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	Leica LAS X, bioDAQ, Clampex (10.7), Logitech C920x HD Pro Webcam camera were used for data collection.
Data analysis	Data were processed and analyzed by GraphPad Prism 10.2.0 and 10.3.1. All code used in this manuscript is available at https://github.com/YTwTJ/Organ-Specific-Sympathetic-Innervation-Defines-Visceral-Functions. For the organ topographical mapping by Python (3.9.17), we imported the VoxelMorph (version 0.2) library, OpenCV (4.5.1) library and 'SciPy' (1.11.1) package. scRNA-seq analysis was performed in Python (3.8.18) using ScanPy (1.9.2), or in R (4.1.1) using Seurat (4.2.1). 'PyDESeq2' (0.4.4) package was used for differential gene expression analysis in scRNA-seq data. 'Harmony' (0.0.9) package was used to integrate seqFISH datasets. Computer vision-based video analysis for bile flow used OpenCV (4.5.1) library. Histological images were quantified by ImageJ (1.54k). Electrophysiological data was analyzed in Clampfit (10.7).

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. scRNA-seq data from CG-SMG cells are available at the Gene Expression Omnibus under accession number GSE278457. The organ innervation and seqFISH data were deposited at Zenodo (#13306861, #13883320).

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 statistical methods were used to predetermine the sample sizes. Sample size is similar to recent publication in the neuroscience field (PMID: 35082448, PMID: 35650438, PMID: 39261733).
Data exclusions	For behavioral and histological experiments, all data were included after verification of virus expression.
Replication	All behavioral and physiological measurements were replicated by more than three animals at multiple time points.
Randomization	Randomization was performed when allocating animals to experimental groups. Chemical administration sequences such as vehicle and CNO were randomized, and didn't lead to any difference in the results.
Blinding	Data collection was not blinded, but data analysis was performed with the experiment conditions blinded. Data from different experimental conditions were automated collected and analyzed using software or custom code, so blinding is less relevant.

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

Primary antibodies: rabbit anti-CHAT antibody (1:500; Abcam, ab178850), chicken anti-NeuN antibody (1:500; Millipore, ABN91), chicken anti-Th antibody (1:500; AvesLab, TYH-0020), goat anti-alpha-smooth muscle actin (1:1000; Novus Biologicals, NB300-978), rabbit anti-glucagon antibody (1:500; Abcam, ab92517), rat anti-mCherry (1:500; Invitrogen, M11217). Secondary antibodies from Jackson Immunoresearch (1:500): donkey anti-chicken 488 (703-545-155), donkey anti-chicken 647 (703-605-155), donkey anti-rabbit 647 (711-605-152), donkey anti-rat Cy3 (712-165-150), donkey anti-goat 647 (705-605-147), and donkey anti-rabbit 488 (711-545-152).

Validation

All antibodies were purchased from and validated by commercial vendors in the USA. The validation data are available on the manufacturer's website:
rabbit anti-CHAT: this antibody has been cited in at least 54 publications.
chicken anti-NeuN: the validation statement from vendor:"This chicken polyclonal antibody specifically detects Neuronal nuclei antigen (NeuN). It targets an epitope within the first 97 amino acids from the N-terminal region.".
chicken anti-Th: this antibody has been cited in at least 85 publications.
goat anti-alpha-smooth muscle actin: this antibody has been cited in at least 64 publications.
rabbit anti-glucagon: this antibody has been cited in at least 81 publications.
rat anti-mCherry: this antibody has been cited in at least 100 publications for immunohistochemistry.
donkey anti-chicken 488: this antibody has been cited in at least 1084 publications.
donkey anti-chicken 647: this antibody has been cited in at least 206 publications.
donkey anti-rabbit 647: this antibody has been cited in at least 794 publications.
donkey anti-rat Cy3: this antibody has been cited in at least 146 publications.
donkey anti-goat 647: this antibody has been cited in at least 263 publications.
donkey anti-rabbit 488: this antibody has been cited in at least 1519 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

Mice of at least 7 weeks old were used for data collection. Ai32 (#024109), and C57BL/6J (#000664) lines were acquired from the Jackson Laboratory. RXFP1-Cre line was developed in our previous publication (PMID: 33057193). SHOX2-Cre animals were generously provided by Y. Chen (Tulane University, PMID: 23620086). TH-Cre mice were a gift from D. Anderson (California Institute of Technology). Animals were housed in a facility with controlled temperature (71-75°F) and humidity (30-70%), following a 13:11 hour light-dark cycle. Animals had ad libitum access to chow and water.

Wild animals

No wild animals were used in the study.

Reporting on sex

Both male and female adult mice were used for data collection, without observable phenotype differences in this study.

Field-collected samples

No field collected samples were used in the study.

Ethics oversight

All experimental procedures were carried out in accordance with US NIH guidelines for the care and use of laboratory animals and approved by the Caltech Institutional Animal Care and Use Committees (IACUC; protocol #1694 and 1866).

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