Spatial Mapping of Theta and Gamma Oscillation Networks: Integrating Single-Nucleus Transcriptomics and Spatial Gene Expression in Hippocampal and Adjacent Cortical Circuits during Spatial Object Recognition

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Introduction

Scientific Question

The Vann and Albasser (2011) journal *Hippocampus and neocortex: recognition and spatial memory* identifies overlapping networks of brain structures involved in both spatial and recognition memory, challenging the traditional view that these functions are strictly segregated. It highlights that specific oscillatory patterns, particularly theta and gamma oscillations, are essential for these memory processes. Theta rhythms are traditionally associated with spatial navigation and memory, while gamma oscillations are important for local circuit computation and feature binding in recognition memory [1]. However, in their research, they are unable to characterize the cellular and molecular underpinnings of these overlapping networks or define how these oscillatory activities are spatially organized within brain region circuits due to technical limitations. In this paper, these gaps are filled by using integrated single-nucleus and spatial transcriptomics to identify and map marker genes for theta and gamma oscillation, determining if theta and gamma oscillation networks are spatially organized within brain circuits during spatial object recognition, and if their respective marker genes correlate with the specific cell types theoretically associated with these oscillations. Understanding how these oscillatory networks are organized at the cellular level provides insight into how brain regions integrate spatial object recognition (SOR) information during memory formation.

Data Selection

The Vanrobaeys et al. (2023) SOR dataset is ideal for addressing this question because:

1. It includes transcriptomics data from hippocampal and adjacent cortical regions during a spatial object recognition task
2. It provides single-nucleus RNA-seq data that can identify specific neuronal subtypes associated with oscillatory activity
3. It provides spatial Visium data that enables mapping of oscillatory networks within hippocampal and adjacent cortical subregions
4. The combination allows us to correlate cell-type distributions with the expression of key genes involved in theta and gamma oscillations

Results

snRNA-seq Analysis

Analysis of home cage (HC) control and SOR-trained single-nucleotide RNA sequencing data revealed neuronal subtypes responsible for theta and gamma wave oscillations and their additional markers. The data was first filtered for multiplets (see Fig. 1) and mitochondrial percentage. This filtered data was then clustered into cell types and subtypes and plotted on UMAP (Fig. 2). The resulting plot was analyzed with Seurat to determine the cluster that most differentially expressed markers for theta and gamma brain wave oscillations. Genes Hcn1 and Pvalb were chosen as markers for theta and gamma brainwaves; their use as such is widely established in previous scientific literature. Hcn1 encodes a hyperpolarization-activated ion channel that regulates neuronal excitability and contributes to the pacing of theta oscillations in brain regions like the hippocampus [2]. Pvalb encodes parvalbumin, a calcium-binding protein expressed in fast-spiking interneurons that synchronize network activity and are essential for generating gamma oscillations [3]. This analysis highlighted two distinct clusters responsible for highly differential expression of these markers between HC and SOR groups, and presumably theta and gamma wave oscillations due to learning (Fig. 3). Subsequent analysis of neuronal cell type using canonical markers revealed that the identified theta oscillating cell cluster had high expression for cell type markers Slc17a7, Grin1, Rbfox3, and Snap25, while gamma oscillating cell cluster had high expression for cell type markers Gad1 and Gad2 (Fig. 4).

Visium Analysis

Analysis of Visium data revealed the spatial organization of theta and gamma oscillation spots. This analysis was broken into two parts: first, using only the widely established markers Hcn1 and Pvalb, and second, using the cell type markers identified in the previous snRNA-seq analysis in addition to the widely established markers in a multi-marker test. Gene expression data was first filtered, then each spot was analyzed using ScanPy and SciPy and plotted on the brain coronal section using ScanPy's spatial plotting function. Theta oscillations were shown to be widely distributed and diffused using only the Hcn1 gene marker (Fig. 5). The use of multiple markers identified in the previous analysis (Hcn1, Slc17a7, Grin1, Rbfox3, and Snap25) revealed a more concentrated distribution of theta oscillation cells, with greater expression seen in certain areas corresponding to theta oscillations such as the cortex and CA1, CA2, and CA3 hippocampal regions as identified in Vanrobaey's paper (Fig. 6) [4]. Gamma oscillations showed a much more conserved region of expression, with both single (Pvalb) and multi-marker (Pvalb, Gad1, and Gad2) analyses showing high, localized expression in a region not identified within the paper (Figs. 7 and 8).

Integrated Analysis

The integration of the two previous data sets, snRNA-seq and Visium, allowed for the investigation of the correlation between the Hcn1 and Pvalb marker genes and the specific cell types theoretically associated with those oscillations. Pyramidal neurons are a primary site of

theta oscillations, as found in recent studies [5]. AI identified Neurod6, Camk2a, Slc17a7, and Satb2 as being high-quality markers for this cell type. Next, a pivotal study by Bartos et al. identified the role of inhibitory interneurons in generating gamma oscillations [6]. AI identified Gad1, Gad2, Sst, and Vip as markers for this cell type. With the cell type markers in hand, the Visium data were deconvoluted with the use of the snRNA-seq data to determine spot cell type proportions for pyramidal neurons and inhibitory interneurons, which were plotted on the coronal brain section (Figs. 9 and 10). Cell proportions for each spot were analyzed using Spearman's correlation with their corresponding theta or gamma marker. Hcn1 expression was positively correlated with pyramidal neuron identity, with a correlation coefficient of $\rho = 0.24$. Pvalb expression was positively correlated with inhibitory interneuron identity, with a correlation coefficient of $\rho = 0.18$.

Discussion

The integrated analysis of snRNA-seq and spatial transcriptomics data provides novel insights into the cellular and spatial organization of oscillatory networks in the hippocampus and adjacent cortical regions during spatial object recognition memory formation. The findings support and extend the observations made by Vann and Albasser (2011) regarding the overlapping networks involved in spatial and recognition memory processes, while providing insights into cellular and molecular resolution previously unavailable.

The snRNA-seq analysis identified distinct neuronal cell types associated with theta and gamma oscillations. The theta oscillation-associated cluster showed high expression of Hcn1 alongside glutamatergic excitatory neuron markers (Slc17a7, Grin1, Rbfox3, and Snap25), confirming that pyramidal neurons are the primary generators of theta rhythms in the hippocampus during SOR tasks. This is consistent with previous studies suggesting that pyramidal cells coordinate hippocampal theta oscillations, but this analysis provides transcriptomic confirmation and identifies additional marker genes co-expressed with Hcn1 in these cells [7].

In comparison, gamma oscillation-associated cells formed a distinct cluster characterized by high expression of Pvalb and GABAergic interneuron markers (Gad1 and Gad2). This finding aligns with previous findings that fast-spiking parvalbumin-positive interneurons generate gamma oscillations through inhibitory feedback circuits [6]. The clear segregation of these cell types in our clustering analysis suggests functionally distinct roles in oscillatory dynamics during memory formation.

The Visium spatial analysis revealed patterns in the distribution of theta and gamma oscillation-associated gene expression. Theta oscillation markers showed a somewhat diffuse expression, though it was concentrated in cortical regions and the CA1, CA2, and CA3 hippocampal subregions. This expression pattern is consistent with findings that theta oscillations coordinate communication across distributed brain networks during spatial navigation and memory encoding [8].

In contrast, gamma oscillation-associated gene expression was localized, forming discrete regions of high expression. This spatial concentration of gamma oscillation generating interneurons suggests localized information processing that occurs during object recognition, supporting the binding by synchrony hypothesis, where gamma-synchronized neural assemblies integrate multiple aspects of experience into one memory [9].

The multi-marker approach revealed more informative spatial patterns than single-gene analyses, further cementing the results and showing the importance of considering a wide scope of transcriptional signatures over individual genes when mapping interacting neural circuits. The distinct spatial organizations of theta and gamma networks provide a structural basis for their different computational roles, with theta contributing to global coordination and gamma to local information processing.

The integrated analysis results in moderate positive correlations between theta markers and pyramidal neuron proportions ($\rho = 0.24$) and between gamma markers and inhibitory interneuron proportions ($\rho = 0.18$). While these correlations are not overwhelming, they provide support for the relationship between these specific cell types and wave oscillatory functions. The correlations also suggest that there are likely additional cell types that contribute to these wave oscillating memory circuits, or nuances such as cell states or input from the surrounding environment, that impact the expression of oscillatory wave-associated genes.

These findings provide a cellular and molecular framework for understanding how the hippocampus integrates spatial and object information during memory formation. The overlapping yet distinct spatial distributions of theta and gamma networks offer a potential explanation for how binding spatial context with object features during SOR tasks occurs. This integrated circuit model explains how the hippocampus can simultaneously process integrated information streams, challenging traditional views of strict functional segregation This builds on the findings of Vann and Albasser's research by demonstrating that these overlapping functional networks are underpinned by distinct cell types with specific spatial organizations that facilitate the integration of spatial context and object features during memory formation.

Figures

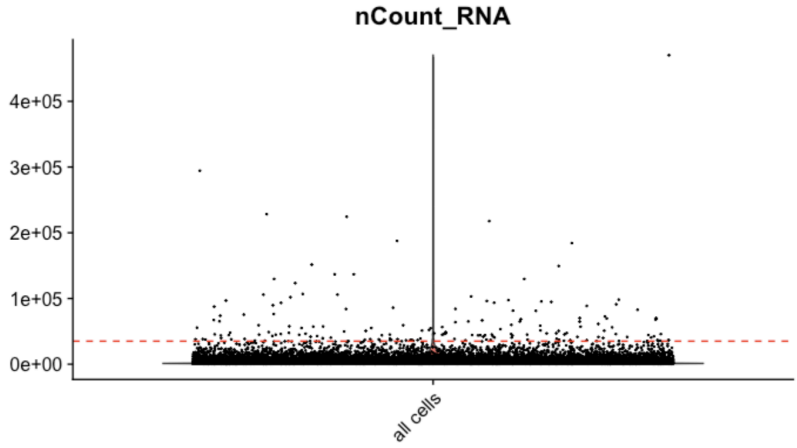

Figure 1. RNA count plot for each cell used to determine an arbitrary multiplet cutoff of < 35,000 per cell.

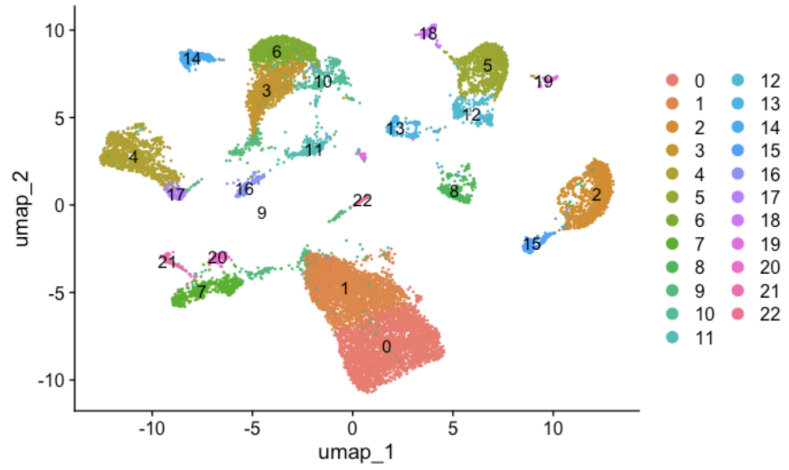

Figure 2. Clustering of cell types and subtypes using UMAP to plot and Seurat to analyze differential transcription of all genes.

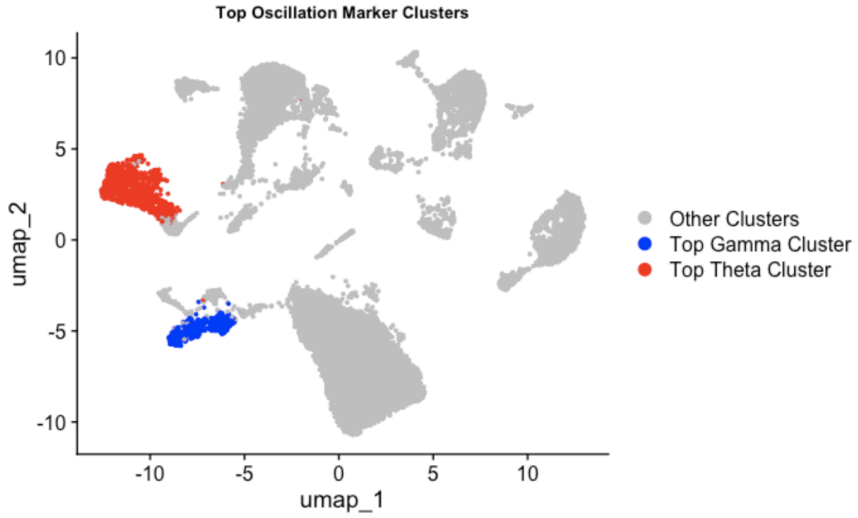

Figure 3. Identified the top differentially expressed cluster for theta oscillation (Hcn1) and gamma oscillation (Pvalb) marker genes.

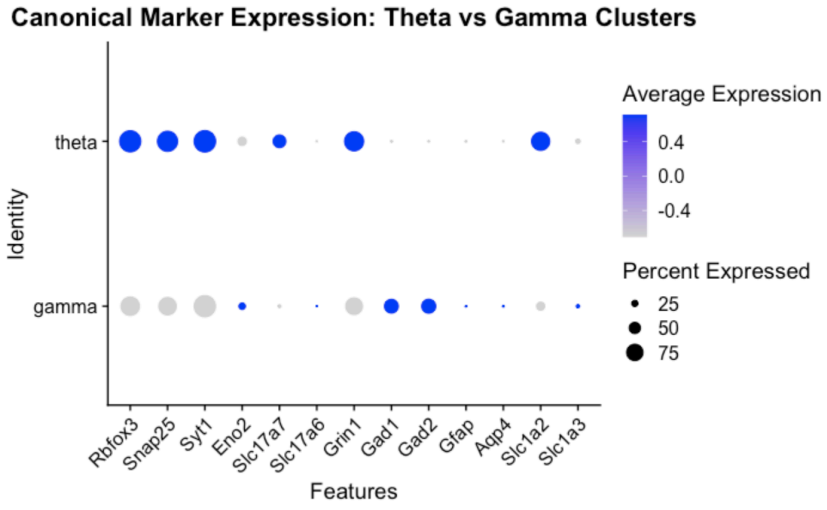

Figure 4. Most informative data from cell type canonical marker analysis of identified theta and gamma oscillation clusters.

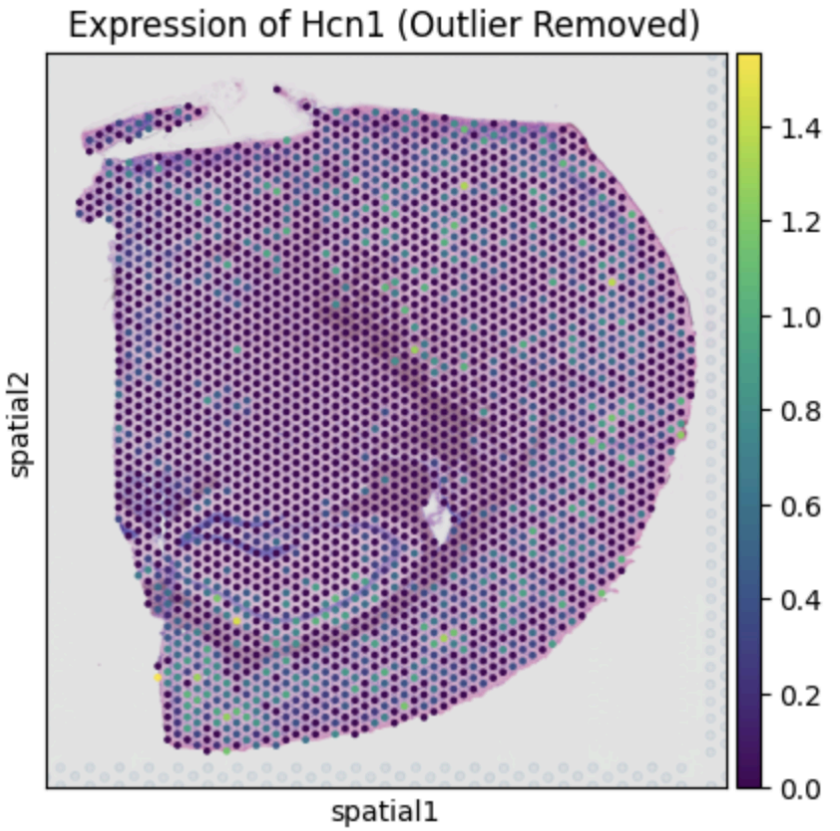

Figure 5. Spatial organization of theta oscillations on mouse coronal brain section using Hcn1 marker gene.

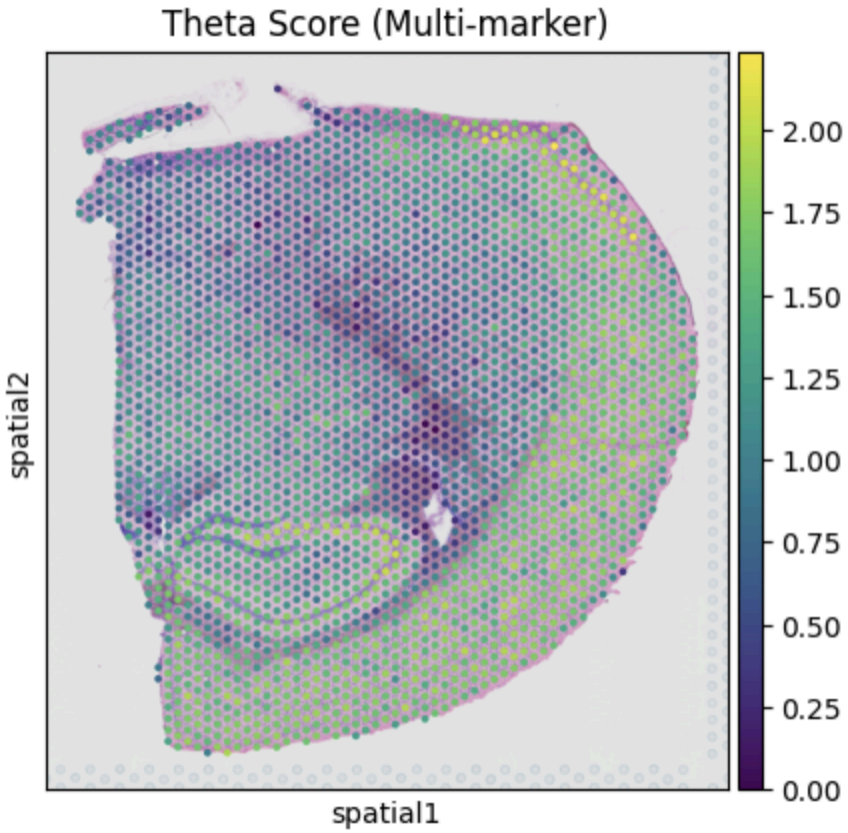

Figure 6. Spatial organization of theta oscillations on mouse coronal brain section using multi-marker gene expression (Hcn1, Slc17a7, Grin1, Rbfox3, and Snap25).

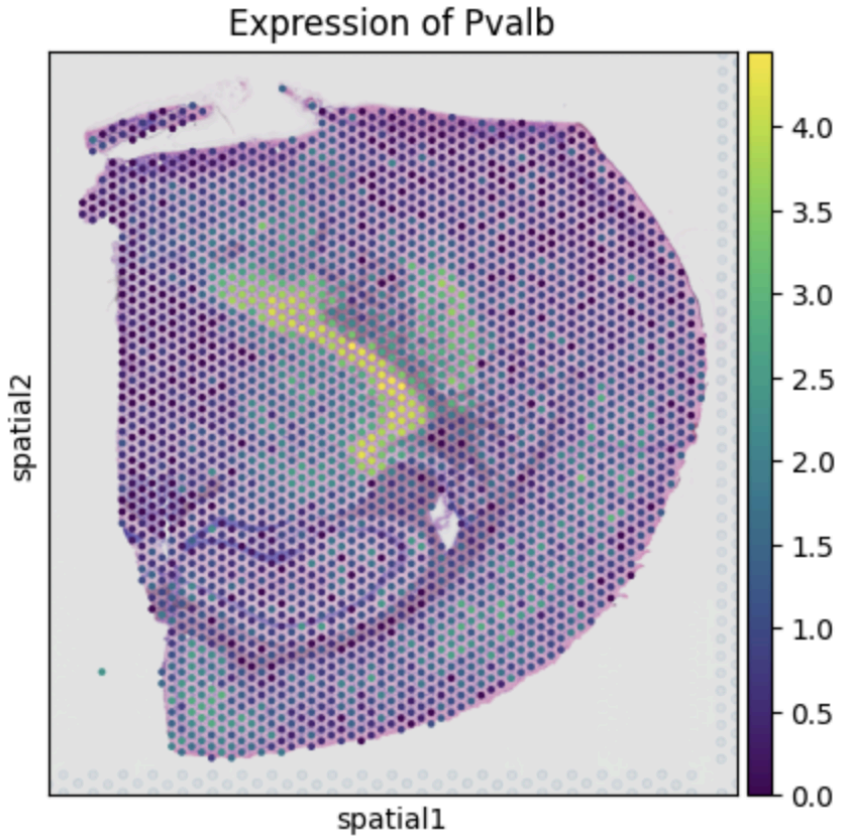

Figure 7. Spatial organization of gamma oscillations on mouse coronal brain section using Pvalb marker gene.

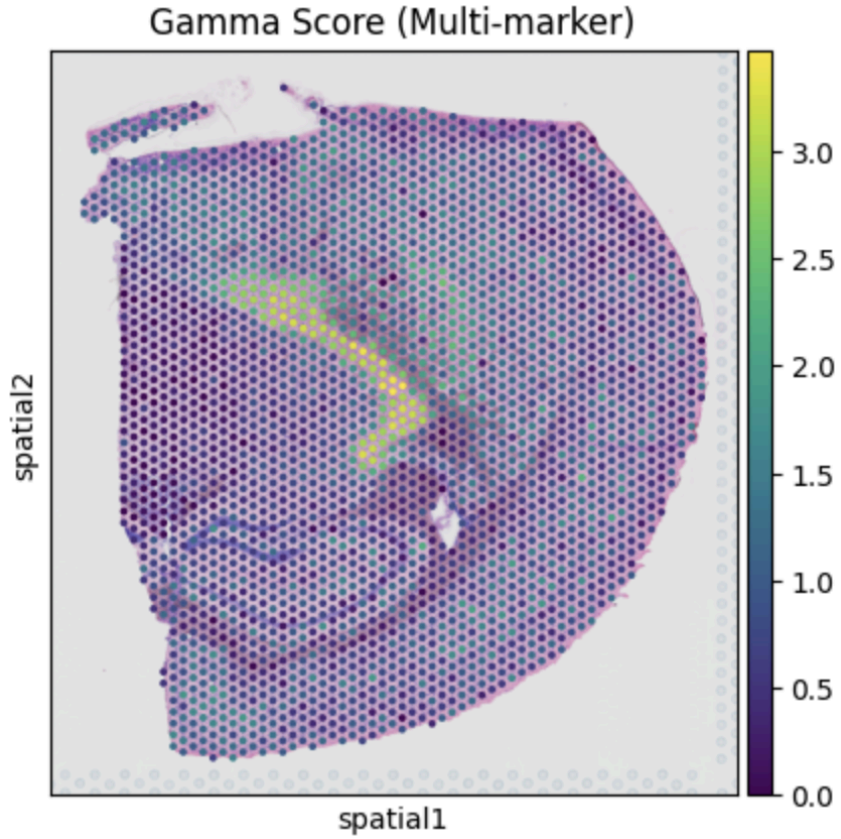

Figure 8. Spatial organization of gamma oscillations on mouse coronal brain section using multi-marker gene expression (Pvlab, Gad1, Gad2).

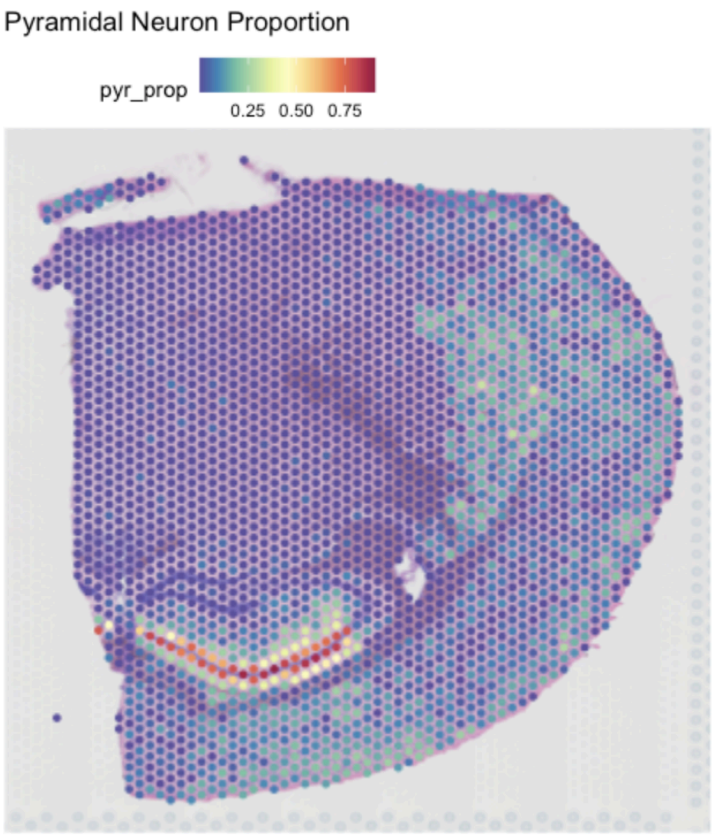

Figure 9. Deconvolution of Visium data using snRNA-seq data to determine the pyramidal neuron proportion of each spot using marker genes (Neurod6, Camk2a, Slc17a7, and Satb2).

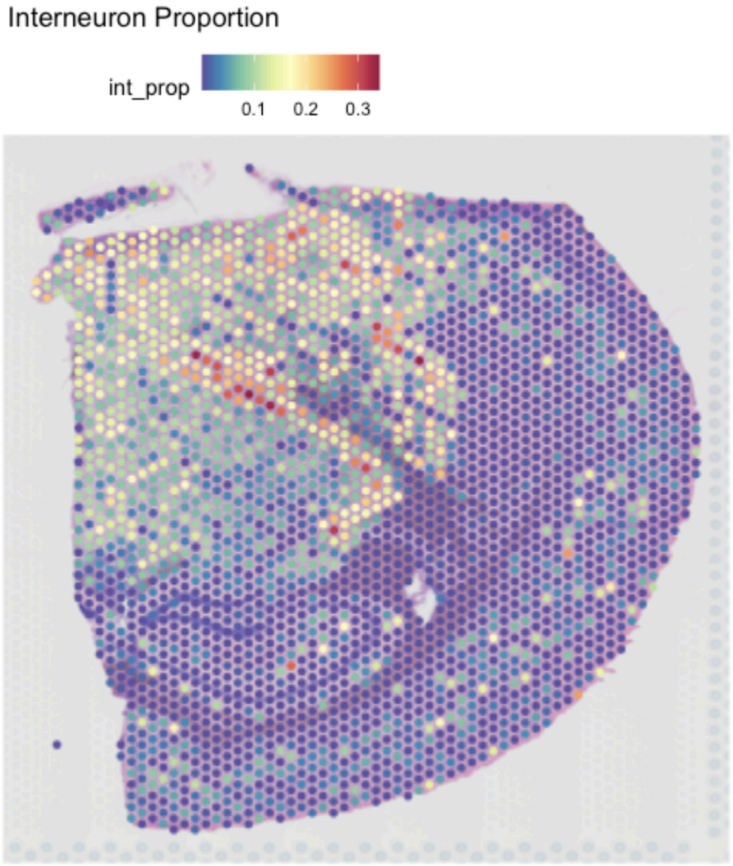

Figure 10. Deconvolution of Visium data using snRNA-seq data to determine the inhibitory interneuron proportion of each spot using marker genes (Gad1, Gad2, Sst, and Vip).

References

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