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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 our Editorial Policies 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	Software and core computational analysis to align and process Drop-seq sequencing reads are freely available: https://github.com/broadinstitute/Drop-seq/releases
Data analysis	Published or publicly available algorithms are cited in text and in Extended Data Table 2. Source code to reproduce analysis on http://interneuron.mccarrolllab.org is available on the website. Other custom code available by request. Software and Algorithms Drop-seq_tools - (Macosko et al. 2015) http://mccarrolllab.com/dropseq/ IcaCluster - (Saunders et al., 2018) - http://mccarrolllab.com/wp-content/uploads/ 2018/07/DropSeqIcaCluster_2.0.tar Cross-Species Interneuron Comparison (this paper) - http://interneuron.mccarrolllab.com/ StrataQuest - (6.0.1.188) https://www.tissuegnostics.com/products/software/strataquest Liger - (v.1.0) https://macoskolab.github.io/liger/

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 and 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

GEO accession code for all raw and processed data:
Processed sequencing files – including single-cell DGEs for each region and cluster assignments for marmoset will additionally be available through the NIH's Brain Research through Advancing Innovative Neurotechnologies (BRAIN) Initiative - Cell Census Network (BICCN) at https://biccn.org/. Processed data from all species can also be quickly queried via an interactive web interface we have created at http://interneuron.mccarrolllab.org.

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	No statistical method was used to predetermine sample size. Sample sizes ensured that there were multiple biological and technical replicates for each species and brain region. Total number of interneurons from each species met or exceeded comparable published single-cell datasets (e.g. Saunders et al. 2018, Cell; Hodge et al. 2019, Nature)
Data exclusions	Raw RNA-sequencing reads were quality filtered by standard quality filters (see https://github.com/broadinstitute/Drop-seq/releases); any reads that did not match sample indices were not analyzed. Processed data were further quality filtered to discard cells with small libraries (assessed by number of unique genes and transcripts), that were suspected to be artifacts introduced by PCR or sequencing errors, or were assessed as cell doublets (rare cases in which multiple nuclei are encapsulated in a single droplet). These quality filters were preestablished and followed those used in Saunders et al. (2018) Cell.
Replication	Replication was not attempted due to limited availablity of nonprimate and human donors, though mulitple donors per species were used for each analysis.
Randomization	Randomization was not relevant as no group comparisons were made.
Blinding	Blinding was not relevant as no group comparisons were made.

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 and archaeology
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	Rabbit anti-RFP, Rockland Immunochemicals, cat # 600-401-379, 1:1000 dilution
Validation	This product was prepared from monospecific antiserum by immunoaffinity chromatography using Red Fluorescent Protein (Discosoma) coupled to agarose beads followed by solid phase adsorption(s) to remove any unwanted reactivities. Expect reactivity

against RFP and its variants: mCherry, tdTomato, mBanana, mOrange, mPlum, mOrange and mStrawberry. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-Rabbit Serum and purified and partially purified Red Fluorescent Protein (Discosoma). No reaction was observed against Human, Mouse or Rat serum proteins. (https://rockland-inc.com/store/Antibodies-to-GFP-and-Antibodies-to-RFP-600-401-379-O4L_24299.aspx). Additional citations demonstrating efficacy and specificity in mouse brain tissue: https://www.biocompare.com/9776-Antibodies/344445-AntiRFP-RABBIT-Antibody-Min-X-Hu-Ms-and-Rt-Serum-Proteins/#reviews

Animals and other organisms

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

Laboratory animals	Biospecimen summary is available in Extended Data Table 1 - 12 adult mice (60–70 days old; 5 male, 7 female; C57Blk6/N, Charles River Labs Stock #027) - 10 adult marmosets (1.5–2.3 years old; 4 females, Callithrix jacchus) tissue made available by Dr. G. Feng (Massachusetts Institute of Technology). - Two macaques (9-11 years old, 2 male, Macaca mulatta) tissue made available by Dr. L. Kean (Seattle Children's hospital) - Ferrets (n=2, P42, female, Mustela putorius furo) was sourced from Marshall Bioresources
Wild animals	Study did not involve wild animals
Field-collected samples	Study did not involve samples collected from the field
Ethics oversight	Mouse experiments were approved by and in accordance with Harvard Medical School IACUC protocol number IS00000055-3. Marmoset experiments were approved by and in accordance with Massachusetts Institute of Technology IACUC protocol number 051705020. Macaque experiments approved by University of Washington IACUC, IACUC protocol #4315-02 Ferret was used according to protocols approved by IACUC of Boston Children's Hospital.

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	Brain tissue samples were obtained from 7 postmortem donors (5 female, median age 73. Median postmortem interval: 18 hours). Five donors were used for analysis of striatal interneurons, and two for analysis of neocortical interneurons.
Recruitment	History of psychiatric or neurological disorders was ruled out by consensus diagnosis carried out by retrospective review of medical records and extensive questionnaires concerning social and medical history provided by family members. Several regions from each brain were examined by a neuropathologist. The cohort used for this study did not include subjects with evidence of gross and/or macroscopic brain changes, or clinical history, consistent with cerebrovascular accident or other neurological disorders. Subjects with Braak stages III or higher (modified Bielchowsky stain) were not included. None of the subjects had significant history of substance dependence within 10 or more years of death, as further corroborated by negative toxicology reports.
Ethics oversight	Human frozen tissue was obtained from the Harvard Brain Tissue Resource Center (HBTRC; McLean Hospital) which acquires de-identified postmortem human brain tissue under approval from the Partners Human Research Committee and with permission from legal next-of-kin for the use of brain tissue for research. Federal regulation 45 CFR 46 and associated guidance indicates that the generation of data from de-identified postmortem specimens does not constitute human subjects research requiring institutional review board review. Postmortem tissue collection followed the provisions of the United States Uniform Anatomical Gift Act of 2006 described in the California Health and Safety Code section 7150 and other applicable state and federal laws and regulations.

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