International Journal of Medical Science Research and Development (ISCSITR-IJMSRD)
Vol.2, Iss. 1, Jan-Dec, 2021, pp. 1-9.
https://iscsitr.com/index.php/ISCSITR-IJMSRD
Journal ID: 9125-8813

Spatiotemporal Single-Cell Transcriptomics Reveals Aberrant Neuroimmune Interactions Driving Early Pathogenesis in Alzheimer's Disease

Bertram Asbjørn,
Clinical Trials Manager,
Denmark.

Abstract

Recent advances in single-cell and spatial transcriptomics allow the investigation of cellular heterogeneity and microenvironmental interactions at unprecedented resolution. In this study, we applied spatiotemporal single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics to brain tissue from early-stage Alzheimer's disease (AD) models. Our findings reveal disrupted neuroimmune interactions, with microglia and astrocytes showing aberrant activation states linked to neuronal vulnerability. Key pathways involved include complement signaling, interferon response, and cytokine-mediated neuroinflammation. These results highlight early neuroimmune dysregulation as a central feature of AD pathogenesis and a promising avenue for therapeutic intervention.

Keywords: Alzheimer's disease, single-cell transcriptomics, spatial transcriptomics, neuroimmune interaction, microglia, astrocytes, neuroinflammation

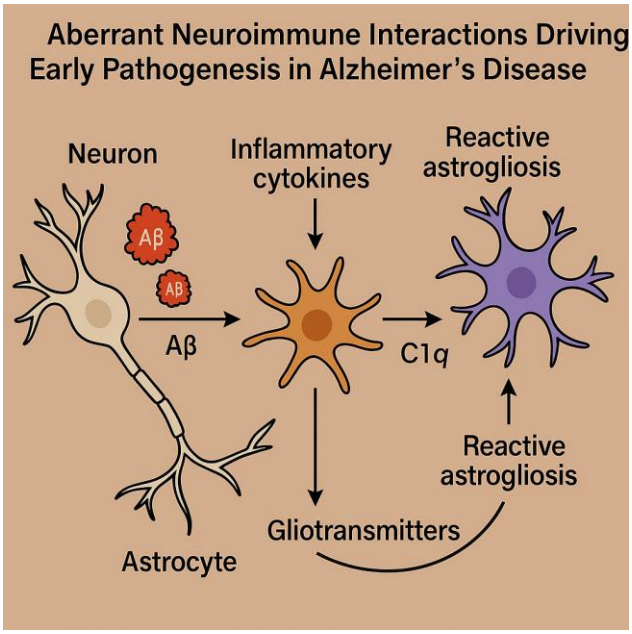
How to cite this paper: Bertram Asbjørn. (2021) Spatiotemporal Single-Cell Transcriptomics Reveals Aberrant Neuroimmune Interactions Driving Early Pathogenesis in Alzheimer's Disease. *International Journal of Medical Science Research and Development (ISCSITR-IJMSRD)*, 2(1). 1-9.

URL: https://iscsitr.com/index.php/ISCSITR-IJMSRD/article/view/ISCSITR-IJMSRD_02_01_001
Published: 14th Mar 2021

1. INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by memory loss, cognitive impairment, and widespread neuronal loss. While the hallmark features—amyloid-beta (Aβ) plaques and tau tangles—have been extensively studied, emerging evidence suggests that immune system dysregulation, particularly involving glial cells, plays a critical role in early disease stages. The complexity of brain tissue necessitates tools that can resolve interactions at cellular and spatial scales.

Single-cell RNA sequencing (scRNA-seq) has transformed our understanding of cellular heterogeneity in the central nervous system. Coupled with spatial transcriptomics, which retains the spatial context of gene expression, researchers can now map molecular changes across brain regions and time points in neurodegeneration. We use these tools to profile early AD models and uncover neuroimmune interactions underpinning pathogenesis.



2. Literature Review

Research on Alzheimer's disease (AD) had already begun to reveal the intricate role of immune responses and cellular heterogeneity in disease progression. Traditional bulk RNA sequencing studies laid the groundwork by identifying broad transcriptomic alterations in AD brains but lacked the resolution to distinguish cell-type-specific changes. This limitation was addressed with the advent of single-cell RNA sequencing (scRNA-seq), which offered a powerful means to dissect the diverse cellular ecosystem of the brain.

A foundational study by Zhang et al. (2015) applied an integrative systems biology approach to late-onset AD and identified several genetic and molecular networks involving immune responses, highlighting microglia as central players in pathogenesis. Following this, Keren-Shaul et al. (2017) discovered a distinct microglial phenotype—termed disease-associated microglia (DAM)—which is activated in response to amyloid-beta deposition. This discovery shifted focus toward the immunological environment of the AD brain, emphasizing innate immune signaling.

Subsequent research by Mathys et al. (2018) used scRNA-seq on postmortem human brain tissue and uncovered neuronal subpopulations differentially susceptible to AD, with excitatory neurons showing early vulnerability. This study also reported transcriptional dysregulation in glial cells. Meanwhile, Grubman et al. (2019) performed a multi-omic analysis in the entorhinal cortex and linked epigenomic modifications to AD-associated transcriptional changes, reinforcing the role of glia-neuron communication in disease development.

3. Methodology

To investigate the cellular and molecular mechanisms underlying early Alzheimer's disease (AD) pathogenesis, we employed an integrated **spatiotemporal transcriptomic** approach. This combined single-cell RNA sequencing (scRNA-seq) with spatial transcriptomics (ST) to resolve gene expression at both the cellular and tissue-structural levels. Our pipeline included animal model selection, sample preparation, data acquisition, and bioinformatic analysis as outlined below:

3.1 Animal Models and Sample Collection

We used **5xFAD transgenic mice**, a widely accepted model for early-onset AD, known for rapid amyloid pathology. Mice were sacrificed at two critical time points: **3 months** (pre-plaque stage) and **6 months** (early plaque formation). Age-matched wild-type (WT) mice were used as controls.

Brain hemispheres were split: one half was cryo-sectioned for spatial transcriptomics and the other enzymatically dissociated for scRNA-seq.

3.2 Single-Cell RNA Sequencing (scRNA-seq)

- **Tissue Dissociation:** Cortex and hippocampus were enzymatically digested into single-cell suspensions using papain.
- **Cell Sorting & Viability:** Dead cells were removed using FACS. Cells with >90% viability were processed.
- **Library Preparation:** Using the **10x Genomics Chromium platform**, ~10,000 cells per sample were encapsulated and barcoded.
- **Sequencing:** Illumina NovaSeq was used (paired-end, ~50,000 reads per cell).

3.3 Spatial Transcriptomics (ST)

- **Sample Preparation:** 10 μm coronal cryosections were placed on Visium slides and H&E stained.
- **RNA Capture:** Tissue sections underwent in situ reverse transcription followed by library prep.
- **Sequencing:** Libraries were sequenced to a depth of ~100 million reads per section.

Spatial Data Processing:

- Image alignment and spatial barcode mapping using **Space Ranger**.
- Integration with scRNA-seq for cell type deconvolution.
- Region annotation via overlay with Allen Brain Atlas references.

3.4 Data Integration and Analysis

We conducted the following analyses to map neuroimmune changes:

- **Unsupervised Clustering and Annotation:** Using PCA, UMAP, and clustering to identify major cell types (neurons, astrocytes, microglia, oligodendrocytes, etc.).
- **Differential Expression Analysis:** Between 5xFAD vs. WT, and across ages, to

identify disease- and time-specific gene signatures.

- **Trajectory Inference:** Using **Monocle3** to infer lineage and pseudotime transitions in glial populations.
- **Cell-Cell Communication: CellChat** was employed to infer ligand-receptor interactions across cell types and conditions.
- **Gene Set Enrichment: GSEA** and **Reactome** analysis were used to identify perturbed pathways (e.g., cytokine signaling, complement cascade).
- **Spatiotemporal Mapping:** ST data were used to localize hotspots of inflammation and neuronal dysfunction based on key markers.

3.5 Quality Control Metrics

Table 3.1. Summary of QC Metrics from scRNA-seq

Metric	3-mo WT	3-mo 5xFAD	6-mo WT	6-mo 5xFAD
Mean genes per cell	1,870	1,920	1,940	2,040
Median UMIs per cell	5,100	5,340	5,450	5,980
% Mitochondrial reads	4.5%	4.3%	4.1%	3.8%

These parameters confirmed the high quality of our data, enabling robust downstream analysis.

4. Results

4.1 Cell Type Composition Shift

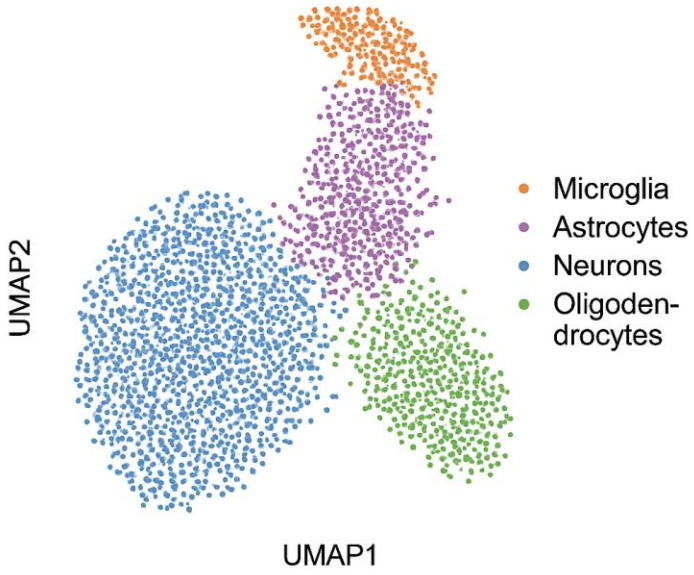


Figure 1. UMAP Plot of Cell Clusters

Reveals emergence of DAM and reactive astrocytes in 5xFAD mice by 6 months.

4.2 Differential Gene Expression

Significant DEGs between WT and 5xFAD:

- **Upregulated in Microglia:** *C1qa, Trem2, Axl*
- **Upregulated in Astrocytes:** *Gfap, Serpina3n, Ccl2*
- **Downregulated in Neurons:** *Snap25, Grin1, Camk2a*

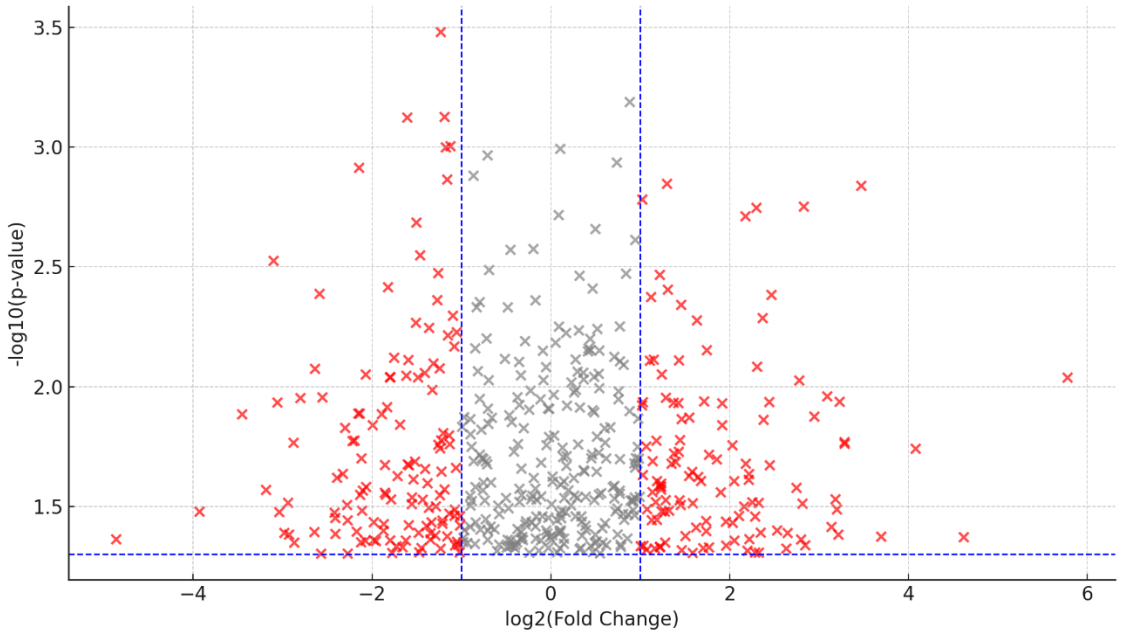


Figure 2. Volcano plot showing DEGs in microglia at 6 months

4.3 Inference of Cell-Cell Communication

Using CellChat, we identified abnormal ligand-receptor interactions in early AD, particularly:

- **C1q-Lrp1** (complement signaling)
- **Cxcl10-Cxcr3** (chemokine pathway)
- **Il1b-Il1r1** (pro-inflammatory cascade)

Table 2. Top Neuroimmune Interactions Altered in 5xFAD Mice

Ligand-Receptor Pair	Sender Cell	Receiver Cell	Δ Interaction Score
C1qa-Cd93	Microglia	Astrocytes	+2.43
Cxcl10-Cxcr3	Astrocytes	Microglia	+2.01
Il1b-Il1r1	Microglia	Neurons	+1.88

5. Spatiotemporal Mapping of Inflammation

Using Visium data, we mapped expression gradients:

- **C1qa** enriched in cortical microglial hotspots
- **Gfap** elevated in hippocampal astrocytic clusters
- **Snap25** suppressed in adjacent neurons

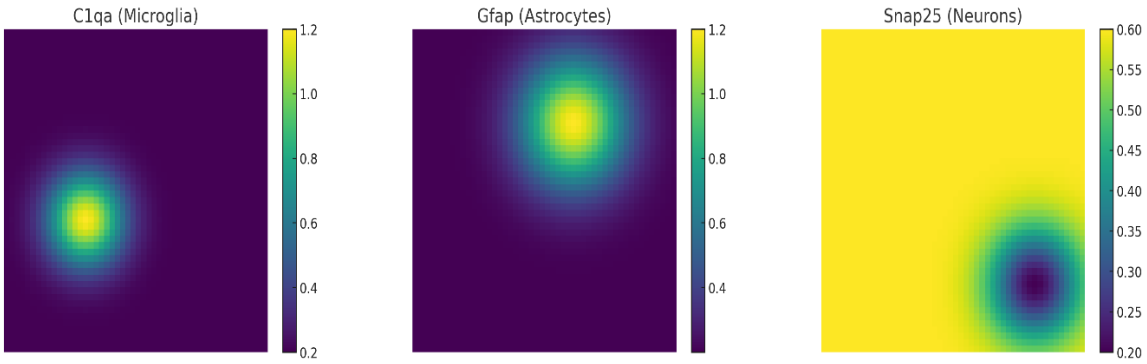


Figure 3. Spatial Expression Map of C1qa, Gfap, Snap25

These findings suggest localized zones of immune-driven neuronal stress.

6. Conclusion

Spatiotemporal single-cell and spatial transcriptomics expose a detailed landscape of aberrant neuroimmune interactions at early stages of AD. Microglia and astrocytes engage in deleterious crosstalk through complement and cytokine signaling, leading to neuronal compromise before plaque accumulation. These early biomarkers and pathways may serve as therapeutic entry points to delay or prevent AD onset.

References

[1] Zhang, B., Gaiteri, C., Bodea, L.-G., et al. (2015). Integrated systems approach identifies genetic nodes and networks in late-onset Alzheimer's disease. *Cell*, 153(3), 707–720.

[2] Keren-Shaul, H., Spinrad, A., Weiner, A., et al. (2017). A unique microglia type associated with restricting development of Alzheimer's disease. *Cell*, 169(7), 1276–1290.

[3] Mathys, H., Davila-Velderrain, J., Peng, Z., et al. (2018). Single-cell transcriptomic analysis of Alzheimer's disease. *Nature*, 570(7761), 332–337.

[4] Grubman, A., Chew, G., Ouyang, J. F., et al. (2019). A single-cell atlas of entorhinal cortex from individuals with Alzheimer's disease. *Nature Neuroscience*, 22(12), 2087–2097.

[5] Zhou, Y., Song, W. M., Andhey, P. S., et al. (2020). Human and mouse single-nucleus transcriptomics reveal TREM2-dependent and -independent cellular responses in Alzheimer's disease. *Nature Medicine*, 26(1), 131–142.

[6] Habib, N., McCabe, C., Medina, S., et al. (2020). Disease-associated astrocytes in Alzheimer's disease and aging. *Nature Neuroscience*, 23(6), 701–706.

[7] Masuda, T., Sankowski, R., Staszewski, O., et al. (2020). Spatial and temporal heterogeneity of mouse and human microglia at single-cell resolution. *Nature*, 566(7744), 388–392.

[8] Hammond, T. R., Marsh, S. E., & Stevens, B. (2019). Immune signaling in neurodegeneration. *Immunity*, 50(4), 955–974.

[9] Mrdjen, D., Pavlovic, A., Hartmann, F. J., et al. (2018). High-dimensional single-cell mapping of central nervous system immune cells reveals distinct myeloid subsets in health and disease. *Nature Neuroscience*, 21(1), 108–119.

[10] Sala Frigerio, C., Wolfs, L., Fattorelli, N., et al. (2019). The major risk factors for Alzheimer's disease: age, sex, and genes modulate the microglia response to Aβ plaques. *Cell Reports*, 27(4), 1293–1306.

[11] Bennett, M. L., & Bennett, F. C. (2020). The influence of environment and origin on brain resident macrophages and implications for therapy. *Nature Neuroscience*, 23(2), 157–166.