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# Mapping the Spatial Epigenomic Landscape of Opioid Addiction and Its Neuronal Circuitry in the Paraventricular Thalamus to Nucleus Accumbens Pathway

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Opioid addiction is a fast-growing public health crisis in the United States. Chronic opioid use results in physical dependence and intense withdrawal symptoms. We have identified the paraventricular nucleus of the thalamus (PVT) to nucleus accumbens (NAc) pathway as critical in mediating opioid withdrawal, with long-term potentiation in this pathway reinforcing opioid-associated memories. Silencing this pathway disrupts opioid-associated memory and offers enduring protection against relapse. Here, we focus on the PVT to NAc pathway, aiming to identify its essential role in opioid withdrawal. To elucidate the cellular heterogeneity and functional complexity within this pathway, we employed single-cell transcriptomic and epigenomic imaging to develop a spatially resolved, single-cell atlas of the PVT-NAc axis. Using Multiome analysis, we observed a subset of withdrawal-induced genes, albeit with modest expression changes, which we subsequently incorporated into our MERFISH gene panel to enhance spatial imaging resolution of the pathway. This high-resolution approach enabled us to identify specific cell types responsive to opioids and withdrawal, as well as gene expression state across various addiction stages. To pinpoint opioid-responsive neuronal populations, we utilized retroAAV tracing, labeling NAc-projecting PVT neurons with unique barcodes. Additionally, we applied single-cell chromatin tracing to investigate structural changes within key genomic loci, such as GRIA1 and BDNF, in thalamic neurons. Our findings reveal distinct chromatin architectures in neuronal versus glial cell types and other cell types, with chromatin folding patterns validated against Hi-C data. This integrative study provides critical insights into the molecular and cellular landscape of opioid addiction.