Bridging the Gap in Synaptic Research: Visualizing Neurexin and Neuroligin Interactions Using Multiomic RNAscope Technology

Ge-Ah Kim [1], Chengxin Zhou [1], Julia Yu [1], Yifan Wang[1], **Debia Wakhloo [1]**, Hamidreza Sepasizangbadi [1], Anushka Dikshit [1], Sonali A. Deshpande [1], Li-Chong Wang[1], Maithreyan Srinivasan[1]
[1]Advanced Cell Diagnostics-A Bio-Techne Brand, , 7707 Gateway Blvd, Newark, CA

INTRODUCTION

Synaptogenesis is a fundamental process of synapse formation that occurs during early development. The formation and regulation of synapses are crucial for all brain functions as they establish connections between neurons. Synaptic disruptions have been implicated in various neurological disorders, such as Alzheimer's, Parkinson's, and Frontotemporal Dementia. Proteins, both on the presynaptic and postsynaptic terminals, play an essential role in the formation of synapses.

Neurexins (NRXs) and Neuroligins (NLGNs) are key protein players in synapse formation and maintenance. NRXs, as presynaptic cell adhesion molecules, are involved in the synaptic specification and differentiation of excitatory and inhibitory synapses. NLGNs, as postsynaptic proteins, regulate synaptogenesis and maintain synaptic stability.

Understanding this interaction would lead to better disease modeling and identifying potential therapeutic targets for pharmacological interventions. However, current methods lack the ability to detect ligand/receptor protein-protein interactions (PPIs) with high specificity and sensitivity, *in situ*. To address this need, we developed a novel assay that modifies the existing RNAscope™ technology to visualize PPIs, proteins, and mRNA *in situ* on the same tissue section. Protein targets were visualized *in situ* along with PPIs/mRNAs using an automated workflow on a Leica BOND Rx instrument. This multiomic assay can interrogate up to 1 PPIs and any combination of mRNA and/or protein targets.

Here, we demonstrate the ability of this assay to detect spatial differences in the level of interaction of the NRX/NGLN protein complex using neurotoxin: MPTP (1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine) injected and WT C57Bl/6J mouse brain tissue. The NRX/NGLN protein complex is known to regulate these terminals; we used RNA probes targeting excitatory presynaptic *(Vglut1/slc17a)*, inhibitory presynaptic *(Vgat1/slc32a1)*, and postsynaptic *(Gabra1)* terminals along with cell profiling conjugated antibodies NEUN and GFAP.

The ability to detect and visualize protein-protein interactions with other mRNA molecules in the same tissue section offers a valuable tool for multi-omics analysis and accurate study of complex brain processes such as synaptogenesis in development, learning, memory, and disease.

METHOD

Figure 1. Multiomic LS RNAscope Fluorescent Assay workflow. FFPE sections are first pretreated, followed by application of conjugated antibodies and target RNA specific probes. RNA transcripts and protein-protein proximity/interaction appears as a punctate dot.

RESULTS

1. Multiomic co-detection of mRNAs, proteins and protein-protein interaction in Mouse Brain

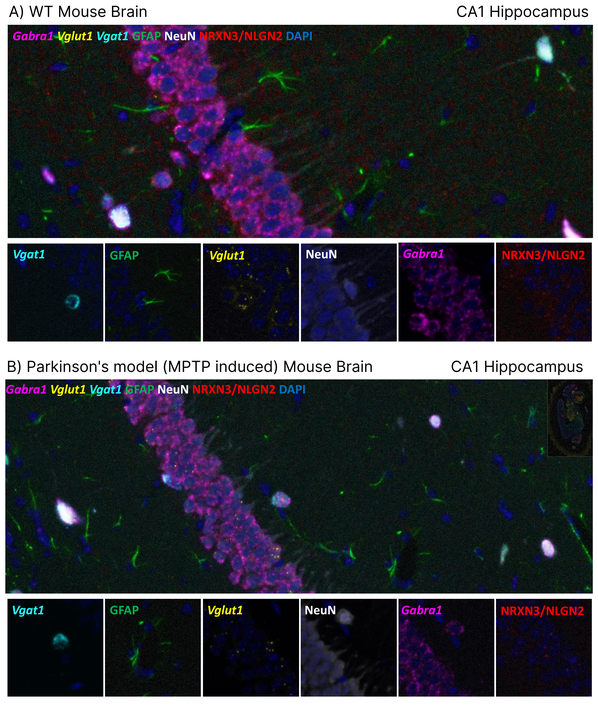
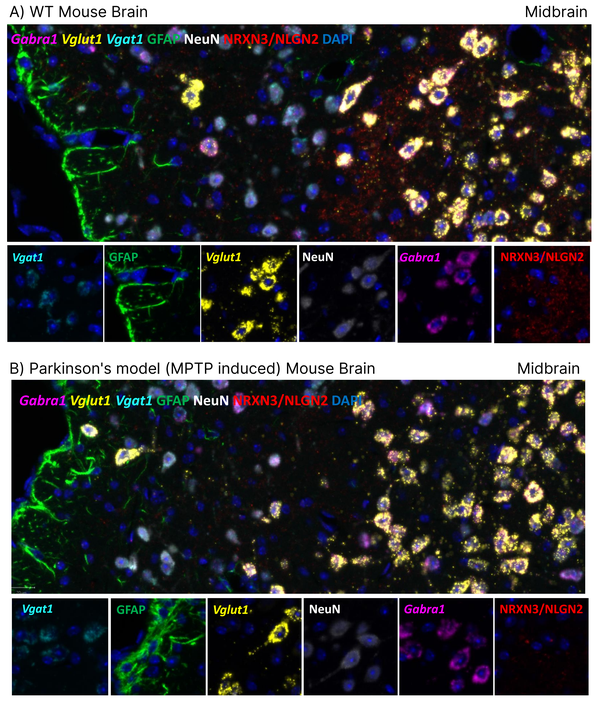


Figure 2. Multiomic LS RNAScope assay indicates reduced interaction between Neurexin and Neuroligin synaptic partners in CA1 Hippocampus region of Parkinson's model mouse brain. Co-localization of 6 markers including 1 PPI, 3 mRNAs, and 2 proteins A) WT mouse CA1 hippocampus region shows all markers B) MPTP injected mouse brain shows reduced PPI signal

2. Midbrain region shows reduced synaptic connections in Parkinson's mouse, indicated by NRXN3/NLGN2 protein-protein interaction using Multiomic LS RNAscope assay



C) Reduced synaptic interaction in Parkinson's Model Mouse Brain observed by NRXN3/NLGN2 interaction in Multiomic LS RNAscope assay

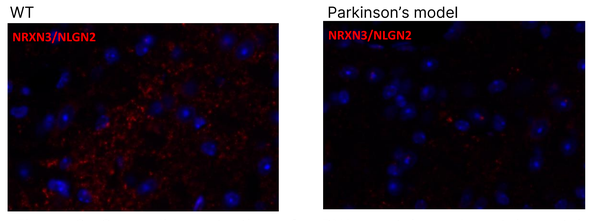


Figure 3. Same-slide detection of mRNA, protein and protein-protein interaction using Multiomic LS RNAscope assay indicate reduced synaptic connections using NRXN3/NLGN2 in midbrain region of Parkinson's mouse model . A) Midbrain region of WT mouse with 6 targets B) Midbrain region of Parkinson's mouse with 6 targets C) Differences between NRXN3/NLGN2 interaction signal in midbrain region of WT and Parkinson's mouse model

3. NRXN3/NLGN2 interaction is significantly reduced in Parkinson's mouse model neurons within the olfactory bulb

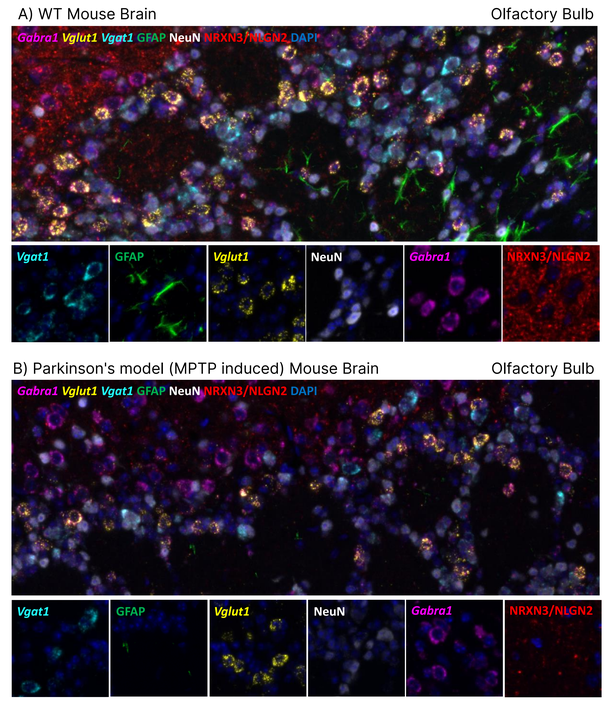


Figure 4. Same-slide detection of 6 targets: two mRNA, two protein and one protein-protein interaction using Multiomic LS RNAscope assay indicate reduced synaptic connections using NRXN3/NLGN2 in olfactory bulb of Parkinson's mouse model . Olfactory bulb of A) WT mouse B) Parkinson's mouse with 6 targets

CONCLUSIONS

- This assay enables simultaneous detection of up to 6 targets including mRNA, protein and protein-protein proximity using immunofluorescence.
- This assay was used to demonstrate expression of region-specific differences in synaptic partners, NRXN3 and NLGN2 in Parkinson's mouse model brain.
- This technology can provide meaningful insights into spatial progression of disease pathology.