

## ***Nr4a1 Activation Suppresses Cocaine-induced Behavior via Epigenetic Regulation of Homeostatic Target Genes.***

*MD Carpenter, Q Hu, SI Lombroso, KS Czarnecki, H Song, ME Wimmer, RC Pierce, EA Heller*

A core feature of addiction is the propensity for relapse caused by the aggregation of neuroadaptations during abstinence (Shaham & Hope, 2005). To combat relapse, endogenous homeostatic mechanisms may restore and even reverse normal function to reward-related brain areas (Huang et al 2011; Keramati et al, 2017). To identify a master regulator of homeostatic gene expression, we profiled global transcriptomic changes in brain reward areas at early and late abstinence following cocaine self-administration in mice. Using this approach, we identified a key role for the transcription factor, nuclear receptor subfamily 4 group A member 1 (Nr4a1), in epigenetic regulation of homeostatic target gene expression and cocaine-evoked behavior.

**Methods:** Mice self-administered either saline or cocaine (0.7 mg/kg/infusion) on a fixed-ratio 1 schedule during daily 2-hour sessions for 21 consecutive days. Mice were sacrificed 1- and 28-days after the last cocaine session, brains were removed, and nucleus accumbens (NAc) was collected. Single-sample sequencing (S3EQ) (Xu & Heller, 2018) was used to analyze changes in gene expression and histone modifications. CRISPR/dCas9-mediated gene activation and repression was used to regulate *Nr4a1* in vivo. Cocaine conditioned place preference (i.p., 10 or 5 mg/kg) was used to assess cocaine reward behavior following CRISPR/dCas9-mediated *Nr4a1* expression in NAc.

**Results:** Cocaine self-administration and abstinence regulated expression of the transcription factor, *Nr4a1*, and its target genes, *Cartpt* and *Vmat2*. Sustained activation of *Cartpt* and *Vmat2* at late abstinence was coupled with depletion of the repressive histone modification, H3K27me<sub>3</sub>, in the presence of the activating marks, H3K27ac and H3K4me<sub>3</sub>. CRISPR-mediated gene activation (dCas9-VP64) or repression (dCas9-KRAB) in NAc was sufficient to activate or repress expression of *Nr4a1* and target genes. Cocaine conditioned place preference was attenuated by *Nr4a1* activation, and enhanced by its repression. Importantly, dCas9-VP64 activated *Nr4a1* expression to the level evoked endogenously by cocaine self-administration.

**Discussion:** This work provides evidence of the relevance of *Nr4a1* and target genes, *Cartpt* and *Vmat*, in cocaine reward. We identified a molecular mechanism of action of *Nr4a1*. Specifically, *Nr4a1* was sufficient to recapitulate cocaine-activation of *Cartpt* via loss of repressive histone modifications in the presence of extant activating modifications. Our findings provide evidence that epigenetic editing of endogenous homeostatic genes is a potential strategy to combat cocaine addiction.

### **References:**

- Shaham, Y. & Hope, B. T. *Nat. Neurosci.* 8, 1437–1439 (2005)  
Keramati, M., Durand, A., Girardeau, P., Gutkin, B. & Ahmed, S. H. *Psychol. Rev.* 124, 130–153 (2017).  
Huang, Y. H., Schlüter, O. M. & Dong, Y. *Behav. Brain Res.* 216, 9–18 (2011).  
Xu, S. J. & Heller, E. A. *J. Neurosci. Methods* 308, 62–73 (2018).

*Financial support is kindly acknowledged from Charles E Kaufman Foundation Young Investigator Award (E.A.H), Whitehall Foundation Grant (E.A.H), NIH-NIDA Avenir Director's Pioneer Award (E.A.H, DP1 DA044250) and Research Supplements to Promote Diversity in Health-Related Research (M.D.C, DP1 DA044250-01), T32 Predoctoral Training Grant in Pharmacology (M.D.C, T32GM008076), NIDA Research Project Grant (C.R.P R01 NIDA R01 DA33641, NIDA R01 DA15214), NIDA K01 Mentored Research Scientist Career Development Award (M.E.W. DA039038). The cocaine used in this study was kindly provided by the NIDA drug supply program.*