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Corresponding author(s): DBPR NMETH-A45405A

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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	The detailed sample preparation and imaging methods of mouse brain data were detailed in the Methods section. We use the TeraConverter module of Vaa3D to downsample the raw data, and use the TeraVR module of Vaa3D to reconstruct the single neuron full morphology of fMOST brains. The TeraConverter and TeraVR software is available through the GitHub release page of vaa3d.org (https://github.com/Vaa3D/).
Data analysis	All analysis was done following the algorithms described in detail in the Methods section. The code is written in C++ and is available at https://github.com/Vaa3D/vaa3d_tools/tree/master/hackathon/mBrainAligner.

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 raw and TeraFly converted fMOST image datasets of all mouse brains used in this study, as well as the CCFv3 registered single neuron reconstructions, are available at BICCN's Brain Image Library (BIL) at Pittsburgh Supercomputing Center (www.brainimagelibrary.org). The VISoR whole mouse brain data and LSFM partially imaged brain data will be available upon request. The single neuron reconstructions, the CCFv3 registered version of these reconstructions, as well as 3D

navigation movie-gallery of these data are available at SEU-ALLEN Joint Center, Institute for Brain and Intelligence (https://braintell.org/projects/fullmorpho/). The neuron reconstructions are also released through NeuroMorpho.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	57 fMOST mouse brains with 31 brains contain digitally reconstructed neurons. This is so far all the data collected in the US BRAIN's BICCN project. It's sufficient to lead to a determination that systematic errors surpassed statistical errors.
Data exclusions	We used all 1708 neurons reconstructed so far from 31 fMOST brains to conduct the soma localization analysis. All 63 neurons with somas in VPM and VPL, and all 162 neurons with apical dendrites distributed in the L1 layer of cortex were selected for axon projection analysis. There is no further exclusion criteria was applied. 31 fMOST brains containing reconstructed neurons were excluded from the network training dataset to ensure that there is no overlap between the training and testing datasets.
Replication	We successfully tested against 31 fMOST, 1 VISoR and 1 MRI brains.
Randomization	Not relevant here.
Blinding	All annotators worked in isolation and were blinded to group allocation.

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