Received: 25 July 2025 | Revised: 11 December 2025 | Accepted: 11 December 2025

DOI: 10.1002/epi.70079

Epilepsia®

COMMENTARY

Spatial transcriptomics in epilepsy research: Early successes, opportunities, and challenges

Donald J. Phillips[1,2] | **Autumn S. Ivy**[3,4,5] | **Samuel Guzman**[6] | **Saman Hazany**[7,8,9] | **Xiangmin Xu**[10,11]

[1]Department of Pediatrics, Rady Children's Hospital of Orange County, Orange, California, USA

[2]Department of Pediatrics, School of Medicine, University of California, Irvine, Irvine, California, USA

[3]Michael V. Johnston Center for Developmental Neuroscience, Kennedy Krieger Institute, Baltimore, Maryland, USA

[4]Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

[5]Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

[6]Department of Pathology, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA

[7]Department of Radiology, VA Greater Los Angeles Healthcare System, Los Angeles, California, USA

[8]Department of Radiology, Keck School of Medicine of USC, Los Angeles, California, USA

[9]Department of Radiology, David Geffen School of Medicine at UCLA, Los Angeles, California, USA

[10]Department of Anatomy and Neurobiology, School of Medicine, University of California, Irvine, Irvine, California, USA

[11]Center for Neural Circuit Mapping, University of California, Irvine, Irvine, California, USA

Correspondence
Donald J. Phillips, Rady Children's Hospital of Orange County, 1201 W. La Veta Avenue, Orange, CA 92868, USA.
Email: dphillips@choc.org

KEYWORDS
bioinformatics, multimodal, multiomics, RNA sequencing, spatial biology, spatial transcriptomics, translational

1 | INTRODUCTION

Epilepsy affects more than 70 million people worldwide[1] and remains an important cause of global disability and mortality.[2] Despite treatment advances, >30% of all epilepsies remain refractory to medication.[3–5] Improving the mechanistic understanding of epilepsy pathogenesis remains a critical unmet need.[6] Epilepsy is often conceptualized as a disease of brain networks, not only as derangements within a connectome of neurons and network hubs,[7] but also as pathological changes within an interactome of molecular processes among neurons and surrounding glia.[8] Considering this conceptual framework,[9] understanding the spatial relationship and interaction among cells within epilepsy networks is crucial.

Transcriptomics, as a method of characterizing cellular gene expression through sequencing mRNA, has rapidly advanced understanding of human brain cellular diversity. Transcriptomic sequencing technology has quickly evolved through three stages: bulk sequencing, single-cell RNA sequencing (scRNA-seq), and spatial transcriptomics (ST; Figure 1).[11] The evolution from bulk to single-cell sequencing[12] has revealed thousands of regionally specific brain cell types,[13] with individual cortical areas

Donald J. Phillips and Autumn S. Ivy contributed equally to this work.

Social Media Statement: Spatial transcriptomics can unlock the molecular architecture of epilepsy, driving discovery toward a deeper understanding of epileptogenesis and precision therapies.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2026 The Author(s). *Epilepsia* published by Wiley Periodicals LLC on behalf of International League Against Epilepsy.

demonstrating >100 unique neuronal and nonneuronal subtypes.[14] However, epilepsy is foundationally a network disease. Although single-cell transcriptomics can unlock new insights into epilepsy pathogenesis, spatially resolved gene expression characterization is needed for comprehensive molecular understanding of altered circuit function. ST addresses this by profiling single-cell gene expression in intact tissue, preserving spatial context critical for understanding neuronal–glial interactions, defining epileptogenic zone boundaries, and determining network-level expression changes. As ST becomes more accessible, building expertise and research communities to catalyze its use in epilepsy discovery is essential.

At the 2024 American Epilepsy Society annual meeting, our team organized an Investigators Workshop highlighting ST in epilepsy research. We now formally summarize and expand that presentation in this article.

Key points

- ST measures RNA expression and resolves cellular subtypes while preserving spatial context in brain and other tissues.
- ST has the potential to provide highly resolved molecular–spatial characterization of hyperexcitable cells and networks in individual epilepsy patients.
- ST can integrate with other clinical and diagnostic modalities for new insights into the molecular characterization, diagnosis, and treatment of epilepsy.
- Multicenter collaboration catalyzes ST by increasing sample sizes, leveraging regionalized expertise, and defraying high individual experiment costs.

2 | ST TECHNOLOGIES

ST technologies are categorized as either imaging-based or sequencing-based, with advantages and limitations of each summarized in Table 1.

Imaging-based approaches use fluorescent probes to visualize specific genes directly in tissue sections. These methods achieve subcellular resolution (<300 nm) and include in situ sequencing (e.g., fluorescence in situ sequencing [FISSEQ]) and in situ hybridization (e.g., multiplexed error-robust fluorescence in situ hybridization [MERFISH]). MERFISH labels RNA with error-robust barcodes detected through successive rounds of fluorescent hybridization, whereas FISSEQ directly sequences RNA within fixed tissue with higher signal-to-noise ratio but lower throughput. Both strategies excel at detecting individual RNA molecules within single cells but are limited to preselected gene panels.

Sequencing-based approaches use spatial barcodes on specialized arrays to capture mRNA from tissue sections, followed by next generation sequencing to profile the entire transcriptome. Spatial information is restored by aligning sequencing data with tissue images.[12,15,16] Although these methods typically have lower resolution than imaging approaches, they offer unbiased,

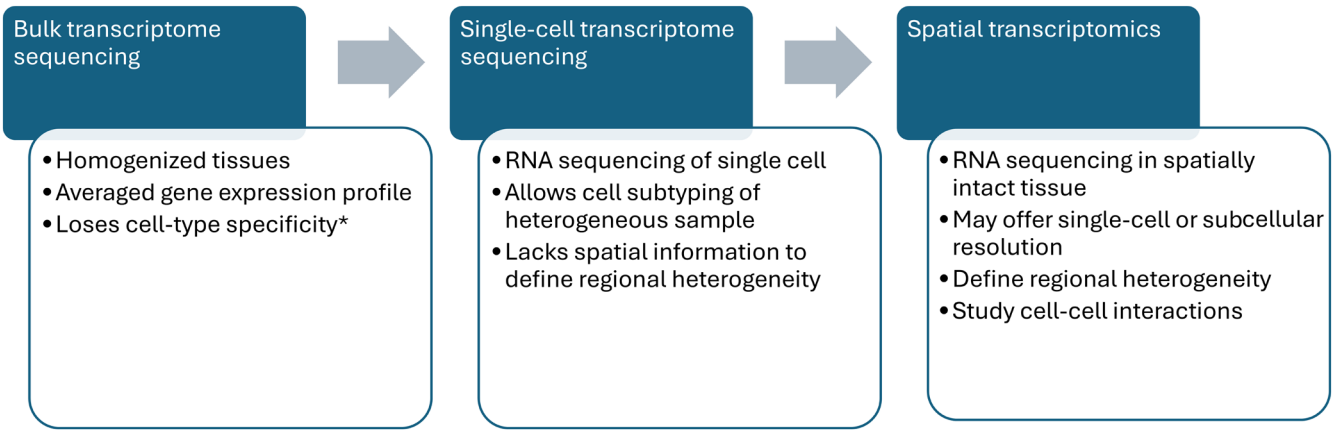

FIGURE 1 Evolution of transcriptomic technology. Transcriptomic technology has evolved through the stages of bulk transcriptome sequencing, single-cell transcriptome sequencing, and spatial transcriptomics (ST), with each step offering deeper characterization of the heterogeneity of complex biological systems. Bulk techniques study homogenized tissues, yielding an averaged gene expression profile from the entire sample. *Cell-type specificity is lost unless cellular subtype is selected for prior to bulk sequencing.[10] Single-cell RNA sequencing characterizes a cell's individual transcriptome, allowing for subtyping of heterogeneous cellular populations, but lacks the spatial information to define regional heterogeneity. ST allows the RNA sequencing and/or visualization of thousands of RNA molecules within spatially intact tissues.

TABLE 1 Comparison of major methodologies in spatial transcriptomics.

	Imaging-based	Sequencing-based
Examples	MERFISH, seqFISH+, STARmap, MERSCOPE, Xenium, CosMx	Visium/Visium HD, Stereo-seq, GeoMX, DBiT-seq, Seq-Scope
Techniques employed	FISSEQ, ISH	Spatially barcoded arrays, next generation sequencing
Number of genes profiled	• Limited/targeted • 500–6000 (targeted) • Preselected genes of interest • Limited by specificity	• Unlimited/unbiased • Whole transcriptome • 10–20 K+ (all expressed genes)
Spatial resolution	• Higher • Subcellular to single cell • ≤300 nm	• Lower • Multicellular to single cell • .5–100 μm
Sensitivity	• Higher • Can detect individual RNA molecules • Good for low-abundance transcripts • Limited by optical crowding, spectral discrimination	• Variable • Dependent upon sequencing depth • May be insensitive to low-abundance transcripts • Limited by template diffusion, probe accessibility
Throughput	• Lower • Slower imaging cycles • Smaller tissue area/cycle	• Higher • Larger tissue areas/cycle
Quantification	• Absolute • Direct molecular counting	• Relative • Uses UMIs to address PCR amplification bias
Cell segmentation	• Easier • Aided by high resolution • Can utilize membrane staining	• More challenging • Lower resolution • Computational methods required
Discovery potential	• Limited • Uses preselected genes • Insensitive to novel transcripts	• High • Unbiased approach • Can identify novel gene expression
Cost per sample	• High • Higher upfront capital costs • Costly reagents • Scales with number of genes, coverage area	• High • Scales with depth of sequencing, coverage area
Application	• Subcellular localization • Smaller tissue areas • Single molecule detection • Specific gene(s) of interest	• Discovery-oriented studies • Whole transcriptome profiling • Larger tissue sections • Novel transcript identification

Note: MERSCOPE: Vizgen. Xenium: 10x Genomics. CosMx spatial molecular imager: NanoString. Visium/Visium HD: 10x Genomics. Stereo-seq: STOmics. GeoMX digital spatial profiler: NanoString.

Abbreviations: DBiT, deterministic barcoding in tissue; FISH, fluorescence in situ hybridization; FISSEQ, fluorescent in situ RNA sequencing; ISH, in situ hybridization; MERFISH, multiplexed error-robust FISH; PCR, polymerase chain reaction; seqFISH+, sequential FISH; STARmap, spatially resolved transcript amplicon readout mapping; UMI, unique molecular identifiers.

genome-wide gene expression profiling without requiring preselection of target genes. Recent advances have improved resolution, sensitivity, and scalability to accommodate larger samples.

Individual platforms within both categories continue to evolve rapidly (Figure 2). Furthermore, hybrid approaches that combine the advantages of both methods are being developed, with some platforms adding multiomic (e.g., transcriptomic + proteomic) capabilities. A schematic of ST workflows is provided in Figure 3.

3 | ST STUDY DESIGN

ST has been successfully applied to mouse and human brain tissue in both physiological[20–22] and pathological[23] conditions, including epilepsy.[24] However, ST requires careful study design due to high costs, lengthy processing times, and lack of technical standardization. Four key considerations guide ST study design (Table 2): experimental question, experimental groups, available resources, and anticipated results.

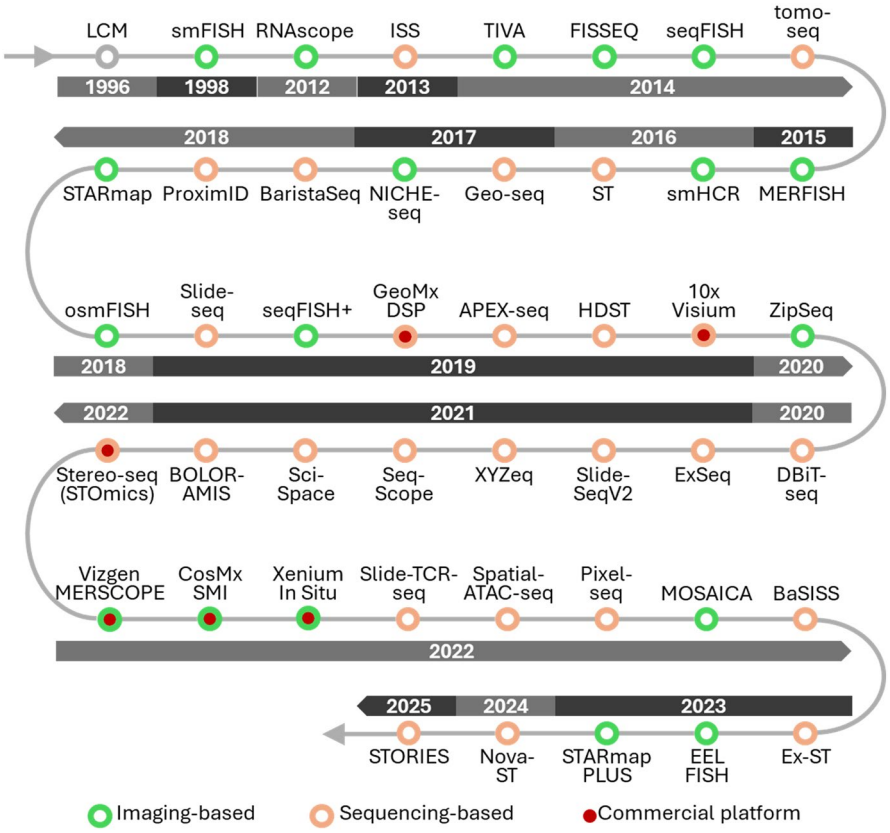

FIGURE 2 Evolution of spatial transcriptomics (ST) techniques. The timeline indicates key technologies and years of publication. APEX, ascorbate peroxidase; ATAC, assay for transposase-accessible chromatin; BaristaSeq, barcode in situ targeted sequencing; BaSISS, barcoding with amplification of specific in situ sequences; BOLARAMIS, barcoded oligonucleotides ligated on RNA amplified for multiplexed and parallel in situ analyses; DBiT, deterministic barcoding in tissue; DSP, digital spatial profiling; EEL, enhanced electric; ExSeq, expansion sequencing; Ex-ST, expansion ST; FISH, fluorescence in situ hybridization; FISSEQ, fluorescent in situ RNA sequencing; Geo-seq, geographical position sequencing; HDST, high-definition ST; ISS, in situ sequencing; LCM, laser capture microdissection; MERFISH, multiplexed error-robust FISH; MOSAICA, multiomic single-scan assay with integrated combinatorial analysis; NICHE, nicking enzyme-assited reaction technology; osmFISH, ouroboros single-molecule RNA FISH; seqFISH, sequential FISH; smFISH, single-molecule RNA FISH; smHCR, single-molecule hybridization chain reaction; STARmap, spatially resolved transcript amplicon readout mapping; Stereo-seq, spatial enhanced resolution omics-sequencing; STORIES, spatiotemporal omics energies; TCR, T-cell receptor; TIVA, transcriptome in vivo analysis; tomo-seq, RNA tomography.[17]

The experimental question determines the optimal ST approach (Figure 4). Data-driven studies aim to characterize total spatial gene expression without specific hypotheses, making them discovery-oriented and best suited for nontargeted, sequencing-based methods that profile the whole transcriptome. These studies have generated valuable transcriptomic atlases.[20,21,26,27] Hypothesis-driven studies test specific predictions and typically utilize imaging-based ST methods for higher spatial resolution and sensitivity.[28,29] These require appropriate controls, which may leverage existing mouse[30,31] or adult human[32] atlas datasets, or investigators may incorporate their own ST control group. Choosing appropriate control tissue is nontrivial, given variability among different brain regions and intersubject variability. Additional considerations, including species, sample type (fixed versus frozen), platform availability, and budget, ultimately guide platform selection.

3.1 | Anticipated results

Investigators must consider a priori the types of data they will obtain and how they may be used to address their research questions. Critical preanalytical considerations include tissue quality, RNA integrity, and sample processing protocols, as RNA degradation significantly impacts data quality and interpretation. Surgical samples present unique challenges due to inherent variability in tissue handling, ischemic time, seizure activity prior to resection, and patient-specific factors (e.g., antiseizure medications, seizure frequency). Standardized protocols for tissue collection, processing, and storage are essential to

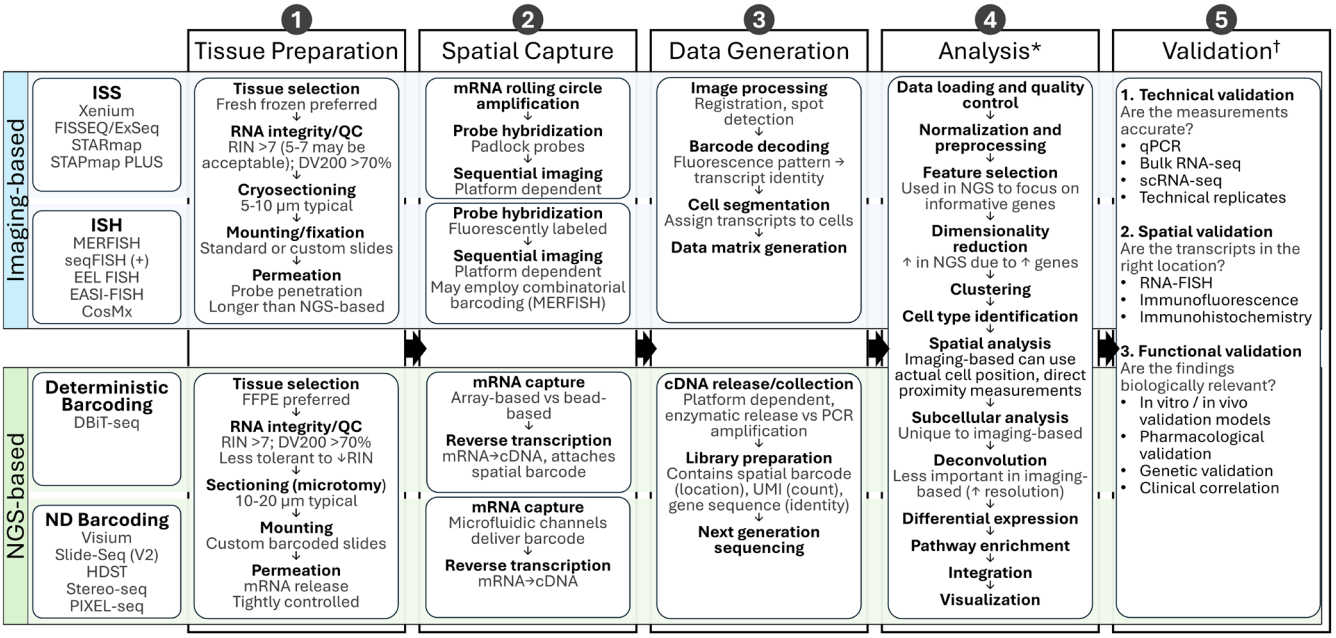

FIGURE 3 Schematic overview of spatial transcriptomics (ST) technologies used in epilepsy research. Image-based ST technologies are divided into two categories: In situ sequencing (ISS), which amplifies mRNA, or in situ hybridization (ISH), which detects target sequences by hybridization of complementary fluorescent probes. The output data of each technique is visualized using multiple fluorescence-based imaging techniques. Each image capturing the mRNA is further processed analyzed to create a gene expression matrix. Next generation sequencing (NGS)-based ST techniques employ two types of barcoding. Deterministic barcoding utilizes microfluidic channel chips to deliver barcodes to the surface of a tissue slide by reverse transcription and ligation through crossflow. Barcoded samples then undergo sequencing for further analyses. In nondeterministic (ND) barcoding, a barcoded array is used to barcode transcripts according to their location in lattice spots. Barcoded samples are then sequenced to extract the spatial expression profile.[18] Data generation descriptions are summarized from Williams et al.[19] *A detailed explanation of ST analysis methods is beyond the scope of this article. General steps are provided for reference. †Validation is also discussed in Table 3. DBiT, deterministic barcoding in tissue; DV200, perentage of RNA fragments larger than 200 nucleotides; EASL, expansion-assisted interative fluorescence; EEL, enhanced electric; ExSeq, expansion sequencing; FFPE, formalin-fixed, paraffin-embedded; FISH, fluorescence in situ hybridization; FISSEQ, fluorescent in situ RNA sequencing; HDST, high-definition ST; MERFISH, multiplexed error-robust FISH; ND, non-deterministic; PIXEL, polony-indexed library sequencing; QC, quality control; qPCR, quantitative polymerase chain reaction; RIN, RNA integrity number; seqFISH, sequential FISH; STARmap, spatially resolved transcript amplicon readout mapping; UMI, unique molecular identifiers.

minimize technical variability. Additionally, batch effects arising from processing samples across different times or conditions must be carefully controlled and computationally corrected during analysis.

ST experiments frequently generate petabytes of data, which undergo multiple steps of data processing, so collaboration with a bioinformatics specialist experienced in large datasets is essential. Computational expertise is especially critical in higher order, inferred datasets like cell–cell interaction, which may be critical to understanding epileptic circuits. Quality control metrics should assess RNA integrity numbers, tissue morphology, sequencing depth, and spatial mapping accuracy before downstream analysis. Once differential gene expression lists, cell subtypes, and their spatial locations are identified, one can perform gene ontology analyses and gene set enrichment analyses to identify features of gene expression that provide insights into changes in molecular and cellular function.[25,33] Lastly, data validation is critical in ST and should be considered during study design (Table 3).[19,34]

4 | ST IN TRANSLATIONAL AND CLINICAL EPILEPSY RESEARCH

ST in preclinical and clinical epilepsy research remains nascent. Although bulk and single-cell techniques have been used in epilepsy models to define cellular heterogeneity,[35,36] glial dysfunction,[37,38] immune infiltration,[39] and interneuron dysfunction,[40] our literature search revealed few publications applying ST to the neurobiology of epilepsy (Table 4). Fewer of these studies utilized spatial data in their analyses, and we failed to find any ST studies assessing cell–cell interaction networks in epilepsy.

Two preclinical studies utilized a mouse kainic acid model of temporal lobe epilepsy (TLE). The first integrated

TABLE 2 Considerations for designing spatial transcriptomics experiments.

	Key questions	Possible guidance
Experimental question	• What biological question is being tested? • Hypothesis driven, discovery driven, or both? • Is the spatial content in the dataset crucial to answering the question? • Does the question necessitate single cell resolution?	• Consider imaging-based techniques if hypothesis-driven, or if single cell/subcellular resolution is needed • Consider sequencing-based methods if discovery-driven • Consider cheaper nonspatial technique if spatial content not needed
Experimental groups	• What is the study population? • What are potential sources or heterogeneity? • What is the control/baseline group or dataset?	• Choose tissue sources to limit heterogeneity • Consider possible confounders, e.g., medication effects • Consider how to source control tissue, age/sex matching, etc. • Leverage biobanks • Consider strengths and limitations of atlases and normal datasets
Available resources	• Size and availability of study tissue? • Species of study sample? • Fresh frozen or FFPE? Anticipated RNA integrity?	• Species, tissue source, and RNA integrity will all determine availability of commercial platforms • Budget may also influence platform choice
Anticipated results	• What types of data will be generated? • What resources will be needed for analysis?	• Understand data-processing capabilities and limitations at study onset, vendor-integrated versus independent • Leverage multicenter expertise/collaboration

Note: Listed for each consideration are key questions driving each decision, as well as possible guidance for each category.

Abbreviations: FFPE, formalin-fixed, paraffin-embedded.

scRNA-seq, single-nucleus RNA sequencing, and ST to create a high-resolution atlas of hippocampal cellular subtypes, uncovering important TLE-induced molecular changes underlying epilepsy pathogenesis, including glial inflammatory responses, suppressed γ-aminobutyric acidergic synapses, and reactive neurogenesis.[43] The second identified specific overexpression of C-C chemokine ligand 5 in seizure hippocampi and functionally validated this finding through maraviroc-induced pathway blockage, preventing microglia activation and neuron degeneration in the seizure mouse.[42] The first study was limited by gene panel size, and both were limited by model generalizability.

Two studies applied sequencing-based ST (10x Genomics Visium) to surgically resected human focal cortical dysplasia (FCD). The first study implicated dysplastic neuronal mTOR and autophagy pathways and balloon cell inflammatory pathways in FCD pathogenesis.[24] The second synthesized a transcriptional catalog of type IIb FCD and identified noncanonical markers of signaling and neurotransmitter pathways in cytomegalic dysplastic neurons, noting *SLC6A5* as a specific marker.[44] Both studies were limited by small sample sizes, tissue heterogeneity, and lack of well-established control data. Finally, Busch et al., employed sequencing-based ST (GeoMx) to study comorbid memory loss in human TLE.[41] Utilizing spatial data, investigators localized the strongest molecular signal to hippocampus CA3, an anatomical substructure critical to memory formation. *BDNF* was identified as a central hub in regulating CA3-related networks.

As such early efforts expand, ST has the potential to reach beyond pathological characterization, translating to aid biomarker discovery, surgical decision-making, and therapeutic development.

5 | ST IN CLINICAL PRACTICE

Histopathological diagnosis remains the gold standard in determining etiology and likely outcomes of various structural epilepsies. This is perhaps best demonstrated in TLE[46,47] and FCD.[48–50] Current histopathological classification criteria rely upon features such as cellular morphology and density to diagnose and stratify. However, molecular techniques are increasingly incorporated into diagnostic practice.[51] Molecular biomarkers have transformed clinical oncology, uncovering new treatment pathways and driving tumor reclassification,[52,53] as well as defining the borders between healthy and diseased tissue. Molecular techniques like single-cell transcriptomics have revealed mTOR/MAP kinase[54] and other pathways involving synaptic function and calcium dynamics alterations in the formation of FCD.[55] ST may likewise alter how structural epilepsy

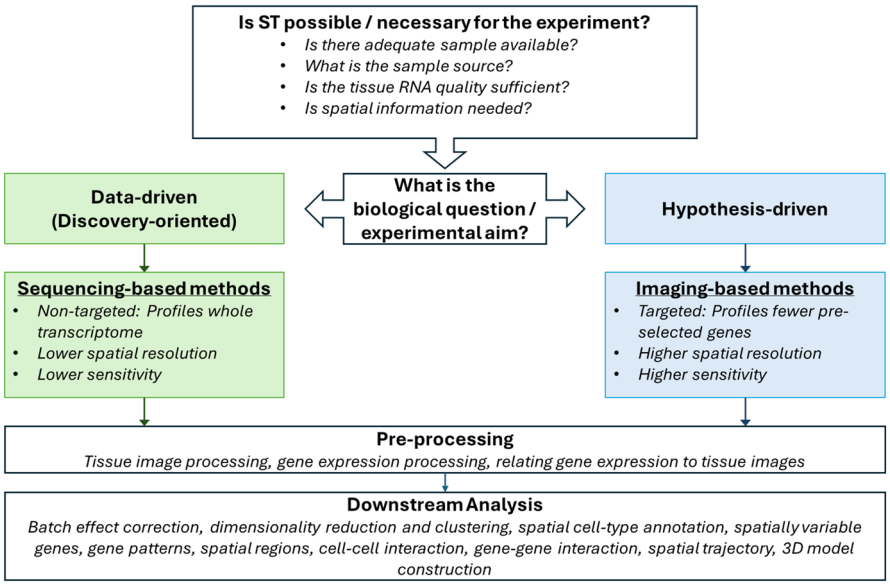

FIGURE 4 Algorithm for study design considerations in spatial transcriptomics (ST). Outlined are possible considerations regarding whether ST is possible and/or necessary to answer the proposed biological question and whether a data-driven (discovery-oriented) or hypothesis-driven (hypothesis testing) strategy is preferred. Data-driven studies are nontargeted and profile the whole transcriptome within the test sample. They are often used to generate hypotheses. Hypothesis-driven studies employ targeted gene profiles at higher sensitivity and spatial resolution. Details regarding preprocessing and downstream analysis are beyond the scope of this paper, but typical steps are listed for reference. Although evolving rapidly, current software tools for analysis steps are also listed, for reference: dimensionality reduction (Scanpy, Giotto, SPATA, STUtility), spatial cell type annotation (cell2location, stereoscope, DestVI, AdRoit), spatially variable genes (Scanpy, SpatialDE, SPARK, GPcounts), gene patterns (stLearn, SpatialDE, SPATA, MERINGUE), spatial regions (MULTILAYER, RESEPT), cell–cell interaction (SpaOTsc, DIALOGUE, SVCA), gene–gene interaction (GCNG, SpaOTsc, MISTy, MESSI), spatial trajectory (SPATA), three-dimensional (3D) model (STUtility, STAGATE).[19,25]

TABLE 3 Data validation in ST.

Validation type	Methods	Assessed/confirmed	Epilepsy example
Technical/measurement	qPCR, bulk RNA-seq, scRNA-seq, technical replicates	Accuracy in gene expression, cell typing/subtyping	Verify upregulation of DEGs in dysplastic neurons
Spatial	RNA-FISH, immunohistochemistry	Transcript/protein localization	Confirm glial or neuronal origin in zone of interest
Functional	Pharmacological intervention, genetic manipulation, electrophysiology, clinical correlation	Biological relevance, mechanistic predictions	Test whether drug blocking ST-identified pathway reduces seizure activity

Note: A detailed review of data validation techniques is beyond the scope of this commentary; however, categories and example methods are briefly tabulated.
Abbreviations: DEG, differentially expressed gene; FISH, fluorescence in situ hybridization; qPCR, quantitative polymerase chain reaction; RNA-seq, RNA sequencing; scRNA-seq, single-cell RNA sequencing; ST, spatial transcriptomics.

is diagnosed, potentially forcing a deeper understanding of epilepsy pathogenesis on an individual patient level.

5.1 | Multimodal integration

ST holds transformative potential for integration with conventional diagnostic modalities (magnetic resonance imagining [MRI], positron emission tomography [PET], electroencephalography [EEG]) to generate noninvasive molecular biomarkers. Building on MRI–transcriptomics integration in Alzheimer disease,[56] similar frameworks could link preoperative neuroimaging to spatial molecular profiles, enabling molecular phenotyping of epileptogenic zones prior to surgery. Combining ST with multielectrode array recordings, which have revealed altered neuro- and gliogenesis in TLE,[57] could elucidate molecular mechanisms of hyperexcitability with subcellular resolution.

TABLE 4 Summary of published studies performing ST in various animal and human epilepsy models.

Study details	Tissue/ species	Epilepsy model	ST platform	Key findings	Genes/pathways	Limitations
Busch, et al., 2022,[41] USA	Human	TLE	GeoMx	Numerous neocortical and hippocampal DEGs between memory groups. CA3 with strongest molecular signal, critical to memory formation. *BDNF* was identified as a central hub in regulating CA3-related networks.	111 DEGs in CA3 between memory groups. *BDNF* identified as central hub.	Sample size, limited to ILAE type 1 HS, unclear contribution of chronic seizures on the upregulation of neurodegenerative genes.
Zhang et al., 2023,[42] China	Mouse	TLE, KA	10x Visium	Characteristic hippocampal inflammatory gene signatures involving glia activation, apoptosis, immune regulation. Specific overexpression of *Ccl5* was noted throughout the entire seizure hippocampus. Blockade of *Ccl5*/*Ccr5* signaling via maraviroc prevented microglia activation and neuron degeneration in seizure mice.	*Ccl5* highest DEG expression; ↑ chemokine-related *Mcp1*, *Ccl12*; ↑ glial *Lcn2*, *Spp1*.	Model not fully representative of human TLE, may have differential effect on inflammatory pathways; *Ccl5*/*Ccr5* role in epilepsy unknown, not specific to epilepsy.
Liu et al., 2024,[43] China	Mouse	TLE, KA	Xenium (mouse brain panel, 247 genes)	High-resolution ST atlas of mouse hippocampus with TLE revealing potential intrinsic mechanisms driving TLE-associated inflammatory activation and altered cell interactions.	↑ glial *Spp1*, *Trem2*, *Cd68*; ↑ neuronal *Penk, Sorcs3, Plekha2*; ↓ glial *Tle4, Sipa1l3*.	Model not fully representative of human TLE, may have differential effect on inflammatory pathways; ST analysis limited to KA injection site; limited to 247 genes.
Wang et al., 2024,[24] China	Human	FCD IIb	10x Visium	DN regions demonstrate upregulation in the mTOR signaling pathway, as well as autophagy, ubiquitin–proteasome, & membrane potential regulation pathways. Balloon cell regions demonstrated upregulation in inflammatory and complement activation pathways.	DNs: *NEFH*, *NEFL*, *NEFM*, *ENC1*, *NRGN*, *VSNL1*, *OLFM1*, *CCK*, *CHN1*, *UCHL1*, *CRYM*, *YWHAH*, *MDH1*, *SNAP25*, *TUBB2A*, *NSF*, *GABRD*, *GAP43*, *NPTX2*, *BASP1*; BCs: *VIM*, *CRYAB*, *EFEMP1*, *TNC*, *CLU*, *IGFBP7*, *SPARC*, *GPNMB*, *PTGDS*, *MAOB*, *MGST1*, *APOE*, *ANXA1*, *SERPINA3*, *AEBP1*, *GSN*, *GJA1*, *C3*, *CSRP1*, *AQP4*.	Heterogeneity of resected tissue; unclear localization of tissue sources; small sample size; variability among samples, especially BCs; lack of control tissue/normal atlas with lobar/ sublobar specificity; lack of single-cell RNA sequencing data/cell subtype analysis; lack of tissue genomic testing.
Paliwal et al., 2025,[44] Canada	Human	FCD IIb	10x Visium	Generation of anatomical transcriptional catalog of type IIb FCD, identified noncanonical markers of signaling and neurotransmitter pathways in CDNs.	↑ CDN *SLC6A5*, *ARHGAP36*, *PDYN* (proved more specific marker of CDN than *NEFL*). Novel ↑ CDN *GlyT2*, *SLC32A1*.	Small sample size: Single ST case with immunohistological validation from 8 additional cases; lack of control tissue.

TABLE 4 (Continued)

Study details	Tissue/species	Epilepsy model	ST platform	Key findings	Genes/pathways	Limitations
Han et al., 2025,[45] China	Human	TLE	10x Visium	Using single cell TLE and ST human Alzheimer datasets, comparing differentially expressed genes and spatial transition tensor (STT)-identified spatial stability genes, *NPY* and *GFAP* were identified as deregulated genes in TLE.	*NPY*; *GFAP*.	Comparison of spatial and nonspatial datasets; control dataset from Alzheimer cohort; reanalysis study: no new ST data generated.

Abbreviations: BC, balloon cells; CDN, cytomegalic DN; DEG, differentially expressed genes; DN, dysplastic neuron; FCD, focal cortical dysplasia; HS, hippocampal sclerosis; ILAE, International League Against Epilepsy; KA, kainic acid; ST, spatial transcriptomics; STT, spatial transition tensor; TLE, temporal lobe epilepsy.

Nonspatial transcriptomic profiling from patch-clamp recordings[58] and stereotactic EEG studies[59] has demonstrated the feasibility of molecular–electrophysiological integration; incorporating spatial context could define molecular changes across epileptic networks. Conversely, well-established electrophysiological and neuroimaging techniques could help validate emerging ST findings (Table 3).

However, substantial challenges remain. Data harmonization across platforms with different resolutions, coordinate systems, and formats requires sophisticated coregistration. Computational interpretability of integrated high-dimensional datasets, namely, millions of gene measurements combined with electrophysiological and imaging data, demands advanced analytical frameworks. Although deep learning shows promise,[60] limited training datasets in epilepsy constrain current applications. Standardized data-sharing protocols, common data elements, and collaborative consortia are essential to build infrastructure for robust multimodal integration and realize ST's translational potential.

6 | CONCLUSIONS AND FUTURE DIRECTIONS

Spatial transcriptomics, as part of a multiomic framework, represents a paradigm shift from descriptive pathology to mechanistically informed, spatially resolved molecular medicine in epilepsy. By revealing molecular disruptions, dysregulated cellular subtypes, and aberrant signaling pathways within epileptic circuits, ST can transform histopathological pattern recognition into mechanistic understanding.

Clinically, colocalizing ST-derived molecular signatures with electrophysiological markers of hyperexcitability (EEG, intracranial recordings) and structural/metabolic abnormalities (MRI, PET) may improve prediction of treatment response, stratification of surgical candidates, and delineation of resection boundaries. Integration with artificial intelligence (AI) can identify previously unrecognized molecular subtypes and disease patterns from large-scale spatial transcriptomic datasets, enabling precision therapies. This mechanism-based, AI-augmented approach holds promise for improving seizure freedom rates, reducing surgical morbidity, and identifying disease-modifying therapies for patients with drug-resistant epilepsy.

AUTHOR CONTRIBUTIONS

Donald J. Phillips: Conceptualization (co-lead); writing—original draft (co-lead); writing—review and editing (equal); visualization (main). **Autumn S. Ivy:**

Conceptualization (co-lead); writing—original draft (co-lead); writing—review and editing (equal). **Samuel Guzman:** Writing—original draft (supporting); writing—review and editing (equal). **Saman Hazany:** Writing—original draft (supporting); writing—review and editing (equal). **Xiangmin Xu:** Conceptualization (supporting); writing—original draft (supporting); writing—review and editing (equal).

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

DATA AVAILABILITY STATEMENT

This commentary synthesizes published literature; no new data were generated. Readers can contact the corresponding author for details on accessing source data from original studies.

ORCID

Donald J. Phillips https://orcid.org/0009-0000-2817-4481
Autumn S. Ivy https://orcid.org/0000-0003-1775-6253
Samuel Guzman https://orcid.org/0000-0001-7029-978X
Saman Hazany https://orcid.org/0000-0002-4864-2710
Xiangmin Xu https://orcid.org/0000-0002-5828-1533

REFERENCES

1. Thijs RD, Surges R, O'Brien TJ, Sander JW. Epilepsy in adults. Lancet. 2019;393(10172):689–701.
2. GBD 2016 Epilepsy Collaborators. Global, regional, and national burden of epilepsy, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 2019;18(4):357–75.
3. Duncan JS, Sander JW, Sisodiya SM, Walker MC. Adult epilepsy. Lancet. 2006;367(9516):1087–100.
4. Kwan P, Brodie MJ. Early identification of refractory epilepsy. N Engl J Med. 2000;342(5):314–9.
5. Perucca E, Brodie MJ, Kwan P, Tomson T. 30 years of second-generation antiseizure medications: impact and future perspectives. Lancet Neurol. 2020;19(6):544–56.
6. Weaver DF, Pohlmann-Eden B. Pharmacoresistant epilepsy: unmet needs in solving the puzzle(s). Epilepsia. 2013;54(Suppl 2):80–5.
7. Royer J, Bernhardt BC, Larivière S, Gleichgerrcht E, Vorderwülbecke BJ, Vulliémoz S, et al. Epilepsy and brain network hubs. Epilepsia. 2022;63(3):537–50.
8. Noebels J. Pathway-driven discovery of epilepsy genes. Nat Neurosci. 2015;18(3):344–50.
9. Mahoney JM, Mills JD, Muhlebner A, Noebels J, Potschka H, Simonato M, et al. 2017 WONOEP appraisal: studying epilepsy as a network disease using systems biology approaches. Epilepsia. 2019;60(6):1045–53.
10. Raus AM, Fuller TD, Nelson NE, Valientes DA, Bayat A, Ivy AS. Early-life exercise primes the murine neural epigenome to facilitate gene expression and hippocampal memory consolidation. Commun Biol. 2023;6(1):18.
11. Zhao SH, Ji XY, Yuan GZ, Cheng T, Liang HY, Liu SQ, et al. A bibliometric analysis of the spatial Transcriptomics literature from 2006 to 2023. Cell Mol Neurobiol. 2024;44(1):50.
12. Marx V. Method of the year: spatially resolved transcriptomics. Nat Methods. 2021;18(1):9–14.
13. Siletti K, Hodge R, Mossi Albiach A, Lee KW, Ding SL, Hu L, et al. Transcriptomic diversity of cell types across the adult human brain. Science. 2023;382(6667):eadd7046.
14. Johansen N, Somasundaram S, Travaglini KJ, Yanny AM, Shumyatcher M, Casper T, et al. Inter-individual variation in human cortical cell type abundance and expression. Science. 2023;382(6667):eadf2359.
15. Rao A, Barkley D, França GS, Yanai I. Exploring tissue architecture using spatial transcriptomics. Nature. 2021;596(7871):211–20.
16. Wang Y, Liu B, Zhao G, Lee Y, Buzdin A, Mu X, et al. Spatial transcriptomics: technologies, applications and experimental considerations. Genomics. 2023;115(5):110671.
17. Huizing GJ, Samaran J, Capocefalo D, Audit A, Peyré G, Cantini L. STORIES: learning cell fate landscapes from spatial transcriptomics using optimal transport. Nat Methods. 2026;23:522–31.
18. Junaid M, Lee EJ, Lim SB. Single-cell and spatial omics: exploring hypothalamic heterogeneity. Neural Regen Res. 2025;20(6):1525–40.
19. Williams CG, Lee HJ, Asatsuma T, Vento-Tormo R, Haque A. An introduction to spatial transcriptomics for biomedical research. Genome Med. 2022;14:68.
20. Fang R, Xia C, Close JL, Zhang M, He J, Huang Z, et al. Conservation and divergence of cortical cell organization in human and mouse revealed by MERFISH. Science. 2022;377(6601):56–62.
21. Zhang M, Eichhorn SW, Zingg B, Yao Z, Cotter K, Zeng H, et al. Spatially resolved cell atlas of the mouse primary motor cortex by MERFISH. Nature. 2021;598(7879):137–43.
22. Allen WE, Blosser TR, Sullivan ZA, Dulac C, Zhuang X. Molecular and spatial signatures of mouse brain aging at single-cell resolution. Cell. 2023;186(1):194–208.e18.
23. Wang C, Acosta D, McNutt M, Bian J, Ma A, Fu H, et al. A single-cell and spatial RNA-seq database for Alzheimer's disease (ssREAD). Nat Commun. 2024;15(1):4710.
24. Wang Y, Wang Y, Guo L, Shen C, Fu Y, Wei P, et al. Spatial transcriptomics in focal cortical dysplasia type IIb. Acta Neuropathol Commun. 2024;12(1):185.
25. Yue L, Liu F, Hu J, Yang P, Wang Y, Dong J, et al. A guidebook of spatial transcriptomic technologies, data resources and analysis approaches. Comput Struct Biotechnol J. 2023;21:940–55.
26. Gabitto MI, Travaglini KJ, Rachleff VM, Kaplan ES, Long B, Ariza J, et al. Integrated multimodal cell atlas of Alzheimer's disease. Nat Neurosci. 2024;27(12):2366–83.
27. BRAIN Initiative Cell Census Network (BICCN). A multimodal cell census and atlas of the mammalian primary motor cortex. Nature. 2021;598(7879):86–102.

28. Sun ED, Zhou OY, Hauptschein M, Rappoport N, Xu L, Navarro Negredo P, et al. Spatial transcriptomic clocks reveal cell proximity effects in brain ageing. Nature. 2025; 638(8049):160–71.
29. Hahn O, Foltz AG, Atkins M, Kedir B, Moran-Losada P, Guldner IH, et al. Atlas of the aging mouse brain reveals white matter as vulnerable foci. Cell. 2023;186(19):4117–4133.e22.
30. Lein ES, Hawrylycz MJ, Ao N, Ayres M, Bensinger A, Bernard A, et al. Genome-wide atlas of gene expression in the adult mouse brain. Nature. 2007;445(7124):168–76.
31. Zhang M, Pan X, Jung W, Halpern AR, Eichhorn SW, Lei Z, et al. Molecularly defined and spatially resolved cell atlas of the whole mouse brain. Nature. 2023;624(7991):343–54.
32. Huuki-Myers LA, Spangler A, Eagles NJ, Montgomery KD, Kwon SH, Guo B, et al. A data-driven single-cell and spatial transcriptomic map of the human prefrontal cortex. Science. 2024;384(6698):eadh1938.
33. Du J, Yang YC, An ZJ, Zhang MH, Fu XH, Huang ZF, et al. Advances in spatial transcriptomics and related data analysis strategies. J Transl Med. 2023;21(1):330.
34. Longo SK, Guo MG, Ji AL, Khavari PA. Integrating single-cell and spatial transcriptomics to elucidate intercellular tissue dynamics. Nat Rev Genet. 2021;22(10):627–44.
35. Pfisterer U, Petukhov V, Demharter S, Meichsner J, Thompson JJ, Batiuk MY, et al. Identification of epilepsy-associated neuronal subtypes and gene expression underlying epileptogenesis. Nat Commun. 2020;11(1):5038.
36. Lake BB, Ai R, Kaeser GE, Salathia NS, Yung YC, Liu R, et al. Neuronal subtypes and diversity revealed by single-nucleus RNA sequencing of the human brain. Science. 2016;352(6293):1586–90.
37. Leonard J, Wei X, Browning J, Gudenschwager-Basso EK, Li J, Harris EA, et al. Transcriptomic alterations in cortical astrocytes following the development of post-traumatic epilepsy. Sci Rep. 2024;14(1):8367.
38. Qian Z, Qin J, Lai Y, Zhang C, Zhang X. Large-scale integration of single-cell RNA-Seq data reveals astrocyte diversity and transcriptomic modules across six central nervous system disorders. Biomolecules. 2023;13(4):692.
39. Hou Y, Chen Z, Wang L, Deng Y, Liu G, Zhou Y, et al. Characterization of immune-related genes and immune infiltration features in epilepsy by multi-transcriptome data. J Inflamm Res. 2022;15:2855–76.
40. Dudok B, Klein PM, Soltesz I. Toward understanding the diverse roles of Perisomatic interneurons in epilepsy. Epilepsy Curr. 2022;22(1):54–60.
41. Busch RM, Yehia L, Blümcke I, Hu B, Prayson R, Hermann BP, et al. Molecular and subregion mechanisms of episodic memory phenotypes in temporal lobe epilepsy. Brain Commun. 2022;4(6):fcac285.
42. Zhang Z, Li Y, Jiang S, Shi FD, Shi K, Jin WN. Targeting CCL5 signaling attenuates neuroinflammation after seizure. CNS Neurosci Ther. 2023;29(1):317–30.
43. Liu Q, Shen C, Dai Y, Tang T, Hou C, Yang H, et al. Single-cell, single-nucleus and xenium-based spatial transcriptomics analyses reveal inflammatory activation and altered cell interactions in the hippocampus in mice with temporal lobe epilepsy. Biomark Res. 2024;12(1):103.
44. Paliwal A, Faust K, Borhani OA, Liu M, Omoto L, Kamski-Hennekam ER, et al. Dysmorphic neurons express markers of inhibitory glycinergic signaling in focal cortical dysplasia IIb. Brain Pathol. 2025;36(2):e70043.
45. Han L, Liu J, Song Y, Wang S, Zeng Z, Shi H. Landscape of spatial disruption of homeostasis in the middle temporal gyrus of epileptic patients. Brain Res. 2025;1860:149688.
46. Blümcke I, Pauli E, Clusmann H, Schramm J, Becker A, Elger C, et al. A new clinico-pathological classification system for mesial temporal sclerosis. Acta Neuropathol. 2007;113(3):235–44.
47. Na M, Ge H, Shi C, Shen H, Wang Y, Pu S, et al. Long-term seizure outcome for international consensus classification of hippocampal sclerosis: a survival analysis. Seizure. 2015;25:141–6.
48. Blümcke I, Thom M, Aronica E, Armstrong DD, Vinters HV, Palmini A, et al. The clinicopathologic spectrum of focal cortical dysplasias: a consensus classification proposed by an ad hoc task force of the ILAE diagnostic methods commission. Epilepsia. 2011;52(1):158–74.
49. Najm IM, Sarnat HB, Blümcke I. Review: the international consensus classification of focal cortical dysplasia - a critical update 2018. Neuropathol Appl Neurobiol. 2018;44(1):18–31.
50. Najm I, Lal D, Alonso Vanegas M, Cendes F, Lopes-Cendes I, Palmini A, et al. The ILAE consensus classification of focal cortical dysplasia: An update proposed by an ad hoc task force of the ILAE diagnostic methods commission. Epilepsia. 2022;63(8):1899–919.
51. Pang JMB, Byrne DJ, Bergin ART, Caramia F, Loi S, Gorringe KL, et al. Spatial transcriptomics and the anatomical pathologist: molecular meets morphology. Histopathology. 2024;84(4): 577–86.
52. McNamara C, Mankad K, Thust S, Dixon L, Limback-Stanic C, D'Arco F, et al. 2021 WHO classification of tumours of the central nervous system: a review for the neuroradiologist. Neuroradiology. 2022;64(10):1919–50.
53. Sahm F, Aldape KD, Brastianos PK, Brat DJ, Dahiya S, von Deimling A, et al. cIMPACT-NOW update 8: clarifications on molecular risk parameters and recommendations for WHO grading of meningiomas. Neuro-Oncology. 2025;27(2):319–30.
54. Baldassari S, Ribierre T, Marsan E, Adle-Biassette H, Ferrand-Sorbets S, Bulteau C, et al. Dissecting the genetic basis of focal cortical dysplasia: a large cohort study. Acta Neuropathol. 2019;138(6):885–900.
55. Chung C, Yang X, Bae T, Vong KI, Mittal S, Donkels C, et al. Comprehensive multi-omic profiling of somatic mutations in malformations of cortical development. Nat Genet. 2023;55(2):209–20.
56. Wu J, Chen Y, Wang P, Caselli RJ, Thompson PM, Wang J, et al. Integrating transcriptomics, genomics, and imaging in Alzheimer's disease: a federated model. Front Radiol. 2022;1:777030.
57. Ammothumkandy A, Ravina K, Wolseley V, Tartt AN, Yu PN, Corona L, et al. Altered adult neurogenesis and gliogenesis in patients with mesial temporal lobe epilepsy. Nat Neurosci. 2022;25(4):493–503.
58. Fuzik J, Zeisel A, Máté Z, Calvigioni D, Yanagawa Y, Szabó G, et al. Integration of electrophysiological recordings with

single-cell RNA-seq data identifies neuronal subtypes. Nat Biotechnol. 2016;34(2):175–83.
59. Checri R, Baldassari S, Baulac S. Detection of brain somatic mutations from stereo-EEG electrodes in focal epilepsy: current advances and future perspectives. Rev Neurol. 2025;181(5):425–31.
60. Yoon JH, Lee H, Kwon D, Lee D, Lee S, Cho E, et al. Integrative approach of omics and imaging data to discover new insights for understanding brain diseases. Brain Commun. 2024;6(4):fcae265.

How to cite this article: Phillips DJ, Ivy AS, Guzman S, Hazany S, Xu X. Spatial transcriptomics in epilepsy research: Early successes, opportunities, and challenges. Epilepsia. 2026;67:1589–1600. https://doi.org/10.1002/epi.70079