570

High-plex spatial multiomic technology at the single-cell level in mouse FFPE brain tissue

Katrina van Raay[1], Alyssa Rosenbloom[1], Brian Birditt[1], Ashley Heck[1], Kimberly Young[1], Tiên Phan-Everson[1], Zachary Lewis[1], Giang Ong[1], Shilah Bonnett[1], Tushar Rane[1], Megan Vandenberg[1], Mithra Korukonda[1], Aster Wardhani[1], Patrick Danaher[1], Trieu My Van[1], Vik Devgan[1], Rudy Van Eijsden[1], Carl Brown[1], Rustem Khafizov[1], David Ruff[1], **Margaret Hoang**[1], Gary Geiss[1], Caleb Stokes[2], Joseph M Beechem[1]

[1]NanoString® Technologies, Seattle, Washington, USA [2]Department of Immunology, University of Washington, Seattle, Washington, USA

NanoString Technologies
530 Fairview Avenue North, Seattle, WA 98109

Abstract

Spatially resolved, single-cell transcriptomics and proteomics in mouse neural models reveal neurobiological mechanisms relevant to human disease. However, spatial-omic protocols are analyte-specific and fail to capture multiomic information within the same single cell. We develop a multiomic workflow on a Spatial Molecular Imager (SMI), a single-cell spatial biology platform that leverages cyclic in situ hybridization chemistry to enable high-plex detection of proteins and RNAs at subcellular resolution. We measured 68 proteins using the Mouse Neuroscience protein panel (Neural Cell Typing and Alzheimer's Pathology) and 1,000 RNA targets (Mouse Neuroscience RNA panel) on the same 5 µm thick FFPE section of the mouse brain.

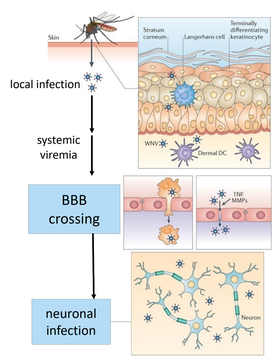
Our multiomic workflow demonstrates significant benefits to cell segmentation using cell-type specific markers (GFAP, Iba1, NeuN, in addition to a pan soma marker S6 and nuclear stain DAPI) in neural tissue. As a result, the number of transcripts captured within a single cell increased, most notably transcripts distal to cell bodies. This improved the quality of cell typing within the brain, including the detection of rare cell types and states.

To validate the multiomic workflow, we profiled the uninfected mouse brain and the brain with West Nile Virus (WNV)-infected encephalitis. As expected from WNV encephalitis, we observed persistent neuroinflammation that includes the recruitment and activation of CD8+ T cells and microglia. We captured the major cell types that comprise inflammatory nodules in post-WNV mouse brain (neurons, microglia, and astrocytes) and identified the signaling pathways that underlie persistent microglial-driven neuroinflammation after WNV encephalitis, which illuminates key aspects of neurodegeneration, neurodevelopment, cell state and signaling, including numerous ligands and receptors involved in neuron-glia communication. Our analysis identified pathways related to inflammation and cellular damage in neurons, astrocytes, microglia and T cells.

Taken together, we used the CosMx™ SMI platform to show, for the first time, that large numbers of mouse neuronal cells can be profiled with both protein and RNA at single-cell resolution in a spatial context. This integrated system maximizes the information content per single cell to enable a mechanistic understanding of infectious disease pathology and inflammatory response in the brain.

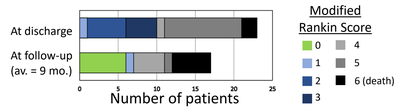
Neuroinflammation in West Nile Virus (WNV) Encephalitis

1. Neuroinvasive infection



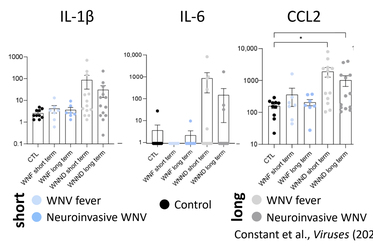
Suthar et al., *Nat. Rev. Microbiol.* (2013)

2. Persistent neurologic symptoms



Santini et al., *Pathogens* (2022)

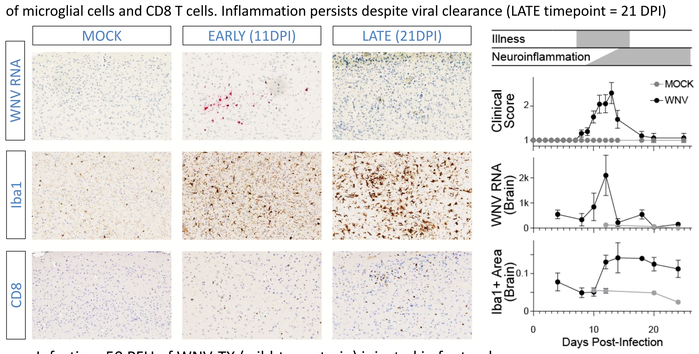
3. Chronic neuroinflammation



Constant et al., *Viruses* (2022)

A Mouse Model of WNV-triggered Neuroinflammation

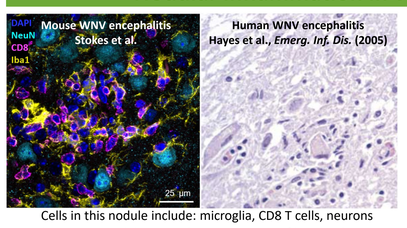
Peripheral infection leads to viral replication in brain (EARLY timepoint = 11 DPI), accompanied by expansion of microglial cells and CD8 T cells. Inflammation persists despite viral clearance (LATE timepoint = 21 DPI)

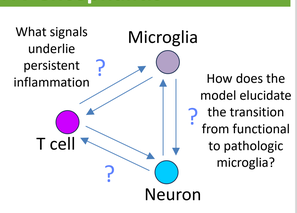


Infection: 50 PFU of WNV-TX (wild-type strain) injected in footpad

Dr. Caleb Stokes et al., Seattle Children's

Nodular inflammation in WNV encephalitis

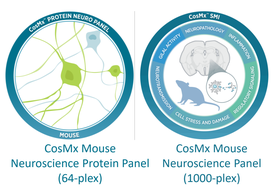




Cells in this nodule include: microglia, CD8 T cells, neurons

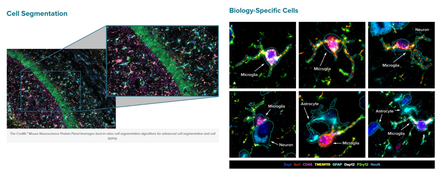
The mouse model recapitulates symptoms of human disease, such as the inflammatory nodules consisting primarily of microglia. We hypothesize these nodules represent a key "inflammatory niche" of the disease.

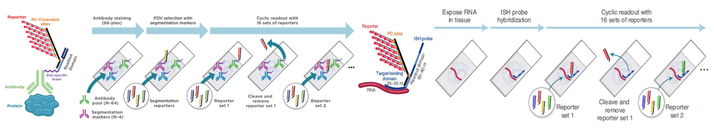
Approach: Multiomic Spatial Profiling of Inflammation in collaboration with Dr. Caleb Stokes at Seattle Children's

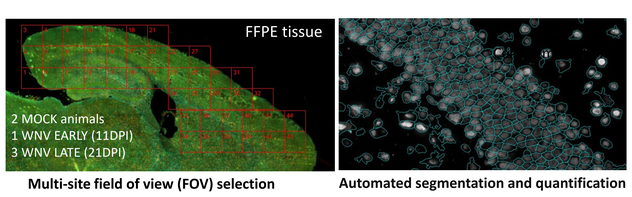


CosMx Mouse Neuroscience Protein Panel (64-plex)

CosMx Mouse Neuroscience Panel (1000-plex)





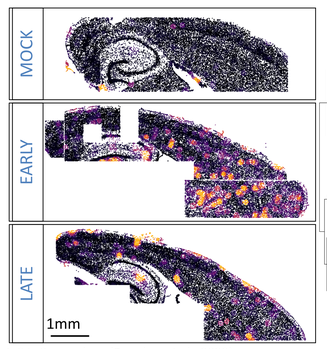


Multi-site field of view (FOV) selection

Automated segmentation and quantification

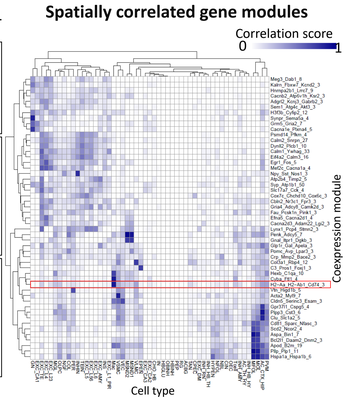
Inflammatory Transcripts are Spatially Circumscribed

Module #21 genes: H2-Aa, H2-Ab1, CD74



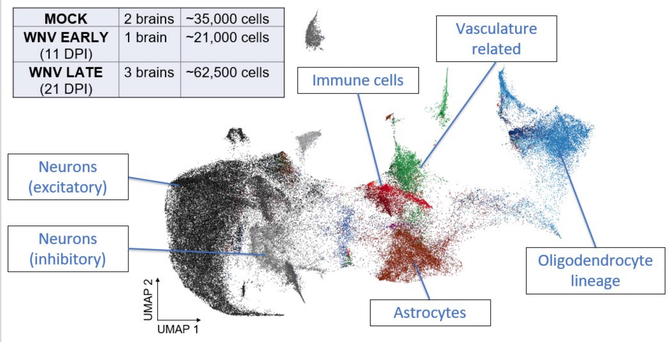
InSituCor analysis (Danaher et al., 2023)

Spatially correlated gene modules

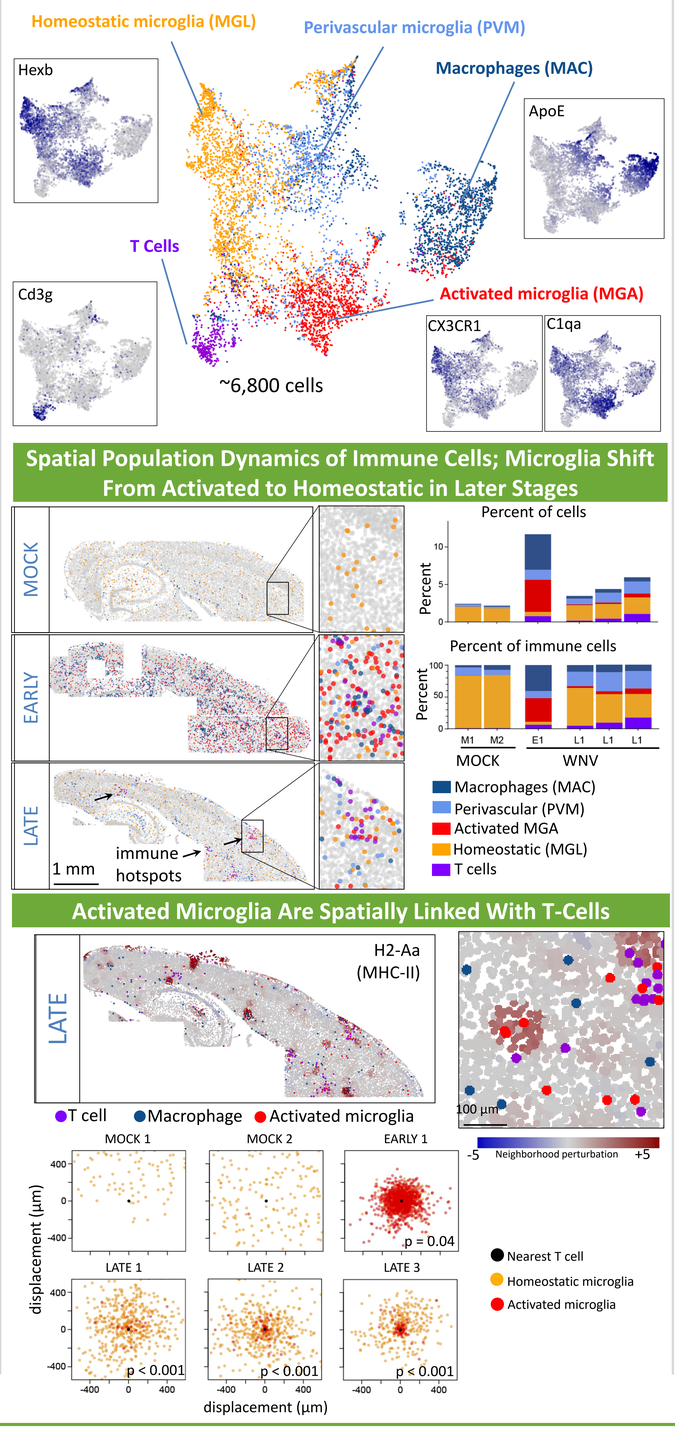


Transcription-based Clustering Identifies Major Cell Types

MOCK	2 brains	~35,000 cells
WNV EARLY (11 DPI)	1 brain	~21,000 cells
WNV LATE (21 DPI)	3 brains	~62,500 cells



Diverse Immune Cell Populations in WNV Encephalitis

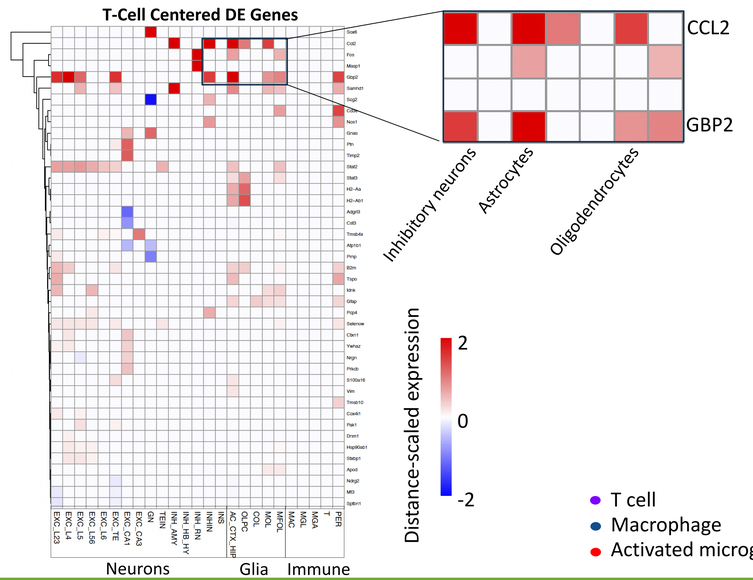


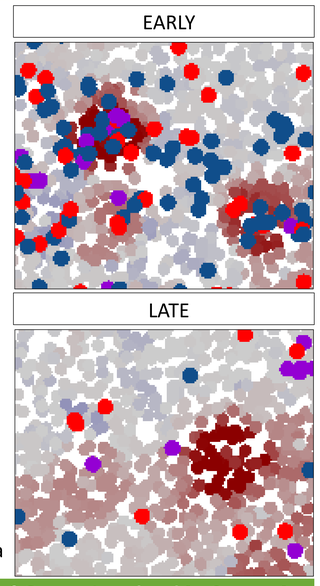
Spatial Population Dynamics of Immune Cells; Microglia Shift From Activated to Homeostatic in Later Stages

Activated Microglia Are Spatially Linked With T-Cells

Co-expression of CCL2 and GBP2 in Inhibitory Neurons, Astrocytes, and Oligodendrocytes Drives T-Cell Recruitment in WNV Encephalitis

T-Cell Centered DE Genes

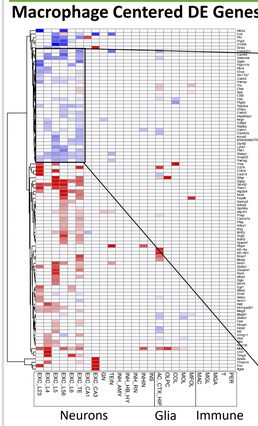




- T cell
- Macrophage
- Activated microglia

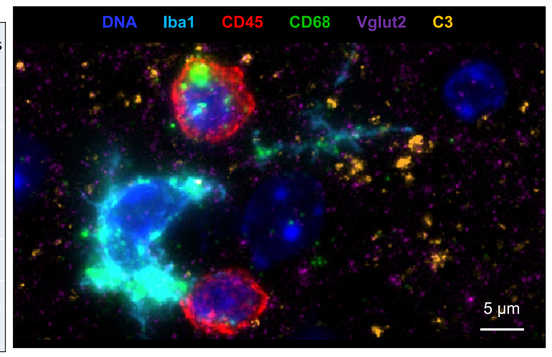
Downstream Consequences of Microglial Activity Include Downregulation of Genes Involved in Synaptic Transmission and Signaling Pathways

Macrophage Centered DE Genes

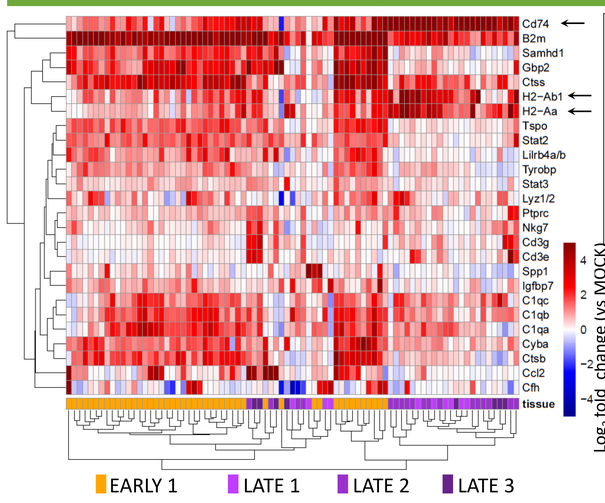


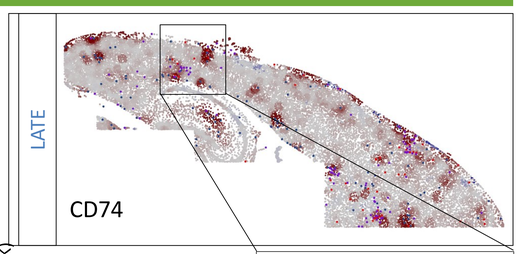
Gene Ontology of Downregulated Gene Cluster

1	Transmission Across Chemical Synapses R-HSA-112315
2	Neuronal System R-HSA-112316
3	Opioid Signaling R-HSA-111885
4	CaMK IV-mediated Phosphorylation Of CREB R-HSA-111932
5	Signal Transduction R-HSA-162582
6	Post NMDA Receptor Activation Events R-HSA-438064

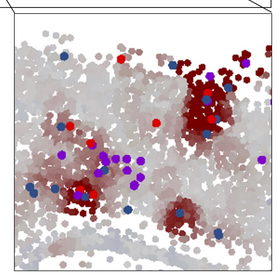


Gene Expression Changes Within Immune Hotspots

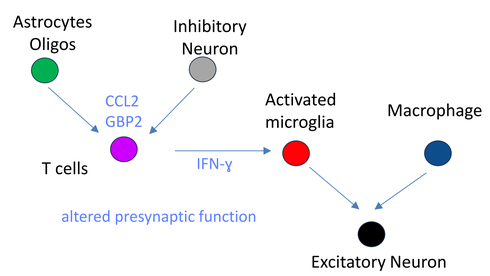




- T cell
- Macrophage
- Activated microglia



Drivers of Nodular Inflammation in WNV Encephalitis



Conclusions

By utilizing a spatial multiomics approach, we:

- Profile **64 proteins and 1000 genes** in a mouse model of WNV-triggered Neuroinflammation
- Identify key cell types involved in the inflammatory response, including **diverse immune cell populations**
- Reveal how spatial dynamics of **immune cell populations change over time**
- Demonstrate a spatial relationship between **activated microglia and T-cells**
- Reveal **key gene expression signatures** in neurons, astrocytes, and oligodendrocytes involved in the **recruitment of T-cells**
- Implicate microglial activation in the **downregulation of essential neuronal functions** such as synaptic transmission and signaling pathways
- Characterized **changes in gene expression** across the timeline of neuroinflammation, identifying a **marked increase in CD74** at late stages

Scan here to download or learn more