

Neuron subtype-specific molecular profiling and gene editing in a mouse model of cocaine addiction

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Addiction is a complex and devastating disease that hijacks homeostatic reward signaling, resulting in progressive loss of control over drug consumption and aversive withdrawal symptoms following prolonged abstinence. Recent studies have suggested that epigenetic modifications, particularly DNA methylation and histone methylation, regulate the long-term storage and maintenance of memory, and that defects of certain epigenetic factors affect drug-induced behaviors. We therefore hypothesize that epigenetic modifications in midbrain dopamine (DA) neurons play essential roles in regulating behavioral responses to drugs of abuse. However, cell heterogeneity in brain still represents a technical hurdle that impedes molecular profiling and editing at neuron-subtype resolution.

To overcome these challenges, we first developed an *in vivo* neuron subtype-specific nuclear tagging method to isolate intact DA nuclei for high-throughput analyses such as RNA-seq and whole genome bisulfite sequencing (WGBS), thereby enabling profiling of gene expression and DNA methylation changes after chronic cocaine administration. Surprisingly, we found that chronic cocaine administration results in repression of MHC class I genes, and that among repressed genes, changes in non-CpG methylation rather than CpG methylation are more tightly linked to gene repression. Second, to probe for the functional role of DNA methylation in DA neurons, we have adapted a cre-inducible CRISPR/Cas9 strategy to disrupt expression of epigenetic modifying enzymes in DA neurons. This strategy, applied to a mouse model of cocaine IV self-administration, will allow for both behavioral profiling of addictive phenotypes *in vivo*, and transcriptome/methylome profiling *ex vivo* with neuron-subtype specificity. Results of these studies will not only enrich our understanding of DNA methylation in DA neurons in the context of addiction, but also expand the molecular toolbox for this burgeoning field.