nature neuroscience

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Manuscript Number: NN-A46662

Manuscript Type: Article

Main Figures: 8

Supplementary Figures: 8

Supplementary Tables: 1

Supplementary Videos:

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
+ -	1	2 way anova	Results para 1	13,12,14, 10,10,11 13,14,13	mice	Fig legend	mean +/- SEM	fig legend	p=0.034	Results para 1	F4,100 = 2.7	Results para 1

		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 #
+ -	5b	paired t-test	Results section, paragraph 6	8, 12,12 ,11 ,10	neurons	Results section, paragraph 6	mean +/- SEM	Results section, paragraph 6	p=0.0057, p = 0.0014, p = 0.0013, p = 0.0026, p = 0.0028, p = 0.0003	Results section, paragraph 6	t7 = 3.93, t11 = 4.217, t11 = 4.284, t9 = 4.108, F4, 48 = 4.704, t19 = 4.426	Results section paragraph 6
+ -	5f	one-sample t test, one-way ANOVA, unpaired t-test	Results section, paragraph 6	8,12,12,11,10	neurons	Results section, paragraph 6	mean +/- SEM	Results section, paragraph 6	p=0.0057, p = 0.0014, p = 0.0013, p = 0.0026, p = 0.0028, p = 0.0003	Results section, paragraph 6	t7 = 3.93, t11 = 4.217, t11 = 4.284, t9 = 4.108, F4, 48 = 4.704, t19 = 4.426	Results section, paragraph 6
+ -	6b	two way anova	Results section, paragraph 7	11,11,11, 11	mice	fig legend	mean +/- SEM	fig legend	p=0.021	Results section, paragraph 7	F1,40 = 5.80	Results section, paragraph 7
+ -	6c	t test	Results section, paragraph 8	8,8	mice	fig legend	bar-and-whisker plots	fig legend	p = 0.013	Results section, paragraph 8	t14 = 2.84	Results section, paragraph 8
+ -	7	t-test; t-test, repeated one way anova	Results section, paragraph 9	10,9; 8,8; 8,8	rats	fig legend	mean +/- SEM	fig legend	p=0.28; p=0.018; p=0.044	Results section, paragraph 9	t17=0.28; t14=2.32;F1,14= 4.91	Results section, paragraph 9
+ -	8b	two way anova	Results section, paragraph 10	13,14,15, 15,16,15, 11,12	mice	fig legend	mean +/- SEM	fig legend	p=0.0001	Results section, paragraph 10	F3,103 = 20.51	Results section, paragraph 10
+ -	8e	two way anova	Results section, paragraph 11	11,10,10, 12,8,10,1 ,2,11	mice	fig legend	mean +/- SEM	fig legend	p=0.0001	Results section, paragraph 11	F3,76 = 20.00	Results section, paragraph 11
+ -	S1	one way anova	Results section, paragraph 1	13,10,13	mice	fig legend	mean +/- SEM	fig legend	p=0.014	Results section, paragraph 1	F2,33=4.90	Results section, paragraph 1
+ -	S2	two way anova	Results section, paragraph 2	6,6,6,6	mice	fig legend	mean +/- SEM	fig legend	p=0.71	Results section, paragraph 2	F24,180=0.82	Results section, paragraph 2
+ -	S4a	one way anova	Results section, paragraph 4	26,20,18 (pVTA) and 18,14,16 (aVTA)	sections	fig legend	mean +/- SEM	fig legend	p=0.97; p=0.02	Results section, paragraph 4	F2,23 = 0.03; F2,31 = 4.40	Fig legend
+ -	S4b	one way anova	Results section, paragraph 4	16,12,12	sections	fig legend	mean +/- SEM	fig legend	p=0.48	Results section, paragraph 4	F2,21 = 0.75	Fig legend

+ -	S4c	one way anova	Results section, paragraph 4	14,12,15	sections	fig legend	mean +/- SEM	fig legend	p=0.049	Results section, paragraph 4	F2,21 = 0.75	Fig legend
+ -	S4d	one way anova	Results section, paragraph 4	14,19,19	sections	fig legend	mean +/- SEM	fig legend	p=0.020	Results section, paragraph 4	F2,67 = 3.15	Fig legend
+ -	S5	t test	Results section, paragraph 5	6,7	mice	fig legend	mean +/- SEM	fig legend	p=0.020	fig legend	t12=2.7	fig legend
+ -	S7a	t test	Results section, paragraph 6	8,7	neurons	Results section, paragraph 6	mean +/- SEM	Results section, paragraph 6	p = 0.0015, p = 0.0051	Results section, paragraph 6	t7 = 5.048, t6 = 4.298	Results section, paragraph 6
+ -	S7b	one-sample t test, one-way ANOVA, unpaired t-test	Results section, paragraph 6	8,12,12,11,10, 7, 12, 12, 10, 7	neurons	Results section, paragraph 6	mean +/- SEM	Results section, paragraph 6	p=0.0057, p = 0.0014, p = 0.0013, p = 0.0026, p = 0.0028, p = 0.0003, p = 0.0106, p = 0.0014, p = 0.0013, p = 0.1361, 0.0128, p = 0.0045, p = 0.0005	Results section, paragraph 6	t7 = 3.93, t11 = 4.217, t11 = 4.284, t9 = 4.108, F4, 48 = 4.704, t19 = 4.426, t6 = 3.662, t11 = 4.217, t11 = 4.284, t9 = 1.637, t6 = 3.501, F4,43 = 4.404, t15 = 4.372	Results section, paragraph 6
+ -	S8	t test	Results section, paragraph 7	6,8	mice	Results section, paragraph 7	mean +/- SEM		p=0.73	Results section, paragraph 7	t13=0.21	Results section, paragraph 7
+ -							mean +/- SEM					
+ -							mean +/- SEM					
+ -							mean +/- SEM					

Representative figures

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

   If so, what figure(s)?

   yes
   Fig.2, Fig. 3, Fig. 4, Fig. 5, Fig 6, Fig. 7, Fig. S3, Fig. S4, Fig. S5, Fig. S6

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 #)?

   There is no statement in the original manuscript, however, this can be added if needed. All the representative images have been chosen because they reflect the average results observed (not the best looking image). The PCR experiments was replicated 3 times, the in situ was replicated 3 times with 2 techniques (radioactivity and fluorescence), the electrophysiology was replicated in two separate cohorts of mice, the CRF immunohistochemistry has been repeated twice. The ISH/IHC in human was replicated in 3 different human subjects

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.	There is no statement in the original manuscript, however, this can be added if needed. Sample size was determined to be able to detect at least a 50% change in mean with a sigma =30% of the mean. In all the experiments described the N exceed this requirement.
2. Are statistical tests justified as appropriate for every figure? Where (section, paragraph #)?	yes
a. If there is a section summarizing the statistical methods in the methods, is the statistical test for each experiment clearly defined?	statistical tests are defined for every results.
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 #)?	all the test were parametric and tested for normality. page 33
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 #)?	the estimate of variance is represented on each figure by the SEM and were similar for each group of data. the sem is described in each figure
d. Are tests specified as one- or two-sided?	tests are two-sided
e. Are there adjustments for multiple comparisons?	no as there was no exploratory post-hoc analysis requiring such adjustment.
3. Are criteria for excluding data points reported? Was this criterion established prior to data collection? Where is this described (section, paragraph #)?	no data point was 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 #)?	For randomnization. Use of randomness calculator on the website random.org was used to determine order of animals. this is not described in the manuscript but can be added if necessary.
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 #)?	All experiments were performed double blinded. Whether it was brain samples, brain sections or individual, groups were always coded by 2 individual independently and the code was revealed only for the statistical analysis. This statement will be added if necessary.

6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included? Where (section, paragraph #)?	yes, ONLINE METHODS paragraph 1
7. Is the species of the animals used reported? Where (section, paragraph #)?	yes, ONLINE METHODS paragraph 1
8. Is the strain of the animals (including background strains of KO/ transgenic animals used) reported? Where (section, paragraph #)?	yes, ONLINE METHODS paragraph 1
9. Is the sex of the animals/subjects used reported? Where (section, paragraph #)?	yes, ONLINE METHODS paragraph 1
10. Is the age of the animals/subjects reported? Where (section, paragraph #)?	yes, ONLINE METHODS paragraph 1
11. For animals housed in a vivarium, is the light/dark cycle reported? Where (section, paragraph #)?	yes, ONLINE METHODS paragraph 1
12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported? Where (section, paragraph #)?	yes, ONLINE METHODS paragraph 1
13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)? Where (section, paragraph #)?	yes, ONLINE METHODS paragraph 1
14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported? Where (section, paragraph #)?	animals had no prior history
a. If multiple behavioral tests were conducted in the same group of animals, is this reported? Where (section, paragraph #)?	yes, figure 6 and 8
15. If any animals/subjects were excluded from analysis, is this reported? Where (section, paragraph #)?	yes ONLINE METHODS paragraph statistics
a. How were the criteria for exclusion defined? Where is this described (section, paragraph #)?	Only animals with correct cannulae placement were included ONLINE METHODS paragraph 4

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 #)?

Different numbers of animals between groups and between the beginning and end of the study are a result of the loss of data due to improper cannula placement, improper brain perfusion, damaged brain samples, or computer failure during testing
OLINE METHODS Paragraph statistics

Reagents

1. Have antibodies been validated for use in the system under study (assay and species)?

yes, the CRF antibody has been tested in a transgenic rat expressing GFP only in CRF neurons, and this antibody had virtually 100% colocalization with GFP. 100% of GFP colocalized with CRF and vice versa.

a. Is antibody catalog number given?

Where does this appear (section, paragraph #)?

yes online methods paragraph 8 & 9

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

Where does this appear (section, paragraph #)?

no but this can be added

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 #)?

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.

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. Is computer source code/software provided with the paper or deposited in a public repository? Indicate in what form this is provided or how it can be obtained.

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 #)?

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?

    a. If yes, is the number rejected and reasons for rejection described?

    Where (section, paragraph #)?

2. Is the number of blocks, trials or experimental units per session and/or subjects specified?

    Where (section, paragraph #)?

3. Is the length of each trial and interval between trials specified?

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.

5. Is the task design clearly described?

    Where (section, paragraph #)?

6. How was behavioral performance measured?

7. Is an ANOVA or factorial design being used?

8. For data acquisition, is a whole brain scan used?

    If not, state area of acquisition.

    a. How was this region determined?

9. Is the field strength (in Tesla) of the MRI system stated?

    a. Is the pulse sequence type (gradient/spin echo, EPI/spiral) stated?

    b. Are the field-of-view, matrix size, slice thickness, and TE/TR/flip angle clearly stated?

10. Are the software and specific parameters (model/functions, smoothing kernel size if applicable, etc.) used for data processing and pre-processing clearly stated?

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 #)?

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 #)?

13. How were anatomical locations determined, e.g., via an automated labeling algorithm (AAL), standardized coordinate database (Talairach daemon), probabilistic atlases, etc.?

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