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Main Figures: 6

Supplementary Figures: 6

Supplementary Tables: 0

Supplementary Videos: 2

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 #	
+ -	4c	Mann-Whitney U	Fig. legend	26 hemispheres	from 15 birds	Fig legend	Tukey box plots, first and third quartile, median, mean and 1.5 interquartile range	Fig legend	p=0.0009; p = 0.0006	fig legend	Z = -3.31; Z = 3.44	fig legend
+ -	4d	Mann-Whitney U	Fig. legend	5,8	13 birds	results line 220-222	Tukey box plots, first and third quartile, median, mean and 1.5 interquartile range	results line 220-222	p = 0.01	results line 220-222	U = -3.81, Z = 2.56	results line 220-222
+ -	5e	unpaired t-test	Fig legend	7,7 7,7	14 birds	fig legend	scatter plots with mean values	figure legend	p = 0.0004 p = 0.07	fig legend	t(12) = -4.8 t(12) = 1.9	fig legend
+ -	5f	paired t-test	fig legend	13 syllables	12 birds	fig legend	Tukey box plots, first and third quartile, median and 1.5 interquartile range	fig legend	p = 0.0019	fig legend	t(11) = -4.0	fig legend
+ -	6f	paired t-test	fig legend	5	learning rates from 5 birds pre & post	fig legend	Tukey box plots, first and third quartile, median, mean and 1.5 interquartile range	fig legend	p = 0.008	fig legend	t(4) = 4.8	fig legend
+ -	Fig 1e	two-sample t test	Figure legend	5, 9, 6 cells	cells from 3, 5, 2 birds	Figure legend	error bars are mean +/- SEM	Figure legend	0.005, 0.02	Figure legend	t(10) = 3.5, t(9) = 2.6	Figure legend
+ -	Fig 1f	two-sample t test	Figure legend	7, 9, 7 cells	cells from 4, 5, 3 birds	Figure legend	error bars are mean +/- SEM	Figure legend	0.007, 0.03	Figure legend	t(10) = 3.3, t(6) = 2.7	Figure legend
+ -	Fig 1g	two-sample t test	Figure legend	7, 9, 7 cells	cells from 4, 5, 3 birds	Figure legend	error bars are mean +/- SEM	Figure legend	0.04	Figure legend	t(7) = 2.5	Figure legend
+ -	Fig 1h	two-sample t test	Figure legend	7, 9, 7 cells	cells from 4, 5, 3 birds	Figure legend	error bars are mean +/- SEM	Figure legend	0.04, 0.01	Figure legend	t(7) = 2.5, t(9) = 3.2	Figure legend
+ -	Sup Fig 5a	paired t-test	fig legend	12 song	12 birds	Figure legend	Tukey box plots, first and third quartile, median and 1.5 interquartile range	Figure legend	0.001	Figure legend	t(11) = 4.34	Figure legend
+ -	Sup Fig 5a	two-sample t-test	Figure legend	24 song	24 birds	Figure legend	Tukey box plots, first and third quartile, median and 1.5 interquartile range	Figure legend	0.02	Figure legend	t(22) = 2.49	Figure legend
+ -	Fig 4f-h	regression	Figure legend	13 birds	song comparison & cell number	figure legend	error bars are mean +/- SEM	figure legend	.004, .9, .25	fig legend	R ² (12) = 0.5, 0.09, 0.03	Fig legend
+ -	Sup Fig 2e	K-S Testt	Figure legend	16, 43 cells	1 bird/condition	figure legend	NA	NA	0.9914	figure legend	KS Stat 0.122	figure legend

► Representative figures

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

If so, what figure(s)?

Fig: 1b-d, 2b-e, 3a-b, 4e, 5b-d, 6b & e
Supp Fig: 2, 3a-e, 4a, 5c-d

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:

1b-d, results par 1-3
 2b-e, results par 4-5
 3a-b, results par 6
 4e, results par 7-8
 5b-d, results par 10-11
 6b & e, results par 12-13
 S2, results par 4
 S3a-e, results par 6
 S4a, results par 9-10
 S5c-d, results par 10

► 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 sample size calculation was performed, however; sample sizes for data shown in figures 4, 5, 6, supp figs 1 and 5 resulted in > 0.95 - 0.99 power of detection in all the shown comparisons.

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

Where (section, paragraph #)?

only standard statistical measures have been used, including paired and unpaired two-sample t tests and nonparametric tests.

- 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)?

yes. described in statistical methods statement

Where is this described (section, paragraph #)?

- c. Is there any estimate of variance within each group of data?

not described

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?

all t-tests are two-tailed

- e. Are there adjustments for multiple comparisons?

no adjustments required

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 dot-plots (with central and dispersion statistics displayed) or to box-and-whisker plots to show data distributions.

Tukey box-plots have been used

4. Are criteria for excluding data points reported?
Was this criterion established prior to data collection?
Where is this described (section, paragraph #)?
- The criteria to discard whole-cell recording, which is shown in the method section, was determined prior to data collection. no other data points were discarded.
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 #)?
- In the whole-cell recording, recorded cells were randomly chosen from retrogradely labeled neurons. Juvenile and adult birds were randomly assigned to experimental and control groups.
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 #)?
- No blinding was done in the whole-cell recording (which was impossible). Cell counts and behavioral analysis was done blind to experimental group
7. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included?
Where (section, paragraph #)?
- It is shown in Methods par 1
8. Is the species of the animals used reported?
Where (section, paragraph #)?
- It is shown in Methods par 1
9. Is the strain of the animals (including background strains of KO/transgenic animals used) reported?
Where (section, paragraph #)?
- NA
10. Is the sex of the animals/subjects used reported?
Where (section, paragraph #)?
- It is shown in Methods par 1
11. Is the age of the animals/subjects reported?
Where (section, paragraph #)?
- it is reported in methods, figure legends and results sections
12. For animals housed in a vivarium, is the light/dark cycle reported?
Where (section, paragraph #)?
- no
13. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported?
Where (section, paragraph #)?
- no
14. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)?
Where (section, paragraph #)?
- no

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

Where (section, paragraph #)?

each experimental is described in the method section

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

Where (section, paragraph #)?

yes, methods and results paragraphs 12-13

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

Where (section, paragraph #)?

NA

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)?

NA

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

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 #)?

NA

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.

- 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 deposition

Provide a Data availability statement in the Methods section under "Data availability", which should include, where applicable:

- 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 #)?

The data that support the findings of this study, and any associated custom software used in data analysis, are available from the corresponding authors upon reasonable request.

► 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.

Song detection software is freely available. LabVIEW codes for acquisition of imaging frames and sound for calcium imaging experiments is included in Supplemental Software.

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.

NA

▶ Human subjects

1. Which IRB approved the protocol?

Where is this stated (section, paragraph #)?

NA

2. Is demographic information on all subjects provided?

Where (section, paragraph #)?

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

Where (section, paragraph #)?

4. 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 #)?

▶ 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?

NA

- 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