Nuclear transcriptome and 3D genome mapping in dopaminergic neurons of adult human midbrain

Sergio Espeso Gil[1a], Tobias Halene[1a], Bibi Kassim[1], Nadejda Tsankova[2], Yan Jiang[1]* and Schahram Akbarian[1]* ([a]Co-first authors, *Co-senior authors)

[1]Department of Psychiatry and Friedman Brain Institute, [2]Department of Neuropathology, Icahn School of Medicine at Mount Sinai, New York, NY 10029 USA.

Monoamine systems play a key role in the pathophysiology of neurological and psychiatric disease. This includes drug abuse and other disorders of reward and addiction. Of note, there is a large literature exploring monoamine pathways in the context of adaptive mechanisms inside the cell nucleus, including changes in gene expression and alterations in chromatin structure and function. However, such types of studies were largely focused on forebrain structures that receive input from monoaminergic neurons. Specifically for the human brain, comprehensive genome-scale and cell-type specific mappings of the transcriptome and epigenome is lacking for many of the relevant brainstem areas, including the substantia nigra (SN) and ventral tegmental area (VTA).

Here, we introduce novel protocols and procedures to comprehensively map the nuclear transcriptome and open chromatin-enriched chromosomal conformations from as little as 20,000 SN dopaminergic neurons. Our protocol includes immunotagging and subsequent fluorescence-activated sorting and separation of nuclei extracted from frozen-thawed midbrain, using Nurr1 and NeuN antibodies, followed by nuclear RNA-seq and a 3D genome mapping method with significant modifications from conventional Hi-C 3D protocols. The Figure attached to this abstract shows representative browser tracks for a 1.3 megabase wide portion of chromosome 11, centered on the *TYROSINE HYDROXYLASE* (TH) gene locus. We expect that our approaches and protocols presented here will be useful for the exploration of (epi)genomic regulation in dopaminergic neurons in the adult human brain, with important implications for the neurobiology and genetics of drug addiction.

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