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Induced pluripotent stem cells (iPSCs) derived cerebral organoids to model the impact of methamphetamine exposure on HIV latency.

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Background: Drugs of abuse such as methamphetamine (METH) aggravate the symptoms of HIV associated neurocognitive disorders (HAND). Our hypothesis is that exposure to drugs of abuse causes inflammatory responses leading to the emergence of HIV from latency and exacerbates HAND.

Methods: To introduce microglia into the organoids, we developed a method using iPSCs to obtain hematopoietic progenitors (iHSC) and cocultured the iHSCs with neural progenitors (NPC) to form 3D embryoid bodies. We used single cell RNA-sequencing (scRNA-seq) to comprehensively analyze the cell types within organoids and evaluated the differential gene expression and epigenetic changes after METH exposure.

Results: We observed highly ramified IBA-1+ microglial cells home uniformly to organoids (oMGs) as early as day 25 of iHSC:NPC cocultures. scRNA-seq revealed distinct cell types in organoids, such as oligodendrites, astrocytes, oMGs and other inhibitory and excitatory neurons. Key markers that distinguished oMGs from other cell types were, AIF1, P2RY13, CX3CR1, CD163, ITGAX, CD4. We also noticed a fraction of the oMGs entered latency in the organoids, as these cells could be reactivated using TNFa, as quantified by both HIV-1 transcripts and HIV-1 Tat expression. In organoids treated with METH, we observed increased expression of chemokine genes such as CCL3, CCL4L2, CCL4, CCL3L1, IL1B in microglia, compared to untreated organoids.

Conclusions: Our methods yield cerebral organoids with well dispersed microglia and allow to model their interactions with different cell types during HIV infection and METH exposures. We are currently evaluating the impact of METH on replication-competent and single-round viruses in the organoids.