Abstract for 6th Chinese Computational and Cognitive Neuroscience Conference (CCCN 2024) (July 3-5, 2024)

Whole Brain Cell Type Mapping by Combining *In Situ* Molecular Labeling and Viral Tracing

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Cell type mapping through single cell RNA sequencing and spatial transcriptomics have dramatically expanded our knowledge boundary of cellular organization in mouse brain. Parallelly, with viral sparse labeling and high resolution optical whole brain imaging, brain-wide long-range projections of thousands of neurons in many brain areas have been reported. However, linking molecular identities to long-range projections in the whole brain is a long-lasting challenge for mouse brain cell type mapping. Here, we utilized fluorescence in situ hybridization (FISH) targeting to multiple canonical neuronal cell-type markers as well as single neuronal viral tracing in all continuous thick slices of entire mouse brains to resolve above-mentioned challenge. With proteins and RNAs crosslinked in hydrogel transformed thick brain slices, whole brain neuronal projections could be resolved through inverted light-sheet microscopic imaging and three-dimensional reconstruction. Then all slices were molecularly labeled with hybridization chain reaction (HCR-FISH) targeting to RNAs of cell type markers e.g., neurotransmitter vesicle transporter, neuromodulator synthase and neuropeptide. We applied this strategy to VTA dopaminergic neurons to catalog the subtypes of these neurons with integrated profiling of molecular markers and whole brain projections.