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Last updated by author(s): Jan 9, 2019

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Data were collected with previously published custom MatLab script (Pollak Dorocic, et al. 2014). Cell counts, images and distances where acquired manually with open source software (ImageJ 2.0) and readily available software (Leica Microsystems LAS AF and Zeiss Zen system).
Data analysis	Data were analyzed and visualized with R (version 1.0.153) and MatLab (2018a). Adobe Illustrator CS6 and Photoshop CS6 were used in the production of data figures. The online sunburst diagrams were created using the open online resource available at the Allen Institute for Brain Science (Lein, E.S., et al. Genome-wide atlas of gene expression in the adult mouse brain. Nature 445, 168-176 (2007)), and part of the computer code adapted from Zhang, S., et al. Organization of long-range inputs and outputs of frontal cortex for top-down control. Nature neuroscience 19, 1733-1742 (2016), licensed under the Apache License Version 2.4. 3D-illustrations were constructed from images freely available from the Allen Institute for Brain Science, as accessed through the API. Schematics of coronal sections with detected RV-eGFP labeled neurons were generated using the WholeBrain suite in R (Furth, D., et al. An interactive framework for whole-brain maps at cellular resolution. Nature neuroscience 21, 139-149 (2018)).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data supporting the findings in this study are available in Supplementary Table 1, and from the corresponding author upon request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size was not statistically predetermined, but the number of animals used was based on our previously published work utilizing rabies virus systems for whole-brain tracing of monosynaptic inputs (Pollak Dorocic, I., et al. A whole-brain atlas of inputs to serotonergic neurons of the dorsal and median raphe nuclei. Neuron 83, 663-678 (2014) and Furth, D., et al. An interactive framework for whole-brain maps at cellular resolution. Nature neuroscience 21, 139-149 (2018)).
Data exclusions	Exclusion criteria were not pre-established. Experimental animals with suboptimal targeting of starter neurons to the subregions of the mPFC were excluded. RV-eGFP expressing neurons in the CP, and in the ACB, were excluded from all statistical analyses due to unspecific labeling, which was confirmed experimentally (Supplementary Fig. 5).
Replication	Reproducibility was ensured by sampling from multiple biological replicates, i.e., from multiple mice. No results are included that were not observed in multiple animals.
Randomization	Animals where not randomized due to the necessity of a genetic construct (PV-Cre, SST-Cre, VIP-Cre mice).
Blinding	Investigators were not blind to subject groups because knowledge of experimental conditions was required during data collection and evaluation.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Host, Target, Company, Catalogue #, Lot #, Dilution, Manufacture, Validation profile

Rabbit, Parvalbumin, Swant, PV27 (previously 25), 5.10, 1:1000, https://www.swant.com/pdfs/PV27_Rabbit_anti_Parvalbumin.pdf

Guinea Pig, Parvalbumin, Synaptic systems, 195 004 - 1:1000, https://www.sysy.com/products/parvalbumin/facts-195004.php

Rat, Somatostatin, Millipore, MAB354, 2326265, 1:500, http://www.merckmillipore.com/SE/en/product/Anti-Somatostatin-Antibody-clone-YC7,MM_NF-MAB354

Goat, Choline Acetyltransferase, Millipore, AB144P, 2280814,2558394, 1:300, http://www.merckmillipore.com/SE/en/product/Anti-Choline-Acetyltransferase-Antibody,MM_NF-AB144P

Rabbit, FoxP2, Abcam, ab16046, GR91556-1, 1:1000, https://www.abcam.com/foxp2-antibody-ab16046.html

Rabbit, Calretinin, Swant, 7697 (previously 7699), 18299, 1:1000, https://www.swant.com/pdfs/Rabbit_anti_calretinin_7697.pdf

Goat, V5, Bethyl Lab., A190-119A, A190-119A-4, 1:1000, https://www.bethyl.com/product/A190-119A

Goat, Somatostatin, Santa Cruz, sc-7819, B0413, 1:250, https://www.scbt.com/scbt/product/somatostatin-antibody-d-20?bvstate=pg:2/ct:r
,
Chicken, V5, Abcam, ab9113, GR203305-4, 1:500, https://www.abcam.com/v5-tag-antibody-ab9113.html

Rabbit, VIP, Immunostar, 20077, 1513001, 1:200, http://www.immunostar.com/shop/content/pdf/20077-1744002.pdf

Guinea Pig, Parvalbumin, Swant, GP 72, 1:1000, https://www.swant.com/pdfs/GP72_Guinea_pig_anti_parvalbumin.pdf

Rabbit, GFP, Invitrogen, A6455, 1900253, 1:1000, https://www.thermofisher.com/antibody/product/GFP-Antibody-Polyclonal/A-6455

Rabbit, Calbindin- 28K, Millipore, AB1778, 2390391, 1:500, http://www.merckmillipore.com/SE/en/product/Anti-Calbindin-D-28K-Antibody,MM_NF-AB1778

Mouse, NeuN, Millipore, AB377, 2279235, 1:500, http://www.merckmillipore.com/SE/en/product/Anti-NeuN-Antibody-clone-A60,MM_NF-MAB377

Secondary Antibodies;

Host, Target, Fluorophore, Company Code #, Dilution
Donkey, Rabbit, Cy3, Jackson, 711-165-152, 1:1000
Donkey, Rabbit, Cy5, Jackson, 711-175-152, 1:1000
Donkey, Rabbit, DyLight 405, Jackson, 711-475-152, 1:500
Donkey, Rabbit, DyLight 488, Jackson, 711-545-152, 1:1000
Donkey, Guinea Pig, Cy3, Jackson, 706-165-148, 1:1000
Donkey, Guinea Pig, Cy5, Jackson, 706-175-148, 1:1000
Donkey, Guinea Pig, DyLight 405, Jackson, 706-475-148, 1:500
Donkey, Rat, Cy3, Jackson, 712-165-153, 1:1000
Donkey, Rat, Cy5, Jackson, 712-175-153, 1:1000
Donkey, Goat, Cy3, Jackson, 705-165-147, 1:1000
Donkey, Goat, Cy5, Jackson, 705-175-147, 1:1000
Donkey, Goat, Alexa Fluor 405, Jackson, 705-475-147, 1:500
Donkey, Chicken, Cy3, Jackson, 703-165-155, 1:1000
Donkey, Chicken, Cy5, Jackson, 703-175-155, 1.1000
Donkey, Mouse, Cy3, Jackson, 715-165-151, 1.1000
Donkey, Mouse, Cy5, Jackson, 715-175-151, 1.1000

Validation

The specificity of the primary and secondary antibodies was validated by the manufacturers. Validation profiles for all primary antibodies can be found in the links provided.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

HEK 293T cells were obtained from ATCC (#CRL-11268); BHK-B19G2 and BHK-EnvARGCD2 cells were described in Wickersham et al., Nature Protocols 2010 Mar;5(3):595-606 and were originally derived from BHK-21 cells obtained from ATCC (#CCL-10).

Authentication

Cell lines were not specifically authenticated.

Mycoplasma contamination

Cell lines were tested for mycoplasma contamination by ATCC but not subsequently.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Adult (2-6 months old) male and female wildtype (wt; C57/BL6), PV-Cre (Jax Stock No. 008069), SST-Cre (Jax Stock No. 013044) and VIP-Cre (JAx Stock No. 010908), tdTomato reporter (Jax Stock No. 007905), and Lhx6-eGFP (MMRC Stock No. 000246-MU), mice were used.

Wild animals

This study did not involve wild animals.

Field-collected samples

This study did not involve field-collected samples.

Ethics oversight	All procedures were performed in accordance with the guidelines of the Stockholm Municipal Committee for animal experiments.

Note that full information on the approval of the study protocol must also be provided in the manuscript.