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Reporting Summary

Nature Research 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 Research policies, see <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

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	Cor	ifrmed
	\boxtimes	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	\boxtimes	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	\boxtimes	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.
	\boxtimes	A description of all covariates tested
	\boxtimes	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	\boxtimes	A full description of the statistics including <u>central tendency</u> (e.g. means) or other basic estimates (e.g. regression coefficient) AND <u>variation</u> (e.g. standard deviation) or associated <u>estimates of uncertainty</u> (e.g. confidence intervals)
	\boxtimes	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>
\boxtimes		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
	\boxtimes	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
\boxtimes		Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated
	\boxtimes	Clearly defined error bars State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on <u>statistics for biologists</u> may be useful.

Software and code

Policy information about availability of computer code

Data collection	Animal Behavior: Audacity software (http://www.audacityteam.org) Matlab (R2015b, MathWorks) - custom script Ethovision XT 8 (Noldus Information Technology) ANY-maze (Stoelting Europe)
	Histology and Microscopy:
	Pannoramic Viewer (3D HISTECH Ltd.)
	Developer XD (Definiens AG) - custom script
	Fiji ImageJ (https://imagej.net/)
	ZEN software (Carl Zeiss Microscopy GmbH)
	Electrophysiology:
	Clampfit software (Molecular devices)
	Neural Population Sequencing:
	STAR (Version 2.4.0d)
	TopHat (Version 2.0.9)
	HTSeq (Version 0.5.4p3)

edgeR (Version 3.7)

Calcium Imaging:

Statistics

NVista HD (Version 2, Inscopix)

Data analysis

GraphPad Prism [®] (Version 7, GraphPad Software Inc.)

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 (Fig. 1-5, Supplementary Fig. 3-5, 7-13) are available from the corresponding author upon reasonable request. Data Supporting Fig. 1,2 are from Allen Mouse Brain Connectivity Atlas (http://connectivity.brain-map.org/). For data Supporting Supplementary Fig. 6 are Accession Codes section.

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 predetermine sample sizes. Sample size were similar as reported in previous publications4,31,59,60.			
Data exclusions	Out of the SCH 23390 cohort, 9 out of 31 animals were excluded from the analysis due to misplaced or blocked infusion cannulae.			
Replication	All methods used in the manuscript underwent rigorous testing for reproducibility when established. Histological, electrophysiological and imaging experiments were replicated. Behavioral experiments were replicated using different methodologies.			
Randomization	All animals and samples were randomly assigned to the experimental groups.			
Blinding	Data collection and analysis were not performed blind to the conditions of the experiments. Data was acquired, processed and analyzed by automated workflows, except Fig. 1e, f, Supplementary Fig. 3a, Supplementary Fig. 4a,b, Supplementary Fig. 5a,b, Supplementary Fig. 7a,b,c,d, Supplementary Fig. 10e, Supplementary Fig. 11b.			

Reporting for specific materials, systems and methods

Materials & experimental systems

Methods

n/a	Involved in the study		
\mathbf{X}	Unique biological materials		

Antibodies	

Eukaryotic cell lines

Palaeontology

Animals and other organisms

Human research participants

n/a	Involved in the study

\boxtimes	ChIP-seq
\sim	cim seq

- Flow cytometry
- MRI-based neuroimaging

Antibodies

Antibodies used	Primary antibodies used:	
	mouse antibody to PKCd (BD Biosciences, 610398 1:500, LOT: n/a) published results for immunohistochemistry in mouse	
	chicken antibody to TH (Abcam, ab 7963, 1:1000, LOT: 2387494) published results for immunohistochemistry in mouse	
	goat antibody to SST (Santa-Cruz, sc-7819, 1:500, LOT: n/a) antibody was tested rigorously for functionality in mouse central amygdala	
	rabbit antibody to DsRed (Living Colors, 632496; 1:1000; LOT: n/a, to optimize visualization of AAV::DIO-M3 and AAV::DIO-M4 expression, both tagged with mCherry fluorophore). published results for immunohistochemistry in mouse	
	Secondary antibodies used:	
	Thermo Fisher Scientific, 1:1000; Alexa Fluor published results for immunihistochemistry; validated binding to primary mouse AB	
Validation	Antibodies were pre-established published commercial antibodies.	

Eukaryotic cell lines

Policy information about <u>cell lines</u>			
Cell line source(s)	NIH-3T3		
Authentication	The identity of NIH-3T3line has not been authenticated specifically		
Mycoplasma contamination	The NIH-3T3 cell line has been tested for contamination		
Commonly misidentified lines (See <u>ICLAC</u> register)	No commonly misidentified cell lines were used		

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research			
Laboratory animals	Male C57BL/6J mice (1-4 months old, Charles River Laboratories) Male TH::Cre mice (1-4 months old, Jax #008601) Male PKCδ::GluCl-CRE BAC transgenic mice (1-4 months old, mmrrc #11559) Male SST-IRES-Cre mice (1-4 months old, Jax #13044) Rosa::IoxP-STOP-IoxP-td-Tomato (Jackson #007905) were crossed to either PKCδ::GluCl-CRE BAC transgenic mice or SST-IRES- Cre mice for double transgenic male offsprings TH::Cre mice were crossed to Male PKCδ::GluCl-CRE BAC transgenic mice for double transgenic male offsprings.		
Wild animals	No wild animals were used		
Field-collected samples	No filed collected samples were used		

Flow Cytometry

Plots

Confirm that:

 \bigotimes The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

All plots are contour plots with outliers or pseudocolor plots.

A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

D1R expression in CEI neurons (Supplementary Figure 6):

PKCd::Cre or SST::Cre mice were crossed to Rosa::loxP-STOP-loxP-td-Tomato animals and the offspring used for neural population sequencing. Males (2-5 months old) were decapitated, brains extracted on ice and 1mm thick brain sections cut in ice-cold Hibernate A Low Fluorescence solution (BrainBits). The central amygdala was extracted using biopsy punchers (1mm

	diameter; Integra Miltex) and enzymatically dissociated following standard procedure (Papain Dissociation System, Worthington Biochem). For each animal, the central amygdala was extracted once on each side and then pooled.
	RNAi mediated knock-down efficiency (Supplementary Figure 10): NIH-3T3 cells were stably transfected with a construct expressing a td-Tomato transgene harboring target sites of D1R and several control shRNAs in its 3'UTR These cells were transduced with ASGE vectors harboring either D1R or control shRNAs. Cells were finally collected and td-Tomato reporter knockdown in GFP-shRNA expressing cells was quantified using flow cytometry.
Instrument	BD FACSAria III
Software	BD FACSDiVa Software
Cell population abundance	D1R expression in CEI neurons (Supplementary Figure 6):
	Approximately 1000 td-Tomato+ cells were retrieved with FACS sorting. Proportionally, SST-cre/tdTomato and PKCd-cre/tdTomato mice yielded approximately 20% positive cells. Note that these numbers underestimate the typical fraction of PKCd+ and SST+ cells in CEA, as conservative gating settings were used, to restrict sampling to unambiguously identified positive cells.
	RNAi mediated knock-down efficiency (Supplementary Figure 10):
	Abundance and post-sort fractions are described in Supplementary Figure 8
Gating strategy	D1R expression in CEI neurons (Supplementary Figure 6) : 1st gate: FSC-A vs. SSC-A
	2nd gate: FSC-A vs. FSC-W - singlet/aggregate separation. 3rd gate: mCherry (target channel - 610/20 nm) vs. PE-Cy7 (non-target channel - 780/60 nm) - positive/negative separation in aspect of mCherry, while avoiding high auto-fluorescent events.
	RNAi mediated knock-down efficiency (Supplementary Figure 10): 1st gate: FSC-A vs. SSC-A
	2nd gate: FSC-A vs. FSC-W - singlet/aggregate separation.
	3rd gate: two channels, tdTomato (target channel - 610/20 nm) vs. GFP (non-target channel - 470/90 nm), were split into four groups: GFP pos/tdTomato neg, GFP pos/tdTomato pos, GFP neg/tdTomato pos, GFP neg/tdTomato neg

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.