Title: Impact of chronic cocaine use on the human striatum methylome

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Background: Cocaine dependence is a chronic relapsing disorder whose development and trajectory is impacted by multiple genetic and environmental factors. Recently, several studies have identified epigenetic marks that are associated with the acquisition of compulsive drug seeking in animal models, but little is known about the role of epigenetics in human cocaine dependence. Of particular interest is DNA methylation as it represents a mitotically stable mark that has been shown to be altered by environmental experience.

Methods: We used Reduced Representation Bisulfite Sequencing (RRBS) on post mortem nucleus accumbens (NAc) and caudate (CD) tissue from 25 dependent cocaine users and 25 drug-free controls to identify genome wide differentially methylated regions (DMRs). We validated and replicated in an independent sample of 36 individuals a DMR within the *TH* gene using targeted deep bisulfite sequencing and used fluorescence activated cell sorting (FACS) to separate neuronal from non-neuronal nuclei to identify cell-type specificity of our results. Using RRBS data generated from mice that chronically self-administer cocaine, we back translated our results. In addition, we used a combination of transcriptional assays, genome editing, and Luciferase experiments to elucidate the impact of methylation on *TH* expression.

Results: Our study identified numerous DMRs in both striatal sub regions, including three CpG dinucleotides within exon 8/9 of *TH* that are more methylated in the cocaine group than in controls. Methylation at this locus negatively correlates with expression of *TH* in the cocaine group. We validated the results and expanded them across a CpG island within *TH.* We also replicated the increased methylation and its association with expression in a

second independent cohort, and found a similar increase in methylation in the NAc of chronically self-administering mice. This hypermethylation appears to be specific to neuronal nuclei and impedes enhancer activity at this locus.

Ongoing: We have identified hypermethylation at a locus within the *TH* gene, which is associated with chronic cocaine dependence in humans and self-administration in animals, and which appears to have regulatory potential. Our ongoing work examines the relationship between methylation and transcription factor binding as a mechanism of transcriptional control.

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