Cocaine-induced histone methylation on Egr3 and Nab2 promoters

R. Chandra[1], B. Evans[1], M. Mcglincy[1], A. Chow[1], K. K. Cover[2], M. Engeln[1], T. C. Francis[1], M. Lobo[1]

[1]Anatomy and Neurobiology, Univ. of Maryland Sch. of Med., Baltimore, MD; [2]Department of Pharmacology, University of Maryland School of Medicine, Baltimore, MD, USA.

An epigenetic modification plays an important role in transcriptional changes in the nucleus accumbens (NAc) in cocaine addiction. However, there is little information about the role of histone demethylation in the NAc, after exposure to drugs of abuse. We previously demonstrated that the transcription factor, Egr3, is upregulated in D1-MSNs and down-regulated in D2-MSNs after repeated cocaine exposure. It is postulated that Egr3 regulates its co-repressor; Nab2 and they both act together as a feedback mechanism to repress Egr3 transcription. Consistent with this, we observed a reduction of Nab2 in D1-MSNs and an increase of Nab2 in D2-MSNs after repeated cocaine. In order to further investigate the molecular regulation of Egr3 and Nab2 we examined the histone lysine demethylase (Kdm1a) enzyme as well as its demethylation targets H3K4me and H3K9me in NAc after repeated cocaine. We observed an increase in Kdm1a mRNA in D1-MSNs and a reduction in D2-MSNs after repeated cocaine. To further assess if Kdm1a might alter methylation on Egr3 and Nab2 promoters after cocaine we performed chromatin immunoprecipitation (ChIP) for histone marks, H3K4me3 and H3K9me2. Repeated cocaine caused changes in H3K4me3 and H3K9me2 on promoters of Egr3 and Nab2. We further found that Kdm1a is enriched on the Egr3 and Nab2 promoters after repeated cocaine. To determine if Kdm1a is responsible for bidirectional induction of Egr3 and Nab2 in D1-MSNs vs. D2-MSNs we are developing a CRISPR-Cas9 approach to target specific methylation sites on the Egr3 and Nab2 promoters. In this approach we will alter methylation on the Egr3 and Nab2 promoters by using light-inducible heterodimerizing proteins CRY2-KDM1A and dCas9-CIB1, along with Egr3 and Nab2 gRNAs. This approach will allow us to understand the precise epigenetic mechanisms regulating expression of Egr3 and Nab2 in MSN subtypes during cocaine-mediated behaviors.