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Using a Cas1sf derived system for CRISPR epigenome editing of fentanyl regulated hub genes

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CRISPR epigenome editing, using CRISPR-activation or CRISPR-interference (CRISPRa/i) allows for more physiologically relevant manipulation of gene expression. Recent advances in CRISPR have paved the way for using a Cas12f derived system (CasMINI) instead of Cas9. The advantages of this system are twofold - for one dead (d)CasMINI is less than half the size of dCas9 allowing for packaging in adenosine-associated viruses (AAVs) instead of the previously used lentiviruses. Further, this construct allows the possibility of including multiple guide constructs for manipulation of multiple genes at the same time. The other advantage is the improved efficiency of Cas12 systems compared to Cas9 in mammalian cells. We are utilizing a CRISPRi system in which a KRAB domain is fused to dCasMINI in one construct, as well as CRISPRa in which VP64 is fused to dCasMINI. A separate construct with guide RNA is also used to guide the CRISPRa/i to endogenous gene targets. With this system, we target genes previously found to be differentially expressed in mouse nucleus accumbens (NAc) D1/D2 MSNs following forced abstinence from fentanyl. We have validated that for the first time in Neuro2a cells that the dCasMINI fusion gene can express at the mRNA and protein level and have validated its ability to up or downregulate expression of gene targets. We are also validating a new light-inducible Opto-dCasMINI system. The potential to use these tools to alter transcription of specific gene(s) in a cell type-specific manner in the brain will allow routine use of AAV CRISPR epigenome editing tools.