DANISH SINGLE-CELL NETWORK NEWSLETTER

December - 2023

THIS MONTH'S HIGHLIGHTS:

The first complete cellular map of adult mouse brain

BRAIN Initiative Cell Census Network (BICCN)

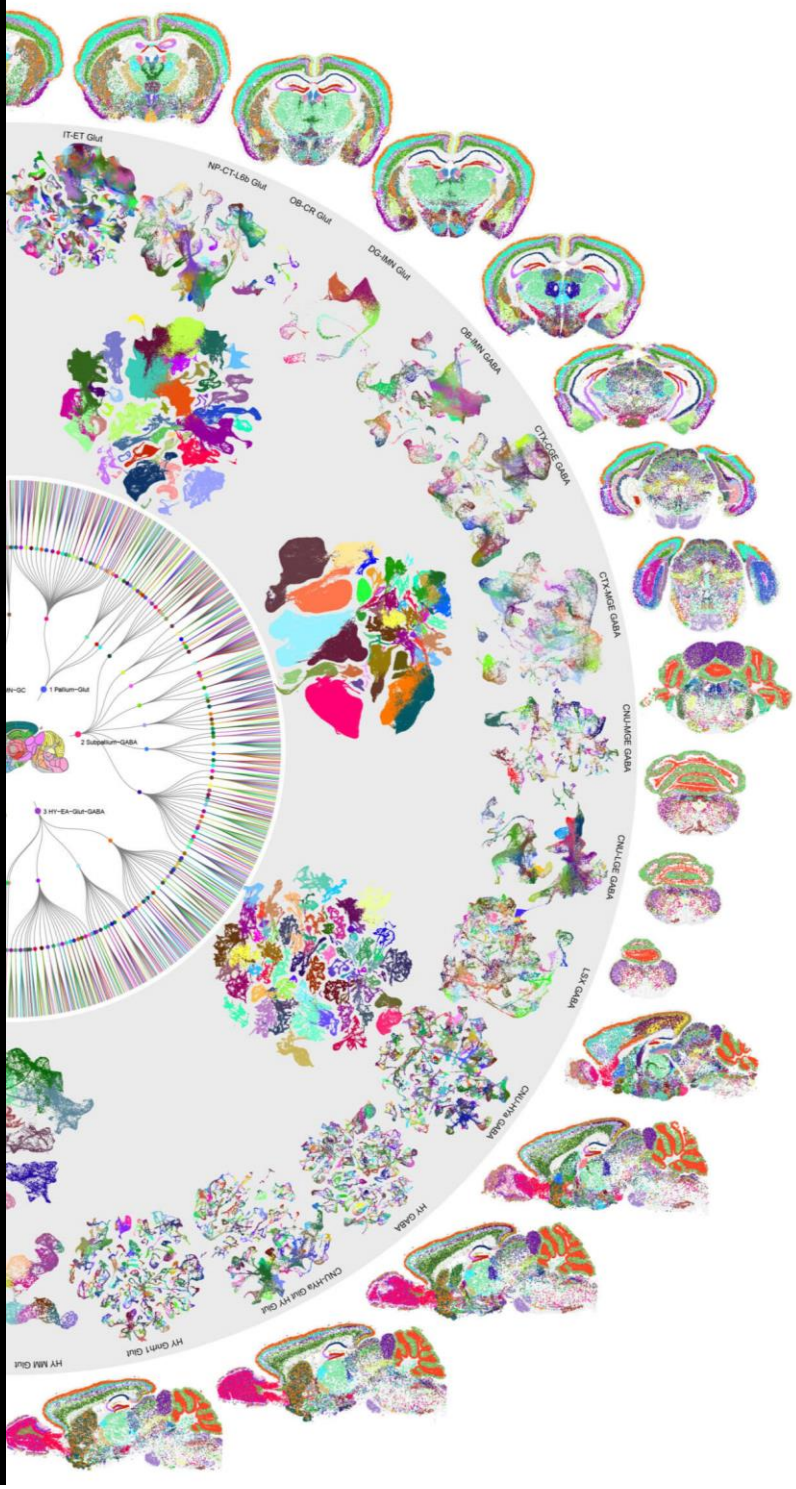
Large scale multi-omic analysis of the human brain

BRAIN Initiative Cell Census Network (BICCN)

COVER IMAGE

Author: Cindy van Velthoven, Zizhen Yao and Hongkui Zeng

Title: [Cell-type atlas of whole mouse brain feauring over 5300-clusters]



UNIVERSITY OF COPENHAGEN

HOLIDAY GREETINGS FROM THE NEWSLETTER TEAM

In the midst of the holiday season, we send you our warmest wishes for a peaceful and reflective time. As the year draws to a close, we'd like to share a collection of impactful papers utizlising the single-cell and spatial transcriptomic technologies.

By that this month, our newsletter takes a different tone, by not exclusivly bringing you individual articles. Specifically we want to shed light on two recently published collections that have caught our attention. Both collections are projects carried out by the National Institute of Health's **B**rain **R**esearch through **A**dvancing **I**nnovative **N**eurotechnologies (**BRAIN**) **I**nitiative - **C**ell **C**ensus **N**etwork (**BICCN**).

The first collaborative effort unveils the first complete cellular map of the adult mouse brain. This is a series of 10 papers published in Nature, taking on a multi-omics approach of systematically collecting and curating data through epigenomic and single-cell and spatial transcriptomic characterization of over 32 million cells across the entire adult mouse (*mus musculus)* brain. Here we will list the collection of papers thematically for you to dive in to:

Transcriptomic and spatial atlas:
A high-resolution transcriptomic and spatial atlas of cell types in the whole mouse brain
Molecularly defined and spatially resolved cell atlas of the whole mouse brain
The molecular cytoarchitecture of the adult mouse brain
Spatial atlas of the mouse central nervous system at molecular resolution

Epigenetic profiling and gene regulatory elements:
Single-cell DNA methylome and 3D multi-omic atlas of the adult mouse brain
Single-cell analysis of chromatin accessibility in adult mouse brain
Brain-wide correspondence of neuronal epigenomics and distant projections
Conserved and divergent gene regulatory programs of the mammalian neocortex

And lastly, papers on brain-wide spinal projecting neurons and the vertebrate retina:
A transcriptomic taxonomy of mouse brain-wide spinal projecting neurons
Evolution of neuronal cell classes and types in the vertebrate retina

We're equially excited to highlight the **BICCN** collection on the human brain, a collaborative effort featured in various Science journals. This compilation offers a comprehensive overview of cell types and functions of the human brain. The collection features over 21 research articles, 2 introductory articles and a perspective paper. These are too many to list here, but we strongly encourage you to probe the collection and unravel its insights.

For this last issue of the year we will, as per usual, bring you short introductions and key points from various papers. In this issue, all featured papers have their origin in one of the two listed collections.

Lastly, thank you for following our newsletters every month, and we look forward to bringing you more exciting developments in the coming year. May your holidays be filled with friends and family, and may the new year usher in even more discoveries and breakthroughs.

Wishing you a joyous holiday season and a scientifically inspiring new year!

Best regards,

The Danish Single-cell Network newsletter team

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A high-resolution transcriptomic and spatial atlas of cell types in the whole mouse brain

Yao, Z. *et al. Nature* (2023), https://doi.org/10.1038/s41586-023-06812-z

Yao et al. presents a groundbreaking study that integrates single-cell RNA-sequencing (scRNA-seq) and multiplexed error-robust fluorescence in situ hybridization (MERFISH) data to create a comprehensive cell-type atlas for the entire adult mouse brain. The atlas, organized into four hierarchical levels, reveals a high degree of correspondence between transcriptomic identity and spatial specificity for each cell type, uncovering unique features of cell-type organization across different brain regions. The consortium identified 34 classes, 338 subclasses, 1,201 supertypes, and 5,322 clusters within the whole mouse brain, establishing a benchmark reference atlas. The study systematically analyzed both neuronal and non-neuronal cell types, highlighting a dichotomy between the dorsal and ventral parts of the brain, with the former containing fewer yet highly divergent neuronal types and the latter featuring more numerous and closely related neuronal types. The spatial specificity of cell types was found to strongly correlate with their transcriptomic identity, emphasizing the importance of anatomical specialization in cell-type classification.

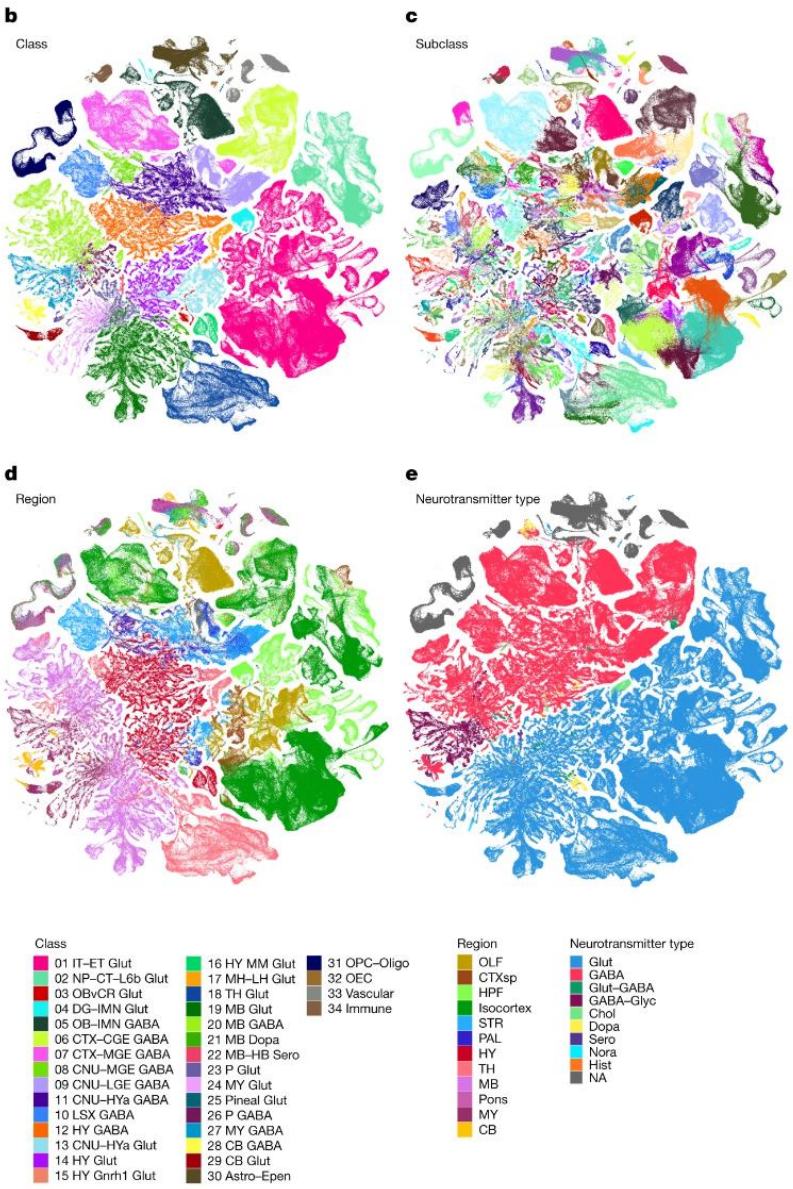

Figure 1. UMAP representation of all cell types colored by class (b), subclass (c), brain region (d) and major neurotransmitter type (e).

Furthermore, the study unveiled extraordinary diversity in neurotransmitter and neuropeptide expression patterns across different cell types, emphasizing the complexity of intercellular communication in the brain. Transcription factors were identified as major determinants of cell-type classification, revealing a combinatorial transcription factor code that defines cell types throughout the brain. The atlas establishes a foundational resource for investigating cellular and circuit function, development, and evolution of the mammalian brain, providing a guidepost for future studies and facilitating cross-species comparative analyses.

Single-cell analysis of prenatal and postnatal human cortical development

Velmeshev, D. and Perez, Y. et. al. Science (2023), https://www.science.org/doi/10.1126/science.adf0834

The study focuses on the meticulously regulated process of cortical development in humans, spanning prenatal and postnatal stages. Leveraging single-nucleus RNA sequencing (snRNA-seq) and single-nucleus chromatin accessibility data, over 700,000 profiles was sourced from 169 tissue samples from 106 donors. he study uncovered several critical branching points in the trajectory, notably between two major groups of excitatory neurons: L2-3, L4, and L5-6-IT (Ex1) and L5 and L6 (Ex2), as well as between L4 and L2-3 or L5-6-IT (Ex3). Beyond classifying genes based on their age of appearance, the authors delved into the dynamic expression patterns of lineage-specific genes. Two predominant patterns emerged: transient expression and burst expression. Transcriptional regulator MN1 was identified as specific to L2-3, L5-6-IT, and L4 neurons, while noncoding RNAs *CYP1B1-AS1* and *LINC00507* were enriched in L2-3 neurons. *HS3ST4,* specific to L5 neurons, was also recognized as a putative regulator. Using a spatial transcriptomic apporach with at panel of 140 genes, early emerging lineage-specific genes associated with excitatory leyer-specific markerss were identified. Markers of L4 neurons, such as hippocalcin (*HPCA*) and gremlin 2 (*GREM2*), were specifically highlighted for their layer-restricted expression during the second trimester, indicative of the early specification of L4 neuronal identity. The same pattern holds true for the other excitatory neuron layers. Subsequently the team set out to investigate lineage-specific developmental gene programs and thei relation risk factors of brain disorders. 2796 lineage specific genes for cell-types were identified and categorized into five groups based on the expression age of onset. The genes were overlayed with rare variants linked to Autism Spectrum Disorder (ASD), Schizophrenia (SCZ), Bipolar Disorder (BPD) and Alzheimer's Disease (AD). Notably, a substantial enrichment of genes linked to the risk of ASD, SCZ, and BPD was observed during the second trimester, extending into the third trimester for ASD and BPD risk genes. In contrast, the expression pattern of AD risk genes remained relatively constant, exhibiting a distinct trajectory from neurodevelopmental and psychiatric disorders. ASD risk genes were notably enriched in deep-layer intratelencephalic projection neurons (L5-6-IT) and L5 neurons. Lastly, the authors provided specific examples of female-specific ASD-HC (high confidence ASD risk) genes, such as the subplate-specific transcription factor NR4A2, the neuronal transcription factor MEF2C, neurexin 2 (NRXN2) involved in axon guidance and synaptogenesis, and PCDH15 encoding a cell adhesion molecule in female L6 neurons. These findings lent strong support to the ASD female protective effect hypothesis and proposed that the fine-tuning of cortical cell lineages by sex-specific developmental programs could contribute to the male bias in ASD pathogenesis.

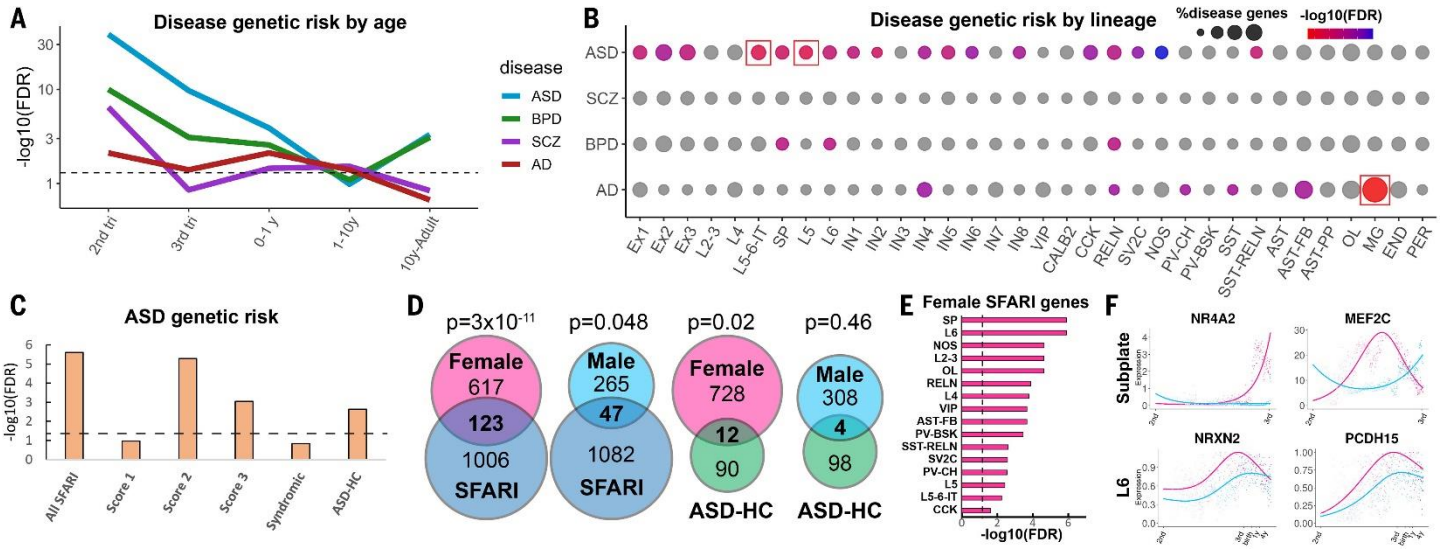

Figure 2. Lineage enrichment of brain-disorder risk genes

Single-cell DNA methylome and 3D multi-omic atlas of the adult mouse brain

Hanqing, L. and Zeng, Q. et. al. Nature (2023), https://doi.org/10.1038/s41586-023-06805-y

This study presents a comprehensive analysis of cytosine DNA methylation in the adult mouse brain, utilizing single-nucleus methylome sequencing (snmC-seq3) and multi-omic sequencing (snm3C-seq1). The investigation involved profiling 301,626 methylomes and 176,003 chromatin conformation–methylome joint profiles from 117 dissected regions throughout the brain. Through iterative clustering and integration with transcriptome and chromatin accessibility datasets, the authors constructed a methylation-based cell taxonomy, identifying 4,673 cell groups and 274 cross-modality-annotated subclasses. The study identified 2.6 million differentially methylated regions representing potential gene regulation elements and revealed spatial methylation patterns on genes and regulatory elements across brain regions. The integration of chromatin conformation data highlighted diversities in neuronal genes associated with DNA methylation and transcription changes. Cell-type comparisons enabled the construction of regulatory networks, incorporating transcription factors, regulatory elements, and downstream gene targets. The study also predicted alternative gene isoform expression based on intragenic DNA methylation and chromatin conformation patterns. The findings establish a brain-wide, single-cell DNA methylome, and 3D multi-omic atlas, providing insights into cellular–spatial and regulatory genome diversity in the mouse brain. Noteably, the authors developed snmC-seq3 and snm3C-seq1, a new method of single-nucleus methylome- and multi-omic- sequencing, respectively.

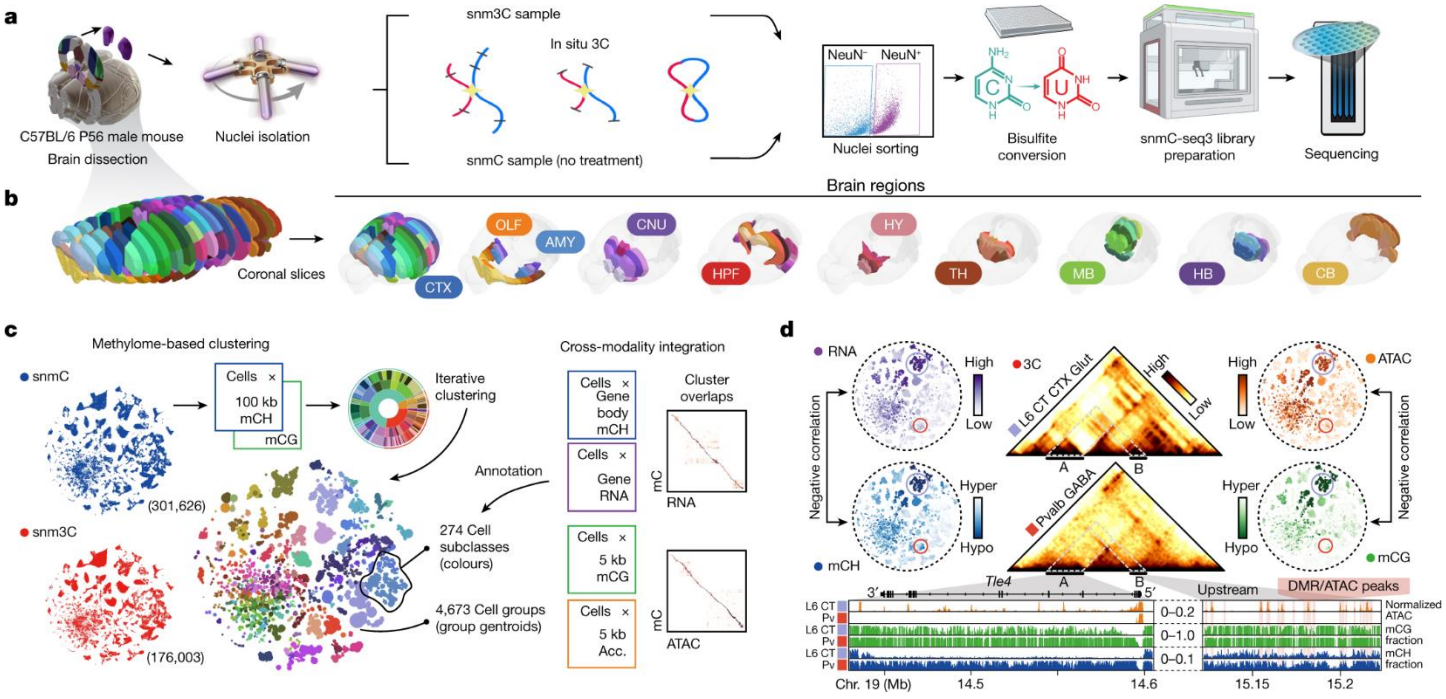

Figure 3. Single-cell DNA methylome and multi-omic atlas chart the cellular and genomic diversity of the whole mouse brain.

New papers from Danish researchers:

Frank, M. *et al.* "Single-cell analysis identifies genes facilitating rhizobium infection in Lotus japonicus" *Nature communications* (2023). https://doi.org/10.1038/s41467-023-42911-1

Ye, Z. *et al.* "High-throughput and scalable single cell proteomics identifies over 5000 proteins per cell" *BioRxiv* (2023). https://doi.org/10.1101/2023.11.27.568953

Kavaliauskaite, G. *et al.* "Automatic quality control of single-cell and single-nucleus RNA-seq using valiDrops" *NAR Genomics and Bioinformatics* (2023). https://doi.org/10.1093/nargab/lqad101

Bendixen, S. *et al.* "Single cell-resolved study of advanced murine MASH reveals a homeostatic pericyte signaling module" *Journal of Hepatology* (2023). https://doi.org/10.1016/j.jhep.2023.11.001

Ma, W. *et al.* "Unveiling the pathogenesis of non-alcoholic fatty liver disease by decoding biomarkers through integrated single-cell and single-nucleus profiles" *MedRxiv* (2023). https://doi.org/10.1101/2023.10.05.23296635

Khodosevich, K. *et al.* "Drug targeting in psychiatric disorders — how to overcome the loss in translation?" *Nature Reviews Drug Discovery* (2023). https://doi.org/10.1038/s41573-023-00847-7

Next Single Cell Seminar

Time and place: 12th January 2024, Panum Faculty Club, room 16.6.16 and Zoom (Link)

9:00 – 09:45
Speaker: TBA
Title: TBA

09:45 – 10:30
Speaker: TBA
Title: TBA

If you would like to announce anything single cell related, e.g. job announcement, event, your published paper, technology development etc., please contact us.

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