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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	Two-photon imaging was performed using ScanImage (v5) software running on MATLAB (2018a). Confocal images were acquired using FluoView software (version 4.02). Electron microscopy images were acquired using Gatan 3View software (version 3.22). Behavioral data was acquired via the continuous monitoring of lever position via a LabJack acquisition device and Ephus (legacy version from original publication) software running on MATLAB (version 2011b, Mathworks) working with custom code running on LabVIEW (version 9.0, National Instruments) to monitor threshold crossing. The behavioral setup was controlled by MATLAB software (Dispatcher).
Data analysis	Analysis of two-photon data was performed using a combination of previously published code from the lab and additional custom code to fit the needs of the current experiments (all in MATLAB), as well as FIJI/ImageJ software (version 2.3, NIH) for reference in dendritic structural analysis. Electron microscopy data was processed and analyzed using a combination of IMOD (version 4.9) running through Cygwin terminal (version 3.3) and Amira (version 2019/2020) software.

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

Data and code available upon request from corresponding author.

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	Sample sizes required for this study were estimated based on pilot studies, but no formal statistical tests were run to pre-determine sample size. However, sample sizes were similar to those reported in similar publications. The intrinsic failure rate of the experiments, the success rate of identifying new dendritic spines over learning, and signal-to-noise ratio of the imaging sensor were critical factors in initial estimates. Pilot experiments were conducted with approximately 5 animals, and sample sizes were estimated accordingly.
Data exclusions	Data were only excluded from the data used in this study if cellular health showed commonly cited signs of deterioration, or if the fluorescent sensor used showed obvious signs of photo-bleaching (i.e visibility was considerably lower on during or across imaging sessions so as to prevent analysis). Parameters for the experiments (e.g. laser power) were selected based on these viability criteria during pilot experiments not presented in this manuscript, whereafter individual occurrences of data exclusion were all recorded and reported in the Methods section under "Data Exclusion"
Replication	Trends in data were evaluated based on pilot experiments (as described in 'Sample Size' above), and reproduction of the data was considered successful if the same trends were observed in the rest of the samples. All of the data presented in this manuscript adhered to this process, and were thus considered successful replications.
Randomization	Animals used in this study were not selected based on any other prerequisite features other than general animal wellbeing (e.g. normal grooming and social behavior, no obvious infections, etc.) for allocation into a particular experimental group. Control experiments presented in this study were performed on randomly selected mice and interleaved with learning-group mice. When possible, equal numbers of mice from each cage were used for each group so as to minimize batch effects of each cohort.
Blinding	When possible, independent analyses were performed blinded. Electron microscopy analyses - after localization of the target portion of the tissue - were performed blinded to the labels from 2-photon data by individuals removed from the 2-photon analysis. Un-blinding was only performed after data analysis was complete. Experimenters were not blinded to data collection conditions, as different hardware or software settings were central to the experimental design. However, decision parameters accessible during the experiments (e.g. dendrite selection) were: 1) optimized for data quality only, 2) conceptually many steps removed from the central parameters used in this study (e.g. movement-relatedness), making it extremely unlikely that the data could be biased in one predictable direction, and 3) impossible to use as a priori prediction criteria due to learning-related changes. Thus, knowledge of conditions is extremely unlikely to produce bias in our measurements. Analysis of 2-photon data was not blinded to the condition of the experiment. This is due to the fact that the primary experimenters also performed all the analysis; the repeated exposure to and familiarity with imaging fields makes blinding to the experiment ID impossible, and since the experimenter could not be blinded to the experiments themselves (for the reasons described above), they cannot be reasonably blinded during analysis. However, ROI drawing and subsequent analyses were compared across experimenters with nearly identical results.

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	All animal procedures were performed in accordance with guidelines set forth and protocols approved by the UCSD Institutional Animal Care and Use Committee and the National Institutes of Health. Mice (mus musculus, c57bl/6, males and females), 6 weeks and older were group-housed in disposable cages with standard bedding in a temperature- and humidity-controlled room (~21°C and 42% humidity) with a reversed ligh cycle (10am-10pm: dark). All experiments were performed during the dark cycle. After surgeries, animals were singly housed. Male and female mice were selected randomly for surgical preparation, with no further selection criteria other than surgery outcome.
Wild animals	This study did not involve wild animals
Field-collected samples	This study did not involve animals collected from the field
Ethics oversight	UCSD Institutional Animal Care and Use Committee

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