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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	X-ray holographic nano-tomography data was acquired using custom code based on TANGO (https://www.tango-controls.org/about-us/#mission) and SPEC (https://www.certif.com/content/spec/) software packages.
Data analysis	X-ray holographic nano-tomography data was reconstructed using custom code written in Octave (https://www.gnu.org/software/octave/) and the PyHST2 software package (https://software.pan-data.eu/software/74/pyhst2). Fourier shell correlation analysis was performed using custom code (https://github.com/jcesardasilva/toupy/tree/master/toupy/resolution). Stitching of XNH image volumes was performed using ImageJ (https://imagej.net, ver. 1.52p) with the BigWarp and BigStitcher plugins. Alignment of serial EM images was performed with AlignTK (https://mmbios.pitt.edu/software#aligntk, ver. 1.0.2). XNH and EM data were aligned to each other using BigWarp (https://imagej.net/BigWarp). Manual data segmentation of XNH and EM images was performed using ITK-snap (www.itksnap.org, ver. 3.6.0) Manual data annotation (tracing) was performed using CATMAID (https://catmaid.readthedocs.io, ver. 2018.11.09-682-g811c25a) and queried using the pyMaid API (https://pymaid.readthedocs.io, https://github.com/schlegelp/pyMaid, ver. 1.1.2). Neuron segmentation was performed using a custom CNN pipeline (see Methods). Ground truth training data was prepared using Brainmaps (Google) and CATMAID. Neurons were reconstructed from segmentation data using Neuroglancer (https://github.com/google/neuroglancer, ver. 1.1.5).

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

Raw XNH image data from this study are available in the following publicly accessible repositories:
1. BossDB (https://bossdb.org/)
   https://bossdb.org/project/Kuan_Phelps2020
2. WebKnossos (https://webknossos.org/)
   wklink.org/8122 XNH_ESRF_mouseCortex_30nm
   wklink.org/7283 XNH_ESRF_mouseCortex_40nm
   wklink.org/9034 XNH_ESRF_drosophilaBrain_120nm
   wklink.org/6724 XNH_ESRF_drosophilaVNC_50nm
   wklink.org/8452 XNH_ESRF_drosophilaLeg_75nm
3. ESRF (https://data.esrf.fr/public/10.15151/ESRF-DC-217728238) (anonymous login)
   doi: doi.esrf.fr/10.15151/ESRF-DC-217728238

See https://lee.hms.harvard.edu/resources for access to skeleton reconstructions via CATMAID

Source data for figures 2e,f,h,i are provided with the paper

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	Fig. 1: Number of samples to be imaged was determined based on available granted beamtime. Fig. 2: (f,g) Number of annotated neurons (n = 261) was calculated to ensure that a large number of sample points (> 30) exist in each of the 4 sublayers (IIa, IIb, III, V). No a priori statistical power calculations were performed to determine sample size but our sample sizes are larger than those reported in previous publications (ref. 27). Fig. 4: Number of reconstructed neurons (n = 100) included most of the large-diameter neurons present in the prothoracic leg nerve that were amenable to rapid proof-reading. This sample was used to qualitatively demonstrate the variety of neuronal morphologies and was not used for statistical tests.
Data exclusions	Samples that had major alignment artifacts due to warping or damage during X-ray imaging were excluded.
Replication	11 samples were imaged (see Extended Data Table 1), including multiple samples of Drosophila brain and mouse cortex. Several of them were imaged multiple times (see Extended Data Table 2), although for different fields of view. Across these samples, were observed reproducible image quality. Due to limitations in synchrotron beamtime, we did not image the same field of view more than once with the same imaging parameters.
Randomization	This study involved detailed anatomical analysis of nervous tissue samples. In most analyses, fundamental organizational principles of neuronal morphology and connectivity were examined, rather than comparing experimental and control samples. Therefore, randomization was not necessary.
Blinding	Our data was not allocated into groups, thus blinding was not applicable.

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

Animals and other organisms

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

Laboratory animals	Mus musculus, C57BL/6J-Tg(Thy1-GCaMP6s)GP4.3Dkim/J, male, 32 weeks and C57BL/6, male, 28 weeks. Housed up to 4 per home cage at normal temperature and humidity on reverse light cycle. Drosophila melanogaster, w1118 background, female, 1-7 day old adults.
Wild animals	No wild animals were used in this study.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	All experimental procedures were approved by the Harvard Medical School Institutional Animal Care and Use Committee and were performed in compliance with the Guide for Animal Care and Use of Laboratory Animals and the animal welfare guidelines of the National Institutes of Health.

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