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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.

		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
+ -	2l	Friedman's test	Figure 2 legend	2043	axon terminals in 12 samples from 3 mice	Figure 2 legend	mean +/- SEM (error bars)	Figure 2 legend	p = 0.00002498	Figure 2 legend	X2=24, df = 2	Figure 2 legend

		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 #
+ -	2l	Dunn's multiple comparison test	Figure 2 legend	2043	axon terminals in 12 samples from 3 mice	Figure 2 legend	mean +/- SEM (error bars)	Figure 2 legend	p = 0.0429 VGluT2+VGaT + vs VGluT2+ only	Figure 2 legend	rank sum difference = 12	Figure 2 legend
+ -	2l	Dunn's multiple comparison test	Figure 2 legend	2043	axon terminals in 12 samples from 3 mice	Figure 2 legend	mean +/- SEM (error bars)	Figure 2 legend	p < 0.0001 VGluT2+VGaT + vs VGaT+ only	Figure 2 legend	rank sum difference = 24	Figure 2 legend
+ -	2l	Dunn's multiple comparison test	Figure 2 legend	2043	axon terminals in 12 samples from 3 mice	Figure 2 legend	mean +/- SEM (error bars)	Figure 2 legend	p = 0.0429 VGluT2+ only versus VGaT+ only	Figure 2 legend	rank sum difference = 12	Figure 2 legend
+ -	4e	Paired t-test	Figure 4 legend	4	neurons held at -70 mV from 4 mice	Figure 4 legend	mean +/- SEM (error bars)	Figure 4	p = 0.026	Figure 4 legend	t = 4.1, df = 3	Figure 4 legend
+ -	4e	Paired t-test	Figure 4 legend	5	neurons held at -50 mV from 5 mice	Figure 4 legend	mean +/- SEM (error bars)	Figure 4 legend	p = 0.5601	Figure 4 legend	t = 0.64, df = 4	Figure 4 legend
+ -	4f	Paired t-test	Figure 4 legend	10	neurons held at -50 mV from 8 mice	Figure 4 legend	mean +/- SEM (error bars)	Figure 4 legend	p = 0.0002	Figure 4 legend	t = 6.2, df = 9	Figure 4 legend
+ -	4f	Paired t-test	Figure 4 legend	5	neurons held at -70 mV from 5 mice	Figure 4 legend	mean +/- SEM (error bars)	Figure 4 legend	p = 0.0437	Figure 4 legend	t = 2.9, df = 4	Figure 4 legend
+ -	4h	Paired t-test	Figure 4 legend	6	neurons held at -70 mV from 3 mice	Figure 4 legend	mean +/- SEM (error bars)	Figure 4 legend	p = 0.0189	Figure 4 legend	t = 3.4, df = 5	Figure 4 legend
+ -	4h	Paired t-test	Figure 4 legend	6	neurons held at -50 mV from 3 mice	Figure 4 legend	mean +/- SEM (error bars)	Figure 4 legend	p = 0.03	Figure 4 legend	t = 3.0, df = 5	Figure 4 legend
+ -	6f	Paired t-test	Figure 6 legend	5	neurons from 3 mice	Figure 6 legend	Percent change in firing rate	Figure 6 legend	p = 0.1801	Figure 6 legend	t = 1.62, df = 4	Figure 6 legend
+ -	6g	Paired t-test	Figure 6 legend	6	neurons from 3 mice	Figure 6 legend	Percent change in firing rate	Figure 6 legend	p = 0.0245	Figure 6 legend	t = 3.18, df = 5	Figure 6 legend
+ -	6h	Paired t-test	Figure 6 legend	7	neurons from 6 mice	Figure 6 legend	Percent change in firing rate	Figure 6 legend	p = 0.0303	Figure 6 legend	t = 2.995, df = 6	Figure 6 legend
+ -	7e	Paired t-test	Figure 7 legend	7	neurons from 4 mice	Figure 7 legend	Number of spikes	Figure 7 legend	p = 0.0007	Figure 7 legend	t = 5.791, df = 6	Figure 7 legend
+ -	Supp 8b	Paired t-test	Supp Fig 8 legend	23	neurons from 14 mice and rats	Supp Fig 8 legend	mean +/- SEM (error bars)	Supp Fig 8 legend	p = 0.0971	Supp Fig 8 legend	t = 1.733, df = 22	Supp Fig 8 legend
+ -	Supp 8b	Paired t-test	Supp Fig 8 legend	23	neurons from 14 mice and rats	Supp Fig 8 legend	mean +/- SEM (error bars)	Supp Fig 8 legend	p = 0.7624	Supp Fig 8 legend	t = 0.3061, df = 22	Supp Fig 8 legend
+ -	Supp 8d	Two way repeated measures ANOVA	Supp Fig 8 legend	6	neurons from 3 rats	Supp Fig 8 legend	mean +/- SEM (error bars)	Supp Fig 8 legend	p = 0.0005	Supp Fig 8 legend	F = 46.2, df = 1,6	Supp Fig 8 legend

+ -	Supp 8d	Two way repeated measures ANOVA	Supp Fig 8 legend	8	neurons from 3 mice	Supp Fig 8 legend	mean +/- SEM (error bars)	Supp Fig 8 legend	p = 0.0002	Supp Fig 8 legend	F = 43.54, df = 1,8	Supp Fig 8 legend
+ -	Supp 8d	Sidak's multiple comparison test	Supp Fig 8 legend	6	neurons held at -70 mV from 3 rats	Supp Fig 8 legend	mean +/- SEM (error bars)	Supp Fig 8 legend	p = 0.0231 versus TTX	Supp Fig 8 legend	n/a	Supp Fig 8 legend
+ -	Supp 8d	Sidak's multiple comparison test	Supp Fig 8 legend	6	neurons held at -50 mV from 3 rats	Supp Fig 8 legend	mean +/- SEM (error bars)	Supp Fig 8 legend	p = 0.0013 versus TTX	Supp Fig 8 legend	n/a	Supp Fig 8 legend
+ -	Supp 8d	Sidak's multiple comparison test	Supp Fig 8 legend	8	neurons held at -70 mV from 3 mice	Supp Fig 8 legend	mean +/- SEM (error bars)	Supp Fig 8 legend	p = 0.0014 versus TTX	Supp Fig 8 legend	n/a	Supp Fig 8 legend
+ -	Supp 8d	Sidak's multiple comparison test	Supp Fig 8 legend	8	neurons held at -50 mV from 3 mice	Supp Fig 8 legend	mean +/- SEM (error bars)	Supp Fig 8 legend	p = 0.00095 versus TTX	Supp Fig 8 legend	n/a	Supp Fig 8 legend

Representative figures

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

   If so, what figure(s)?

   Figures 1-7 and Supplementary Figures 1-3, 5-9.

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 #)?

   The representative images belong to group data (e.g., that include different animals and neurons). Each figure displaying a representative example shows the group means and standard error of the means. The number of repeating n is described in each figure legend.

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.

For in situ hybridization experiments, ("Data analysis of in situ hybridization studies" section, paragraph 1), the number of analyzed rats was based on previous studies in our lab using radioactive detection of VGluT2 mRNA from rat VTA neurons18,33.

For confocal and electron microscopy experiments ("Fluorescence Microscopy and Three-dimensional Analysis" section, paragraph 1), we analyzed over 2000 mesohabenular axon terminal samples from three mice, which provided statistical power to detect small effects.

For intracellular recording experiments ("Intracellular recordings" section, paragraph 1), light-evoked changes were predicted to either have no change or be abolished by drug application. The expected standard deviation of pre vs. post drug effects used in power calculations was 40, based on analysis of previous results. Using this criteria, a minimum of four pairs of neurons receiving both pre and post drug examination provided adequate power to detect large effects.

For in vivo recording experiments ("Data Analysis" within the "In vivo single-unit recordings of LHb and VTA neuron" section, paragraph 1), sample size was determined as described in the "Intracellular recordings" sections.

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

   Where (section, paragraph #)?

Statistical tests were used in Figures 2,4,6,7, Supplemental Figure 8.

Figure 2 - "Fluorescence Microscopy and Three-dimensional Analysis" section, paragraph 1
Figures 4 and Supplemental Figure 8 - "Intracellular recordings" section, paragraphs 1-2
Figures 6 and 7 - "Statistics" section within "In vivo single-unit recordings of LHb and VTA neurons", paragraph 1

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

Figure 2 - "Fluorescence Microscopy and Three-dimensional Analysis" section, paragraph 1
Figures 4 and Supplemental Figure 8 - "Intracellular recordings" section, paragraphs 1-2
Figures 6 and 7 - "Statistics" section within "In vivo single-unit recordings of LHb and VTA neurons", paragraph 1

   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 #)?

Unless otherwise stated, normality and other assumptions of statistical tests were met. In cases where normality or other assumptions were not met (e.g., sphericity), corrected statistical tests were applied. This is reported for Figure 2 (Fluorescence Microscopy and Three-dimensional Analysis section, paragraph 1) and Figures 6-7 (Statistics section within In vivo single-unit recordings of LHb and VTA neurons, paragraph 1).

   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 #)?

Figure variance (s.e.m.) is described in Figure Legends, Table 1 legend, and Supplemental Figure Legends.

d. Are tests specified as one- or two-sided?	Tests were two-sided.
e. Are there adjustments for multiple comparisons?	Multiple comparisons were made in Figure 2, comparing three groups. No adjustments were made to the Newman-Kuels posthoc test. Multiple comparisons were made in Supplementary Figure 8. Posthoc pariwise comparisons were Sidak adjusted.
3. Are criteria for excluding data points reported? Was this criterion established prior to data collection? Where is this described (section, paragraph #)?	Exclusion criteria were established prior to data collection. Retrogradely labeled neurons localized outside of the VTA were excluded from phenotype analysis (Figure 1; exclusion on "Data analysis of in situ hybridization studies" section, paragraph 1). Neurons recorded outside of the LHb or VTA were excluded from
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 in vitro and in vivo recordings, neurons were randomly sampled ("Intracellular recordings section"; "In vivo recordings" section). Drug tests were randomly assigned ("Intracellular recordings section"; "In vivo recordings" section).
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 #)?	Retrograde tract tracing cell counting was completed blind of injection site ("Data analysis of in situ hybridization studies" section, paragraph 1) and confocal analysis quantification occurred blindly ("Fluorescence Microscopy and Three-dimensional Analysis" section, paragraph 1). Electron microscopy, intracellular and in vivo recordings data collection and analysis were not performed blind to the conditions of the experiments ("Ultrastructural analysis" section, paragraph 1; ."Intracellular recordings" section, paragraph 2; "Data analysis" section, paragraph 1.
6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included? Where (section, paragraph #)?	Animals and surgical procedures section, paragraph 1.
7. Is the species of the animals used reported? Where (section, paragraph #)?	Retrograde tracer injections section, paragraph 1. Virus injections section, paragraph 1. Tissue preparation section, paragraph 1. Confocal and electron microscopy section, paragraph 1. Slice preparation section, paragraph 1. In vivo recordings section, paragraph 1.
8. Is the strain of the animals (including background strains of KO/ transgenic animals used) reported? Where (section, paragraph #)?	Retrograde tracer injections section, paragraph 1. Virus injection section, paragraph 1.
9. Is the sex of the animals/subjects used reported? Where (section, paragraph #)?	Retrograde tracer injections section, paragraph 1. Virus injections section, paragraph 1. Slice preparation section, paragraph 1. In vivo recordings section, paragraph 1.
10. Is the age of the animals/subjects reported? Where (section, paragraph #)?	Virus injections section, paragraph 1.

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

    Where (section, paragraph #)?

Retrograde tracer injections section, paragraph 1.
Virus injections section, paragraph 1.

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

    Where (section, paragraph #)?

Retrograde tracer injections section, paragraph 1.
Virus injections section, paragraph 1.

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

    Where (section, paragraph #)?

No behavioral tests were conducted.

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

    Where (section, paragraph #)?

Surgical details are shown in retrograde tracer injections, paragraph 1 and Virus injections section, paragraph 1. Animals received surgery once and were only used again for the terminal procedure of perfusion or recording.

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

       Where (section, paragraph #)?

No behavioral tests were conducted.

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

    Where (section, paragraph #)?

Animals were not excluded from analysis.

    a. How were the criteria for exclusion defined?

       Where is this described (section, paragraph #)?

n/a

    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 #)?

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?

Where does this appear (section, paragraph #)?

TH - Phenotyping of retrogradely labeled cells by immunocytochemistry and in situ hybridization section, paragraph 1
FG - Phenotyping of retrogradely labeled cells by immunocytochemistry and in situ hybridization section, paragraph 1
mCherry - Immunolabeling for light microscopy section, paragraph 1
VGluT2 - Fluorescence Microscopy and Three-dimensional Analysis section, paragraph 1
VGaT - Fluorescence Microscopy and Three-dimensional Analysis section, paragraph 1
GluR1 - Fluorescence Microscopy and Three-dimensional Analysis section, paragraph 1
GABAA - Fluorescence Microscopy and Three-dimensional Analysis section, paragraph 1
GFP - In vivo recordings section, paragraph 1

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

Where does this appear (section, paragraph #)?

TH - Phenotyping of retrogradely labeled cells by immunocytochemistry and in situ hybridization section, paragraph 1
FG - Phenotyping of retrogradely labeled cells by immunocytochemistry and in situ hybridization section, paragraph 1
VGluT2 - Fluorescence Microscopy and Three-dimensional Analysis section, paragraph 1
VGaT - Fluorescence Microscopy and Three-dimensional Analysis section, paragraph 1
GluR1 - Fluorescence Microscopy and Three-dimensional Analysis section, paragraph 1
GABAA - Fluorescence Microscopy and Three-dimensional Analysis section, paragraph 1
mCherry - Immunolabeling for light microscopy section, paragraph 1.
GFP - in vivo recordings section, paragraph 1

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 #)?

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?

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 #)?

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? — n/a

    Where (section, paragraph #)?

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

    Where (section, paragraph #)?

3. Is the length of each trial and interval between trials specified? — n/a

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. — n/a

5. Is the task design clearly described? — n/a

    Where (section, paragraph #)?

6. How was behavioral performance measured? — n/a

7. Is an ANOVA or factorial design being used? — n/a

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

    If not, state area of acquisition.

    a. How was this region determined? — n/a

9. 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

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

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

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 #)?	n/a
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 #)?	n/a
13. How were anatomical locations determined, e.g., via an automated labeling algorithm (AAL), standardized coordinate database (Talairach daemon), probabilistic atlases, etc.?	n/a
14. Were any additional regressors (behavioral covariates, motion etc) used?	n/a
15. Is the contrast construction clearly defined?	n/a
16. Is a mixed/random effects or fixed inference used?	n/a
a. If fixed effects inference used, is this justified?	n/a
17. 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
18. If the threshold used for inference and visualization in figures varies, is this clearly stated?	n/a
19. Are statistical inferences corrected for multiple comparisons?	n/a
a. If not, is this labeled as uncorrected?	n/a
20. 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
21. Is there correction for multiple comparisons within each voxel?	n/a
22. For cluster-wise significance, is the cluster-defining threshold and the corrected significance level defined?	n/a

Additional comments

Additional Comments