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Cocaine regulation of alternative splicing via changes in histone postranslational modifications.

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Alternative splicing differentiates identity and function of neuronal subtypes and is activity dependent. Defects in neuronal splicing are associated with neurological and psychiatric disorders. The hPTM, H3K36me3, is implicated in splicing and interacts with a variety of splicing-related proteins. The H3K36me3 writer, SETD2, directly interacts with splicing factors. We find that H3K36me3 is causally linked to alternative splicing in the context of cocaine reward behavior in mice. However, we still lack a mechanistic understanding of the interplay between hPTMs and alternative splicing. This project aims to define the regulation of alternative splicing by SETD2 and/or H3K36me3 by decoupling these putative splicing regulators. To accomplish this, we acutely depleted either H3K36me3 and SETD2 and then measured global splicing changes using RNA-sequencing in ex-vivo mouse neurons. We achieved depletion of H3K36me3 using a newly SETD2 inhibitor, EZM0414, and of SETD2 through a dTAG degron at the endogenous locus. In addition, we apply a CRISPR epigenetic editing tool to specifically deposit H3K36me3 and assess the sufficiency of H3K36me3 to drive splicing at target exons. This project will be the first to determine the relative contributions of SETD2 and H3K36me3 to alternative splicing to uncover how these factors might regulate diverse neuronal functions.