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Epigenome editing of nucleus accumbens cell subtype transcripts regulated by fentanyl abstinence

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The opioid use epidemic has significantly impacted the financial and societal health burdens around the world, particularly the United States. Many studies indicate that dysfunctionality in the mesocorticolimbic brain areas are central to understanding opioid use, dependence, and addiction. The nucleus accumbens (NAc) is a critical brain hub for altered molecular processes mediating behavioral responses to opioids. We identified distinct MSN subtype gene expression networks that are altered during fentanyl abstinence including hub genes, which are key drivers of gene expression alterations in MSN subtype specific modules. Currently, we are developing CRISPR epigenome editing tools that will target specific hub genes in MSN subtype modules that are significantly regulated by fentanyl abstinence- 5 days 10µg/ml fentanyl followed by 10 days of abstinence. Since most genes are down-regulated we use CRISPRa (activation) to upregulate expression of hub genes in MSN subtypes. Using a two vector adenoassociated virus (AAV) system we designed single gRNA or multiplex gRNAs, the latter targeting multiple hub genes within one module. gRNAs targeting hub genes or a lacZ control gRNA are cloned into an AAV-gRNA-nlsGFP. The second vector is an AAV-DIO-dCas9-VP64 containing the nuclease dead Cas9 fused the VP64 transcriptional activation domain. We have transfected these vectors along with Cre into Neuro2A cells and demonstrated upregulation of MSN subtype fentanyl abstinence regulated hub genes. We are currently packaging these vectors into AAVs to target NAc MSN subtypes, during opioid exposure and abstinence to determine if upregulating hub genes can influence MSN subtype/fentanyl abstinence gene expression networks