Spatial transcriptomic profiling of spiny projection neurons in the mouse striatum

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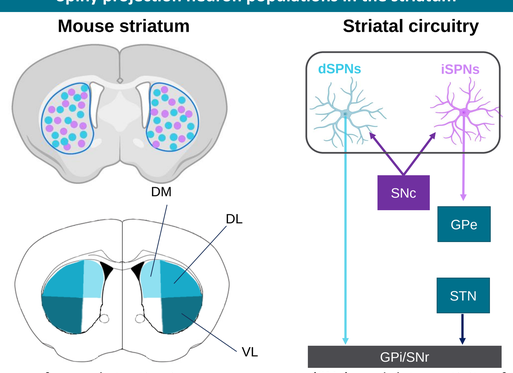
Abstract

Defining the molecular architecture of gene expression within the brain is critical for understanding normal brain function and for developing and testing new hypotheses toward understanding and treating neurological disease. Novel spatial transcriptomic methods enable high resolution *in situ* profiling of individual cell-types while maintaining the organizational context of each population profiled. Here, we use NanoString's GeoMx® Digital Spatial Profiler in combination with the Mouse Whole Transcriptome Assay (WTA) to segment and profile subpopulations of spiny projection neurons (SPNs) in the mouse striatum. SPN subpopulations are generally divided into those that express D1 dopamine receptors (dSPN) and those that express D2 dopamine receptors (iSPN). Recently, it was shown that these subpopulations are more complex than previously thought whereby gene expression in SPNs is, in part, mediated by their position within regional striatal subdivisions.

To analyze SPNs, 10um fresh frozen coronal sections from six C57BL/6J x DBA mice (n=3 female, n=3 male) were placed across two slides. dSPNs and iSPNs were identified using *Drd1* and *Adora2a* RNAScope probes respectively in combination with Syto13 for visualization of all nuclei. Regions of interest (ROIs) were selected in dorsomedial, dorsolateral, and ventrolateral striatum to identify cell-type specific gene expression signatures in regions previously associated with unique functional roles within the striatum. Within each ROI, *Adora2a* and *Drd1* positive neurons were profiled and analyzed separately with average gene detection in each population ranging from 6000-9000+ genes. In *Drd1*-expressing SPNs, 171 genes were differentially expressed in dorsomedial versus dorsolateral striatum, and 356 genes were differentially expressed in dorsolateral versus ventrolateral striatum (Benjamini-Hochberg padj<0.1; |FC|≥1.25). In *Adora2a*-expressing SPNs, 76 genes were differentially expressed in dorsomedial versus dorsolateral striatum, and 335 genes were differentially expressed in dorsolateral versus ventrolateral striatum (Benjamini-Hochberg padj<0.1; |FC|≥1.25).

In conclusion, we have established a workflow enabling whole transcriptome data from spatially mapped SPN populations within healthy mice and demonstrate high precision and accuracy of the approach. We demonstrate functional gene expression differences between SPNs based on spatial localization within the striatum highlighting spatial context's critical importance in transcriptomic analysis. These data and workflow can be used as standards to inform future profiling studies in mouse models of neurological disorders and disease.

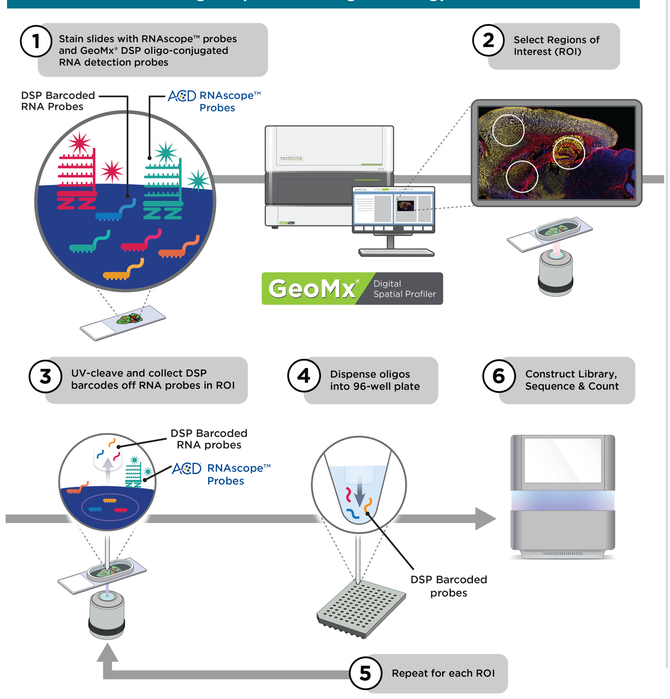
Spiny projection neuron populations in the striatum



- Dopamine from substantia nigra pars compacta (SNc) modulates activity of the striatum
  - Direct pathway SPNs (dSPNs) promote movement (identified with *Drd1* RNAscope)
  - Indirect pathway SPNs (iSPNs) inhibit movement (identified with *Adora2a* RNAscope)

- Three functional subregions of interest within the striatum differ in cortical inputs and gene expression patterns.
  - Dorsomedial (DM) striatum receives convergent input from associative areas and is thought to support goal-directed behavior by representing associations between responses and outcomes.
  - Dorsolateral (DL) striatum is thought to be involved in habitual and skilled performance, and receives sensorimotor input involving the trunk and lower limbs.
  - Ventrolateral (VL) striatum receives sensorimotor input involving the upper limbs and mouth.

GeoMx Digital Spatial Profiling Technology Workflow



Targeted whole transcriptome profiling of dSPNs and iSPNS using spatial localization and RNAscope guided segmentation

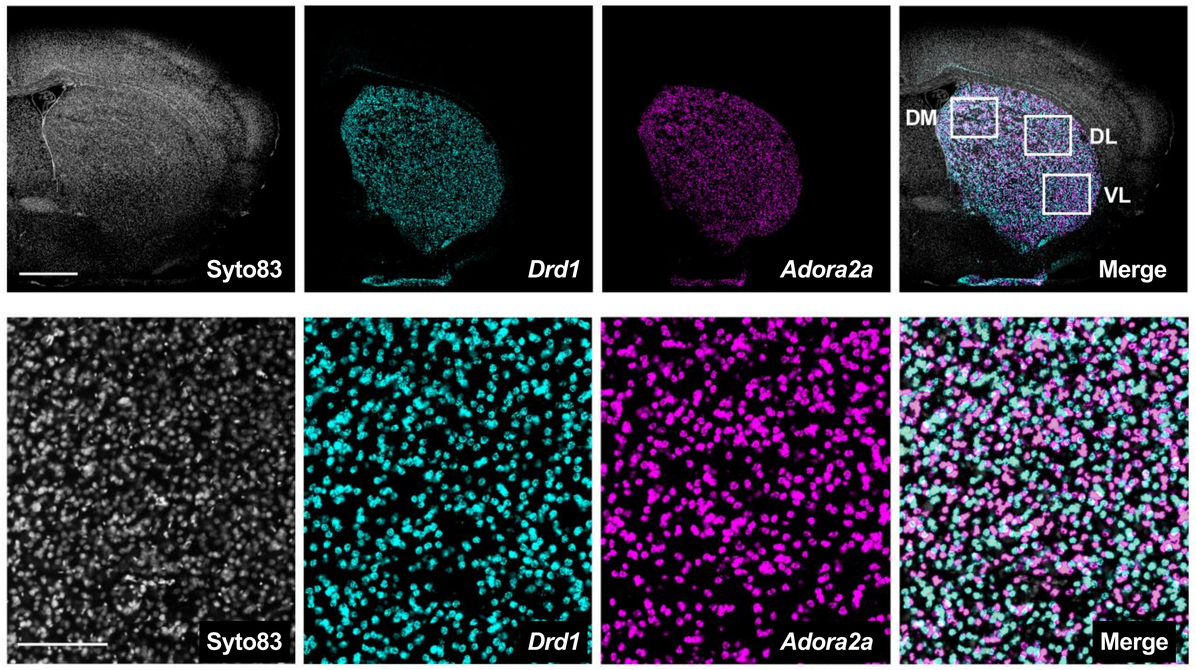


Figure 1. dSPNs and iSPNs in striatal subregions. Representative micrographs of a coronal section of mouse brain through the striatum. Nuclei were visualized with Syto83 (grey), dSPNs were identified by fluorescent *in situ* hybridization for the D1 dopamine receptor (*Drd1*; aqua), and iSPNs were identified by fluorescent *in situ* hybridization for the adenosine A2a receptor (*Adora2a*; purple). The white boxes in the merged micrograph demarcate the dorsomedial (DM), dorsolateral (DL) and ventrolateral (VL) striatal subregions used for sample collection. Scale bar represents 1 mm on top panel; 200 µm on bottom panel.

Over 5000 genes detected in dSPN and iSPN populations, with clear enrichment of canonical marker genes the respective populations

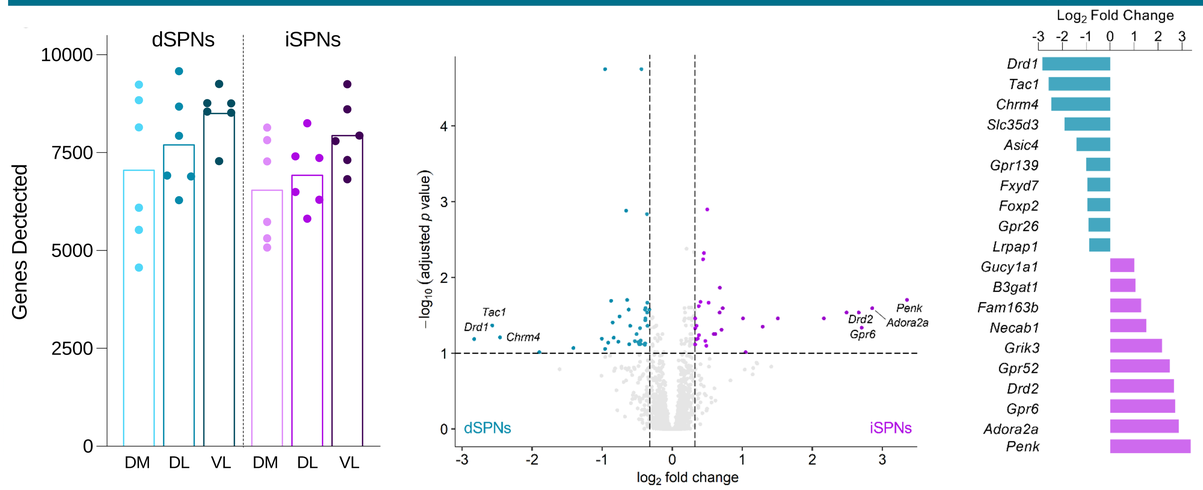


Figure 2. Transcriptional profiles of dSPNs and iSPNs in control mice. (A) Number of genes detected in each sample (n = 6, 3 striatal subregions/mouse). Each point represents an individual mouse and horizontal lines represent means. **(B)** Volcano plot depicting genes differentially expressed in dSPNs and iSPNs; linear mixed model with animal as the repeated measure). Genes significantly enriched in dSPNs (fold change < -1.25, n = 39) are represented by aqua dots and genes significantly enriched in iSPNs (fold change > 1.25, n = 31) are represented by purple dots (BH adjusted *p* value < 0.1). **(C)** The ten most-enriched genes in dSPNs (aqua) and ten most-enriched genes in iSPNs (purple) based on $\log_2$ fold change.

Transcriptional profiles of iSPNs and dSPNs vary by spatial location within the striatum

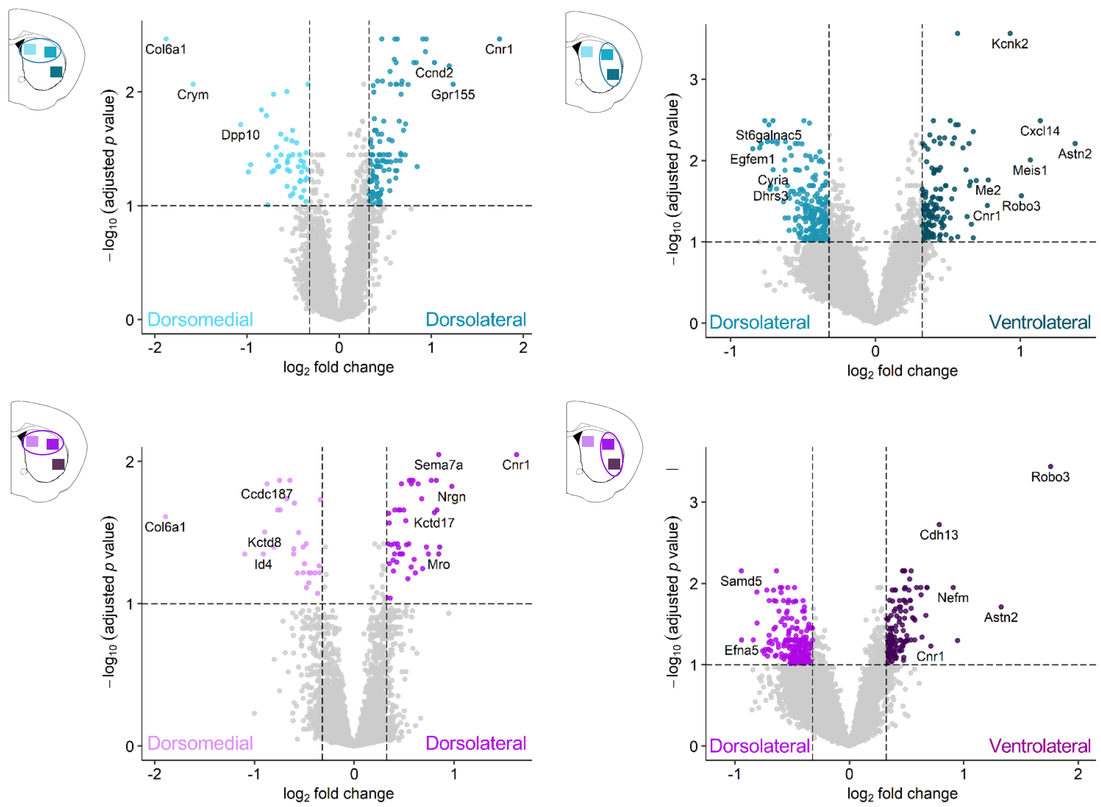


Figure 3. Region-specific striatal gene expression. Volcano plots depicting genes enriched in dSPNs (top, aqua) and iSPNs (bottom, purple) within the three striatal subregions.

Summary of differentially expressed genes by spatial location

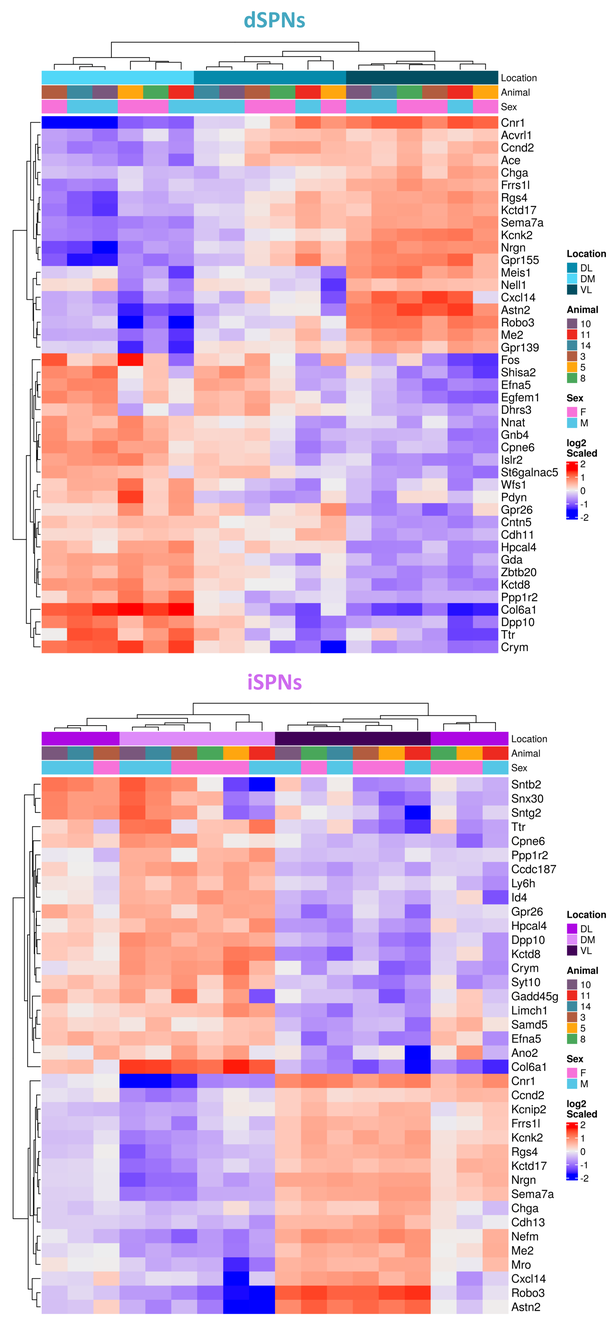


Figure 4. Genes that vary by striatal location in SPNs. Heatmaps listing the top 65 differentially expressed genes in dSPNs (top) and iSPNs (bottom) with unsupervised hierarchical clustering.

Conclusions and next steps

- Spatial transcriptomic profiling using GeoMx DSP enables sub-region and cell-type specific gene expression profiling in the mouse brain.
- Gene expression in striatal SPNs varies dependent on subtype (dSPN and iSPN) and spatial location.
- The same methods can be used to characterize gene expression changes within SPN populations in mouse models of movement and other striatal disorders.
- Genes of interest identified here using GeoMx can be explored at the single-cell level using NanoString's CosMx spatial molecular profiling technology.
- Additional GeoMx profiling approaches for spatial whole transcriptome profiling in mouse brain can be found at NanoString's **Spatial Organ Atlas** website:

https://nanostring.com/products/geomx-digital-spatial profiler/spatial-organ-atlas/

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