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Neuronal Methylome and Hydroxymethylome Profiling of Opioid Use Disorder

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Opioid use disorder (OUD) is influenced by genetic and environmental factors, suggesting a role for epigenetic mechanisms. Alterations in DNA methylation (5mC) have been observed in peripheral and brain tissue of OUD individuals. DNA hydroxymethylation (5hmC), a less studied epigenetic modification, is involved in the demethylation pathway and highly enriched in the brain. However, its role in OUD is unknown. This study aims to investigate neuron-specific genomewide 5mC and 5hmC changes associated with OUD in the human postmortem orbitofrontal cortex (OFC). 38 human male postmortem OFC samples from the VA Brain Bank were included. Fluorescence-activated nuclei sorting was used to isolate neuronal nuclei. Sorted nuclei were processed for reduced representation oxidative bisulfite sequencing. For the differential analysis of 5mC/5hmC, methylKit R package was used. Covariates included race, age of death, postmortem interval, smoking, and post-traumatic stress disorder. A total of 896 5mC and 1579 5hmC marks were identified. In promoter regions, these mapped to genes enriched for Wnt signaling, G-protein signaling, and opioid signaling for 5mC and axon guidance, GPCR, and BDNF signaling for 5hmC. At gene bodies, 5mC and 5hmC differential marks enriched for transcriptional regulation and RNA polymerase II. Differential 5mC and 5hmC marks also showed enrichment in neuron-specific active enhancers. This is the first study to examine neuronal methylomic and hydroxymethylomic profiles of OUD in human postmortem OFC. Our findings suggest that OUD-related changes in 5mC and 5hmC target different genes and pathways, with some overlap. It also reveals a novel important role of 5hmC on OUD.