Mapping transcriptome heterogeneity in the CNS with single cell resolution using highly multiplexed spatial transcriptomics

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NCB|O ERA CHAIR

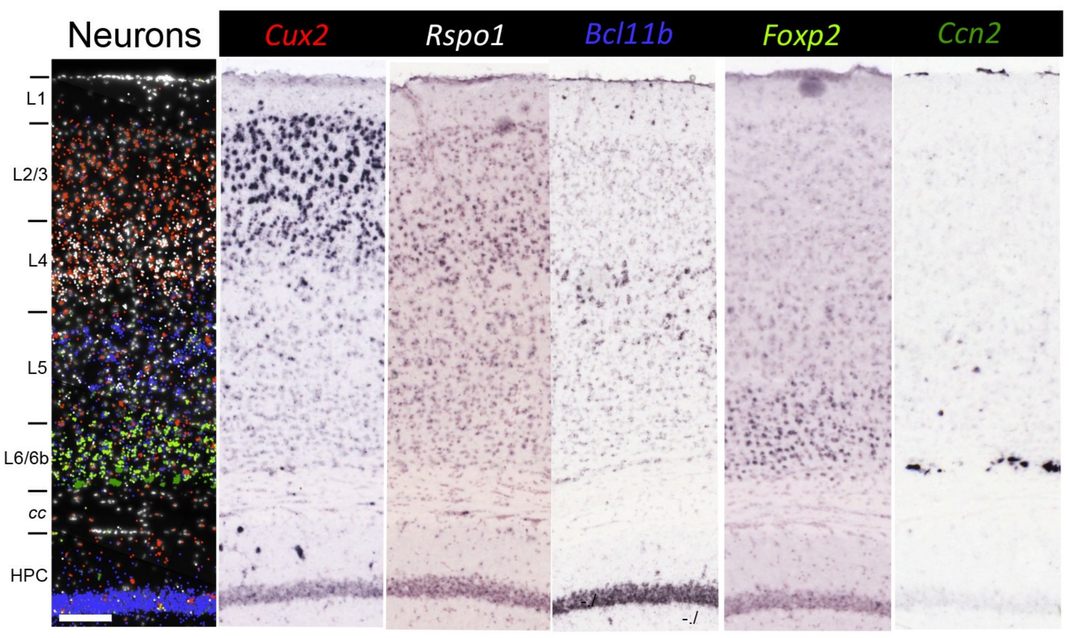
The central nervous system (CNS: brain) is arguably the most complicated organ in mammals, consisting of a multitude of major cell types (including neurons, astrocytes, oligodendrocytes, microglia and vascular cells), whose close functional interactions are responsible for generating thoughts and ideas, and ultimately controlling behaviors. The recent explosion in transcriptome information has revolutionized our understanding of the molecular diversity among cell types in the brain providing crucial insights into the variety of cell-type specific functions needed to produce a working nervous system. Unfortunately, however, critical information on the spatial relationships between these cell types, both during development, as well as in response to injury and/or disease, is lost during single cell RNA-seq experiments because of the need to dissociate the tissue allowing cell isolation, library preparation and sequencing. The ability to map gene expression data onto intact tissue is, therefore, essential to further our understanding of brain function.

OBJECTIVES

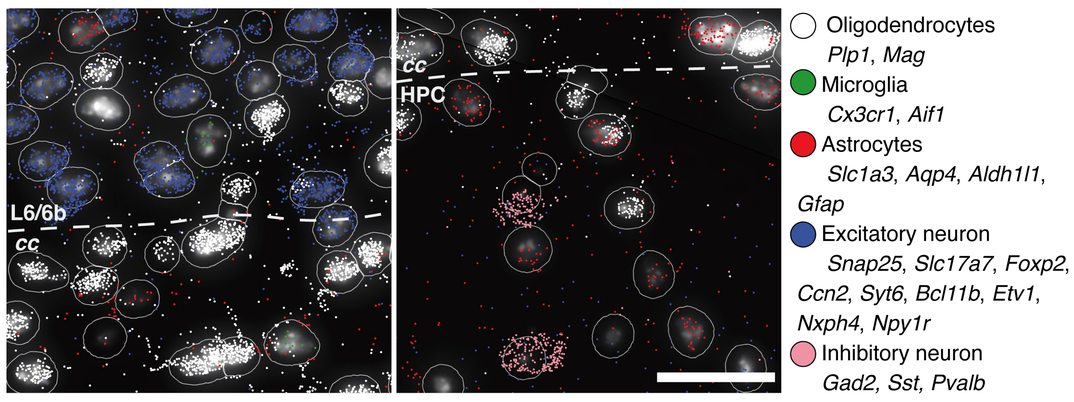
1) To **validate a highly multiplexed single molecule fluorescence *in situ* hybridization (smFISH)** method (Molecular Cartography™ from Resolve Biosciences) for use in complex CNS tissue.
2) To demonstrate the power of Molecular Cartography™ to uncover **changes in gene expression and spatial positioning** of cells in response to **injury and/or disease**, which may be exploitable in therapeutic settings.

MOLECULAR CARTOGRAPHY™ VALIDATION

1) Adult (P56) BL6 mouse brain fixed using Paxgene and 10 µm tissue samples prepared.
2) Molecular Cartography™ ISH (D'Gama et al., Cell Rep, 2021):
   - *multiplexed probe panel against known mRNA markers*



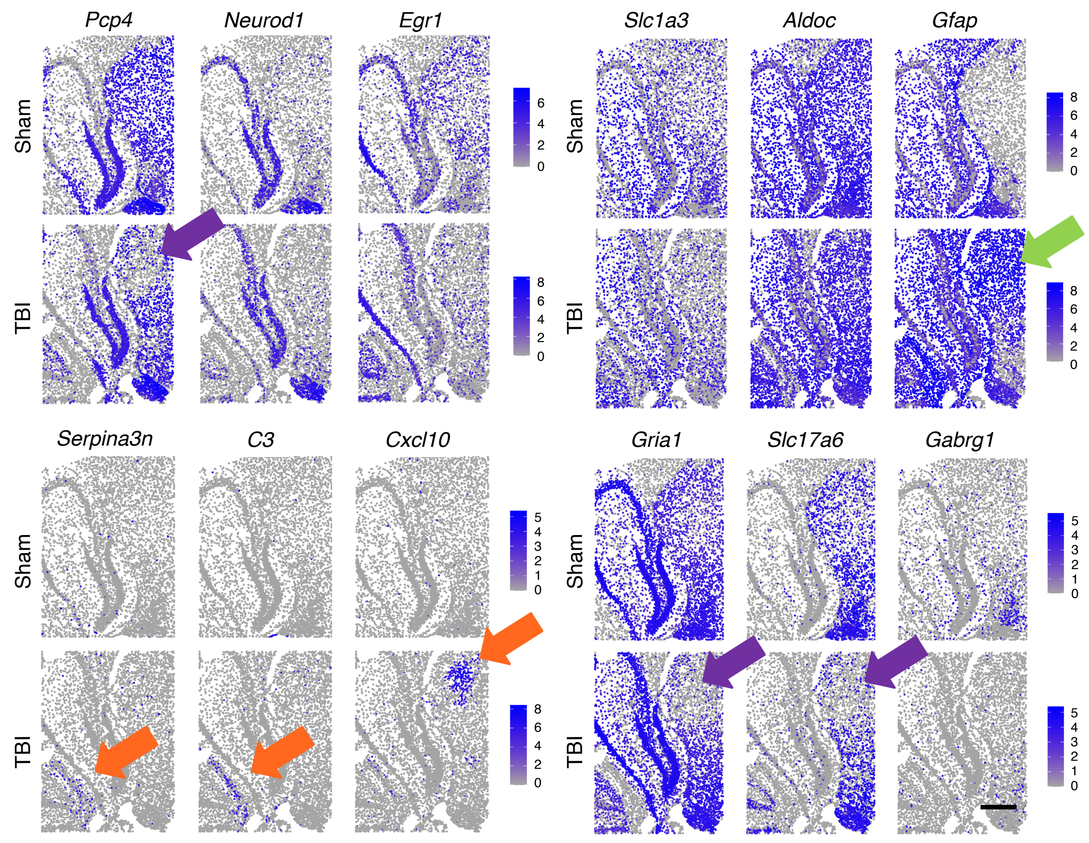
Neuronal cortical layering is accurately captured by Molecular Cartography™, when compared to data from the Allen Brain Atlas. L, Cortical layer; CC, Corpus Callosum; HPC, Hippocampus. Scale bar, 100 µm.



Cell-type identification at single cell resolution across a tissue slice using Molecular Cartography™: individual nuclei are identified based on DAPI signal (fluorescence image) and are used to define the limits of a given cell (white circular profile). Cell types can be identified based on marker mRNAs localized to each nucleus. Each fluorescent 'spot' equates to a probe-mRNA pair, giving an indication of mRNA expression level. Scale bar, 30 µm.

MAPPING INJURY RESPONSE

Traumatic brain injury (TBI) is the leading cause of adult death, below 45 years of age. TBI can be modelled in mice using the **controlled cortical impact** (CCI) model, which leads to secondary hippocampal damage, including in the Dentate Gyrus, which contains the Neural Stem and Progenitor Cells (NSPCs), which are responsible for generating new neurons over the life of the animal. It is thought this hippocampal damage explains the **long-term cognitive impairment** seen in patients. However, the cellular changes underlying **hippocampal dysfunction** remain unclear. To begin to elucidate these changes, adult (P84) BL6 mice received CCI (or a sham procedure) followed by Molecular Cartography™ to check neuronal and glial changes in hippocampus.



Probes against specific mRNAs identified neurons (*Pcp4, Neurod1, Egr1*) and astrocytes (*Slc1a3, Aldoc, Gfap*), inflammatory responses (*Serpina3a, C3, Cxcl10*) and neurotransmitter receptors/pumps (*Gria1, Slc17a6, Gabrg1*). Scale bar, 200 µm. Results demonstrate **neuronal loss** in hippocampus (magenta arrows) and reactive astrogliosis (green arrow). High level multiplexing reveals **different types of reactive gliosis** (orange arrows) occurring in spatially distinct hippocampal regions.

CONCLUSIONS

Molecular Cartography™ can be used to accurately map and quantify gene expression across multiple cell types in the adult mouse CNS.

FUTURE PERSPECTIVES

Molecular Cartography™ technology should be widely applicable in studies of basic biological processes (including development), as well as in mouse models of various diseases/injuries.

We are currently combining 10X Chromium Sequencing and Molecular Cartography™ to refine our understanding of changes to NSPCs in the DG following TBI.

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