Engineering zinc-finger protein and CRISPR/Cas9 constructs to model the epigenetic and transcriptional phenomena that underlie cocaine-related behaviors

Hamilton PJ, Heller EA, Ortiz Torres I, Burek DD, Lombroso SI, Pirpinias ST, Neve RL, Nestler EJ

Multiple studies have implicated genome-wide epigenetic remodeling events in brain reward regions following drug exposure. However, only recently has it become possible to target a given type of epigenetic remodeling to a single gene of interest, in order to probe the causal relationship between such regulation and neuropsychiatric disease (Heller et al., *Nat Neurosci*, 2014). Our group has successfully utilized synthetic zinc-finger proteins (ZFPs), fused to either the transcriptional repressor, G9a, that promotes histone methylation or the transcriptional activator, p65, that promotes histone acetylation, to determine the behavioral effects of targeted in vivo epigenetic reprogramming in a locus-specific and cell-type specific manner. Given the success of our ZFP approaches, we have broadened our technical repertoire to include the more novel and flexible CRISPR/Cas9 technology. We designed guide RNAs to target nuclease-dead Cas9 (dCas9) fused to effector domains to the *fosB* gene locus, a locus heavily implicated in the pathogenesis of drug abuse. We observe that dCas9 fused to the transcriptional activator, VP64, or the transcriptional repressor, KRAB, and targeted to specific sites in the *fosB* promoter is sufficient to regulate *FosB* and *ΔFosB* mRNA levels, in both cultured cells and in the nucleus accumbens (NAc) of mice receiving viral delivery of CRISPR constructs. Next, we designed a fusion construct linking the dCas9 moiety to a pseudo-phosphorylated isoform of the transcription factor CREB (dCas9-CREB(S133D)). CREB binding to the promoter of *fosB* gene has been demonstrated underlie the cocaine-mediated induction of ΔFosB. We observe that viral delivery and targeting of dCas9-CREB(S133D) to the promoter of *fosB* is sufficient to up-regulate ΔFosB mRNA and protein levels in the NAc of mice.