nature portfolio

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Last updated by author(s): Oct 9, 2024

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	Data collection of animal behavior for the conditioned place preference were camera recorded with Ethovision software. Whole-cell patch-clamp recordings were performed using Clampex 10 software. Data from the nerve skin experiments were recorded and anlayzed (discrimination of the spikes) with the Spike2 software.
Data analysis	Most analyses were performed using standard commercial softwares including Microsoft office and Graphpad Prism. Human RNA sequencing data analysis was performed using a dataset that is publicly available in the NCBI database under accession code GSE158380. This dataset has already been analyzed and published in Nature Communications: https://www.nature.com/articles/s41467-021-25125-1

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

As mentionned in the data availability statement, source data are provided as a Source Data file.

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	Power analysis for ANOVA design yields a group size of 8 mice or 6 rats per group with a 95% confidence interval to achieve statistical significance. Samples sizes different to the calculated number are decided based on previous literature using animal models of pain and opioid dependence.
Data exclusions	2 animals were excluded from the cAMP dosage because of aberrant values obtained after time-resolved FRET assays. For behavioral experiments exclusion criteria have been defined before the experiments. The main ones were motor dysfunction after intrathecal injection. In our experiments, one animal was excluded from the analysis.
Replication	Behavior experiments were divided into 2 to 3 sub-experiments that consisted into smaller sample size per group. Results from these sub-experiments were then pulled together to get the final results with full sample size. This method has been specifically designed to avoid reproducibility failures. For in vitro experiments, data were collected from more than three biologically independent experiments. All attempts at replication were successful.
Randomization	All experiments involving animal behavior were randomized into groups that result in equal sample sizes. For pain-related behaviors, baseline values were averaged and classified in ascending order. Animals were then successively assigned to a different experimental group.
Blinding	Investigators were not blinded to the group for immunohistochemistry, RT-PCR, calcium imaging, electrophysiology except for behavioral experiments (genotype or drug treatment). However, animals injected with an opioid or receiving an injury are easily identifiable by the experimenter.

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: The following primary antibodies were employed: anti-CGRP Abcam ab36001 (goat 1:1000), anti-GFAP Abcam ab4674 (chicken 1:1000), anti-Iba1 Wako (rabbit 1:500). The following secondary antibodies were used anti-rabbit AlexaFluor 555/488/647 (donkey 1:1000), anti-goat AlexaFluor 488 (donkey 1:1000) from Life Technologies.

Validation: All antibodies used have been extensively validated in previous researches. For CGRP: the manufacturer's website states it was cited in 126 publications. For GFAP: the manufacturer's website states it was cited in 522 publications. For Iba1: The manufacturer's website states it was cited in 4160 publications.

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: Experiments were performed in male Sprague-Dawley rats (150-170g, Janvier, France), C57BL/6 naive mice (25-30g, Janvier, France), or C57BL/6 mice carrying a homozygous deletion of Flt3 (Flt3KO mice) and their littermates (WT) weighing 25-30 g, and MORmCherry C57BL/6 mice. Animals were maintained in a climate-controlled room on a 12 h light/dark cycle and allowed access to food and water ad libitum.

Wild animals: The study did not involve wild animals.

Reporting on sex: Male and female mice were first considered separately in behavioral procedures. Both sexes showed mechanical hypersensitivity of same intensity after repeated morphine injection and were similarly affected by Flt3 deletion (ANOVA followed by Bonferroni's test, n = 8 for both sexes and genotypes for each experiment, Fig. S1). Thereafter, experiments were performed only on male mice.

Field-collected samples: N/A

Ethics oversight: All the procedures were approved by the French Ministry of Research (authorization #20114-2019040310181741v3)

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