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Life Sciences Reporting Summary

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For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study.

For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

▸ Experimental design

1. **Sample size**

   Describe how sample size was determined.

   > No sample size calculations were performed.

2. **Data exclusions**

   Describe any data exclusions.

   > We excluded fluorescence-based reconstructed neurons that showed abrupt terminations in the ipsilateral hemisphere from analysis. Cells with cell bodies in layer 5 of mouse 7 of the MAPseq data were excluded from analysis, as this sample appears t be contaminated by area LM.

3. **Replication**

   Describe the measures taken to verify the reproducibility of the experimental findings.

   > All findings replicated across animals, subject to the sampling inherent in single cell tracing approaches.

4. **Randomization**

   Describe how samples/organisms/participants were allocated into experimental groups.

   > Group allocation is not applicable in this study, as we do not compare across treatment conditions but report only static connectivity properties.

5. **Blinding**

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   > No blinding was used for fluorescence based single neuron reconstructions. The researcher producing the MAPseq sequencing libraries was blinded to sample identity.

   Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. **Statistical parameters**

   For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	A statement indicating how many times each experiment was replicated
☐	☒	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 any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.*
☐	☒	A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒	Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.

▶ Software

7. Software

    Describe the software used to analyze the data in this study.

> All software used to analyses the data in this study are available from GitHub and http://mouse.vision/han2017. This includes custom pipelines for stiching of the 2-photon-tomography imaging data, atlas aligment and single neuron tracing, as well as MAPseq data analysis (extraction of barcode reads from raw sequencing data, error correction, barcode matching and barocode matrix construction) as well as custom analysis code to produce the figures in the manuscript.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

▶ Materials and reagents

8. Materials availability

    Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

> All materials used in this study are available from standard commercial sources, or from addgene.org.

9. Antibodies

    Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

> No antibodies were used in this study.

10. Eukaryotic cell lines

    a. State the source of each eukaryotic cell line used.

> No eukaryotic cell lines were used in this study.

    b. Describe the method of cell line authentication used.

> No eukaryotic cell lines were used in this study.

    c. Report whether the cell lines were tested for mycoplasma contamination.

> No eukaryotic cell lines were used in this study.

    d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

> No eukaryotic cell lines were used in this study.

▶ Animals and human research participants

11. Description of research animals

    Provide all relevant details on animals and/or animal-derived materials used in the study.

> male and female adult (>8 weeks of age) C57BL/6 mice

12. Description of human research participants

    Describe the covariate-relevant population characteristics of the human research participants.

> This study did not involve human research participants.