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Multi-omics characterization of cocaine use disorder in postmortem human brain

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Cocaine use disorder is a severe psychiatric disorder characterized by excessive and compulsive use of cocaine, as well as difficulties in reducing cocaine intake and remaining abstinent. Due to the chronic effects of cocaine on the organism, individuals with cocaine use disorder have a significantly increased risk for the development of cardiovascular disease and stroke, as well as comorbid psychiatric disorders such as depression, resulting in a substantial burden of disease. Several million people worldwide suffer from cocaine use disorder. Still, the currently available treatment approaches have limited effectiveness and are associated with high relapse rates, highlighting that the successful treatment of cocaine use disorder remains a major challenge. Regarding the etiology of the disease, it is assumed that changes in brain neurocircuits contribute to the development and maintenance of cocaine use disorder. In this context, the “neurocircuitry of addiction” model has been developed, which allows to relate the symptoms of cocaine use disorder to specific regions of the brain, such as the striatum or cortical areas. Molecular changes at the level of epigenetics, transcription, and protein expression are hypothesized to underlie the neuroadaptations and neurocircuit alterations in cocaine use disorder. Despite intensive research on the molecular underpinnings of cocaine use disorder, many aspects remain unclear, for instance, how changes in epigenetics are related to transcriptional and protein-level changes, while also the role of different cell types in the brain in cocaine use disorder is largely unknown. To characterize molecular alterations in the human brain, postmortem brain tissue serves as an important resource for investigating molecular changes using case-control study designs. The aim of the three studies presented was to conduct a comprehensive molecular characterization of cocaine use disorder in the human brain and to provide deeper insights into its pathophysiology, as well as potential new therapeutic approaches for cocaine use disorder.

In the first study, tissue from Brodmann Area 9, a subregion of the prefrontal cortex, from 21 individuals with cocaine use disorder and 21 control subjects was analyzed for genome-wide changes in DNA methylation. The second study represents a multi-omics approach performing integrative analyses of DNA methylation and gene expression data obtained by RNA sequencing. In addition, RNA splicing patterns in Brodmann Area 9 were examined, and the conservation of gene expression signatures in the prefrontal cortex in cocaine use disorder was investigated using replication datasets. The third study represents a comprehensive molecular characterization of cocaine use disorder in the same cohort, but in a striatal brain region, the ventral striatum. Omics-wide datasets of microRNA, RNA, and protein expression were integrated, and cell type-specific transcriptomic changes were identified using single-nuclei RNA sequencing. To investigate the biological role of molecular changes associated with cocaine use disorder, pathway and network analyses were performed, as well as drug repurposing analyses to identify drugs that can reverse the molecular brain changes associated with cocaine use disorder. The epigenome-wide association study in Brodmann Area 9 identified a total of 20 differentially methylated regions associated with cocaine use disorder. In addition, network analyses revealed an enrichment of differential DNA methylation within genes involved in neurotransmission and synaptic signaling. Data integration with information on RNA expression, as performed in the second study, confirmed differential expression of synaptic genes at the RNA level and also suggested altered oxidative phosphorylation and fatty acid metabolism in the brain. With *INPP5E* and *ZBTB4*, two gene candidates were identified that showed significant changes in individuals with cocaine use disorder across analyses of DNA methylation, splicing, and RNA expression while also containing genetic risk variants. Pharmacological compounds that act on the glucocorticoid receptor were identified as potential drugs that could reverse the cocaine use disorder-associated gene expression profile. The study in the ventral striatum confirmed findings of altered oxidative phosphorylation and fatty acid metabolism in the brain of individuals with

cocaine use disorder and represents one of the first studies that included proteomic profiling in multi-omics approaches for addiction research. The transcriptome analysis at the single nuclei level highlighted a particular relevance of striatal astrocytes and medium spiny neurons in cocaine use disorder, suggesting alterations in cell-cell adhesion and glutamatergic neurotransmission.

In summary, the three studies represent an in-depth molecular characterization of cocaine use disorder in postmortem human brain tissue, providing deep insights into the molecular changes in two important regions of the neurocircuitry of addiction: the prefrontal cortex and the ventral striatum. The integration of multiple omics-wide data sets, both at the bulk level and at the single nuclei level using complex biostatistical methods, highlights the additional value that results from integrative analyses of high-dimensional data sets, for example, allowing the identification of changes in RNA expression that are directly linked to altered DNA methylation. Promising targets for future therapeutic approaches in cocaine use disorder could, for example, be related to glutamatergic neurotransmission, fatty acid and mitochondrial metabolism, as well as at (neuro-)immunological targets such as the glucocorticoid receptor. Functional validation of findings from multi-omics studies, for example, using animal or organoid models, depicts the next step to use this knowledge for a better understanding of the underlying disease mechanisms and for developing novel therapeutic approaches for cocaine use disorder.