al Figure 1 And lines 385-3 86	p = 0.0361, 0.0443, and 0.0071, cortex, striatum and cerebellum, respectively	Lines 378-389	(t(degrees) cortex = t6 = 2.688 striatum = t6 =2.536 cerebellum t6 = 4.007	Cortex = line 379 Striatum = line 379 cerebellu m = line 380
+ -	suppl eme ntal Figur e 1a-c	Kolmogorov -Smirnov test		4	yes, 4 mice per group	Suppleme ntal Documen t Suppleme ntal Figure 1 Legend. Last line	Individual animals are plotted within the same plot to show variability	Outlin ed inside supple menta l figure, top of figure	P ≤ 0.0001	Reported in Supplem ental 1 Figure legends, last line.	cortex = D value = 0.48 striatum = D value = 0.4536 cerebellum = D value = 0.407	NR
+	Fig. 2E DAPI	unpaired t- test		4	yes, 4 mice per group	Line 385 and Lines 821-823	Error bars are mean +/- SEM	lines 385-3 86	p = 0.0034, 0.0541, cortex and striatum, receptively	Line 380-381	cortex = t6 = 4.669 striatum t6 = 2.390	cortex = line 381 striatum = line 382
+	Fig. 2E Neun /Calb	unpaired t- test		4	yes, 4 mice per group	Line 385 and Lines 821-823	Error bars are mean +/- SEM	Lines 385-3 86	p = 0.037, 0.128, and 0.039, cortex, striatum and cerebellum, respectively	Lines 381-382	cortex = t6 = 2.662 sttiatum = t6 = 1.764 cerebellum = t6 = 3.328	cortex = line 383 striatum = 383 cerebellu m = 384
+	Fig. 2E s100	unpaired t- test		4	yes, 4 mice per group	Line 385 and Lines 821-823	Error bars are mean +/- SEM	Lines 385-3 86	p = 0.6738 and 0.1814, cortex and striatum, repsectively	Lines 384-385	cortex = t6 = 0.4422 striatum = t6 = 1.512	cortex = line 385 striatum = line 386
+ -	Fig. 3B	unpaired t- test		3	yes, 3 mice per group	Lines 399-400	Error bars are mean +/- SEM	Lines 399-4 00	p = 0.0014	Line 396	t4 = 7.814	line 397
+ -	Fig. 3C	unpaired t- test		3	yes, 3 mice per group	Lines 399-400	Error bars are mean +/- SEM	Lines 399-4 00	P ≤ 0.0001,	Lines 396-397	t4 = 18.29	line 398
+ -	Supp leme ntal Figur e 6 c and d	unpairted ttest	NR	1 animal per dose, 3 brain sections per dose	yes, 3 sections per group	Line 6 of Suppleme ntal Figure legend 6 and Methods line 803-804	Error bars are mean +/- SEM between the 3 sections	Line 6 of Suppl ement al Figure legen d 6	NR	NR	NR	NR

Representative figures

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

If so, what figure(s)?

 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 #)?

Figure 1c-g Figure 2a-c Figure 3a,d Figure 5b,e Supplemental Figure 3a,b Supplemental Figure 4a-c

Figure 1c-g - (n = 4 individual animals per group), Line 825-826 Figure 2a-c (n = 4 individual animals per group), Lines 827-828 Figure 3a (n = 3 individual animals per group), Lines 827-828 and line 400 Figure 3d (n = 2 animals sections per section of intestine/colon) Lines 400-401 Supplemental Figure 3a,b, NR Supplemental Figure 4a-c NR

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.

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

Where (section, paragraph #)?

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

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 #)?

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

e. Are there adjustments for multiple comparisons?

Not done formally, Sample sizes were chosen based on preliminary data and suggested a large effect size. Lines 821-823

Each figure legend outlines the statistics test used and n number. In addition, statistic section 820-837

Yes, Lines 795-812

parametric for all statistics outlined in figure legends as listed above in the statistical figure reporting. Line 832

Data were not tested for normal distribution due to small sample sizes.

Yes, Fig2 and 3 statistics were all two-sided, specified in the figure legends

No multiple comparisons were made

- 3. To promote transparency, Nature Neuroscience 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 dotplots (with central and dispersion statistics displayed) or to box-andwhisker plots to show data distributions.
- 4. Are criteria for excluding data points reported? Was this criterion established prior to data collection? Where is this described (section, paragraph #)?
- 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 #)?

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 #)?

7. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included?

Where (section, paragraph #)?

8. Is the species of the animals used reported?

Where (section, paragraph #)?

9. Is the strain of the animals (including background strains of KO/ transgenic animals used) reported?

Where (section, paragraph #)?

10. Is the sex of the animals/subjects used reported?

Where (section, paragraph #)?

11. Is the age of the animals/subjects reported?

Where (section, paragraph #)?

12. For animals housed in a vivarium, is the light/dark cycle reported?

Where (section, paragraph #)?

13. For animals housed in a vivarium, is the housing group (i.e. number of No animals per cage) reported?

Where (section, paragraph #)?

Mice were randomly assigned to groups of predetermined sample size. All experiments with direct comparisons were performed in parallel to minimize variability. Lines 632-633. To minimize bias, when direct comparisons for quantification Lines

762-765

Manual cell counting in the striatum and cortex was performed by a blinded observer (Fig. 2e). Automated counting was performed of Purkinje cells in the cerebellum (Fig. 2e). Lines 765-767.

Yes, methods, lines 628-632.

Yes, 798-800

Yes, methods, lines 629-631

Yes, methods, lines 629-631

Yes, methods, line 631-632

Yes, line 640

No

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

Where (section, paragraph #)?

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

Where (section, paragraph #)?

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

Where (section, paragraph #)?

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

Where (section, paragraph #)?

a. How were the criteria for exclusion defined?

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.

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. Cell line identity
 - Are any cell lines used in this paper listed in the database of commonly misidentified cell lines maintained by <u>ICLAC</u> and <u>NCBI Biosample</u>?

Where (section, paragraph #)?

 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. All antibodies used within the study are reported in methods section lines 696-751

1 animal in each group did not show any virus transduction in figure

complete lack of expression was observed, possible reason was due to failed retro orbital injection during virus injection. Line 823-825

2 and were excluded. Line 823-825

complete lack of expression. Line 823-825

Yes. 696-751

NA

No

No

No

No

- 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 #)?

Data availability

Provide a Data availability statement in the Methods section under "Data availability", which should include, where applicable: addgene.com • 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 #)?

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.

custom Matlab and Python scripts were used to assist in data analysis

At the stage of publication, novel capsid sequences, will be deposited to Genbank and all viral plasmids will be deposited to addgene.com

Virus production, line 682

 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.

Human subjects

- Which IRB approved the protocol?
 Where is this stated (section, paragraph #)?
- Is demographic information on all subjects provided?
 Where (section, paragraph #)?
- Is the number of human subjects, their age and sex clearly defined?
 Where (section, paragraph #)?
- Are the inclusion and exclusion criteria (if any) clearly specified?
 Where (section, paragraph #)?
- 5. How well were the groups matched?

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

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

Where (section, paragraph #)?

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

Where (section, paragraph #)?

Additional comments

Additional Comments

Code will be deposited to github near publication.

NA