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Fentanyl Exposure Disrupts Cellular and Molecular Integrity in Human Midbrain Organoids

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Studies in animals and humans have shown that Individuals with a history of opioid exposure have higher risks for drug seeking behavior. Although the midbrain plays a pivotal role in drug seeking, the underlying cellular and molecular mechanisms have not been well understood. Here, we hypothesize that a protracted Fentanyl (FTY) exposure disrupts neural function in the midbrain. To examine our hypothesis, we have developed a human midbrain organoids model (hMBOs) derived from human induced pluripotent stem cells. hMBOs were exposed to one month of FTY followed by withdrawal for a certain period (e.g., 1-2 months) during which functional and multiome studies were performed. Our results show that FTY leads to a decrease in the dose-dependent release in Dopamine after FTY withdrawal. Using snRNA/ATAC sequencing analysis, we identified, FOXP2 (decreased expression and TF activity) in GABA neurons, LMX1A (stable expression and decreased TF activity) in Dopaminergic neurons, and FOXP1 (increased expression and TF activity) in Glutamatergic neurons, 3 TFs known to be involved in the lineage state determination of their respective neuronal subtypes. Differentially accessible peaks were enriched for pathways associated with neuron lineage specification, axonogenesis, and synaptic signaling and neuronal TFs including FOXP2, REST, and KLF9. These results demonstrate that a history of FTY exposure can lead to transcriptomic and epigenetic modifications, resulting in midbrain cell state changes which, we speculate, play a role in increased risks for drug seeking.