

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <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>
☒	☐ 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 <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	Two-photon calcium imaging and facial data were collected with Scanbox version 11.0 (Neurolabware) in MATLAB.
Data analysis	All data analysis was performed using MATLAB or Python. Analysis code is available at https://github.com/nguyenr95 . Tracking neurons across days code is available at https://github.com/RichieHakim/ROICaT .

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](#)

Processed imaging and behavioral data is available at <https://research.bidmc.harvard.edu/datashare/DataShareInfo.ASP?Submit=Display&ID=11>. Raw imaging and behavioral data is 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

Life sciences study design

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

Sample size No statistical methods were used to predetermine sample size but our sample sizes were similar to or larger than previous related studies and minimized the number of experimental animals for ethical considerations.

Data exclusions We excluded data in which the targeting of the window did not mostly (>50%) cover visual cortex because our study focused on this region. This criterion was predetermined.

Replication For all experiments, each individual mouse was a replicate (n = 13 mice in total). We replicated the imaged region (lateral visual cortex), behavior, stimulus presentation protocol, and analysis for all mice. We imaged each mouse every day or every other day for 2-14 days.

Randomization Randomization was used when possible. We randomized the sex of mice used for initial surgery. Randomization was not possible for which mice was used for imaging as the surgery had to meet criteria for expression of jGCaMP7s in thousands of neurons across lateral visual cortex. Randomization was not possible for analysis of each mouse as it was completed successively. We used random shuffles in data analysis as needed.

Blinding Experiments did not involve experimenter blinding. The nature of the experiments did not allow for blinding with one experimenter.

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

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	All animals were adult (> P56) transgenic mice. Animals were on a 12-hour dark/light cycle with temperature ranging between 20-22 degrees Celsius and humidity ranging between 30-70%. Strains: Emx1-cre, The Jackson Laboratory; HDC-Cre, The Jackson Laboratory; Vgat-ires-Cre, The Jackson Laboratory.
Wild animals	No wild animals were used in this study.
Reporting on sex	We used 13 mice (8 female, 5 male) in this study. Sex was assigned. Sex was not considered relevant in this study.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	All animal care and experimental procedures were approved by the Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee.

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