

Corresponding Author: Matteo Carandini

Manuscript Number: NN-BC52709C

Manuscript Type: Brief Communication

Main Figures: 3

Supplementary Figures: 9

Supplementary Tables: 0

Supplementary Videos: 0

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 #	
+ -	Fig. 2b	The Wilcoxon signed rank test	online Methods, 'Data analysis'	14	yes	main text, para 7	mean +/- sem	main text, para 7	p=0.0006	main text, para 7	not applicable	
+ -	Fig. 2d	The Wilcoxon signed rank test	online Methods, 'Data analysis'	14	yes	main text, para 8	mean +/- sem	main text, para 8	p=0.005	main text, para 8	not applicable	
+ -	Fig. 2e	The Wilcoxon signed rank test	online Methods, 'Data analysis'	14	yes	main text, para 9	mean +/- sem	main text, para 9	p=0.004	main text, para 9	not applicable	
+ -	Fig. 2f	The Wilcoxon signed rank test	online Methods, 'Data analysis'	14	yes	main text, para 9	mean +/- sem	main text, para 9	p=0.0004	main text, para 9	not applicable	
+ -	Fig. 2h	The Wilcoxon signed rank test	online Methods, 'Data analysis'	10	yes	main text, para 12	mean +/- sem	main text, para 12	p=0.002	main text, para 12	not applicable	
+ -	Fig. 2l	The Wilcoxon signed rank test	online Methods, 'Data analysis'	10	yes	main text, para 12	mean +/- sem	main text, para 12	p=0.002	main text, para 12	not applicable	
+ -	Fig. 2j	The Wilcoxon signed rank test	online Methods, 'Data analysis'	10	yes	main text, para 13	mean +/- sem	main text, para 13	p=0.006	main text, para 13	not applicable	
+ -	Fig. 2n	The Wilcoxon signed rank test	online Methods, 'Data analysis'	10	yes	main text, para 13	mean +/- sem	main text, para 13	p=0.002	main text, para 13	not applicable	
+ -	Supplementary Fig. 1f	The Wilcoxon signed rank test	Fig. legend	9,7	yes	Fig. legend	mean +/- sem	Fig. legend	p>0.05	Fig. legend	not applicable	
+ -	Supplementary Fig. 2f	Spearman's rank correlation	Fig. legend	42	yes	Fig. legend	mean +/- sem	Fig. legend	p=0.047	Fig. legend	not applicable	

+	Supplementary Fig. 4d,e,i	The Wilcoxon signed rank test	Fig. legend	8,7,7	yes	Fig. legend	mean +/- sem	Fig. legend	p=0.383, 0.938, 0.375	Fig. legend	not applicable	
+	Supplementary Fig. 8a-c	The Wilcoxon signed rank test	Fig. legend	14, 10, 10, 50, 30, 36, 15	yes	Fig. legend	mean +/- sem	Fig. legend	p=0.4, 0.7, 0.77, 0.009, 0.006, 0.0009, 0.002	Fig. legend	not applicable	

► Representative figures

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

If so, what figure(s)?

No image.

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 #)?

No image.

► 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.

In each test, the alpha level (0.05) was appropriate for the sample size.
In Online Methods, 'Data analysis'.

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

Where (section, paragraph #)?

Yes, in Online Methods, 'Data analysis'.

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

Yes, in Online Methods, 'Data analysis'.

- 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 #)?

Our test is the Wilcoxon signed rank test, which is non-parametric.
In Online Methods, 'Data analysis'.

- 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 #)?

Not applicable

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

Two-sided

e. Are there adjustments for multiple comparisons?	Not applicable
3. Are criteria for excluding data points reported? Was this criterion established prior to data collection? Where is this described (section, paragraph #)?	Two neurons with narrow spikes (presumably PV cells) are not analyzed. In Online Methods, 'Electrophysiology'.
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 compared conditions with and without optogenetic stimulation. These conditions are randomized in order in a single block In Online Methods, 'Visual Stimulation'.
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 experimenter blinding was done, In Online Methods, 'Initial surgery'.
6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included? Where (section, paragraph #)?	Yes, at the beginning of Online Methods.
7. Is the species of the animals used reported? Where (section, paragraph #)?	Yes, in Online Methods, 'In utero electroporation'.
8. Is the strain of the animals (including background strains of KO/transgenic animals used) reported? Where (section, paragraph #)?	Yes, in Online Methods, 'In utero electroporation'.
9. Is the sex of the animals/subjects used reported? Where (section, paragraph #)?	Yes, in Online Methods, 'Initial surgery'.
10. Is the age of the animals/subjects reported? Where (section, paragraph #)?	Yes, in Online Methods, 'Initial surgery'.
11. For animals housed in a vivarium, is the light/dark cycle reported? Where (section, paragraph #)?	Yes, in Online Methods, 'In utero electroporation'.
12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported? Where (section, paragraph #)?	Yes, in Online Methods, 'In utero electroporation'.
13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)? Where (section, paragraph #)?	No behavioral experiments.

14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported?
- Where (section, paragraph #)?
- Yes, in Online Methods, 'In utero electroporation', 'Initial surgery' and 'Pre-recording surgery.'
- a. If multiple behavioral tests were conducted in the same group of animals, is this reported?
- Where (section, paragraph #)?
- Not applicable.
15. If any animals/subjects were excluded from analysis, is this reported?
- Where (section, paragraph #)?
- All the animals were included in our analysis, as long as data could be successfully obtained (from 23 mice).
- In more detail, 48/96 electroporated mice were not considered for data collection because the target for optogenetic stimulation was not clearly visible. In 25 out of the remaining 48 electroporated, data could not be obtained.
- In Online Methods, 'Initial surgery' and 'Electrophysiology'.
- a. How were the criteria for exclusion defined?
- Where is this described (section, paragraph #)?
- Not applicable.
- 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 #)?
- Not applicable.

► Reagents

1. Have antibodies been validated for use in the system under study (assay and species)?
- Not applicable.
- a. Is antibody catalog number given?
- Where does this appear (section, paragraph #)?
- Not applicable.
- b. Where were the validation data reported (citation, supplementary information, Antibodypedia)?
- Where does this appear (section, paragraph #)?
- Not applicable.
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 #)?
- Not applicable.
- 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.
- Not applicable.

- 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 #)?

Not applicable.

► 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 #)?

Not applicable.

► 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.

Not applicable.

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.

Not applicable.

► Human subjects

1. Which IRB approved the protocol?

Where is this stated (section, paragraph #)?

Not applicable.

2. Is demographic information on all subjects provided?

Where (section, paragraph #)?

Not applicable.

3. Is the number of human subjects, their age and sex clearly defined?
Where (section, paragraph #)?
- Not applicable.
4. Are the inclusion and exclusion criteria (if any) clearly specified?
Where (section, paragraph #)?
- Not applicable.
5. How well were the groups matched?
Where is this information described (section, paragraph #)?
- Not applicable.
6. Is a statement included confirming that informed consent was obtained from all subjects?
Where (section, paragraph #)?
- Not applicable.
7. For publication of patient photos, is a statement included confirming that consent to publish was obtained?
Where (section, paragraph #)?
- Not applicable.

► 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?
- Not applicable.
- a. If yes, is the number rejected and reasons for rejection described?
Where (section, paragraph #)?
- Not applicable.
2. Is the number of blocks, trials or experimental units per session and/or subjects specified?
Where (section, paragraph #)?
- Not applicable.
3. Is the length of each trial and interval between trials specified?
- Not applicable.
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.
- Not applicable.
5. Is the task design clearly described?
Where (section, paragraph #)?
- Not applicable.
6. How was behavioral performance measured?
- Not applicable.
7. Is an ANOVA or factorial design being used?
- Not applicable.

8. For data acquisition, is a whole brain scan used?
If not, state area of acquisition.
- Not applicable.
- a. How was this region determined?
- Not applicable.
9. Is the field strength (in Tesla) of the MRI system stated?
- Not applicable.
- a. Is the pulse sequence type (gradient/spin echo, EPI/spiral) stated?
- Not applicable.
- b. Are the field-of-view, matrix size, slice thickness, and TE/TR/flip angle clearly stated?
- Not applicable.
10. Are the software and specific parameters (model/functions, smoothing kernel size if applicable, etc.) used for data processing and pre-processing clearly stated?
- Not applicable.
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 #)?
- Not applicable.
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 #)?
- Not applicable.
13. How were anatomical locations determined, e.g., via an automated labeling algorithm (AAL), standardized coordinate database (Talairach daemon), probabilistic atlases, etc.?
- Not applicable.
14. Were any additional regressors (behavioral covariates, motion etc) used?
- Not applicable.
15. Is the contrast construction clearly defined?
- Not applicable.
16. Is a mixed/random effects or fixed inference used?
- Not applicable.
- a. If fixed effects inference used, is this justified?
- Not applicable.
17. Were repeated measures used (multiple measurements per subject)?
- Not applicable.
- a. If so, are the method to account for within subject correlation and the assumptions made about variance clearly stated?
- Not applicable.
18. If the threshold used for inference and visualization in figures varies, is this clearly stated?
- Not applicable.
19. Are statistical inferences corrected for multiple comparisons?
- Not applicable.

a. If not, is this labeled as uncorrected?

Not applicable.

20. Are the results based on an ROI (region of interest) analysis?

Not applicable.

a. If so, is the rationale clearly described?

Not applicable.

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

Not applicable.

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

Not applicable.

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

Not applicable.

► Additional comments

Additional Comments

Nothing in particular.