nature portfolio

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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	Commercial software or open-source software used including: Labview (version 2018), OpenEx software (version 2.36), Bonsai-rx software (version 2.8), Clampfit (version 10.2), Zeiss Zen software (black edition, version 2.3), Spike 2 software (version 7.03). The code for tracking locomotion is available on GitHub using the link: https://github.com/thepenglab/posTracker.
Data analysis	Commercial software or open-source software software used including: GraphPad Prism (version 9), Image J (version 1.52), Zeiss Zen software (black edition, version 2.3), Imaris (version 8), Lychnis (version 1.2.5), MATLAB (version 2017b). Custom codes written in MATLAB for 2D image registration can be found in the following link (https://sites.google.com/view/brain-mapping/home/code)

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

Source data are provided with this paper. Images showing the expression of OPRM1 in pain related regions in the brain, spinal cord and sensory neurons from the OPRM1Cre X Ai14 mice are available on Zenodo: https://zenodo.org/records/17110834

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 calculations were made to predetermine sample size. Sample size was chosen based on literature review (PMID: 30532001, PMID: 30209395, PMID: 33442058).
Data exclusions	None.
Replication	All results were successfully replicated. The number of independent biological replications is indicated in the figure legends.
Randomization	Randomization was performed for all behavioral and in vivo imaging experiments involving group comparisons, with mice randomly allocated to experimental groups prior to testing or imaging. For experiments where randomization was not applicable (e.g., longitudinal imaging of the same animals for multiple days), group allocation was predetermined by experimental design. In these cases, potential covariates including age, sex, and litter were controlled by using age- and sex-matched cohorts and, where applicable, littermate controls.
Blinding	For behavioral experiments, experimenters were not blinded to group allocation because injured animals could be readily identified. Behavioral measures, including CPA score and locomotion, were quantified automatically using custom MATLAB-based tracking software. For c-Fos imaging experiments, investigators were blinded to treatment group during image quantification and data analysis. For fiber photometry and electrophysiology experiments, experimenters were not blinded to genotype because only animals of the required genotype were included by experimental design.

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

Antibodies

Antibodies used

Rabbit anti-mCherry (1:1,000, cat. no. 600401379, lot no. 20070, Rockland)
Rabbit anti-cFos (1:1,000, cat. no. 226003, lot no. 6-76, Synaptic Systems)
Mouse anti-HA (1:1000, cat. no. 901514/901513, lot number B365051, Biolegend)
Goat anti-TPH2 (1:500, cat. no. ab121013, lot no. GR176206-57, Abcam)
Alexa 594 conjugated donkey anti-rabbit antibody (1:1000, cat. no. A21207, lot no. 2897798, Invitrogen)
Alexa 647 conjugated donkey anti-rabbit antibody (1:1000, cat. no. A31573, lot no. 2420695, Invitrogen)
Alexa 647 conjugated donkey anti-mouse antibody (1:1000, cat. no. A31571, lot no. 2892404, Invitrogen)
Alexa 647 conjugated donkey anti-goat antibody (1:1000, cat. no. A21447, lot no. 3034156, Invitrogen)

Validation

Rabbit anti-mCherry (1:1,000, 600401379, Rockland), manufacture's website https://www.rockland.com/categories/primary-antibodies/rfp-antibody-pre-adsorbed-600-401-379/?srsltid=AfmBOopAZ_Mvho1IPFCskkoqZSg5lLmHANVDOqTnqvf4jB2SLYlGpfA7

Rabbit anti-cFos (1:1,000, 226003, Synaptic systems), manufacture's website https://sysy.com/product/226003

Mouse anti-HA (1:1000, 901514, Biolegend), manufacture's website https://www.biolegend.com/en-us/products/anti-ha-11-epitope-tag-antibody-11071

Goat anti-TPH2 (1:500, ab121013, Abcam), manufacture's website https://www.abcam.com/en-us/products/primary-antibodies/tph2-antibody-ab121013?srsltid=AfmBOoqpKvP7VwWDbW8wniIC_CmgWszl5mJxnMgm7r04zoU1wxySfDhN&sourceClicked=searchClickQuery-publications

Alexa 594 conjugated donkey anti-rabbit antibody (1:1000, A21207, Invitrogen), manufacture's website https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21207

Alexa 647 conjugated donkey anti-rabbit antibody (1:1000, A31573, Invitrogen) manufacture's website https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31573

Alexa 647 conjugated donkey anti-mouse antibody(1:1000, cat. no. A31571, lot no. 2892404, Invitrogen) manufacture's website https://www.thermofisher.com/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31571

Alexa 647 conjugated donkey anti-goat antibody (1:1000, cat. no. A21447, lot no. 3034156, Invitrogen) manufacture's website https://www.thermofisher.com/antibody/product/Donkey-anti-Goat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21447

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

Mice (1.5 day-10 weeks) from both sexes were used in experiments. Genetically-engineered mouse lines used in this study included Oprm1Cre/+, Vglut2-IRES-Cre (JAX #016963). The Oprm1Cre/+ knock-in mouse line was generated in the Stanford University Transgenic, Knockout and Tumor model Center using conventional ES cell targeting strategies. The Cre recombinase cDNA, followed by the rabbit β-globin poly-A signal, was introduced via homologous recombination immediately after the start codon in exon 1 of the mouse Oprm1 gene. Heterozygous mice were generated by mating chimeric mice to C57BL/6 wild-type mice. Mice were group-housed in a temperature- and humidity-controlled facility maintained according to institutional animal care guidelines, under a 12 h light/12 h dark cycle, with ad libitum access to food and water. All procedures were conducted in accordance with institutional animal care and use committee guidelines.

Wild animals

None.

Reporting on sex

Similar number of male and female mice were used in the experiment. We included the sex of mice for all experiments in the Supplementary table 1.

Field-collected samples	None.
Ethics oversight	All procedures were in accordance with the US National Institutes of Health (NIH) guidelines for the care and use of laboratory animals, and were approved by Stanford University's Administrative Panel on Laboratory Animal Care.

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A