nature portfolio

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Last updated by author(s): Nov 6, 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	1. Ion channel genes and HGCN gene families were obtained from HGNC database (https://www.genenames.org/); 2. The cell adhesion molecule genes were adapted from Paul A. et. al, 2017 (DOI:10.1016/j.cell.2017.08.032); 3. The gene sets with mouse Gene Ontology (GO) annotations were obtained through pyMN (DOI: 0.1038/s41467-018-03282-0); 4. The mouse transcription factor gene list was obtained from AnimalTFDB4 (DOI:10.1093/nar/gkac907)
Data analysis	1. Custom codes used to generate figures and data analysis were deposited to Github (https://github.com/bcmjianglab/cn_project); 2. Scanpy 1.8.1 was used for single cell RNA-seq data analysis (https://scanpy.readthedocs.io/en/stable/index.html); 3. Cellranger 5.0.1 was used to generate 10x single-cell sequencing expression matrix (https://www.10xgenomics.com/support/software/cell-ranger/); 4. Star 2.7. 7a (https://github.com/alexdobin/STAR) and Featurecounts (https://subread.sourceforge.net/) were used to generate Patch-seq expression matrix; 5. Morphology was reconstructed using Neurolucida, and MorphoPy 0.7.1 was used to extract morphological features (https://github.com/berenslab/MorphoPy); 6. MetaNeighbor analysis was conducted on the snRNA-seq dataset utilizing pyMN (https://github.com/gillislab/pyMN); 7. Synergistic core identification was performed with method reported by Okawa et al (https://git-r3lab.uni.lu/satoshi.okawa/synergisticcore); 8. Statics and machine learning were performed with Scikit-learn (ver 1.0.2, https://scikit-learn.org/) in Python 3.7. 9. The figures were created using Plotly 5.6.0, Seaborn 0.11.2, and Matplotlib 3.4.3 in Python 3.7. The final layout and cartoon figures were created in CANVAS X (Canvas GFX). The 3D brain model in Fig. 1a was generated with Brainrender python package (ver 2.1.10).

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

1. Original electrophysiological, morphological, RNA-sequencing data and FISH/immunostaining imaging supporting the findings of this study have been deposited to the Mendeley Data (doi:10.17632/2n8m8k6b2g.1).
2. The raw snRNA-seq and Patch-seq datasets have been deposited in the NCBI database with GEO accession number: GSE273145.
3. Source data are provided as a Source Data file within the paper.

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	No Human participants and human data in current study
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	Sample size was determined based on similar studies in this field or power analysis with a desired power of 0.8 and a 1-alpha of 0.95
Data exclusions	1. In snRNA-seq data, nuclei with > 5% UMIs mapped to mitochondrial genes and those with less than 200 genes detected were excluded from the analysis. Doublets samples were remove using Scrublet (DOI: 10.1016/j.cels.2018.11.005); 2. In Patch-seq data, we performed Patch-seq from 438 cells, 387 neurons passed further quality control (no RNA degradation, genecount>=4000, mapping rate of >= 60%,etc) for further analysis.
Replication	1. Nine samples for single-nucleus extraction and single-nucleus RNA sequencing, and each CN tissue sample was pooled from 8 to 10 mice 2. Patch-seq neurons were acquired from 69 mice. 3. FISH staining were performed on 5-8 consecutive sagittal sections of CN.
Randomization	The study does not involve specific experimental conditions requiring randomization.
Blinding	Regarding the expert cell type classification, cell collection and classification were independantly performed by four individuals. Single-cell RNA sequencing data and FISH imaging were not collected or analyzed in a blinded manner, as the study does not involve specific experimental conditions requiring blinding.

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

1. Anti-Necab2 antibody, Sigma-Aldrich, HPA013998, 1:500
2. Anti-Phgdh antibody, Invitrogen, PA5-54360, 1:400
3. Goat anti-Mouse secondary antibody Alexa Fluor™ 633, Invitrogen, A-21052, 1:500
4. Goat anti-Rabbit secondary antibody Alexa Fluor™ 647, Invitrogen, A-21244, 1:500

Validation

1. https://www.sigmaaldrich.com/US/en/product/sigma/hpa013998
2. https://www.thermofisher.com/antibody/product/PHGDH-Antibody-Polyclonal/PA5-54360
3. https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21052
4. https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21244

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

In this study, we used 222 mice (Mus musculus) in total (121 males and 101 females). Wildtype, GlyT2-EGFP(RRID:MGI:3835459), Sst-IRES-Cre(RRID:IMSR_JAX:013044), Penk-IRES2-Cre(RRID:IMSR_JAX:025112), Gabra6-Cre(RRID:MMRRC_015966-UCD), Ai9 RRID:IMSR_JAX:007909) with C57BL/6 background at the age of postnatal (P)22-28 were used in current study. Penk-Cre mice are a gift from Yong Xu Lab at Baylor College of Medicine, originally from Jackson Laboratory, and Gabra6-Cre mice are a gift from Susumu Tomita Lab at Yale University. Others are from Jackson Laboratory.

Wild animals

N/A

Reporting on sex

Both genders were used in current study

Field-collected samples

N/A

Ethics oversight

All experiments were approved with the animal protocol (AN-7352) by IACUC of Baylor College of Medicine and performed according to the IACUC guidelines at Baylor College of Medicine. All animals were maintained in the animal facility with a light cycle from 6 am to 6 pm daily, with temperatures ranging from 68 to 72 °F and humidity ranging from 30% to 70%.