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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	NIS Elements software. MotorMonitor Software (Kinder Scientific, Poway, CA). FreezeFrame (Actimetrics, Wilmette, IL). Video camera system (ViewPoint, Lyon, France). nVista HD (Inscopix, Palo Alto, CA). Custom code is available upon request.
Data analysis	GraphPad Prism v7. ImageJ 1.49v, NIH. Synaptic analysis was performed in Igor Pro using freely available routines (https://sites.google.com/site/tarotoolsregister/). FreezeView softwares (Actimetrics, Wilmette, IL). Inscopix Data Processing Software (v1.0.0.2273, Inscopix, Palo Alto, CA). MATLAB (Mathworks, Natick MA). Constrained Non-negative Matrix Factorization optimized for microEndoscopic data (CNMF-E) code from Zhou et al., Elife, 2018. CellReg code from Sheintuch et al., Cell Reports, 2017. 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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

The data that support the findings of this study are available from the corresponding author upon request.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-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 pre-determine sample sizes but our sample sizes are similar to those reported in previous publications (see methods).
Data exclusions	At the end of each behavioral experiment (7 weeks following viral surgery), post-mortem control of viral and fiber optics placement was carried out to ensure appropriate targeting. 1 DIO-ChR2 injected mouse was discarded out of 7 animals (Fig. 5). This animal was used as a home cage control on perfusion day (day 21) for c-Fos analysis). Mice showing no obvious calcium transients were not included for behavioral analysis (approximately 50% of implanted mice). Calcium imaging traces without significant transients were excluded from analyses.
Replication	Several of our experiments were replicated using a complementary technique. Immunohistochemical analysis of c-Fos in DLS was reproduced twice in Supplementary Fig. 2a and Supplementary Fig. 2f-j. Rabies (Fig. 2a-b) and Canine (supplementary Fig. 4b) viral tracing as well as slice electrophysiological recordings (Fig. 2c-g) yielded similar results showing monosynaptic CA3 inputs onto DLS SST-INs. Characterization of SST-INs in DLS was reproduced twice by using immunohistochemical detection of SST (supplementary Fig. 5a) as well as by crossing SST-Cre mice with the reporter line Ai14 (supplementary Fig. 5b). Characterization of SST-INs projections to subcortical regions was reproduced twice by injecting an AAV-DIO-Chr2-eYFP into SST-Cre mice as well as Gad2-Cre mice (supplementary Fig. 5f). In vivo calcium imaging: These data were analyzed using two independent methods following a pre-processing step using Inscopix Data Processing Software (v1.0.0.2273, Inscopix, Palo Alto, CA) for motion correction. The pre-processed movies were then analyzed with (i) "constrained non-negative matrix factorization" (CNMF-E)"algorithm for ROI detection, fluorescence trace extraction followed by cell registration using CellReg (Fig. 3h-k). Alternatively, the videos were analyzed with principal component analysis and individual component analysis (PCA-ICA) algorithm (Inscopix Data Processing Software, v1.0.0.2273, Inscopix, Palo Alto, CA) for ROI detection, fluorescence trace extraction followed by cell registration (supplementary Fig. 6h-k). The control of DLS SST-INs on freezing behavior was replicated by using eNpHR3.0 to show an increase in freezing behavior (Fig. 4h) and ChR2 to demonstrate a decrease in freezing behavior (Fig. 5h).
Randomization	Age-matched, male mice (3-4 months old) were used for behavioral experiments. Cagemates were pseudo-randomly assigned to groups during virus injection (i.e., eYFP vs. ChR2).
Blinding	During testing, investigators were not blind to condition. However, results were replicated across several cohorts. Videos for behavioral scoring (i.e., freezing behavior) were analyzed using FreezeView softwares (Actimetrics, Wilmette, IL) during sessions without light application. For sessions in which mice received photostimulation (silencing), the light coming out of the implants prevented automatic scoring and was therefore independently scored by 2 investigators. Other analyses of behavior such as ambulation in the open-field was scored automatically using MotorMonitor Software (Kinder Scientific, Poway, CA). Anxiety assessment in the elevated plus maze and novelty suppressed feeding tests were carried out by 1 investigator blinded to treatment and/or genotype. Sahay (PI) selected individuals in the lab to perform independent scoring.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Rabbit anti c-fos, Calbiochem PC38, 1:10,000 (Antibodyregistry.org: AB_2106755)(discontinued); different batches of rabbit, Santa Cruz SC52, 1:2,000-1:5,000 (Antibodyregistry.org: AB_2106783)(discontinued); rabbit anti-GFP, Life Technologies A11122, 1:500 (Antibodyregistry.org: AB_221569); chicken anti-GFP, Abcam AB13970, 1:2,000 (Antibodyregistry.org: AB_300798); mouse anti-CB, Swant 300, 1:5,000 (Antibodyregistry.org: AB_10000347); goat anti-SST, Santa Cruz SC7819, 1:400 (Antibodyregistry.org: AB_2302603); mouse anti-CR, Swant 6B3, 1:500 (Antibodyregistry.org: AB_10000320); mouse anti-PV, Millipore MAB1572, 1:2,000 (Antibodyregistry.org: AB_2174013); rabbit anti-NPY, Sigma N9528, 1:10,000 (Antibodyregistry.org: AB_260814); goat anti-Chat, Millipore AB144P, 1:400 (Antibodyregistry.org: AB_11214092); mouse anti-RGS14, UC Davis/ NIH NeuroMAB, 1:400 (Antibodyregistry.org: AB_10698026) . Donkey anti-rabbit, anti-mouse, anti-goat and/or anti-chicken Cy3, FITC or Cy5-coupled secondary antibody (Jackson ImmunoResearch, 1:500).

Validation

All antibodies are widely used and we defined the optimal antibody dilution by performing serial dilutions, including negative controls omitting the primary and secondary antibody. Immunohistological data were compared to in situ hybridization data freely available on the Allen Brain Atlas database and each antibody is referenced in the Antibodyregistry.org.

Animals and other organisms

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

Laboratory animals

Male mice were housed four per cage in a 12 hr (7:00 a.m. to 7:00 p.m.) light/dark colony room at 22°C–24°C with ad libitum access to food and water. Age-matched, male mice (2-4 months old) were used for behavioral experiments. Cagemates were randomly assigned to groups during virus injection. Behavioral experiments took place between 8:00 a.m. and 6:00 p.m. All animals were handled and experiments were conducted in accordance with procedures approved by the Institutional Animal Care and Use Committee at the Massachusetts General Hospital and Boston University in accordance with NIH guidelines.

8 week-old C57BL/6J male mice were purchased from Jackson labs (Bar Harbor, ME). Rosa-CAG-LSL-tdTomato-WPRE::deltaNeo (Ai14) (C57BL/6J) mouse line harbors a loxP-flanked STOP cassette allowing transcription of CAG promoter-driven tdTomato following Cre-mediated recombination. Ai14 was purchased from Jackson labs (stock number 007914). Sst-IRES-Cre knock-in (C57BL/6J) mouse line expresses Cre recombinase in somatostatin-expressing neurons. SST-IRES-Cre was purchased from Jackson labs (stock number 028864). Gad2-IRES-Cre knock-in (C57BL/6J) mouse line expresses Cre recombinase in GAD2-expressing neurons. Gad2-IRES-Cre was purchased from Jackson labs (stock number 028867). Rosa-LSL-Tva-lacZ (mixed 129S6;C57BL/6J) mouse line has a loxed-STOP cassette allowing transcription of avian receptor Tva-lacZ fusion gene following Cre-mediated recombination. Rosa-LSL-TVA-lacZ mouse line was generously provided by Pr. Dieter Saur. Tail DNA from all offspring was genotyped by PCR to detect the presence of each transgene separately. All experiments were conducted with 8-16 week-old mice (unless indicated otherwise).

Wild animals

This study did not contain wild animals

Field-collected samples

This study did not contain field-collected samples