

Reporting Summary

Nature Portfolio 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 Portfolio 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
<input type="checkbox"/>	<input checked="" type="checkbox"/> The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
<input type="checkbox"/>	<input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
<input type="checkbox"/>	<input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
<input checked="" type="checkbox"/>	<input type="checkbox"/> A description of all covariates tested
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
<input type="checkbox"/>	<input checked="" type="checkbox"/> 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)
<input type="checkbox"/>	<input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
<input checked="" type="checkbox"/>	<input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
<input checked="" type="checkbox"/>	<input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
<input checked="" type="checkbox"/>	<input type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), 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	No commercial or custom code was used for data collection.
Data analysis	Custom code was used to annotate the data (e.g., our proprietary software ConnectionLens, CL). CL was used to register (warp), threshold, and annotate the connectivity data across the cases. In addition, custom code was used to analyze the data annotated with CL (Louvain community detection, generation of color-coded matrices to visualize the community output). This code is available upon request.

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 Portfolio [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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

The data presented in the paper are available upon request.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender N/A

Reporting on race, ethnicity, or other socially relevant groupings N/A

Population characteristics N/A

Recruitment N/A

Ethics oversight N/A

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

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](https://www.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 Sixty-eight 8-week-old C57Bl/6J (Jackson Laboratories) male mice were used (stated in the Methods section). 17 male MORF3 mice were used from our established breeding colony at UCLA (stated in the Methods section). Table 1 shows the number of repeated injections made in each ROI presented in the paper. For the morphology figure, the number of neurons analyzed for all comparisons is also stated.

Data exclusions In a typical neuroanatomy experiment, cases with missed injection sites are used as control and are not excluded.

Replication All of our connectivity data are rigorously validated to ensure reliability to the extent possible. This includes making repeated injections in the same ROIs (presented in Table 1) and validating a large majority of the data, which is discussed throughout the paper.

Randomization N/A

Blinding N/A

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

☒ ☐ Clinical data

☒ ☐ Dual use research of concern

☒ ☐ Plants

Methods

n/a Involved in the study

☒ ☐ ChIP-seq

☒ ☐ Flow cytometry

☒ ☐ MRI-based neuroimaging

Antibodies

Antibodies used Primary antibodies

Antibodies used	<p>PHAL (1:1000 rabbit anti-Phal antibody, Vector Laboratories, #AS-2300) Cre (1:4000 mouse anti-Cre recombinase antibody, EMD Millipore, #MAB3120) V5 [1:1000 anti-V5, Fortis, #A190-119A (goat) or #A190-120A (rabbit)]</p> <p>Secondary antibodies For PHAL (1:500 concentration of anti-rabbit IgG conjugated with Alexa Fluor® 488 or 647; Invitrogen, 488: #A-21206; 647: #A-31573) For Cre (1:500 concentration of anti-mouse IgG conjugated with Alexa Fluor® 488 or 647; Life Technology, 488: #A-21202; 647: #A-31571) For V5 (1:1000 in house conjugated Fab-Setau-647, details described in the Methods section)</p> <p>Cytoarchitectural background staining NeuroTrace 435/455 (1:500; Invitrogen, #N21479) DAPI (1:500, ThermoFisher Scientific, #D1306) Syto 13 (1:500, ThermoFisher Scientific, #S7575) Propidium iodide (1:500, ThermoFisher Scientific, #P1304MP)</p>
Validation	We have extensively used each of these antibodies and have demonstrated through our publications that they all work well (Zingg et al., 2014; Hintiryan et al., 2016; Bienkowski et al., 2018; Foster et al., 2021; Hintiryan et al., 2021; Benavidez et al., 2021).

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	Sixty-eight 8-week-old C57Bl/6J (Jackson Laboratories) male mice were used (stated in the Methods section). 17 male MORF3 mice were used from our established breeding colony at UCLA (stated in the Methods section).
Wild animals	N/A
Reporting on sex	Only male mice were used in the current study. To our knowledge, sexually dimorphic connections cannot be detected at the mesoscale resolution, which was predominantly used in this project, especially for the thalamus (as opposed to more sexually dimorphic regions of the brain). Notably, in the future we will be combining all of our connectome work to generate the most comprehensive and reliable brain-wide connectome and a large majority of our mesoscale connectivity work has been conducted in male mice. Therefore, it was important to remain consistent with the current project.
Field-collected samples	N/A
Ethics oversight	All procedures were conducted in compliance with regulatory standards outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals and institutional guidelines established by the Institutional Animal Care and Use Committee at the University of Southern California (USC) and at the University of California, Los Angeles (UCLA).

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Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A