THC Modifies Microglial Phenotype And Induces Synaptic Pruning In Frontal Cortex Of Adolescent Mice

Iness Charfi[1, 2], Derek N. Robertson[1], Florian Wünnemann[1, 4], Antônia Sâmia Fernandes do Nascimento[1], Sévérine Leclerc[1], Gregor U. Andelfinger[1, 4], Graziella Di Cristo[1, 3] and Graciela Piñeyro[1, 2]

[1]*CHU Ste-Justine Research Centre, 3175 Côte-Ste-Catherine, Montreal, QC, H3T 1C5, Canada,*
[2]*Department of Pharmacology, Université de Montréal, Montreal, QC H3T 1J4, Canada,*
[3]*Departments of Neuroscience, Université de Montréal, Montreal, QC H3T 1J4, Canada,*
[4]*Department of Pediatrics, Université de Montréal, Montreal, QC H3T 1J4, Canada,*
[5]*Department of Psychiatry and Addictology, Université de Montréal, Montreal, QC H3T 1J4, Canada*

Aim
Adolescence marks a critical period of neurodevelopmental changes, with the prefrontal cortex (PFC) playing a key role in cognitive and emotional maturation. Cannabis use during this stage has been linked to later cognitive deficits, yet the mechanisms whereby cannabinoids affect the adolescent cortex remain poorly understood. This study aims to elucidate cellular effects of cannabinoids in adolescent PFC.

Methods
THC was administered to adolescent male mice over a period of 5 days in an amount equivalent to 4.5 joints, each containing 14% THC. We employed unbiased single-cell transcriptomics to identify cellular targets of THC in PFC, and used immunocytochemistry to validate observations in frontal cortical cells and in primary cultures. Effects of THC on cellular respiration were assessed with a Seahorse®-analyzer mitostress test.

Results
The single-cell transcriptomic approach identified microglia as the cortical cell type most susceptible to treatment. In these cells, THC led to downregulation of genes associated with mitochondrial respiration and upregulation of genes linked to phagocytosis and synaptic pruning ($p<0.05$; FDR-corrected). Immunohistochemistry in microglial PFC confirmed 34% downregulation of respiratory complex I subunit NDUFB8 ($p=0.0006$). This effect, mediated via CB2 receptors, was also observed in microglial cultures, where THC-induced downregulation of complex I subunits was associated with 1.45-fold increase in mitochondrial proton leak. Cultured microglia exposed to THC also displayed 75% increase in VGLUT1 incorporation ($p<0.0001$), indicating heightened phagocytosis of synaptic material. THC-induced increase in phagocytic drive influenced synaptic remodeling in the adolescent PFC as evidenced by enhanced spine removal from Layer 3 pyramidal neurons. Indeed, in these cells, THC reduced apical and basal dendritic spines by 40% ($p=0.0143$) and 30% ($p= 0.0286$), respectively.

Conclusions
These findings support the idea that THC acts on microglia to modify respiration and intensify their phagocytic drive, thereby disrupting the neurodevelopmental remodeling of excitatory synapses in the adolescent PFC.