Ganglionic Eminence Sequencing Unveils Cortical and Hippocampal Inhibitory Neuron Origins

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Abstract:

The ganglionic eminence (GE) plays a critical role in brain development, yet its molecular mechanisms remain elusive. While excitatory neuron (ExN) development in the hippocampus (HPC) and prefrontal cortex (PFC) has been profiled, incorporating the GE allows us to find inhibitory neuron progenitors, since inhibitory neurons (InN) migrate from the GE to other regions. Thus, we combined mid-gestation HPC, PFC and GE gene expression data to reveal genes involved in the developmental trajectories of InN. Here we perform pseudotime analysis on single-nucleus RNA-seq data from the NeMO archive in Scanpy and reveal region and cell type specific markers in InN differentiation. For radial glia, VIM was expressed everywhere, PTN and FABP5 were expressed for the hippocampus and cortex, and NNAT and NTRK2 were active for the GE. We found three paths of early-stage InN in the GE where TPX2 and SOX4 were expressed. These findings can provide insight to neurodevelopmental disease mechanisms.

Samples

1

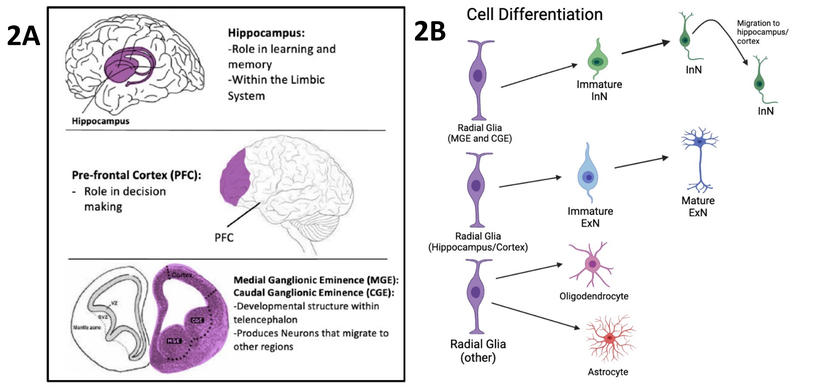
Part of Brain	Sample	Age	# of Cells
Hippocampus			
	GW18_hippocampus	2nd Trimester	6702
	GW18_2_hippocampus	2nd Trimester	10991
Pre-frontal Cortex			
	GW18_PFC	2nd Trimester	15131
Caudal Ganglionic Eminence (CGE)			
	GW18_CGE	2nd Trimester	9302
	GW18_2_CGE	2nd Trimester	5730
Medial Ganglionic Eminence (MGE)			
	GW18_MGE	2nd Trimester	8656
	GW18_2_MGE	2nd Trimester	7174
Total Cells Before Preprocessing			63686
Total Cells After Preprocessing			46357
*Samples orginated from the BICCN data from the Neuroscience Multi-Omic Data Archive			

Why Single Cell?

- Profiling diverse cell types and brain regions individually
- Examining dynamic cell differentiation and development
- Generating comprehensive cellular maps for visual representation
- Gaining insights into disease mechanisms

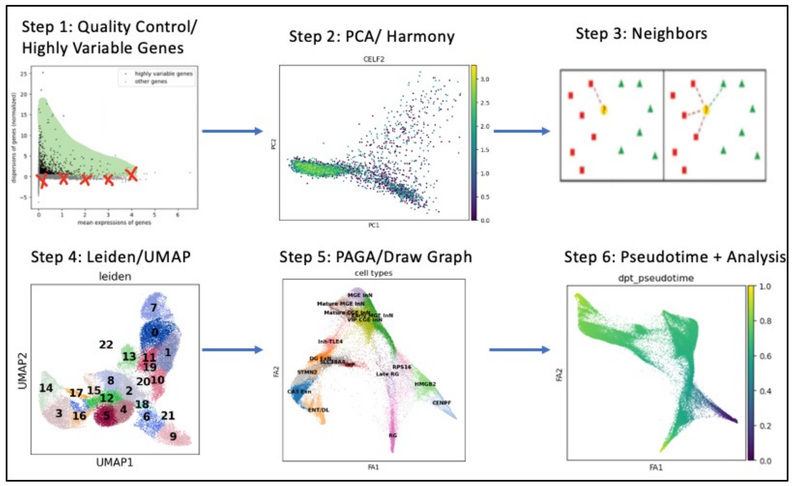
1: Neuroscience Multi-Omic Data Archive BICCN data set collected from 18 weeks gestation. The dataset comprises >17,000 hippocampal cells and >15,000 cells each from the PFC, MGE, and CGE, totaling >63,000 cells. Following detailed quality control and filtering, >46,000 cells were retained for analysis.

Anatomy and Cell Differentiation



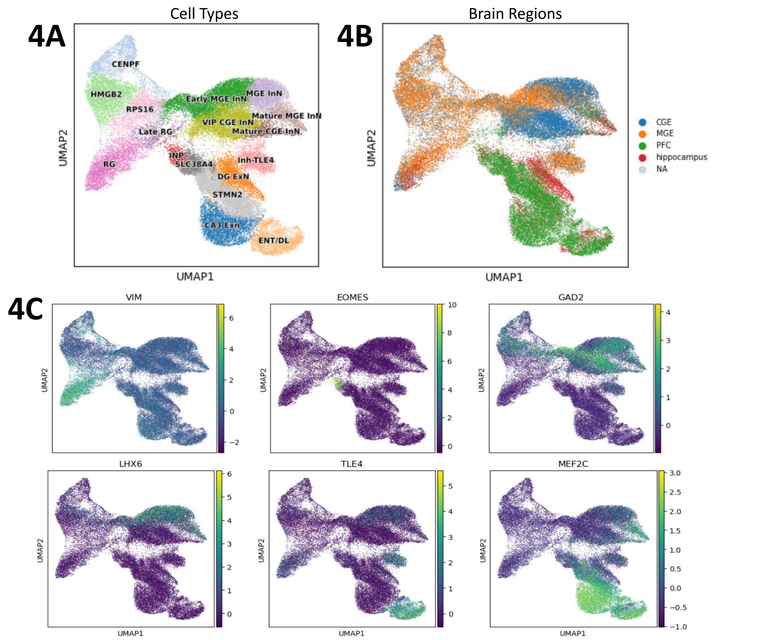
2A: The study profiles hippocampus, pre-frontal cortex (PFC), and ganglionic eminence (GE) brain regions. GE subdivides into MGE and CGE, exclusive to brain development, generating inhibitory neurons which migrate to the cortex and hippocampus. **2B:** All cell types originate from non-neuronal Radial Glia. Hippocampus/PFC host excitatory neuron development, while GE gives rise to migrating inhibitory neurons for the cortex.

Methods Workflow



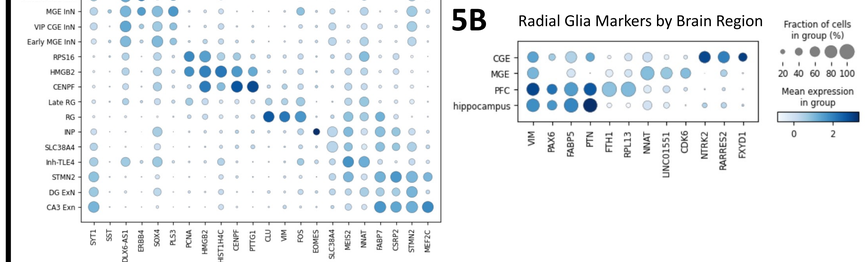
3: The GW18 data was concatenated using Python's Scanpy. Quality control (QC) was conducted to eliminate low-quality genes, retaining 5k highly variable genes. Batch correction was achieved through PCA and harmony. Prior to UMAP visualization, K nearest neighbors were computed. Leiden clustering and UMAP were applied, with known literature markers used to label the clusters by cell type. The paga and draw graph algorithms were utilized for diffusion pseudotime analysis, setting a random radial glia cell as the root node. Subsequently, paths were defined, and gene expression heatmaps were generated to examine cell differentiation trajectories.

Known Markers are used for UMAP and Clustering



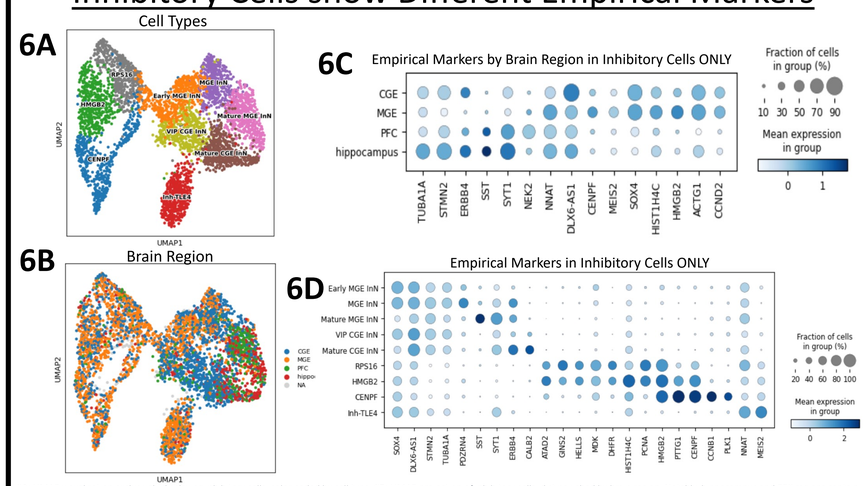
4A: UMAP projection of GW18 data, using Leiden clustering to segregate cells by type. Cells are annotated based on literature markers. **4B:** UMAP projection of the same data annotated by brain region, indicating overlapping regions on the UMAP. **4C:** UMAP visualization reveals gene expression profiles of known markers from literature. Notably, MEF2C marks CA3 excitatory neurons, EOMES characterizes INPs, VIM denotes radial glia, and LHX6 identifies MGE inhibitory neurons.

Empirical Markers Vary by Cell Type and Brain Region



5A: Top two marker genes per cell type/cluster were identified across all brain regions using pseudotime correlations and Wilcoxon tests. Upper panel displays inhibitory paths, while the lower panel represents excitatory paths. **5B:** Focused on Radial Glia cells, VIM consistently shows expression in all radial glia, with shared markers between the hippocampus and PFC and individual markers for the GE.

Inhibitory Cells show Different Empirical Markers



6A: UMAP visualization exclusively displaying inhibitory cells, color-coded by cell type. **6B:** UMAP projection of inhibitory cells, distinguished by brain region. Notably, hippocampus and PFC regions contain mature neurons (6A) post-migration from GE. **6C:** Gene expression of marker genes for specific inhibitory cell types: MGE inhibitory neurons, CGE inhibitory neurons, CENPF inhibitory neurons, and Inh-TLE4 cells. **6D:** Gene expression of marker genes in the same set of inhibitory cells from 6C, categorized by brain region. Some markers are uniformly expressed across all four regions, while others display region-specific patterns. A portion of these marker genes were NOT previously identified in literature.

References:

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3. FJ, WFP. Scanpy: Large-scale single-cell gene expression data analysis. Genome biology. Accessed July 31, 2023. https://pubmed.ncbi.nlm.nih.gov/29409532/.

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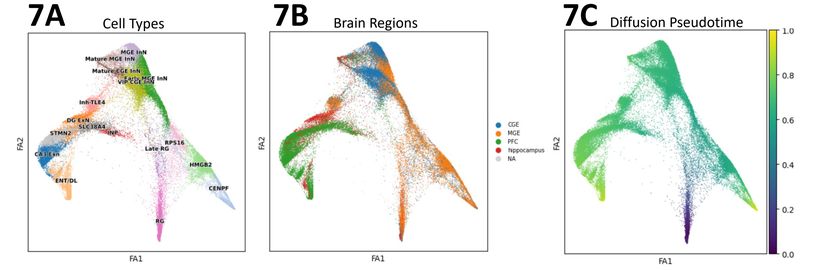
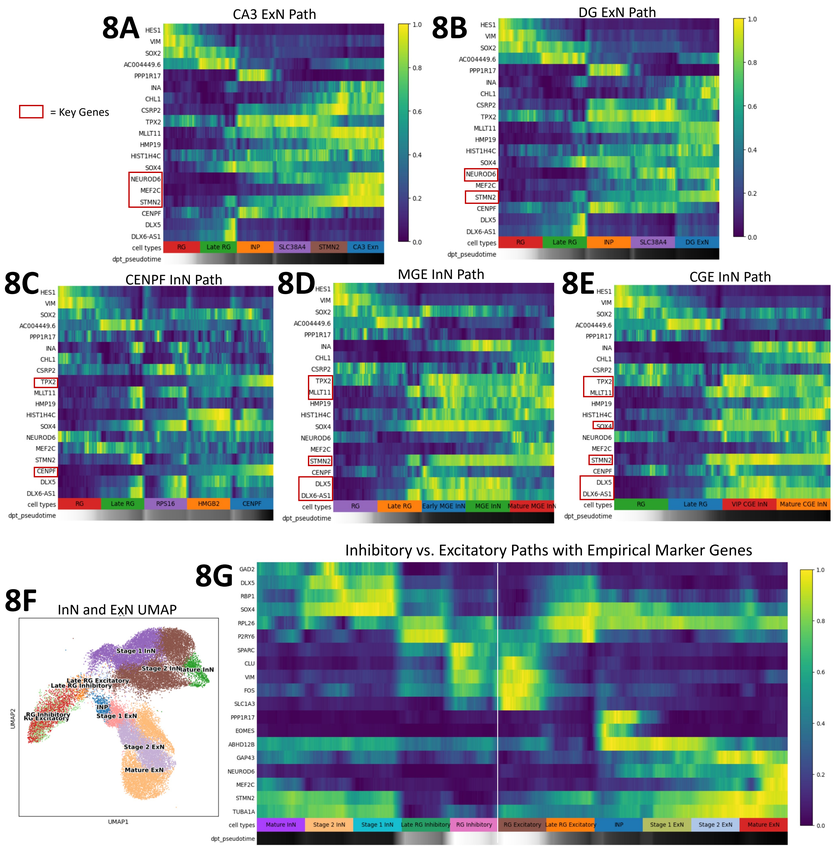
2. Figure 2 created with BioRender.com

Pseudotime Reveals Three Major InN Paths



7A: The dataset underwent draw graph analysis, visually representing separate developmental trajectories annotated with cell type labels. **7B:** Draw graph analysis annotated by brain region, highlighting the origin of migrated inhibitory neurons in the top-middle hippocampus and PFC segment. **7C:** Diffusion Pseudotime analysis was conducted using a random Radial Glia cell as the root node. **7D:** Draw graphs depicting the five investigated development paths: two excitatory paths and three inhibitory paths. A comparison can be made to the pseudotime analysis in 7C.

InN and ExN Paths Show Different Marker Genes



Inhibitory vs. Excitatory Paths with Empirical Marker Genes

8A: Gene expression analysis of marker genes over cell types/dpt pseudotime within the CA3 excitatory path (see 7D) in the hippocampus. Genes that differ across paths are marked by the red box. **8B:** Gene expression analysis following the same method as 8A, focusing on the DG excitatory path (see 7D) in the hippocampus. **8C:** Gene expression analysis using the same method as 8A, showing the CENPF inhibitory path (see 7D) in the GE. **8D:** Same method in 8A with the MGE Inhibitory path (see 7D). **8E:** Same method in 8A with the CGE Inhibitory path (see 7D). **8F:** UMAP depicting aggregated ExN and InN paths **8G:** Path plot depicting gene expression of inhibitory cell types on the left, excitatory cell types on the right, and Radial Glia cells in the middle.

Incorporating the GE provided the origin of InN pre-migration

Our investigation of InN paths in the ganglionic eminence identified three distinct trajectories of cell differentiation for InN and identified markers of neuron development using pseudotime correlation and the Wilcoxon test. This included previously unreported genes as well as known validated markers. The radial glia demonstrated VIM being present in all regions, PTN and FABP5 primarily active in the HPC and PFC, and NNAT and NTRK2 in the GE. Moreover, different cell types exhibited specific marker genes during differentiation, exemplified by MEF2C and NEUROD6 presence in ExN cell differentiation. In contrast, markers like TPX2 and SOX4 were expressed solely in Mature InN. These findings offer valuable insights into neurodevelopmental processes and may hold significant implications for understanding related diseases. Further investigations encompassing diverse gestational age samples could reveal prenatal developmental gene expression variations across these regions, enhancing comprehension of neuronal development.