nature neuroscience

Corresponding Author:	Robert C. Froemke
Manuscript Number:	NN-A47214B
Manuscript Type:	Article

# Main Figures:	8
# Supplementary Figures:	13
# 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.
- 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 page 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, and 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?	PAGE	EXACT VALUE	DEFINED?	PAGE	REPORTED?	PAGE	EXACT VALUE	PAGE	VALUE	PAGE
example	1a	one-way ANOVA	4	9, 9, 10, 15	mice from at least 3 litters/group	4	error bars are mean +/- SEM	4	p = 0.044	4	$F(3, 36) = 2.97$	4
example	results, pg 6	unpaired t-test	6	15	slices from 10 mice	6	error bars are mean +/- SEM	6	p = 0.0006	6	$t(28) = 2.808$	6
+ -	1	Student's unpaired two-tailed t-test	39-40	1f, filled: 34 neurons from 18 rats; open: 20 neurons from 8 rats	yes	39-40	error bars are mean +/- s.e.m.	39-40	1c: p=10-8; 1d: p=0.3; 1e: p=0.004; 1f: left p=0.0009, p=0.02 and p=0.3, p=0.4, right p=0.02, p=10-4, p=0.2, p=0.2	39-40	1c: z=3.0; 1d: z=-0.3; 1e: z=1.1, z=1.8, z=3.4; 1f: z=3.1±0.8, z=−0.2 ±0.1, z=2.0±0.5, z=1.6±0.6, z=−0.7 ±0.2, z=2.9±0.4	39-40
+ -	2	Student's unpaired two-tailed t-test	40	representative recordings	n/a	8-9	error bars are mean +/- s.e.m.	40	2c: p=10-8 and p=10-5	40	n/a	n/a

	FIGURE NUMBER	TEST USED		n			DESCRIPTIVE STATS (AVERAGE, VARIANCE)		P VALUE		DEGREES OF FREEDOM & F/t/z/R/ETC VALUE	
		WHICH TEST?	PAGE	EXACT VALUE	DEFINED?	PAGE	REPORTED?	PAGE	EXACT VALUE	PAGE	VALUE	PAGE
+ -	3	Student's unpaired two-tailed t-test	41	representative recordings	n/a	9	error bars are mean +/- s.e.m.	41	p=0.001 and p=10-15	41	n/a	n/a
+ -	4a,b	Student's paired two-tailed t-test	41-42	4a,b: current clamp 21 cells early, 7 cells late; voltage clamp 16 cells early, 10 cells late	yes	41-42	error bars are mean +/- s.e.m.	41-42	4a: p=0.0003 early, p=0.01 late; 4b: p=0.03 early, p=0.2 late	41-42	n/a	n/a
+ -	4c,d	Student's paired two-tailed t-test	42-43	4c,d: current clamp 47 cells early and 21 cells late from 21 rats; voltage clamp 40 cells early and 16 cells late from 16 rats	yes	42-43	error bars are mean +/- s.e.m.	42-43	4c: p=10-9 early, p=0.0002 late; 4d: p=10-4 early, p=0.1 late	42-43	n/a	n/a
+ -	4e,f	Student's paired two-tailed t-test	43	4e,f: current clamp 22 cells early and 13 cells late from 13 rats; cell-attached 50 cells early and 16 cells late from 22 rats	yes	43	error bars are mean +/- s.e.m.	43	4e: p=10-5 early, p=0.002 late; 4f: p=0.001 early, p=0.7 late	43	n/a	n/a
+ -	5	Student's paired two-tailed t-test	43-44	5c,e: 16 and 21 neurons, 5d,f: 21 and 12 neurons	yes	43-44	error bars are mean +/- s.e.m.	43-44	5c: p=0.006 and p=0.002; 5d: p=0.004 and p=0.04; 5e: p=0.01 and p=0.9; 5f: p=0.3 and p=0.2	43-44	n/a	n/a
+ -	6	Student's unpaired two-tailed t-test	45-46	6a: 4 and 4 cells from 4 rats; 6b: 6 and 8 cells from 7 rats; 6c: 6 and 8 cells from 6 rats	yes	45-46	error bars are mean +/- s.e.m.	45-46	6a: p=10-5 and p=0.3; 6b: p=0.7 and p=0.0006; 6c: p=10-4 and p=0.3	45-46	n/a	n/a
+ -	7	Student's unpaired two-tailed t-test	46-47	7b: left, 6 cells from 3 rats; right, 5 cells from 4 rats	yes	46-47	error bars are mean +/- s.e.m.	46-47	7b: left, p=0.1; right, p=0.3	46-47	n/a	n/a
+ -	8	Student's paired two-tailed t-test and sliding t-test for 8g-h	47-48	8c: 19 and 6 rats, 8d: 11 and 9 rats, 8g: 11 and 6 rats, 8h: 3 and 3 rats	yes	47-48	error bars are mean +/- s.e.m.	47-48	8c: p=10-4, p=0.5; 8d: p=0.01, p=0.04; 8g-h: p=0.04	47-48	n/a	n/a
+ -	Suppl 1	n/a	n/a	representative images and recordings; f: 3 recordings from 3 rats	yes	Suppl Fig 1	error bars are mean +/- s.e.m.	Suppl Fig 1	n/a	n/a	n/a	n/a
+ -	Suppl 2	Student's unpaired two-tailed t-test	Suppl Fig 2	3-4 recordings from 3-4 rats under each anesthetic	yes	Suppl Fig 2	error bars are mean +/- s.e.m.	Suppl Fig 2	p=0.3, p=0.29, and p=0.4	Suppl Fig 2	n/a	n/a
+ -	Suppl 3	Student's unpaired two-tailed t-test	Suppl Fig 3	filled: 34 neurons from 18 rats; open: 20 neurons from 8 rats	yes	Suppl Fig 3	error bars are mean +/- s.e.m.	Suppl Fig 3	a: p=0.009 and p=0.04, APV: p=0.3, 0.4; b: p=0.2 and p=0.03, APV: p=0.7, 0.8	Suppl Fig 3	n/a	n/a
+ -	Suppl 4	Student's unpaired two-tailed t-test	Suppl Fig 4	a-b: 5 cells from three animals; c-d: 6 cells from three animals	yes	Suppl Fig 4	error bars are mean +/- s.e.m.	Suppl Fig 4	a: p=10-4; b: p=0.3; c: p=0.001; d: p=0.05	Suppl Fig 4	n/a	n/a

July 2013

+/-													
+ -	Suppl 5	Student's paired two-tailed t-test	Suppl Fig 5	Suppl 5c: 6 cells	yes	Suppl Fig 5	error bars are mean +/- s.e.m.	Suppl Fig 5	Suppl 5c left, p=0.03; right, p=0.015	Suppl Fig 5	n/a		n/a
+ -	Suppl 6	Student's paired two-tailed t-test	Suppl Fig 6	n=7	yes	Suppl Fig 6	error bars are mean +/- s.e.m.	Suppl Fig 6	p=0.002, p=0.02, p=0.03, p=0.1	Suppl Fig 6	n/a		n/a
+ -	Suppl 7	Student's unpaired two-tailed t-test	Suppl Fig 7	d: 7 cells from five animals	yes	Suppl Fig 7	error bars are mean +/- s.e.m.	Suppl Fig 7	p=10-6	Suppl Fig 7	n/a		n/a
+ -	Suppl 8	Student's unpaired two-tailed t-test	Suppl Fig 8	d: 52 cells from 24 animals	yes	Suppl Fig 8	error bars are mean +/- s.e.m.	Suppl Fig 8	p=0.3	Suppl Fig 8	n/a		n/a
+ -	Suppl 9	Student's paired two-tailed t-test	Suppl Fig 9	n=3	yes	Suppl Fig 9	error bars are mean +/- s.e.m.	Suppl Fig 9	p=0.6, p=0.6, p=0.1, p=0.3	Suppl Fig 9	n/a		n/a
+ -	Suppl 10	Student's paired two-tailed t-test	Suppl Fig 10	Suppl 10d left 16 cells, right 21 cells	yes	Suppl Fig 10	error bars are mean +/- s.e.m.	Suppl Fig 10	Suppl 10d left p=0.04, right p=0.8	Suppl Fig 10	n/a		n/a
+ -	Suppl 11	Student's paired two-tailed t-test	Suppl Fig 11	a: n=14; b: n=6 and n=5	yes	Suppl Fig 11	error bars are mean +/- s.e.m.	Suppl Fig 11	a: p=0.02, p=10-4, p=0.0002, p=0.0003; b: 10-11, p=0.04, p=0.3	Suppl Fig 11	n/a		n/a
+ -	Suppl 12	Student's paired two-tailed t-test	Suppl Fig 12	Suppl 12c-d: 12 rats, 6 rats	yes	Suppl Fig 12	error bars are mean +/- s.e.m.	Suppl Fig 12	Suppl 12c: p=10-4, p=0.6; Suppl 12d: p=0.01, p=0.8	Suppl Fig 12	n/a		n/a
+ -	Suppl 13	Student's paired two-tailed t-test	Suppl Fig 13	c: n=9 paired and 9 unpaired rats; f: n=7 paired and 4 unpaired rats	yes	Suppl Fig 13	error bars are mean +/- s.e.m.	Suppl Fig 13	c: unpaired p=0.5, paired p=0.0009; f:unpaired p=0.02, paired p=0.0009	Suppl Fig 13	n/a		n/a

▶ Representative figures

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

   If so, what figure(s)?

   > Yes: Fig 1c-e, 2b-c, 3a-b, 5a-b, 5e-f, 6a-c, 8a-b, 8e-f, and Supplementary Figs 1a-f, 2a-b, 3a, 3b, 5a-b, 6a-c, 7a-b, 8a-c, 9a, 10a-b, 11a-b, 12b, 13a-b, 13d-e

2. For each representative image, is there a clear statement of how many time s this experiment was successfully repeated and a discussion of any limitations in repeatability?

   If so, on what page(s) is this reported?

   > Yes, each representative experiment is followed by summary data, in most cases showing each individual experiment. This is reported throughout the manuscript and in the legends, and summarized in the statistics in the table above.

▶ Statistics and general methods

1. Is there a justification of the sample size?

   If so, how was it justified?

   On what page(s)?

   Even if no sample size calculation was performed, authors should report why the sample size is adequate to measure their effect size.

   > Yes, power analyses are performed for the behavioral studies as mentioned on page 31, and n's given throughout for physiology are standard sample sizes (3-21) or even higher in the case of Figure 4.

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

   On what page(s)?

   We use Student's t-tests throughout, using paired tests when we are comparing the same cell or animal before/after locus coeruleus pairing.

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

   This is integrated into the specific sections describing analysis of the electrophysiological and behavioral experiments, and the specific statistical test also mentioned in the figure legends.

   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?

   Yes, this is the standard assumption for these data. This is described in the methods.

   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?

   In all cases we report mean and s.e.m. This is described in the methods and in every figure legend.

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

   Yes, two-sided (tailed) in all cases.

   e. Are there adjustments for multiple comparisons?

   No, this was not required for our experimental design.

3. Are criteria for excluding data points reported?

   Was this criterion established prior to data collection?

   On what page(s) is this described?

   Yes, for physiology we exclude cells based on changes in input/series resistance as described on page 26. For behavior, we describe criteria for advancing animals to further stages of training on page 30.

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.

   On what page(s) does this appear?

   Randomization was not used for electrophysiological experiments as stated on page 29. For behavioral experiments after initial shaping, animals were randomly assigned to different tests as stated on page 30.

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, is a statement to this effect included?

   On what page(s)?

   Blinding was not performed, and this is mentioned in the methods on pages 29 and 31.

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

   On what page(s)?

   Yes, all procedures were approved under NYU IACUC protocols. This is stated on page 24.

7. Is the species of the animals used reported?

   On what page(s)?

   Yes, rats on page 2 and 24.

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

   On what page(s)?

   Yes, Sprague-Dawley and Long-Evans on pages 3, 5, 9, 16, 24, and 30.

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

    On what page(s)?

    > Yes, female on page 24.

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

    On what page(s)?

    > Yes, 3-5 months on page 24.

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

    On what page(s)?

    > Yes, 12/12 hours on page 24.

12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported?

    On what page(s)?

    > Yes, 1-2 animals/cage on page 24.

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

    On what page(s)?

    > Yes, it was done at both times as required by the prolonged time course of our studies. This is reported on page 30.

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

    On what page(s)?

    > Yes, the entire set of procedures for each animal is described in the methods section.

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

        On what page(s)?

        > Yes on page 30.

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

    On what page(s)?

    > Yes, we describe criteria for advancing animals to further stages of training on pages 29-30.

    a. How were the criteria for exclusion defined?

        Where is this described?

        > Animals had to achieve hit rates of 80% within 6 weeks of training, and were also excluded if their implants fell out. This is described on page 30.

    b. Specify reasons for any discrepancy between the number of animals at the beginning and end of the study.

        Where is this described?

        > n/a

▶ Reagents

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

    > Yes

    a. Is antibody catalog number given?

        On what page(s) does this appear?

        > We use standard antibodies to YFP and TH, page 25.

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

On what page(s) does this appear?

> These are standard antibodies in wide use.

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

On what page(s)?

> n/a

a. Were they recently authenticated?

On what page(s) is this information reported?

> 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.

1. Are accession codes for deposit dates provided?

On what page(s)?

> n/a

▶ Computer code/software

1. Is there any custom algorithm/software that is integral to the study that has not been previously reported?

If so, is this algorithm/software provided in a usable and readable form for the referees?

Indicate in what form this is provided.

> n/a

▶ Human subjects

1. Which IRB approved the protocol?

Where is this stated?

> n/a

2. Is demographic information on all subjects provided?

On what page(s)?

> n/a

3. Is the number of human subjects, their age and sex clearly defined?

On what page(s)?

> n/a

4. Are the inclusion and exclusion criteria (if any) clearly specified?

    On what page(s)?

    n/a

5. How well were the groups matched?

    Where is this information described?

    n/a

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

    On what page(s)?

    n/a

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

    On what page(s)?

    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?

        On what page(s)?

        n/a

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

    On what page(s)?

    n/a

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

    n/a

4. Is a blocked design used?

    If so, is length of blocks specified?

    n/a

5. Is an event-related design being used?

    If so, how was the design optimized?

    n/a

6. Is the task design clearly described?

    Where?

    n/a

7. How was behavioral performance measured?

    n/a

8. Are any planned comparisons being used?

    n/a

    a.   Are they clearly described?

> n/a

    b.   Is an ANOVA used?

> n/a

9. 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

10. 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

11. Is the software used for data processing and pre-processing clearly stated?

> n/a

12. For any anatomical imaging, is the coordinate space defined?

> n/a

13. How was the brain image template space, name, modality and resolution determined?

> n/a

14. How were anatomical locations determined?

> n/a

15. Is the statistical model and estimation method clearly described?

> n/a

16. Were any additional regressors (behavioral covariates, motion etc) used?

> n/a

17. Is the contrast construction clearly defined?

> n/a

18. Is a mixed/random effects or fixed inference used?

> n/a

    a.   If fixed effects inference used, is this justified?

> n/a

19. 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

20. If the threshold used for inference and visualization in figures varies, is this clearly stated?

> n/a

21. Are statistical inferences corrected for multiple comparisons?

> n/a

    a.   If not, is this labeled as uncorrected?

> n/a

22. 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

23. Is there correction for multiple comparisons within each voxel? n/a

24. For cluster-wise significance, is the cluster-defining threshold and the corrected significance level defined? n/a

▶ Additional comments

Additional Comments n/a