

Multi-Omic Profiling Reveals Cell-Type-Specific Gene Regulatory Networks in The Rat Nucleus Accumbens

Genomic enhancers, through their dynamic accessibility, mediate gene regulatory network (GRN) interactions between transcription factors (TFs) and their target genes. These mechanisms are essential for the transcriptional programs that support cell-type diversity and homeostatic functions. Transcriptional dysregulation within the nucleus accumbens (NAc), a key brain region for reward-related learning and motivated behavior, has been previously associated with substance use disorders (SUDs). However, the specific GRN mechanisms and enhancers mediating their activity remain unresolved at the cell-type level, hindering the development of novel targeted therapeutics. To address this gap, we collected multi-omic data from ~68,000 cells (paired snATAC-seq + snRNA-seq) from 23 rats (Male + Female). Within our data, we identify 10 transcriptionally defined cell-type clusters, recovering known populations of medium spiny neurons (MSNs), interneurons, and glia. Multi-omic analysis with SCENIC+ identified 191 GRN modules comprising expressed TFs, the putative enhancers they bind, and downstream genes whose expression is associated with both enhancer accessibility and TF expression. GRN modules with high cell-type-specific activities include known developmental regulators, such as *Dlx1*, *Fli1*, and *Sox9*. In Grm8-MSNs, the cell type with the highest mu-opioid receptor expression in the NAc, we find that homeodomain family TFs such as *Pbx1*, *Pbx3*, and *Cux2* converge on several enhancers to commonly regulate Grm8-MSN marker genes. Supporting the role of these TFs in driving Grm8-MSN-specific transcription, sequence-to-function modeling with ChromBPNet identifies homeodomain family motifs as key contributors to enhancer accessibility in Grm8-MSNs. Ongoing experiments will use in situ RNA-scope to validate the Grm8-MSN-specific enhancer activity with AAV constructs containing putative enhancer sequences upstream of a minimal promoter driving expression of a fluorescent reporter. Characterizing these enhancers generates mechanistic hypotheses about GRN dysregulation in SUD and enables us to develop new transgenic tools to test these hypotheses using cell-type-specific manipulations.

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