How Adolescent THC exposure may cause short-term and long-term changes in microglia.

Introduction

There is an immense increase of cannabis use in the adult population, climbing from 32.9 million in 2013 to 48.2 million in 2019 [1]. This has only gone up due to products such as vapes, which are heavily marketed to attract younger customers. 8% of eighth graders, 19% of 10th graders, and 22% of 12th graders reported vaping marijuana past year [2]. This is compounded by cannabis being legalized on the state level, with the possibility of it being legalized on the federal level as well. As cannabis is more normalized in society, there is an increase of interest in using components of cannabis as therapeutic treatments in all areas of medicine; such as pain management, nausea relief and appetite stimulation [3]. However, only recently has cannabis lost its original taboo status, which affects how much information we have of cannabis's global effects on the body. One particularly interesting area is how cannabis exposure affects different areas of brain development—more specifically, our brain's primary immune cell, microglia. During adolescence, microglia are a crucial part of neocortical maturation, and Δ9-tetrahydrocannabinol (THC) exposure during this critical time could possibly affect how microglia functions.

Adolescents are particularly vulnerable to the harmful effects of THC, as the human brain continues to develop until age of 25 [4]. Marijuana may have permanent negative effects, especially people who began using marijuana during youth or adolescence [5]. The National Academies of Science Engineering and Medicine reported a strong association between THC consumption and neuropsychiatric issues, including addiction, depression, temporary psychosis, schizophrenia, and impaired brain function [6]. Thus, there is an urgent need to examine the endocannabinoid signaling in order to fill in the gap from the studies about the effects of cannabis on brain development.

Background

THC binds with CB1 and CB2 cannabinoid receptors, two core components of the endocannabinoid system. This signaling complex helps regulate many aspects of the CNS, such as neuronal migration. This is due to how endocannabinoid signals play important regulatory roles in microglia, specialized macrophages of CNS parenchyma that are critically involved in the maintenance of adult brain homeostasis. Microglia are stubborn, long living cells with a diverse population within the human brain. Research has determined that disruptions in microglial homeostasis are a key role in neurodegenerative and psychiatric disorders. Additional literature also suggests that adolescent THC administration induces a persistent neuroinflammatory state that may disrupt the normal developmental process including synaptic pruning and maturation. We hypothesized that adolescent THC exposure may cause both short- and long-term changes in the microglia, which leads to a dysregulated microglial activation that contributes to the persistent effect of THC.

Objective

There are two main goals with this project. The first is to observe the persistent alterations in the endocannabinoid signaling in microglia caused by THC exposure in adolescents. The second is to define which receptor (Cb1 or Cb2) mediates the long term effects of adolescent THC exposure.

Approach

Project 1: Observe the persistent changes in microglia due to adolescent exposure of THC. First, mice will be administered with THC (5mg/kg) according to our protocol from PND 30 to PND 43. Then, assessment will be made at two different time points. To study the acute effect, we will sacrifice the mice and isolate the brain after 6 days from the last THC injection at PND 49, and purify microglia from the whole brain. To assess the persistent effect, we will isolate the brain after 27 days from the last THC injection at PND 70, and prepare microglia from the whole brain. Brain dissociation and isolation of neural cells will be achieved by an optimized method using the Adult Brain Dissociation kit (Miltenyi Biotec), which will be followed by flow cytometry cell sorting of the microglia. Once the microglia has been collected, we will perform the following analyses.

Aim1.1: Use RNA-seq to assess what parts of the transcriptome have been affected by the administration of THC. It is expected to see changes in pro-inflammatory genes.
Aim1.2: Confirm what changes we are seeing with proteomic analysis, such as western blots and/or ELIZA assays.

Aim1.3: Use immunohistochemistry to visualize phenotypic changes in microglia post exposure.

Project 2: Define the CBR (Cb1 or Cb2) that mediate the long term effects of adolescent THC *exposure.* In male and female mice starting using our THC protocol outlined in project 1, but this time with additions of either a Cb1R antagonist or Cb2R antagonist. This way we can determine the pathology more clearly, and possibly figure out a way to prevent harmful THC effects.

Aim2.1: Use RNA-seq to see how both antagonists have changed the overall effect of THC has on the transcriptome.
Aim2.2: To uncover possible mechanisms underlying persistent modifications in ECB signaling.

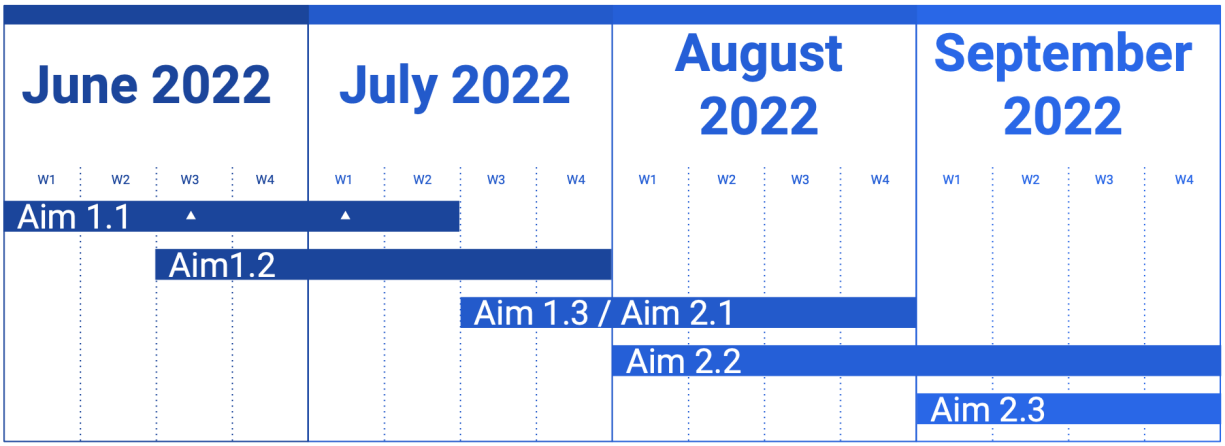
Aim2.3: To see if microglia will retain the persistent effect of THC with the two antagonists are used in tandem with THC treatment.

Student Responsibilities

Students will be responsible for preparing all treatments needed for the planned experiments. Students will be expected to weigh and inject the vehicle treatments for each experiment. Students will also assist with collection of the mouse brains for microglia cell sorting, assist with the brain dissociation of each mouse, and later extract RNA for molecular analyses. Students will

also be responsible for analyzing any RNA-seq and immunohistochemistry data from experiments.

Timeline



Budget

Item	Quantity	Catalog	Price
Adult Brain Dissociation kit	1 kit	Miltenyi Biotec 130-107-677	1,250.00
Isoflurane	25g	VWR TCC2486-25G	179.00
Syringes w/ 27G needle	1 box	Fisher Scientific 14-826-87	52.80
RNeasy Micro Kit	2 kits	Qiagen 74004	1,116
Animal Husbandry	N/A	N/A	~200.00
Mice from Jackson Laboratories	24 mice	/000664	776.64
Taqman Fast Advanced Master Mix	2 x 5mL	Applied Biosystems/44-449-63	992.00

Total: 4,566

References

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