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	R (v 4.2.1) and python (v3.8.20, v3.11.8, v3.11.10, and v3.12.4) were used to process and analyze single-nuclear RNA and ATAC Sequencing data
Data analysis	All datasets were generated as described in the Methods. Raw sequencing data were processed using standard pipelines. For multiome libraries, alignment, barcode calling, and quantification for both gene expression and chromatin accessibility assays were performed with Cell Ranger ARC (v2.0.2) against the human reference genome (hg38, GENCODE v42). Nuclear fractions were computed using DropletQC (v0.0.0.9000) to assess RNA quality and confirm low ambient RNA levels. Gene expression and ATAC matrices were separated using Scanpy (v1.9.6). Ambient RNA removal for gene expression matrices was performed using CellBender (v0.2.0). ATAC peak calling was carried out with MACS2 (v2.2.7). MEA recordings and stimulation were performed using the MaxWell Biosystems MaxOne device with the MaxLive recording software. Spike sorting and electrophysiology analyses were performed using Kilosort2 and SpikeInterface (v0.101.0), followed by rigorous automated and manual curation as detailed in the Methods. Custom analyses for single-unit classification, assembly detection, drift quantification, and activation analyses were performed using reproducible pipelines in Python and R, and all steps are fully documented in the accompanying scripts. Processed matrices were further analyzed using custom workflows following the procedures detailed in the Methods. For each major analysis, we explicitly report the tools, statistical approaches, and parameters used. All custom Python and R scripts used for preprocessing, quality control, and downstream analyses are provided for full transparency. Standalone tools used:

Cell Ranger ARC v2.0.2
CellBender v0.2.0
DropletQC v0.0.0.9000
MACS2 v2.2.7
Kilosort2
SpikeInterface v0.101.0
Scanpy v1.9.6
MaxLive recording software v 25.1.8.1

Major R and Python libraries:
Seurat (v4.3.0)
Harmony (v0.1.1)
SCTransform v2 (v0.4.1)
Signac (v1.10.0)
scDblFinder (v1.12.0)
DESeq2 (v1.44.0)
scMultiMap (v1.0.1)
SCENIC+ (v1.0a1)
Augur (v1.0.3)
RRHO2 (v1.0)
hdWGCNA (v0.4.03)
ggplot2 (v3.4.4)
WaveMAP (v0.1.12)
sklearn (v1.9.0)
NumPy (v2.4.6)
SciPy (v1.18.0)
pandas (v3.0.3)
matplotlib (v3.10.9)

Programming languages:
R (v4.2.1)
Python (v3.8.20, v3.11.8, v3.11.10, and v3.12.4)

All custom code used in this study is deposited and publicly available at:
https://github.com/BioinformaticsMUSC/Moore_etal_MEA

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

All raw MEA snRNA-seq/snATAC-seq and IVS snRNA-seq data were deposited to GEO archive under the accession number GSE288939. The data presented in this manuscript are available through interactive Shiny applications that enable users to explore the datasets in detail, including gene expression profiles, dimensionality reduction and clustering results, cell type annotations, marker gene expression, and additional features. Users can also generate custom visualizations directly from the datasets.

These applications include data from MEA excitatory, inhibitory, and non-neuronal cells:
https://biocm-moore-etal-mea-multiome-subglia.share.connect.posit.cloud/
https://biocm-moore-etal-mea-multiome-inhneurons.share.connect.posit.cloud/
https://biocm-moore-etal-mea-multiome-excneurons.share.connect.posit.cloud/

These applications include data from IVS excitatory, inhibitory, and non-neuronal cells:
https://biocm-moore-etal-ivs-snrna.share.connect.posit.cloud/
https://biocm-moore-etal-ivs-excneurons.share.connect.posit.cloud/
https://biocm-moore-etal-ivs-inhneurons.share.connect.posit.cloud
https://biocm-moore-etal-ivs-subglia.share.connect.posit.cloud

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 sex-based analyses were performed.
Reporting on race, ethnicity, or other socially relevant groupings	No ethnicity-based analyses were performed.
Population characteristics	Human brain tissue was obtained from fully consented adult patients undergoing surgical treatment for epilepsy at your institution. Tissue collection was conducted under IRB protocol STU 092014-026. Only non-pathological cortical tissue was used: no seizure foci, and tissue with tumors or cortical dysplasia was excluded. Samples were obtained during standard anterior temporal lobectomies, and only small portions of resected anterior temporal cortex were used for research.
Recruitment	Surgical epilepsy patients who consented to donate excess cortical tissue.
Ethics oversight	The UT Southwestern Medical Center Institutional Review Board reviewed and approved all procedures involving human surgical tissue (IRB protocol STU 092014-026). Because the study utilized excess cortical tissue obtained during clinically indicated epilepsy surgeries, all donors provided informed consent for research use.

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 sample size for the datasets we generated because of the limited availability of human brain surgical samples. However, our sample sizes for single nuclear RNA-seq is similar to other published studies using human brain surgical samples such as PMID 33686299 and PMID 34051145.
Data exclusions	We excluded units with signal to noise ratios less than 2, inter-spike interval violations greater than 3%, and firing rates less than 0.1 Hz. Unit pairs with the highest correlation values at an absolute lag time of 2 ms or less in any of these 4 groups were excluded from further analysis to ensure a focus on unit pair correlations with distinct nonzero synaptic delay times. Data were filtered for RNA/ATAC QC thresholds, ambient RNA and doublets. This exclusion criteria was pre-establishe and necessary to remove poor quality data.
Replication	Findings were not replicated due to the nature and limited availability of human brain surgical tissues.
Randomization	Subjects were not randomly selected for inclusion in the study based on the availability of human tissue. However, tissue pieces were randomized for processing for RNA-seq. All known technical and biological covariates were included in the statistical models, and therefore randomization is not relevant.
Blinding	Tissue collection required knowledge of the clinical context and surgical source to ensure that only non-pathological anterior temporal cortex from consented epilepsy patients was included, and to appropriately assign samples to the correct developmental stage and experimental workflow. Similarly, electrophysiological recordings and sequencing library preparation required awareness of sample identity for accurate processing and quality control.

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

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.