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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	All data were collected with commercially available software reported in the methods. More information is available upon request.
Data analysis	Data were analyzed with commercially available, open-source and custom made code. Descriptions of these analyses are found in the methods. In cases that there are published descriptions of the methods, full references are included. Custom 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 datasets generated during and/or analyzed during the current study will be made available upon reasonable request.

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 were not predetermined and based on similar studies in the literature (Beyeler et al., 2016; Namburi et al., 2015). Sample size is reported in the legends and methods.
Data exclusions	Animals were excluded based on histological verification. For the pharmacology experiments involving bilateral cannula injections, all animals with one or both cannula placements not targeting the BLA were excluded from analysis by an experimenter blind to the experimental manipulation. For tone-sucrose association behavior, behavioral outliers were excluded after using Grubbs test (with a P value threshold of 0.05). A total of 4 mice out of 91 mice used in Figure 1g, h were excluded. Data from each individual mouse (with the excluded outliers identified) is available upon request. Grubbs test was also used to discard outliers from the NGS data in Figure E3a and c. Data from one sample was discarded across all 19 samples. Data from each sample (with the outlier identified) is available upon request. In multisite photometry experiments, mice that did not acquire the task were excluded. Two out of 7 mice were excluded based on these criteria for tone-air puff association, and two out of 7 mice were excluded for tone-sucrose association. Data from each individual mouse (with the non-learners identified) is available upon request. In in situ validation of CRISPR-cKO of Nt gene, 6 out of 19 mice from control groups were excluded from the plot due a power outage of our freezer which made these tissues undesirable for in situ hybridization. In situ validation of Vglut2 mRNA was only done on a group of randomly selected mice. Freezing videos recorded during the test session were lost for 5 control and 3 CRISPR mice, therefore, they were excluded from the analysis. For the in vivo electrophysiological recording, 4 control mice and 1 CRISPR mice were excluded due to the optrode placement. One of 6 mice was excluded from the NT sensor in vivo recording due to its fiber placement. One neurons from BLA-CeA group was excluded for the analysis except the basal firing due to the loss of its signal. Other than fiber placement, one of 13 mice was excluded from the terminal photometry experiment due to the poor quality of its anticipatory eye closure video recording.
Replication	Experiments included in Figure 1g and h were repeated twice across two different institutes (MIT and Salk). Experiments included in Figure 2c and d were repeated four times by the same investigator. Experiments included in Figure 3h were repeated 3 times means using 3 coverslips of neuron cultures, each coverslip was analyzed with 30-40 ROIs and the averaged response of 30-40 ROIs are plotted as a single circle, the bars represent average of 3 coverslips. Inset: repeated 3 times means using 3 coverslips of neuron cultures, inset showing one representative coverslip. Experiments included in Figure 3i were repeated 3 times means using 3 coverslips of neuron cultures, each coverslip was analyzed with 30-40 ROIs and the averaged response of 30-40 ROIs are plotted as a single open circle, the solid rectangles represent average of 3 coverslips. Experiments included in Figure E7b were repeated 4 times using 4 batches of HEK293T cells, each time cells were tested in 3 individual wells, each well contains 10 image fields. Experiments included in Figure E7c were repeated 3 times means using 3 coverslips of transfected HEK293T cells and 30-40 cells from each coverslip are analyzed; the lines with shading indicate average and s.e.m. of 30-40 ROIs from a single representative coverslip. Experiments included in Figure E7d were repeated 3 times means using 3 coverslips of transfected HEK293T cells and 30-40 cells from each coverslip are analyzed; the response of each cell are plotted as a single circle and the bars represent average of all cells from 3 coverslips. Experiments included in Figure E7f were repeated 3 times; means measured 3 times using one bath of HEK293T cells stably expressing NT1.0 sensor. The spectrum is plotted using data from 1 representative measurement. Experiments included in Figure E9 were repeated by 4 different investigators across 56 animals with 1-3 cells per animal. Results involving freezing scoring and histological quantifications were independently evaluated by at least two investigators. All other experiments included in the manuscript, except in Figure E1, were repeated with multiple cohorts by multiple investigators. However, data evaluation was not performed independently across each cohort.
Randomization	Mice in each cage were randomly divided into experimental and control groups, with 2 experimental and 2 control mice in cage of 4, or 2 experimental and 1 control, or 1 experimental and 2 control mice in a cage of 3. All the conditioning stimuli were counter-balanced across mice. For experiments involving multiple conditioning boxes, approximately equal number of mice belonging to the experimental and the control groups were conditioned in each box. Same number of mice from experimental and control groups were tested together at a time. For experiments other than those involving mice, samples were randomly allocated into experimental groups.
Blinding	During behavioral testing investigators were not always blind to the group affiliation (experimental vs control) given familiarity with the subjects. However, for histology, optogenetic experiments, and ex vivo electrophysiological recordings, the experimenters were blinded to the group assignment of the animals (experimental vs control). During electrophysiological data processing and analysis experimenters were blinded to the group affiliation until the point that all data was processed such that group comparisons could be made.

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

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	The HEK293T cell line was from ATCC (Cat. CRL-3216).
Authentication	The cell line was authenticated by visual inspection of the cell morphology under microscope and the analysis of the growth curve.
Mycoplasma contamination	The cell line was not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	This study did not involve commonly misidentified cell lines.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Group housed male and female mice of C57 strain, between the ages of 8-20 weeks were used for all the experiments.
Wild animals	No wild animals were used in this study
Field-collected samples	No field-collected samples were used in this study
Ethics oversight	IACUC Salk Institute for Biological studies and MIT

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