nature portfolio

Corresponding author(s): Grégory Scherrer

Last updated by author(s): May 17, 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	Matlab (2019b) to control a camera for recording mouse behavior; IDAS (Inscopix Data Acquisition Software) for recording calcium activity of rACC-Pn neurons; Clampfit 10 for collecting slice electrophysiology data; Zeiss Zen software for acquiring confocal microscopy images.
Data analysis	Extract (https://github.com/schnitzer-lab/EXTRACT-public) running on Matlab (2019b) for calcium imaging analysis; Ethovision XT15 and DeepLabCut for analyzing videos of mouse behavior; R 4.0.3 for data processing, statistical tests and figure preparation; Stimfit 0.14.9 and Clampfit 11.2 for analyzing electrophysiology data; Seurat v4.0 for analyzing RNA-seq data; Fiji for analyzing confocal microscopy images. The R code for generating clusters and visualizations from raw count matrices; for processing Ca2+ imaging, behavioral, and electrophysiology data, is available at https://github.com/ScherrerLab/PontineNucleus2024/.

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

Single-cell RNA sequencing data are available through the Gene Expression Omnibus (GEO) with accession numbers GSE267264 for SMART-seq and GSE267265 for 10x Genomics. All of the raw data for behavioral, electrophysiological, and immunohistochemical analyses are provided in the main text or supplementary materials and source data file. Processed Ca2+ imaging data can be accessed at https://github.com/ScherrerLab/PontineNucleus2024/. Raw Ca2+ imaging data are available upon request due to the large file size.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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	Sample sizes for mouse behavior and electrophysiology experiments were determined using the power analysis ('pwr' R package). Specifically, the function 'pwr.t.test' was used, with a significance level of 0.05, a power of 0.80, and effect sizes estimated from pilot experiments and/or previous studies using similar methods. This power analysis was used to determine sample sizes for experiments establishing placebo analgesia conditioning (PAC)-induced analgesic effect (Fig.1), comparing calcium activity before and after PAC (Figs.2 and 6), determining the electrophysiological properties of rACC-Pn neurons (Fig.3), and establishing the effect of photoinhibition of Pn neurons on PAC-induced analgesic effect (Fig.5). The function 'pwr.anova.test' was used to determine sample sizes in optogenetic experiments that determined the contribution of the rACC-Pn pathway to the PAC-induced analgesic effect, thermal pain, and mechanical pain (Fig.4). Sample sizes for histology experiments were determined based on similar studies in the field (Wang et al., 2018).
Data exclusions	No data were excluded.
Replication	In Fig.1h, j, and Fig.5f, two mice in each group were performed with similar results. In Fig.5h, 6a-c, three independent repeats were performed with similar results and representative images were shown. In Extended Data Fig.3a-c, d-e, and 12b, two independent repeats were performed with similar results.
Randomization	For the histology experiment, samples were allocated by randomly selecting brain slices containing regions of interest. This approach ensured unbiased representation across experimental groups. For all other experiments, samples and animals were randomized into groups .
Blinding	Experimenters were blinded to experimental groups before and during all mouse behavior experiments. Calcium imaging data were analyzed by two independent researchers using two different analysis pipelines at two universities.

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

Methods

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

Antibodies

Antibodies used

Anti-GFP antibody; Invitrogen; A11122; 2273763
Streptavidin-AF555; Fisher; S32355; 2207533

Validation

All antibodies listed above are commercially available. Specificity has been validated by previous studies from our lab and other several independent studies. Below are some published studies:
Wang, D., Tawfik, V. L., Corder, G., Low, S. A., François, A., Basbaum, A. I., & Scherrer, G. (2018). Functional Divergence of Delta and Mu Opioid Receptor Organization in CNS Pain Circuits. Neuron, 98(1), 90–108.e5.
Corder, G., Ahanonu, B., Grewe, B. F., Wang, D., Schnitzer, M. J., & Scherrer, G. (2019). An amygdalar neural ensemble that encodes the unpleasantness of pain. Science, 363(6424), 276–281.
Wei XP, Collie M, Dempsey B, Fortin G, Yackle K. A novel reticular node in the brainstem synchronizes neonatal mouse crying with breathing. Neuron. 2022 Feb 16;110(4):644-657.e6. doi: 10.1016/j.neuron.2021.12.014. Epub 2022 Jan 7.
Wu ST, Chen JY, Martin V, Ng R, Zhang Y, Grover D, Greenspan RJ, Aljadeff J, Su CY. Valence opponency in peripheral olfactory processing. Proc Natl Acad Sci U S A. 2022 Feb 1;119(5):e2120134119. doi: 10.1073/pnas.2120134119.

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

C57BL/6 wild-type mice (Stock #: 000664), TRAP2 mice (FosCreERT2, Stock #: 030323), and PvalbCre mice (Stock #: 017320) mice were purchased from Jackson Laboratory. Oprd1Cre mice were generated at Stanford Transgenic, Knockout and Tumor Model Center (TKTC) using standard gene targeting procedures. Mice were housed at a maximum of 5 per cage and maintained on a 12-hr light/dark cycle in a temperature-controlled environment with ad libitum access to food and water. Male or female mice with an age range of 8–12 weeks were used for experiments.

Wild animals

No wild animals were used.

Reporting on sex

For histology, RNA-seq, and some mouse behavior experiments, both male and female mice were used. All other experiments were performed in male mice.

Field-collected samples

No field collected samples were used in the study.

Ethics oversight

All procedures followed animal care guidelines approved by the Administrative Panel on Laboratory Animal Care (APLAC) of Stanford University, by the Institutional Animal Care and Use Committee (IACUC) of the University of North Carolina at Chapel Hill, and by the International Association for the Study of Pain.

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