In situ validation and spatial mapping of diverse striatal cells identified by scRNA-seq in the mouse brain at single-cell resolution

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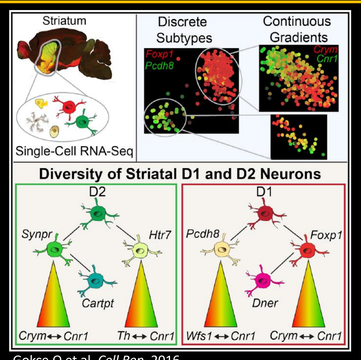
Introduction

Characterizing the transcriptomic profiles of individual cells by single-cell RNA sequencing (scRNA-seq) has become a universal tool to identify both known and novel cell types and to understand tissue structure and function, ushering in a new era of single cell biology. This has proven to be especially true in complex organs with high cellular heterogeneity, such as the mammalian brain. However, scRNA-seq utilizes dissociated cells and results in the loss of spatial organization of the cell population being analyzed. Validation and spatial mapping of scRNA-seq results can be obtained using assays that retain spatial organization, such as RNA *in situ* hybridization (ISH). Therefore, in this study, we sought to validate and spatially map the diverse cell types in the mouse striatum that have been previously identified by scRNA-seq (Gokce *et al*, *Cell Rep*, 16(4):1126-1137, 2016) using the RNAscope multiplex fluorescent RNA ISH assay. We validated the major and minor gene signatures identified by scRNA-seq, including discrete D1 and D2 medium spiny neuron (MSN) subtypes: Drd1a/Foxp1, Drd1a/Pcdh8, Drd2/Htr7, and Drd2/Synpr. Further cellular heterogeneity within the MSN subpopulations was marked by a transcriptional gradient, which we could spatially resolve with RNA ISH, revealing that cells highly expressing one end of the gradient were located in a region adjacent to cells highly expressing the other end of the gradient, with a small overlapping region containing co-expressing cells. Lastly, we validated heterogeneity within non-neuronal striatal cell types, including vascular smooth muscle cells, endothelial cells, microglia, macrophages, and oligodendrocytes.



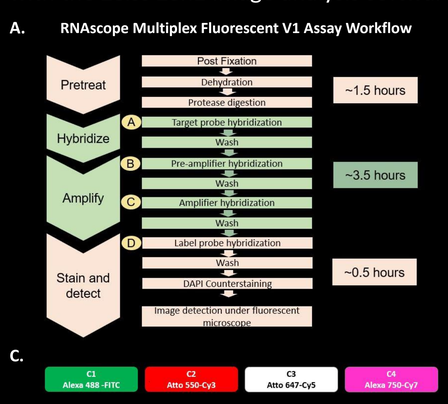
Gokce O et al, *Cell Rep*, 2016

RNAscope Technology and Experimental Design

Tissue preparation: Sagittal sections (10 µm thick) of fresh frozen brain tissue from 6 week old C57/BL6 male mice were purchased from Acepix.

RNAscope *in situ* hybridization: The RNAscope Multiplex Fluorescent V1 Assay was used for gene expression analysis in the brain, with a focus on the striatum.

Imaging and quantification: Images were acquired using either the Zeiss Axio Z1 fluorescent slide scanner microscope with the Zeiss Zen2 image analysis software or Perkin Elmer Vectra Polaris imaging system.



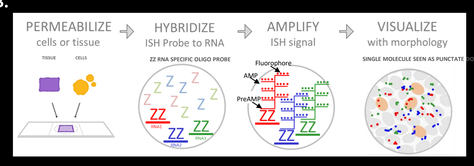
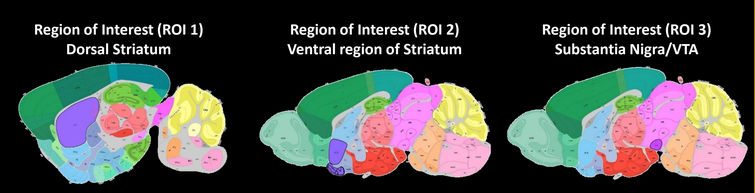


Figure 1. Assay workflow and brain regions analyzed in this study. (A, B) RNAscope Multiplex Assay workflow. (C) Fluorophores used in this study. (D) Regions of the brain selected for analysis in this study.



Allen Mouse Brain Atlas, 2004

Results

Striatal Medium Spiny Neuronal Sub-Types

Drd1a Medium Spiny Neurons

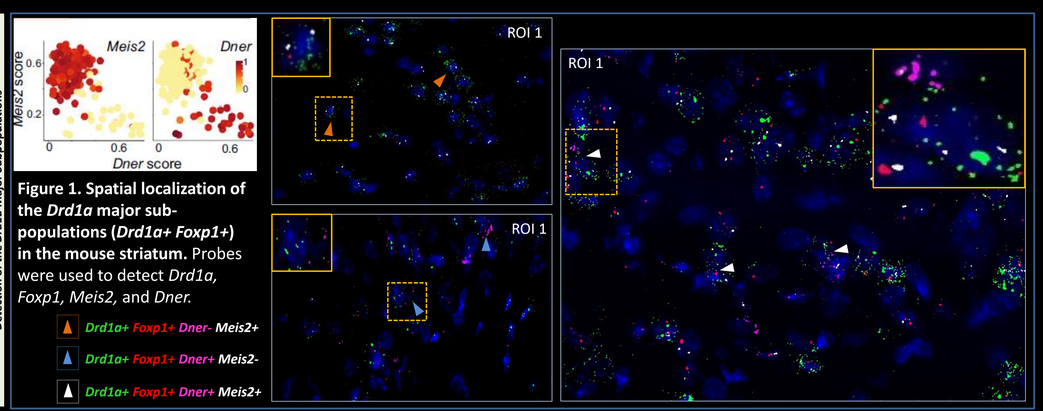


Figure 1. Spatial localization of the *Drd1a* major sub-populations (*Drd1a+ Foxp1+*) in the mouse striatum. Probes were used to detect *Drd1a, Foxp1, Meis2,* and *Dner.*

Drd1a+ Foxp1+ Dner- Meis2+
Drd1a+ Foxp1+ Dner+ Meis2-
Drd1a+ Foxp1+ Dner+ Meis2+

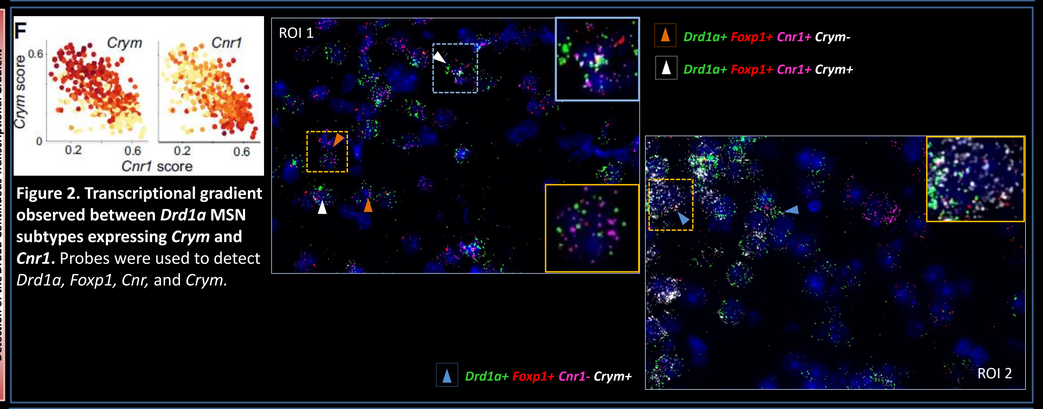


Figure 2. Transcriptional gradient observed between *Drd1a* MSN subtypes expressing *Crym* and *Cnr1*. Probes were used to detect *Drd1a, Foxp1, Cnr,* and *Crym.*

Drd1a+ Foxp1+ Cnr1+ Crym-
Drd1a+ Foxp1+ Cnr1+ Crym+
Drd1a+ Foxp1+ Cnr1- Crym+

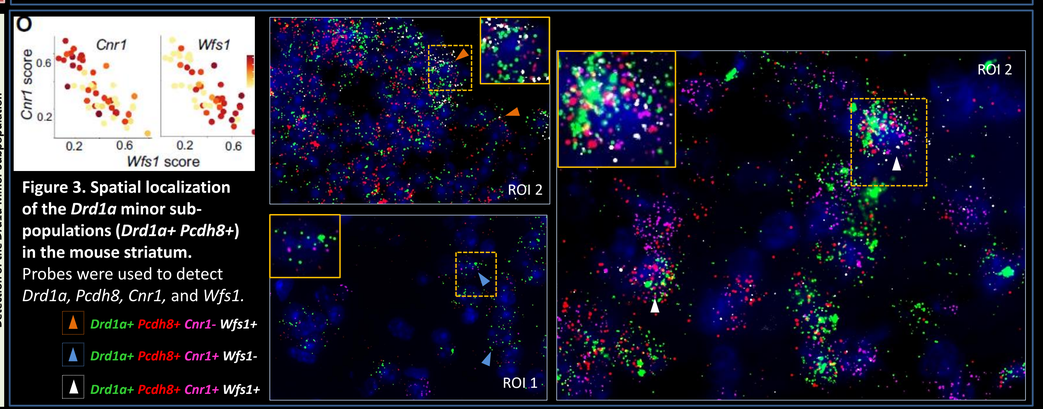


Figure 3. Spatial localization of the *Drd1a* minor sub-populations (*Drd1a+ Pcdh8+*) in the mouse striatum. Probes were used to detect *Drd1a, Pcdh8, Cnr1,* and *Wfs1.*

Drd1a+ Pcdh8+ Cnr1- Wfs1+
Drd1a+ Pcdh8+ Cnr1+ Wfs1-
Drd1a+ Pcdh8+ Cnr1+ Wfs1+

Drd2 Medium Spiny Neurons

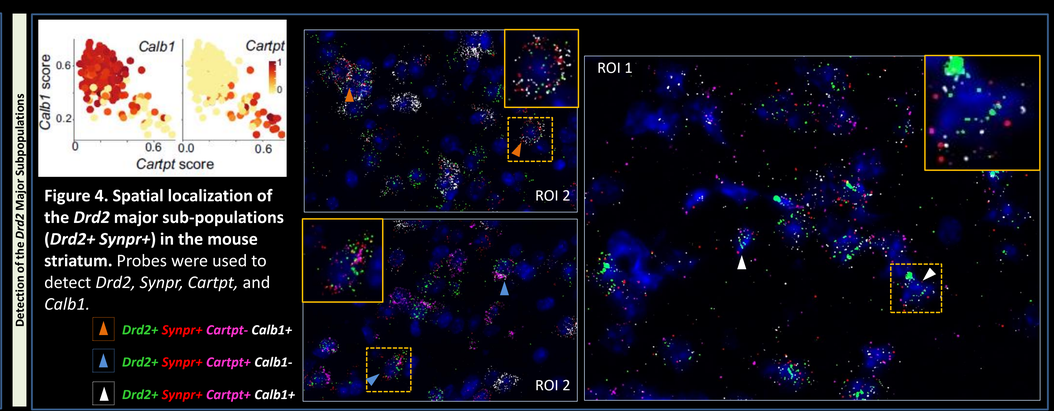


Figure 4. Spatial localization of the *Drd2* major sub-populations (*Drd2+ Synpr+*) in the mouse striatum. Probes were used to detect *Drd2, Synpr, Cartpt,* and *Calb1.*

Drd2+ Synpr+ Cartpt- Calb1+
Drd2+ Synpr+ Cartpt+ Calb1-
Drd2+ Synpr+ Cartpt+ Calb1+

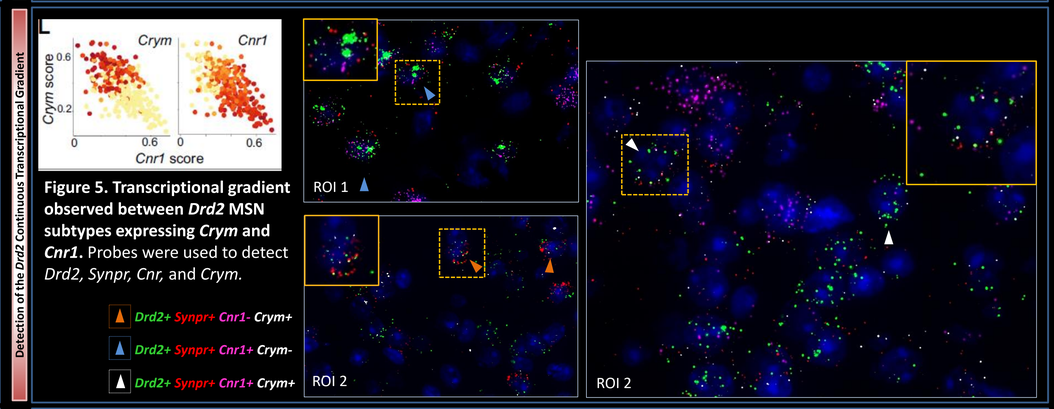


Figure 5. Transcriptional gradient observed between *Drd2* MSN subtypes expressing *Crym* and *Cnr1*. Probes were used to detect *Drd2, Synpr, Cnr,* and *Crym.*

Drd2+ Synpr+ Cnr1- Crym+
Drd2+ Synpr+ Cnr1+ Crym-
Drd2+ Synpr+ Cnr1+ Crym+

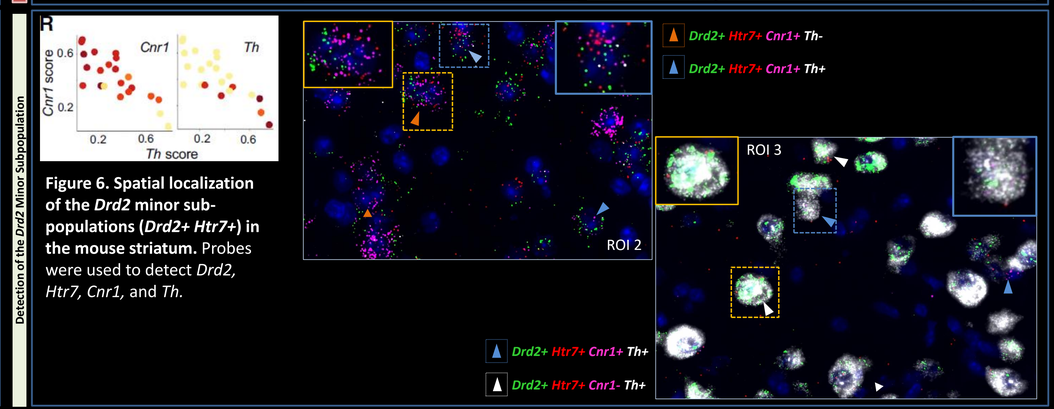
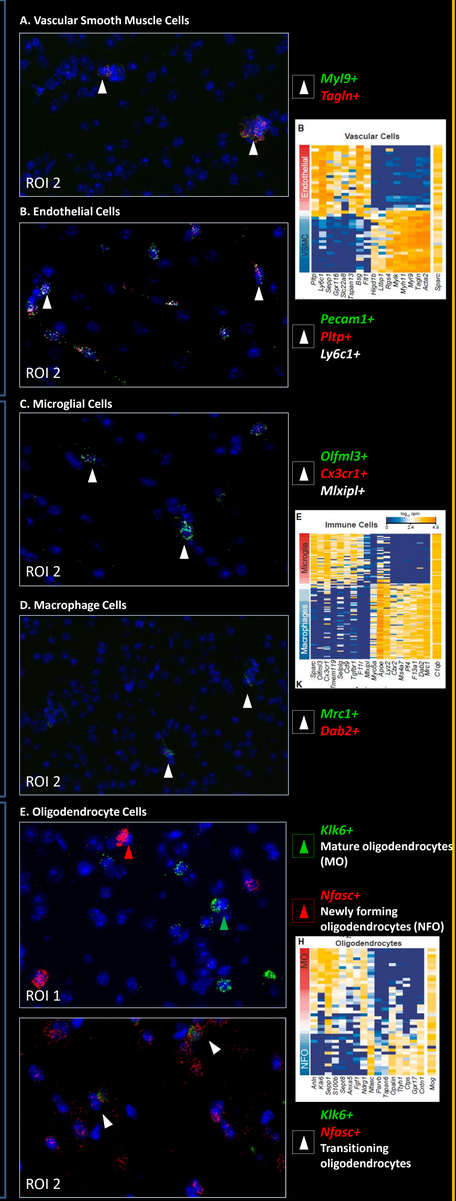


Figure 6. Spatial localization of the *Drd2* minor sub-populations (*Drd2+ Htr7+*) in the mouse striatum. Probes were used to detect *Drd2, Htr7, Cnr1,* and *Th.*

Drd2+ Htr7+ Cnr1+ Th-
Drd2+ Htr7+ Cnr1+ Th+
Drd2+ Htr7+ Cnr1+ Th+
Drd2+ Htr7+ Cnr1- Th+

Non–Neuronal Striatal Cell Types

Figure 7. Validation of non-neuronal cell types identified in the striatum.



Conclusion

Taken together, we have demonstrated the capabilities of a multiplexed *in situ* transcriptomic approach for the validation and spatial mapping of scRNA-seq results in the highly complex and heterogenous mouse striatum. Single-cell transcriptomics combined with spatial mapping with RNA ISH holds great promise in resolving heterogeneous tissues at cellular resolution and providing insights into cellular organization and function of diverse cell types in healthy and disease states.

References

- Gokce O, Stanley GM, Treutlein B, Neff NF, Camp JG, Malenka RC, Rothwell PE, Fuccillo MV, Sudhof TC, Quake SR. Cellular Taxonomy of the Mouse Striatum as Revealed by Single-Cell RNA-Seq. *Cell Rep*. 2016;16(4):1126-1137.
- Allen Mouse Brain Atlas, 2004.