Engineering CRISPR constructs as tools for gene-targeted transcriptional reprogramming in mammalian brain to elucidate the causal pathogenic mechanisms of drug abuse

Hamilton PJ[1], Lardner CK[1], Lorsch ZS[1], Conkey A[1], Neve RL[2], Heller EA[3], Nestler EJ[1]

[1]Fishberg Department of Neuroscience and Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA; [2]McGovern Institute for Brain Research, Massachusetts Institute of Technology, Cambridge, MA, USA; [3]Department of Systems Pharmacology and Translational Therapeutics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Drug addiction is a chronic, debilitating syndrome with a substantial body of evidence indicating that epigenetic and transcriptional mechanisms are associated with disease progression. However, a major obstacle in efforts to understand and devise treatments for addiction stem from an inability to determine causality between enrichment of an epigenetic modification or transcription factor binding at a specific gene and the pathogenesis of addiction. In an effort to determine this causality, our group has successfully utilized synthetic zinc-finger proteins (ZFPs) fused to epigenetic editing moieties to determine the neural and 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 flexible CRISPR/Cas9 technology. We have designed a fusion construct linking the nuclease-dead Cas9 (dCas9) moiety to a pseudo-phosphorylated isoform of the transcription factor CREB (dCas9-CREB(S133D)) and designed guide RNAs (gRNAs) to target the *Fosb* gene locus, a locus implicated in drug addiction pathogenesis. CREB binding to the promoter of *Fosb* gene has been demonstrated to underlie the cocaine-mediated induction of ΔFosB. We observe that viral delivery and targeting of dCas9-CREB(S133D) to the *Fosb* promoter is sufficient to up-regulate ΔFosB mRNA and protein levels in the NAc of mice as well as potentiate cocaine conditioned place preference, indicating a causal role for CREB binding to *Fosb* in the progression of cocaine responses. Having utilized these tools at the well-understood *Fosb* locus, we capitalized on the intrinsic flexibility of CRISPR to design gRNAs targeting dCas9-CREB(S133D) to the previously unexplored, CREB-regulated gene *Zfp189* – which we have observed to be induced in NAc by cocaine self-administration. The targeted recruitment of CREB to *Zfp189* will allow us to identify the causal transcriptional and behavioral consequences of this interaction within the brain's reward regions.

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