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Cocaine-related DNA methylation of *IRX2* alters local 3D chromatin architecture in human brain cells.

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Background: Epigenetic mechanisms are of particular interest in understanding the biological basis of cocaine use disorders, as they may mediate the long-term effects of chronic cocaine abuse on brain cell functioning. Although animal research has built a strong case for the roles of DNA methylation and chromatin remodeling in cocaine dependence, little is known about the relationship between these phenomena in human patients.

Methods: We used RRBS to identify cocaine -related differential methylated CpG clusters (DMCs), in post mortem striatum samples from dependent cocaine users, and FACS to investigate cell-type specificity. We also used a mouse model of cocaine seeking, transcriptomics and chromatin conformation capture (3C) to explore the genomic regulation of the *IRXA* gene cluster. Our *in vitro* studies use dCas9-DNMT3a epigenome editing to investigate causal relationships between methylation and chromatin function.

Results: Among >100 DMCs, we found hypomethylation within *IRX2* in the caudate nucleus, which we validated in neuronal nuclei and replicated in an independent cohort, and which contains a potential CTCF binding site in both human and mouse. We also found increased expression of *IRX2* and altered co-expression with its neighbor, *IRX1*. Moreover, we identified a large chromatin loop in human cells, which is related to *IRX2* methylation, and may mediate its influence on gene expression.

Conclusions: Cocaine dependence is associated with widespread alterations in DNA methylation in the human striatum, including decreased methylation within *IRX2* in the caudate nucleus. This appears to be associated with dysregulated gene expression, and may drive three-dimensional chromatin looping.