Investigating the Effect of Δ9-tetrahydrocannabinol on Brain-Derived Neurotropic Factor Levels in Depressed Male Rats

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Abstract

Introduction: Despite the legalization of cannabis in Canada, researchers have yet to understand the extent of its side effects on neural development. This is especially true when considering the impact of cannabis on those with mental illnesses including depression. One vital protein involved in the growth and differentiation of neurons in the brain is the brain-derived neurotropic factor (BDNF). The aim of our proposed study is to evaluate how the neural health of depressed individuals is impacted by Δ9-tetrahydrocannabinol (THC) consumption, the primary psychoactive component of cannabis, in comparison to those without depression by comparing their respective BDNF levels.

Methods: To test this, we will use olfactory bulbectomised rats as models for depression and measure the BDNF concentration in their frontal cortices, hippocampi, and amygdalae following controlled exposure to THC for 21 days via intraperitoneal injections. BDNF will be detected via Western blot and quantified using BDNF specific ELISAs.

Conclusion: Our study could influence how society views the risks associated with the use of cannabis amongst individuals with depression. Given that BDNF baseline levels are lower in individuals with depression versus those without, the potentially significant effect of BDNF on overall neural development could possibly be impacted by recurrent THC dosages. Our study could impact the frequency of usage of THC amongst depressed individuals.

Keywords: amygdala; bdnf; bilateral olfactory bulbectomy surgery; cannabis; depression; hippocampus; THC

Introduction

On October 17, 2018, the Canadian government legalized the purchase and use of recreational cannabis by individuals 19 or older [1]. However, studies have shown that cannabis has an effect on the long-term memory and adolescent brain development of both rodents and humans [2-4]. Nevertheless, there have been insufficient studies investigating the effect cannabis has on the brain development of people with depression [5]. This is concerning since up to one-third of individuals aged 18-24 have reported using cannabis and they also have the highest prevalence of major depressive episodes [6]. Therefore, it is important we focus on understanding how cannabis affects the brain development and cognitive ability of those suffering from mental illnesses [6, 7].

When studying cannabis’ effect on the brain, researchers focus on its main psychoactive component, Δ9-tetrahydrocannabinol (THC). Segal-Gavish et al. showed that schizophrenic mice produced less brain-derived neurotrophic factor (BDNF) following THC administration compared to wild-type (WT) [8]. BDNF is a neurotrophic protein that acts on the central nervous system to support the development and maintenance of neurons and synapses [9]. BDNF is principally active in the basal forebrain and hippocampus, and is thought to be vital for long-term memory and maintaining normal neural development. Its overexpression partially counteracts the harmful effects of THC on the brain [8, 10]. We want to test how strongly depressed rats can counteract the negative effects of cannabis, compared to WT, by measuring BDNF levels in the hippocampus and amygdala of WT and depressed rats after THC administration. According to previous studies, depression in rats can be modelled by conducting olfactory bulbectomy, or a surgical removal of the olfactory bulbs [11, 12]. Hence, we shall use that model to induce depression. We hypothesize that male adolescent rats with induced depression will have a lower surge in BDNF levels upon THC exposure in comparison to WT rats, based on previous studies which seem to indicate less pronounced changes in BDNF levels in depressed rats when exposed to THC [11].

Methods

Before the experiment, a population of 100 healthy male adolescent rats, 42 to 51 days postnatal, will be kept at room temperature (22 ± 2°C) and given ample food and water for a week to allow them to acclimatize to the laboratory environment. Males were chosen because...
literature indicates pronounced neurological effects of cannabis use among male adolescents [13]. The rats will be weighed daily between 9:00 A.M. and 9:30 A.M., and only those 180g to 300g will be used for the study in accordance with ElBatsh et al. [11]. Half of the remaining rats will be selected to receive the bilateral olfactory bulbectomy (OB), identical to that conducted by ElBatsh et al., to induce depression [12]. The remaining half will be subjected to a sham surgery involving the same drilling procedure but without olfactory bulb removal. The rats will be given two weeks to recover, during which time they will be weighed daily between 9:00 A.M. and 9:30 A.M. [11]. This weighing process shall be repeated after THC administration begins to compare their growth.

The bulbectomised (OB) rats will be subjected to an open field test on a circular arena, as performed by ElBatsh et al., to determine their level of neophobia (fear of new or unfamiliar surroundings) after surgery [12]. The manifestation of neophobia will be an indicator of depression, and therefore, an indicator of a successful OB surgery [12]. Those not manifesting neophobia will be excluded from the study. The OB and WT rats will each be further divided into 2 groups: those receiving THC and those that are not. Those receiving THC will be injected intraperitoneally with a 2.0 mg/kg dose of THC every 48 hours, in accordance with Elbatsh et al. [11]. Intraperitoneal injections would permit us to control the amount of THC the rats are exposed to. The rats not receiving THC will receive a 1.0 mg/kg vehicle treatment (of cremophor, ethanol, and saline in a ratio of 1:1:18 respectively) intraperitoneally every 48 hours, serving as a negative control [11]. These THC and vehicle doses will be administered to the rats for a period of 21 days (Figure 1).

**Figure 1:** Overview of the experiment. Week 0 marks the start of THC and vehicle administration. After three weeks of treatment, BDNF level data collection begins.
Results
After the 21 days, BDNF levels of each rat exhibiting neophobia will be analyzed by extracting the hippocampi, frontal cortices, and the amygdalae. These will be dissected and stored at -80°C. The proteins in the hippocampus and amygdala will be evaluated via Western blot and a BDNF-specific ELISA kit to detect and quantify the presence of BDNF. For the primary antibody in the Western blot, anti-BDNF antibody in blocking solution will be used. We anticipate a surge in BDNF levels after the administration of THC. This surge would be greater in WT rats as opposed to the depressed ones, representing how effectively BDNF counteracts the negative effects of THC.

Discussion
The results will be used to compare between the WT-THC (non-depressed rats) and the OB-THC (bullectomized/depressed rats) groups. The controls are represented by the WT-Vehicle and the OB-Vehicle groups which help us control for the baseline BDNF content in the OB and WT groups without the influence of THC. Statistical significance can be evaluated via a two-way ANOVA and a Tukey’s post-hoc test, with significance set to p < 0.05. As with any study, there are a few limitations to consider. In our case, although BDNF is an extremely vital factor in promoting the healthy development of neurons and synapses, its concentration in the brain is not the sole measure of a cognitive function or long-term memory. For instance, TrkB receptors (to which BDNF binds) and thyroid hormone receptors are both very important in terms of promoting brain development in individuals suffering from mental illnesses like depression who often resort to marijuana consumption as part of recreational/therapeutic use. Following the legalisation of cannabis in Canada, this can help educate Canadians and marijuana consumers regarding the harms of THC on aspects like brain development and long-term memory, particularly on depressed adolescents. BDNF is an essential component of the brain development process. It manifests higher baseline levels when exposed to THC as a negative feedback response. These BDNF surges are expected to be lower in individuals with depression versus those without; thus, suggesting that constant THC exposure to depressed individuals increases the risk of cognitive deficiencies. Our study could impact the frequency of usage of THC amongst individuals with depression.

Conclusions
Our results may shed light on the detrimental effects of THC on neural development in individuals suffering from mental illnesses like depression who often resort to marijuana consumption as part of recreational/therapeutic use. Following the legalisation of cannabis in Canada, this can help educate Canadians and marijuana consumers regarding the harms of THC on aspects like brain development and long-term memory, particularly on depressed adolescents. BDNF is an essential component of the brain development process. It manifests higher baseline levels when exposed to THC as a negative feedback response. These BDNF surges are expected to be lower in individuals with depression versus those without; thus, suggesting that constant THC exposure to depressed individuals increases the risk of cognitive deficiencies. Our study could impact the frequency of usage of THC amongst individuals with depression.

List of Abbreviations
Brain-derived neurotrophic factor (BDNF): A growth factor of the neurotrophic family encoded by the BDNF gene that is found in the hippocampus, cortex, and basal forebrain.
Enzyme-Linked Immunosorbent Assay (ELISA): An assay method used for detecting and quantifying the presence of a ligand (hormone, protein, antibodies, etc.) using a ligand-specific antibody linked to an enzyme that generates a measurable product.
Olfactory Bullectomy (OB): A surgery involving the removal of the olfactory bulbs. Commonly used to induce depression in rat models.
A9-tetrahydrocannabinol (THC): A cannabinoid that is classified as the main psychoactive component of cannabis.

Conflicts of Interest
The author(s) declare that they have no conflict of interests.

Ethics Approval and/or Participant Consent
All article types: This proposal did not require ethics approval, as it was a protocol designed for a case competition.

Authors’ Contributions
SD: made contributions to the design of the study, drafted the manuscript, and gave final approval of the version to be published. HAS: contributed to study design and planning, drafted the manuscript, and gave final approval of the version to be published. JB: contributed to the design of the study, revised the manuscript critically, and gave final approval of the version to be published. SA: contributed to the design of the study, revised the manuscript critically, and gave final approval of the version to be published.

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