Incorporation of spatial mapping and confirmation of gene signatures by a multiplex *in situ* hybridization technology into single cell RNA sequencing workflows

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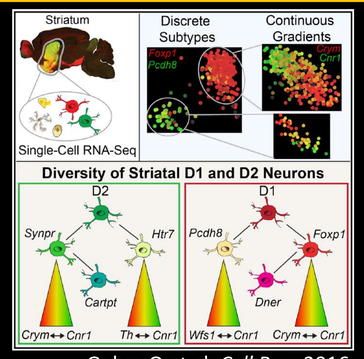
Introduction

Complex and highly heterogenous tissues such as the brain are comprised of multiple cell types and states with exquisite spatial organization. Single-cell RNA sequencing (scRNA-seq) is now being widely used as a universal tool for classifying and characterizing known and novel cell populations within these heterogenous tissues, ushering in a new era of single cell biology. However, the use of scRNA-seq presents some limitations due to the use of dissociated cells which results in the loss of spatial context of the cell populations being analyzed. Incorporating a multiplexed spatial approach that can interrogate gene expression with single cell resolution in the tissue context is a powerful addition to the scRNA-seq workflow. In this study, we used the RNAscope Multiplex Fluorescent and RNAscope HiPlex *in situ* hybridization (ISH) assays to confirm and spatially map the diverse striatal neurons that have been previously identified by scRNA-seq in the mouse brain (Gokce *et al*, *Cell Rep*, 16(4):1126-1137, 2016). We confirmed the gene signatures of two discrete D1 and D2 subtypes of medium spiny neurons (MSN): *Drd1a/Foxp1, Drd1a/Pcdh8, Drd2/Htr7*, and *Drd2/Synpr*. The heterogenous MSN subpopulations were marked by a transcriptional gradient, which we could spatially resolve with RNA ISH. Numerous striatal non-neuronal cell populations identified by scRNA-seq, including vascular cells, immune cells, and oligodendrocytes, were also confirmed with the multiplex ISH assay. Finally, the spatial relationship between the D1 and D2 MSN subtypes identified by Gokce *et al.* was visualized using the RNAscope HiPlex assay, which allows for detection of up to 12 RNA targets simultaneously in intact tissues.

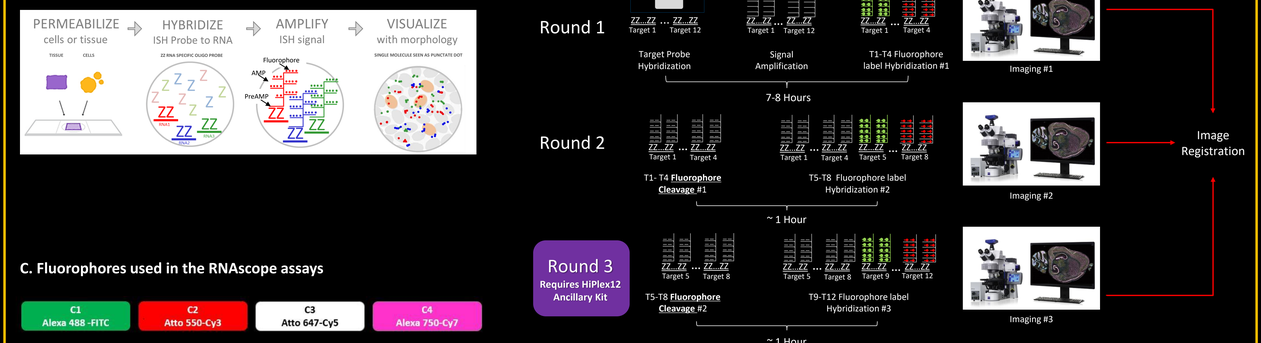


Gokce O et al, *Cell Rep*, 2016

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 and the RNAscope HiPlex 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 and HiPlex assay workflows. (C) Fluorophores used in this study.



Results

Striatal Medium Spiny Neuronal Sub-Types

RNAscope Multiplex Fluorescent Assay (up to 4 targets)

Figure 2. Spatial resolution of the *Drd1a* cell types. (Upper) Major/Minor *Drd1a* sub-populations. (Lower) *Drd1a* major sub-type expressing *Foxp1, Dner and Meis2*

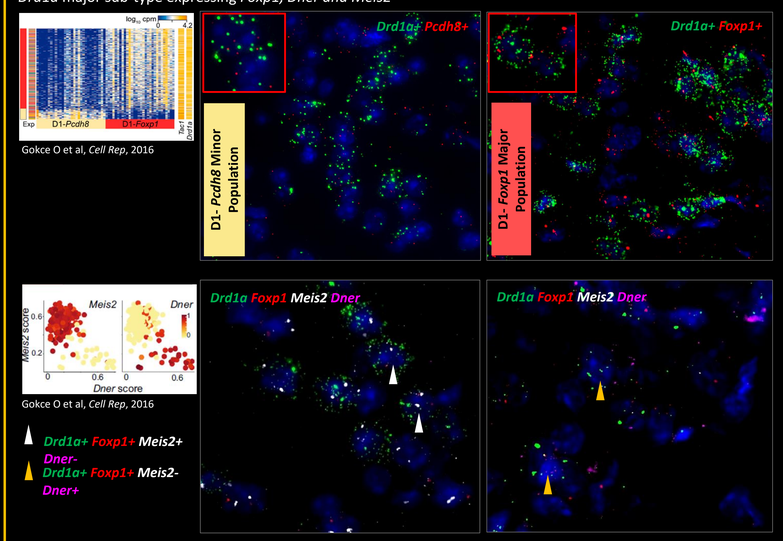
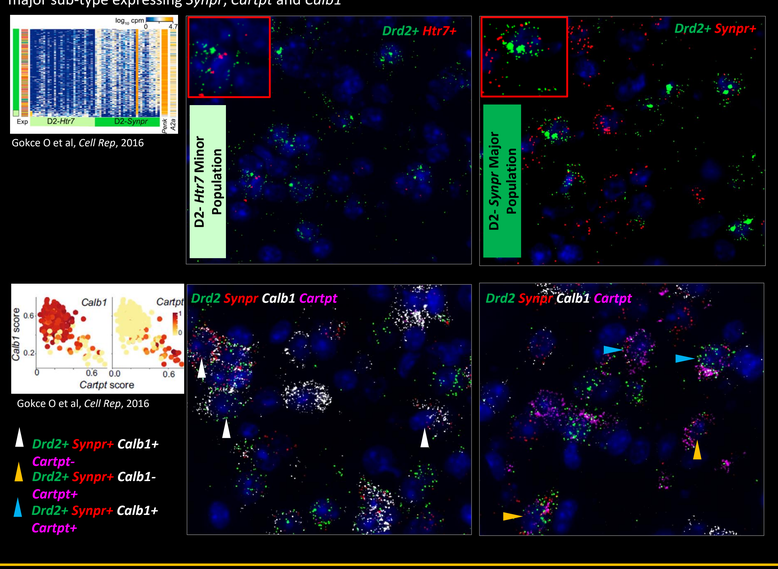


Figure 3. Spatial resolution of the *Drd2* cell types. (Upper) Major/Minor *Drd2* sub-population. (Lower) *Drd2* major sub-type expressing *Synpr*, *Cartpt* and *Calb1*



RNAscope HiPlex Assay (up to 12 targets)

Figure 4. Spatial mapping of all the *Drd1a/Drd2* striatal sub-populations in mouse brain. Spatial resolution of up to 12 target genes at the single cell level was performed with the RNAscope HiPlex assay in the mouse striatum.

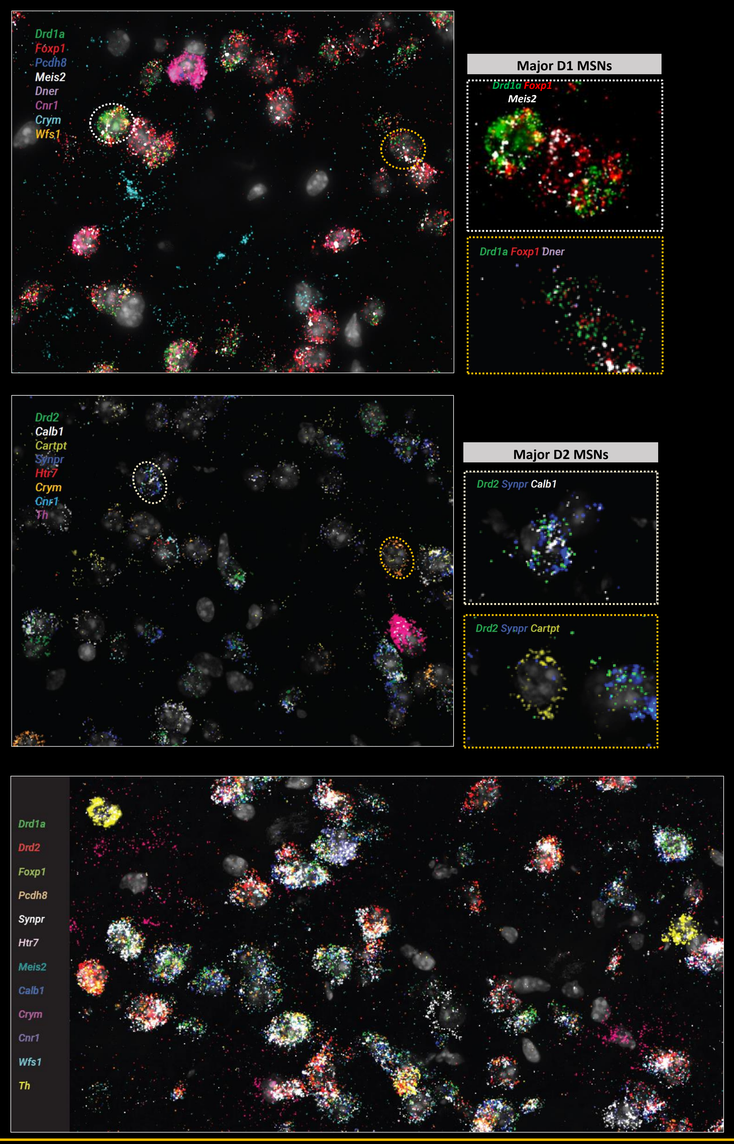


Figure 5. Detection of the major and minor D1 and D2 subtypes simultaneously in the mouse brain. (A) Visualization of the D1 (Red) and D2 (Green) major and minor populations on the same sagittal section.

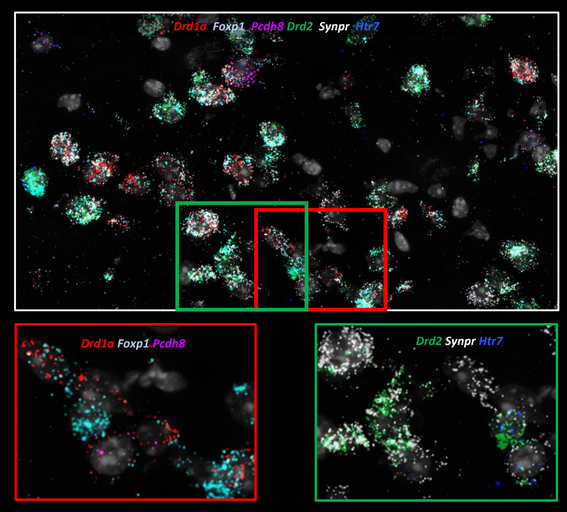
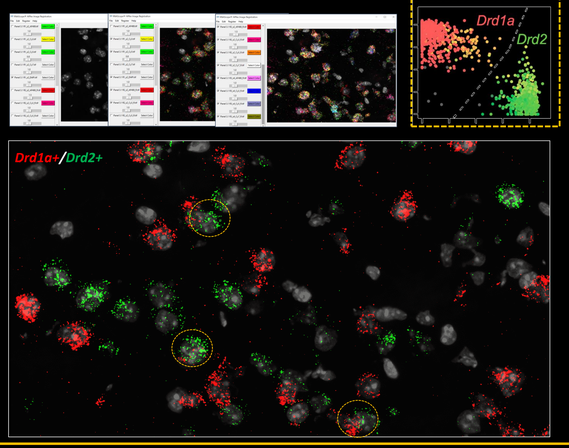
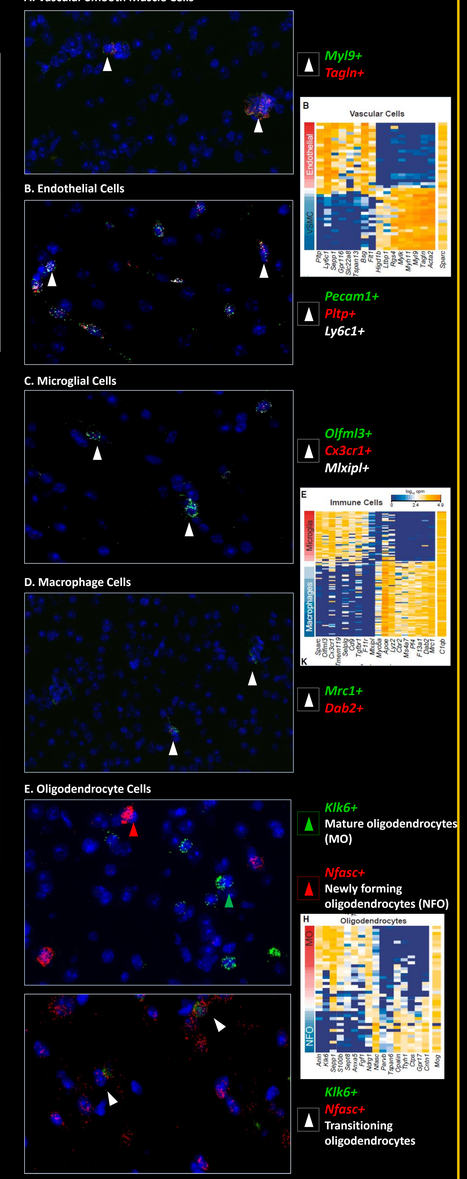


Figure 6. Detection of rare cells co-expressing both *Drd1a* and *Drd2* in the mouse striatum. Detection of cells co-expressing *Drd1a* and *Drd2* (marked by yellow circles) were identified in the same section/field of view as in Figs. 4 and 5 using the HiPlex Image registration software.



Non–Neuronal Striatal Cell Types

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



Conclusion

In conclusion, we have demonstrated the utility of two multiplexed RNAscope ISH assays for the confirmation and spatial mapping of scRNA-seq transcriptomic results in the highly complex and heterogenous mouse striatum at the single cell level. Incorporating spatial mapping by the RNAscope technology into single cell transcriptomic workflows complements scRNA-seq results and provides additional biological insights into the cellular organization and functional states of diverse cell types in healthy and disease tissues.

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.