Analyzing the molecular basis underlying anatomic and functional complexity of the mouse brain with MERSCOPE™

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Introduction

The development of spatially resolved genomic assays enables molecular analysis of tissues, with the potential of revealing how single-cell gene activity orchestrates the structure of complex tissues like the nervous system. Here, we use the MERSCOPE™ Platform to generate a transcriptionally defined and spatially resolved single-cell mouse brain atlas. By performing multiplexed error-robust fluorescence *in situ* hybridization (MERFISH) assays with a 500-gene panel designed for cell typing, **we obtained over two million cells with precise gene expression and spatial information across the mouse brain**. Clustering analysis of the gene expression data resolved all major cell populations as well as detailed neuron and non-neuron subtypes across different brain regions. By assessing the relationship between molecular and anatomic features of identified cell types, we found that both excitatory and inhibitory neuron subtypes exhibit significant variation in gene expression and spatial distribution along multiple axes of different brain structures. Furthermore, the high-resolution spatial transcriptomic data enabled us to assess the spatial relationship and cell-cell interactions across different cell types. **Altogether, our work not only created a molecularly defined and spatially resolved mouse brain cell atlas, but also demonstrated the power of MERFISH measurements generated by the MERSCOPE™ Platform in analyzing the molecular basis underlying the anatomic and functional complexity of the nervous system.**

Materials and Methods

MERSCOPE™ is a spatial transcriptomic platform enabling high-throughput MERFISH assays, resulting in the simultaneous measurement of hundreds of RNA species across a whole tissue slice (FIGURE 1)[1]. In this study, we generated a mouse brain cell atlas of 16 sagittal and 11 coronal tissue sections.

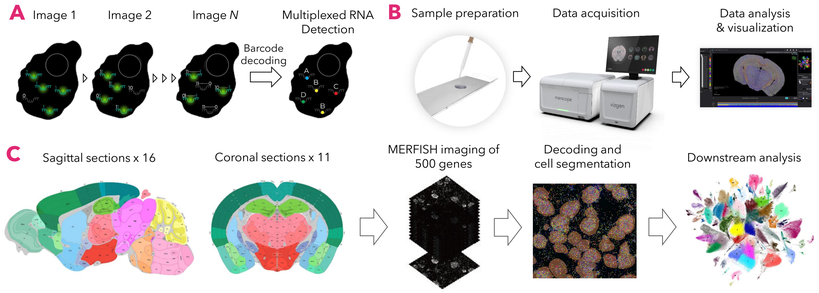

Figure 1. The MERSCOPE™ Platform enables the generation of a molecular tissue atlas. (A) Target RNA species are labeled with a tile of oligo probes containing different barcodes, which are fluorescently detected in sequential rounds of imaging. (B) MERSCOPE™ is an end-to-end platform for MERFISH measurements. (C) Using the MERSCOPE™ Platform to generate a mouse brain cell atlas.

Results

MERSCOPE™ faithfully captures gene expression in mouse brain

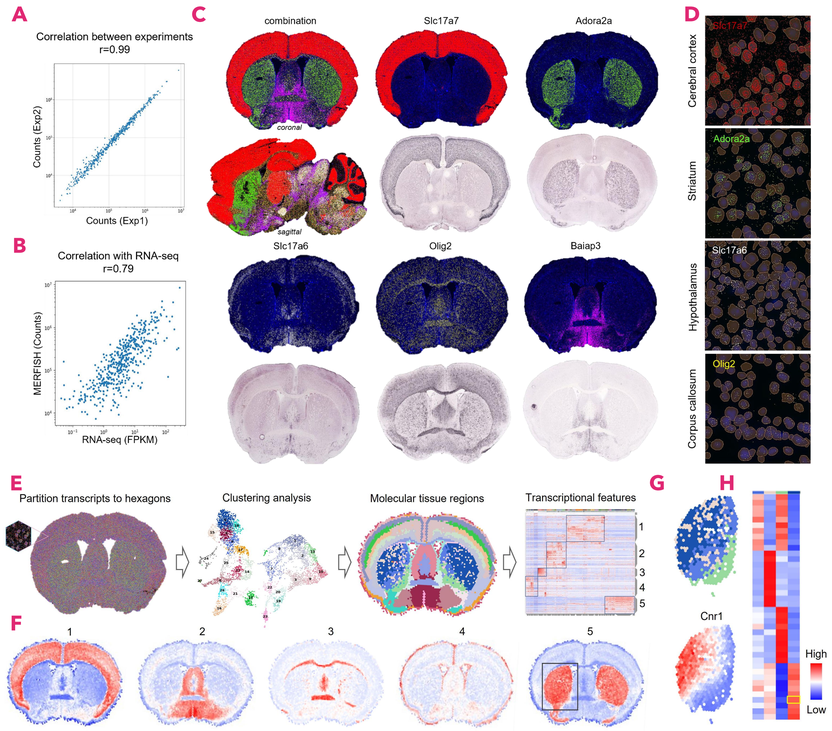

Figure 2. MERSCOPE™ faithfully detects the gene expression profile in mouse brain. (A) Correlation of MERFISH data from (A) adjacent sagittal brain sections and (B) a coronal section with mouse brain bulk RNA-seq data. (C) Expression pattern of *Slc17a7*, *Adora2a*, *Slc17a6*, *Olig2* and *Baiap3* as detected by MERSCOPE™ (upper panels) and *in situ* hybridization (lower panels). (D) Different transcripts are enriched in cells of different brain regions. (E) Molecular tissue region identification using spatial distribution of different transcripts. (F) Heatmaps showing different groups of genes are enriched in different molecular tissue regions (defined in E). (G) Gene expression heterogeneity in striatum subregions. Upper panel, four molecular tissue regions identified in striatum, shown by different colors. Lower panel, *Cnr1* is highly expressed in one of the four tissue regions. (H) Heatmap showing different groups of genes specifically enriched in four molecular tissue regions of striatum; expression levels are color-coded.

MERFISH reveals distribution of major cell types in mouse brain

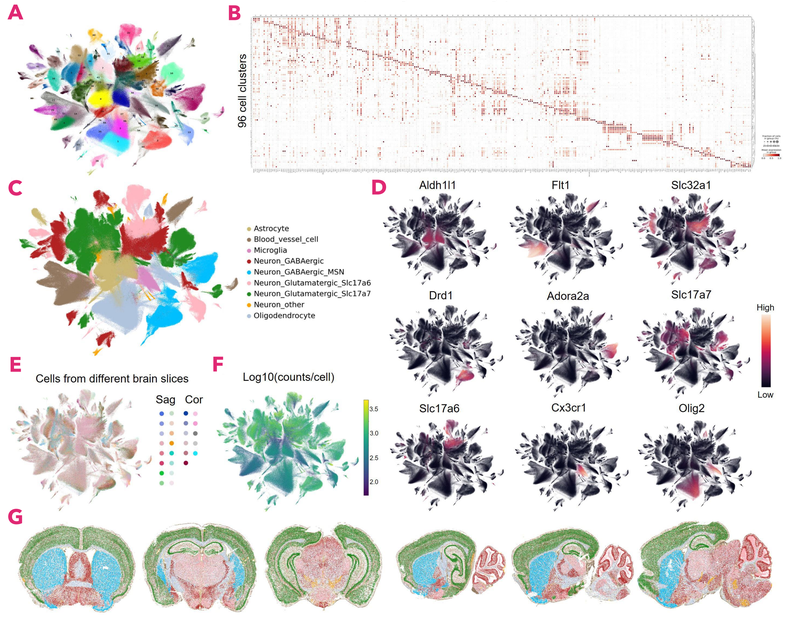

Figure 3. Molecularly distinct cell types and their spatial distribution across mouse brain. (A) UMAP showing the cell clusters (represented by different colors) revealed from unsupervised clustering analysis of MERSCOPE™ data. (B) Dot plot showing expression of top marker genes across different cell clusters. (C) Cell clusters are grouped into major cell populations based on established marker genes. (D) UMAP showing expression level of major cell population marker genes across all the cells. The expression level is color-coded. (E) UMAP showing the cells from different brain slices. Sag, sagittal sections; Cor, coronal sections. (F) UMAP showing the number of transcripts detected in each cell. The data are log-transformed. (G) Plotting major cell populations (same colors as C) on different coronal and sagittal sections.

Neuronal diversity underlying anatomic heterogeneity of mouse brain

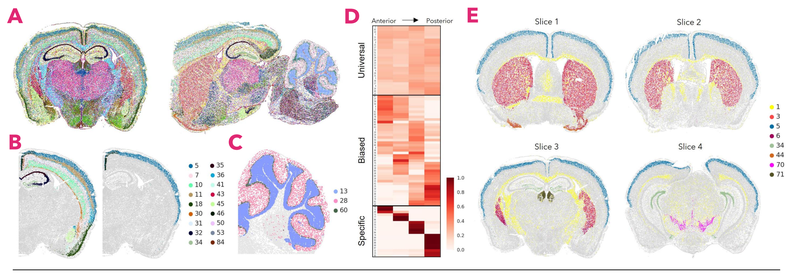

Figure 4. MERSCOPE™ data reveal the spatial distribution of molecularly defined cell clusters underlying anatomic heterogeneity of mouse brain. (A) Plotting the spatial distribution of all cell clusters on one coronal and one sagittal section. Different cell clusters are shown in different colors. (B) Spatial distribution of *Slc17a7*+ cells on a coronal section. On the right, only cluster 5 and 46 are shown. (C) Spatial distribution of cluster 13, 28 and 60 in cerebellum. (D) Heatmap showing the proportion of each cluster across four coronal sections along the anterior-posterior axis. Cell clusters with similar patterns are grouped together. (E) Spatial patterns of eight cell clusters across different coronal mouse brain sections.

MSN diversity underlie the anatomic heterogeneity of striatum

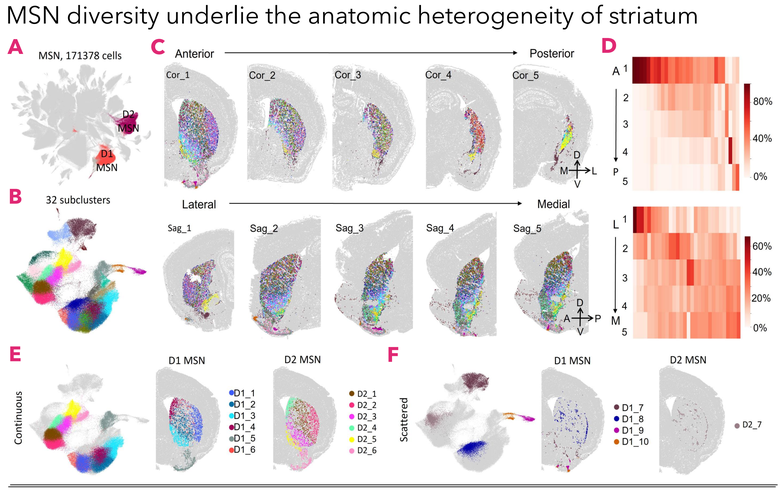

Figure 5. Medium spiny neuron subtypes exhibit distinct spatial distribution patterns in striatum. (A) UMAP showing D1 and D2 medium spiny neuron (MSN) clusters identified in initial clustering analysis. (B) UMAP showing the 32 subclusters of MSNs. Different subclusters are shown in different colors. (C) Spatial distribution of different MSN subclusters in coronal (upper) and sagittal (lower) sections. (D) Heatmap showing the proportion of different MSN subclusters along anterior-posterior (upper) and medial-lateral (lower) axis. MSN subclusters with continuous (E) or scatter (F) spatial patterns. UMAP showing the gene expression relationship of MSN subclusters (left panels). The spatial distribution of continuous or scatter D1 and D2 MSN subclusters in a coronal section are shown on the right panels.

References

1. Spatially resolved, highly multiplexed RNA profiling in single cells (2015). https://science.sciencemag.org/content/348/6233/aaa6090.full

Neuronal diversity in diencephalon and mesencephalon

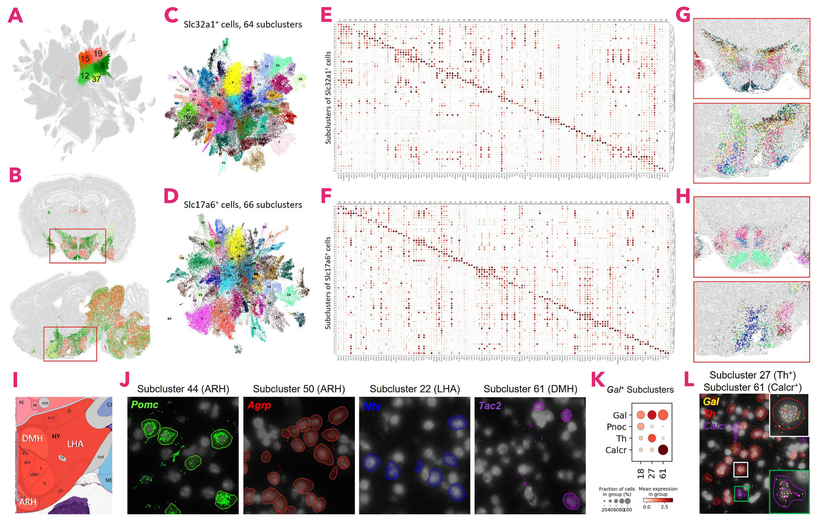

Figure 6. MERSCOPE™ reveals neuronal diversity in the diencephalon and mesencephalon of mouse brain. (A) UMAP showing three GABAergic (green) and two glutamatergic (red) neuron clusters representing diencephalon and mesencephalon neurons in the initial clustering analysis. (B) Spatial distributions of diencephalon and mesencephalon neuron clusters in one coronal and one sagittal section. UMAP showing the subclusters of GABAergic (C) and glutamatergic (D) neurons in diencephalon and mesencephalon. Different clusters are shown in different colors. Dot plot showing the expression of top marker genes across different GABAergic (E) and glutamatergic (F) subclusters. Spatial distribution of GABAergic (G) and glutamatergic (H) subclusters in hypothalamus on coronal and sagittal sections. The regions correspond to the boxed regions in B. (I) Illustration of different subregions of hypothalamus on a coronal section. (J) The spatial distribution of four different GABAergic subclusters in corresponding hypothalamic subregion. The transcripts of subcluster markers are also shown. ARH, arcuate hypothalamic nucleus; LHA, lateral hypothalamic area; DMH, dorsomedial nucleus of hypothalamus. (K) Dot plot showing the expression of different marker genes in three *Gal*+ subclusters. (L) GABAergic subclusters 27 and 61 are both distributed in DMH but with different gene expression features. Different subclusters and mRNA species are shown in different colors. The two boxed cells are enlarged and shown as the inserted panels.

Anatomic and functional insights into mouse brain

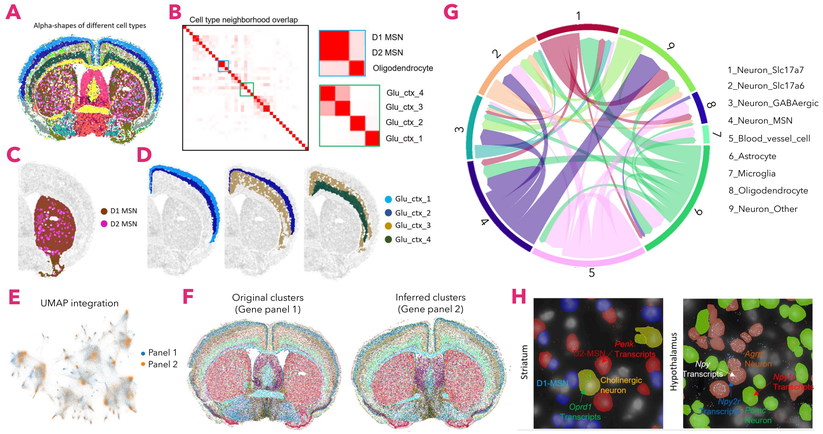

Figure 7. MERSCOPE™ data provides anatomic and functional insights into the nervous system. (A) The neighborhoods of different cell clusters of a coronal section as calculated by alpha-shape analysis; different clusters are shown in different colors. (B) Heatmap showing cell cluster neighborhood overlap across different cell clusters; range of overlap is color-coded. The two boxed regions are enlarged and shown on right. Corresponding cell types of these two regions are also listed. (C) The neighborhoods of D1 and D2-MSNs are highly overlapped in striatum (blue box in B). (D) Different cortical glutamatergic neuron clusters exhibiting different spatial relationships with other clusters (green box in B). (E) UMAP showing the integration results of MERFISH data generated from two different gene panels. (F) Based on the integration results, the identities of cell clusters could be accurately inferred, as the spatial distribution of different cell clusters are highly consistent across datasets. (G) Integration of MERFISH and scRNA-seq data enable transcriptome-wide analysis of ligand-receptor interaction across molecularly defined cell types. (H) Combining gene expression and spatial information enables *in situ* cell-cell interaction analysis in cell type-specific manner. Selected cell types and transcripts in the striatum (left) and arcuate nuclei of hypothalamus (right) are shown in different colors.

Conclusions

Here, we provide evidence that MERFISH data generated by the MERSCOPE™ Platform enables:

- High-throughput single-cell spatial transcriptomic profiling of mouse brain
- Identification of major cell types and their spatial distribution across various brain regions
- Detailed cell typing in different brain regions
- Insights into the anatomic heterogeneity and functional complexity of mouse brain

Applications enabled by MERSCOPE™

- Map neuron type-specific connection by combine MERFISH with neural tracing
- Link transcriptionally defined neuron types to different functions with neuronal activity staining/imaging
- Analyze cell type-specific transcriptional changes in different disease models

Acknowledgments

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