nature portfolio

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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	
☐	☒	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	No commercial or custom code was used for data collection.
Data analysis	Custom code was employed to annotate and quantify the data using our proprietary software, Outspector, which facilitates imaging registration (warping), threshold setting, annotation, and quantification of connectivity data across cases. Additionally, this code was utilized to construct connectivity matrices. All code used for this study are available at https://github.com/ucla-brain/mpf.

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

Source data are provided in a Source Data file. An online application is available for viewing the reconstructed pathways (https://brain.neurobio.ucla.edu/mpf/). The annotated data for each of the processed MPF cases used in this work is also available at https://github.com/ucla-brain/mpf.

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

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Neuroanatomic tracing experiments (N=129): Male 2-month-old WT (n=95), MORF3 (n=20), VGLUT2 Cre (n=5), CRH Cre (n=5), CRH Cre Ai14 (n=4) Neuroanatomic morphology experiments: 4 male MORF3 mice (2-month-old) CRACM experiments (N=18): ENTl/AUD/DP (n=5, male WT, 2-month old); PIR/AUD/DP (n=6, male WT, 2-month old); DP/PVH (n=7, male, CRH Cre Ai14 mice, 2-month-old) Optogenetic CORT experiments: 15 male WT mice (2-3-month-old) Miniscope calcium imaging experiments (N=12): DP (n=6, male VGLUT2 Cre, 2-month old); ILA (n=6, male VGLUT1 Cre, 2-month old) c-Fos behavior experiments (N=36): WT (n=32, male, 2-3-month-old); TRAP2 CreER Ai14 (n=4, male, 2-3-month-old) Sample sizes for each of the experiments were chosen based on our previous experience and were not predetermined through any statistical measurements. Based on the results of the experiments, sufficient power was achieved to detect significance.
Data exclusions	Neuroanatomic tracing experiments: In a typical neuroanatomy experiment, cases with missed injection sites are used as control and are not excluded. Neuroanatomic morphology experiments: No data exclusions were made. CRACM experiments: Animals that showed recorded neurons outside of the DP were excluded. Optogenetic CORT experiments: five animals were omitted due to missed bilateral DP fibre implantations. Miniscope calcium imaging experiments: No data exclusions were made. c-Fos experiments: No data exclusions were made.
Replication	Neuroanatomic tracing experiments: Tracer injections in each medial prefrontal cortical area were replicated with similar results. Neuroanatomic morphology, CRACM, optogenetic CORT, Miniscope calcium imaging, and cfos experiments were not replicated.
Randomization	Animals used in the optogenetic CORT analysis and c-Fos behavior experiments were randomly assigned to their corresponding groups. Randomization was not performed for the neural tracing experiments since animals were not assigned to different groups. Randomization is necessary for reducing bias and controlling variability. Instead, the data were validated in different ways (see Data Reproducibility section). Randomization was also not necessary for neuroanatomic morphology experiments due to the characteristics of MORF3, we achieve random labeling of neurons and then reconstruct the traceable neurons. Randomization was not necessary for CRACM experiments since there was one group per experiment (ENTl/AUD/DP; PIR/AUD/DP; DP/PVH). Randomization was not necessary for the miniscope calcium imaging experiments since all animals underwent both social and non-social behavior testing.
Blinding	For neuroanatomic tracing, neuroanatomic morphology, CRACM, and miniscope calcium imaging experiments blinding was not necessary because animals were not assigned to different groups. For c-Fos behavior studies, no blinding was necessary to assess the presence or absence of c-Fos activation in the DP. For optogenetic CORT experiments, the researcher assessing the accuracy of the bilateral implant location was blind to the treatment condition of the animal.

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
PHAL (1:1000 rabbit anti-PHAL antibody, Vector Laboratories, #AS-2300)
FG (1:5000 rabbit anti-FG antibody, Millipore Sigma, #AB153-I)
Cre (1:4000 mouse anti-Cre recombinase antibody, EMD Millipore, #MAB3120)
c-Fos (1:1000 rat anti-cFos antibody, Synaptic Systems, 226017)
V5 [1:1000 anti-V5, Fortis, #A190-119A (goat) or #A190-120A (rabbit)]
Oxytocin (1:5000, mouse anti-oxytocin antibody, Millipore Sigma, MAB5296)
Vasopressin (1:5000, rabbit anti-vasopressin antibody, Millipore Sigma, AB1565)
Somatostatin (all information for SS staining)
Streptavidin for biocytin staining (1:1000, ThermoFisher, S21374)

Secondary antibodies
1:500 anti-rabbit IgG conjugated with Alexa Fluor® 488 or 647 (Invitrogen, 488: #A-21206; 647: #A-31573)
1:500 anti-mouse IgG conjugated with Alexa Fluor® 488 or 647 (Life Technology, 488: #A-21202; 647: #A-31571)
1:500 anti-mouse IgG conjugated with Alex Fluor®647 (Jackson ImmunoResearch, #715-605-150)

Cytoarchitectural background staining
NeuroTrace 435/455 (1:500; Invitrogen, #N21479)
DAPI (1:500, ThermoFisher, #D1306)

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

Neuroanatomic tracing experiments: Male 2-month-old WT (n=95), MORF3 (n=20), VGLUT2 Cre (n=5), CRH Cre (n=5), CRH Cre Ai14 (n=4)
Neuroanatomic morphology experiments: 4 male MORF3 mice (2-month-old)
CRACM experiments: ENTl/AUD/DP (n=5, male WT, 2-month old); PIR/AUD/DP (n=6, male WT, 2-month old); DP/PVH (n=7, male, CRH Cre Ai14 mice, 2-month-old)
Optogenetic CORT experiments: 15 male WT mice (2-3-month-old)
Miniscope calcium imaging experiments: DP (n=6, male VGLUT2 Cre, 2-month old); ILA (n=6, male VGLUT1 Cre, 2-month old)
c-Fos experiments: WT (n=32, male, 2-3-month-old); TRAP2 CreER Ai14 (n=4, male, 2-3-month-old)

Wild animals

No wild animals were used in this study.

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. 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

No field-collected samples were used in this study.

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). An ethics statement is also included in the manuscript.

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

Plants

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