nature research

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Last updated by author(s): Jul 3, 2021

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	A custom software written in C++ (v11) with Qt (v5.7.1) development kit was used for controlling devices in the VISoR2 imaging system and acquiring all of the data used in this study. Micro-Manager programmatic interface (MMCore 8.1.1), OpenCV (v3.4.11), VTK (v8.2) and CUDA (v10) libraries were used in the codes. Custom codes, executables and the user guides of the custom software can be accessed at https://github.com/SMART-pipeline .
Data analysis	Custom software written in Python (v3.7) was used for reconstructing the image volumes. Imaris File Converter 9.2.1 was used for converting reconstructed image volumes to IMS format for visualization in Imaris (v9.1-v9.5) and tracing in Lychnis (v1.3.5). ImageJ 1.49q was also used for image analysis. Freesurfer (v4.5.0) and custom Matlab (R2017a) scripts were used for analyzing MRI images. The source codes, executables and user guides of custom software can be accessed at https://github.com/SMART-pipeline .

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

The complete image datasets (raw and processed) of macaque brains exceed 1 petabyte, and therefore are impractical to be fully uploaded to a public data repository. A fraction of the data are available at http://doi.org/10.5281/zenodo.4451992, including image blocks shown in Fig. 3 and Fig. 4 for tracing and exploring

with Lychnis; or through http://smart.bigconnectome.org, with a browser for viewing full size reconstructed 2D images shown in Fig. 1, Fig. 2 and Supplementary Fig. 3. The subsets related to any figure or video in this work are available upon request with feasible data transfer mechanisms (such as physical hard disk drives, cloud storage, or onsite visiting). Morphological data of 8 mouse MD neurons and 2 RE neurons used in this work were from the publicly available MouseLight dataset with neuron IDs: AA0054 (DOI: 10.25378/janelia.5521765), AA0055 (DOI: 10.25378/janelia.5521768), AA0094 (DOI: 10.25378/janelia.5526661), AA0095 (DOI: 10.25378/janelia.5526664), AA0138 (DOI: 10.25378/janelia.5527288), AA0353 (DOI: 10.25378/janelia.5526664), AA0363 (DOI: 10.25378/janelia.7613897), AA0368 (DOI: 10.25378/janelia.7613912), AA0370 (DOI: 10.25378/janelia.7613921) and AA0371 (DOI: 10.25378/janelia.7613924).

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 sample size dependent statistical testing was performed. Animal number was chosen to minimize the usage of macaques for consideration of animal welfare of primates, in the meantime demonstrating the reproducibility of the imaging and reconstruction technology developed in this study.
Data exclusions	No data was excluded from the analyses.
Replication	We performed whole-brain imaging and reconstruction experiments of 20 mice each sectioned into 40~50 slices for technical development, and 2 macaques each sectioned into 240~250 slices with the imaging system and reconstruction algorithm introduced in this study. Related description are listed in the “Statistics and Reproducibility” in the “Methods” section and in the legends of Supplementary Figs..
Randomization	Randomization was not performed as multiple experimental groups across biological samples were not used in this study.
Blinding	Blinding was not performed as multiple experimental groups across biological samples were not used in this study.

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	1. Primary antibodies: Antigen, Vendor, Catalog#, Host species, Clonality, Dilution Glial Fibrillary Acidic Protein (GFAP), Dako, Z0334, Rabbit, P, 1:100 Anti-Tyrosine Hydroxylase Antibody, Millipore, MAB318, Mouse, M, 1:500 2. Secondary antibodies: Name, Vendor, Catalog#, Host species, Target, Clonality, Dilution Alexa Fluor 647-AffiniPure Donkey Anti-Mouse IgG (H+L), Jackson ImmunoResearch, 715-605-151, Donkey, Mouse, P, 1:200 Alexa Fluor 488-AffiniPure Donkey Anti-Rabbit IgG (H+L), Jackson ImmunoResearch, 711-545-152, Donkey, Rabbit, P, 1:200
Validation	Validation information and literature references can be found in the manufacturers' websites (Anti-Tyrosine Hydroxylase Antibody, https://www.emdmillipore.com/US/en/product/Anti-Tyrosine-Hydroxylase-Antibody-clone-LNC1,MM_NF-MAB318; GFAP, https://www.labome.com/product/Dako/Z0334.html). Antibodies were further validated by comparing antibody signals to established scientific articles that performed immunostaining for the same targets.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	The HEK293 cell line used in this study was purchased from American Type Culture Collection (ATCC).
Authentication	None of the cell lines used in this study were authenticated.
Mycoplasma contamination	All cell lines were tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Animals and other organisms

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

Laboratory animals	Three adult, 10-year-old, male rhesus macaques (Macaca mulatta) were used in this study. Eight-week-old male C57BL/6 and Thy1-YFP-H (Jax: 003782) mice were used in this study for prototyping the sample preparation and imaging methods. Mice used in this study were group-housed with a 12-hour light/dark cycle (lights on at 7 a.m.), controlled temperature (20-22°C) and humidity (50%-70%), and free access to food and water.
Wild animals	This study did not involve wild animals.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	1. Macaques were obtained from the breeding colonies of the Primate Research Center of Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ, CAS), which was accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International). The experimental procedures were approved by the Institutional Animal Care and Use Committees (IACUC) of KIZ, CAS (IACUC No. IACUC18018). 2. All mice experiments were carried out following protocols approved by the Institutional Animal Care and Use Committees of the University of Science and Technology of China (IACUC No. USTCACUC1601018).

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

Magnetic resonance imaging

Experimental design

Design type	Anatomical imaging.
Design specifications	Not relevant as this study did not use functional MRI methods.
Behavioral performance measures	Macaques were anesthetized during MRI imaging for MRI-guided injection and for anatomical segmentation of brain areas according to MRI images.

Acquisition

Imaging type(s)	Structural.
Field strength	3.0 Tesla.
Sequence & imaging parameters	We use gradient echo scanning sequence with spiral image type. Scanning field of view is 120 * 120 * 180 mm. Matrix size is 480 * 480 * 180 in pixel. Slice thickness is 0.5 mm. Orientation is zero. Flip angle is 10 degree.
Area of acquisition	A whole brain scan was used.

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software	AFNI 16.3.08, ANTs 2.1.0, and FreeSurfer 4.5.0 were used.
Normalization	Structural MRI volumes were nonlinearly co-registered to template, and the inverse transform was used to project anatomic areas from atlas to individual brain.
Normalization template	NIMH Macaque Template (NMT) was the main template. Anatomic areas were defined in D99 template and warped to NMT before further warped into individual brain.
Noise and artifact removal	Non-uniform intensity normalization of FreeSurfer was used.

Volume censoring	Not applicable. No censoring was performed.

Statistical modeling & inference

Model type and settings	Not applicable.
Effect(s) tested	Not applicable.

Specify type of analysis: ☒ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016)	Not applicable. This study did not require inferences.
Correction	Not applicable. This study did not perform data correction.

Models & analysis

n/a	Involved in the study
☒	☐ Functional and/or effective connectivity
☒	☐ Graph analysis
☒	☐ Multivariate modeling or predictive analysis