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Acute and long-term effects of Δ9-tetrahydrocannabinol on object recognition and anxiety-like activity are age- and strain-dependent in mice

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ABSTRACT

Use of exogenous cannabinoids disrupts the fine-tuned endocannabinoid receptor system, possibly leading to alterations in cognition, memory, and emotional processes that endure long after cannabinoid use has stopped. Long-term adolescent use may uniquely disrupt these behaviors when compared to adult use. The current study explored the acute and long-term behavioral effects of six 10 mg/kg Δ9-tetrahydrocannabinol (THC) injections across the adolescent or early adult period in male inbred C57Bl/6J and DBA/2J mice. The acute and prolonged effects of THC on object memory using the novel object recognition task, unconditioned anxiety in the elevated plus maze and open field, and sedative effects in the open field were examined. Acute THC treatment resulted in anxiogenic activity in both strains, but only caused sedation in B6 mice. Repeated THC treatment resulted in a protracted effect on object recognition, but not unconditioned anxiety, assessed 4 weeks later. In both strains, an adolescent history of THC treatment disrupted later object recognition. Interestingly, in B6 mice an adult history of THC exposure appeared to rescue a deficit in object recognition observed in vehicle-treated adults. Repeated THC administration also produced a protracted effected on CB1R protein expression. Animals treated with THC in adolescence maintained increased levels of CB1R protein expression compared to their adult THC-treated counterparts at five weeks following the last injection. These results indicate that THC use may have long-lasting effects with adolescence being a unique period of susceptibility.

1. Introduction

Cannabis is the most commonly used illicit drug in the United States across all age groups (National Institute on Drug Abuse, 2014). Exogenous cannabinoids found in cannabis, such as Δ9-tetrahydrocannabinol (THC),[1] as well as endogenous cannabinoids (endocannabinoids), bind to cannabinoid receptors (CBRs). These are inhibitory receptors located on many cell types including neurons, microglia, astrocytes, and endothelial cells in the central and peripheral nervous systems. In an unaltered state, endocannabinoids regulate "fine-tuned" synchronous neuronal outputs which maintain system function and contribute to long term potentiation and depression (Freund and Katona, 2007; Svíženská et al., 2008; Chevaleyre and Piskorowski, 2014). Conversely, administration of cannabinoids, such as following cannabis consumption, broadly disrupts this honed regulation. Repeated exposure to cannabinoids may result in alterations in cognition and memory, focus, mood shifts, and inflammatory and pain responses that persist even after prolonged abstinence. Due to the role the endocannabinoid system plays in neurodevelopment, whether adolescent exposure alters the trajectory of cannabinoid effects should also be studied (Freund and Katona, 2007; Svíženská et al., 2008; Volkow et al., 2016; National Academies of Sciences, 2017).

In a recent review of the literature surrounding both the beneficial and detrimental effects of cannabis use in humans, the National Academies of Sciences (2017) made several recommendations to further development of the cannabis research field. These include focusing on the developmental period of adolescence and the use of preclinical studies that examine both acute and chronic exposure to cannabinoids. The adolescent period in mice is conservatively accepted to range in age from postnatal day (PND) 28-42 (Schneider, 2013) wherein many behavioral and neurobiological changes occur in rodents that mimic those seen in humans, including the developmental influence of the cannabinoid system (Lee and Gorzalka, 2012). However, there are accepted caveats that some adolescent-like behaviors fall outside of this range, and there are gender-specific differences which may push developmental "milestones" closer to PND60, which is generally considered as adulthood (Spear, 2000; Casey et al., 2008). Nevertheless, adolescent rodent models are increasingly being utilized to explore the

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[1] C57Bl/6J (B6), cannabinoid receptors (CBR), DBA/2J (D2), elevated plus maze (EPM), novel object recognition (NOR), postnatal day (PND), Δ9-tetrahydrocannabinol (THC).

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neurodevelopmental effects of drug exposure (Casey et al., 2008; Schramm-Sapyta et al., 2009).

The goal of the current study was to characterize whether adolescent and adult mice demonstrate different behavioral consequences following an acute THC injection and a repeated history. THC's effects on memory in a novel object recognition (NOR) task, anxiety-like behavior on the elevated plus maze (EPM), and locomotor activity in the open field were selected as they mimic long-term changes that have been reported in human cannabis users (Freund and Katona, 2007; Svíženská et al., 2008; Volkow et al., 2016). These behaviors exemplify non-spatial memory retrieval, unconditioned anxiety, and sedation behavior, and are independent of motivation to obtain a reinforcer or reward, or to avoid punishment (Cohen and Stackman, 2015; Lee et al., 2015; Mohammad et al., 2016). Importantly, as these tasks require little to no training, they are optimal to run during the relatively short period of rodent adolescence.

THC's ability to alter NOR and EPM has been explored under both acute and repeated injection conditions. In the NOR task, acute THC did not alter object discrimination in adolescent or adult rats (Ciccocioppo et al., 2002; Swartzwelder et al., 2012). Conversely, a repeated adolescent history of THC exposure was shown to reduce novel object discrimination in rats (Quinn et al., 2008; Realini et al., 2011; Zamberletti et al., 2012; but see O'Tuathaigh et al., 2010). However, of these studies, only Quinn et al. (2008) utilized an adult control and found no effect of adult THC history on later object discrimination. This may indicate that there are important age-related differences in behavior following THC exposure.

The National Academies of Sciences (2017) also recommends recording feelings of anxiety and sedation in all clinical studies, as these are symptoms often associated with cannabinoid use. Preclinical studies have examined the effects of a 30 minute acute THC pretreatment on EPM activity. These studies have produced conflicting findings, with some showing anxiogenic effects (Celerier et al., 2006; Schramm-Sapyta et al., 2007) and others showing anxiolytic effects (Rubino et al., 2007; Braida et al., 2007; Fokos and Panagis, 2010) in both adolescents and adults. In part, this disagreement may be due to differences in strain/genotype sensitivity to THC and/or THC dose, with doses under 1.5 mg/kg generally being anxiolytic. A history of repeated injections in rodents has also produced mixed results. Onaivi et al. (1990) found no effect in mice, but an anxiogenic effect in rats, when THC was administered during adulthood. Conversely, Cadoni et al. (2008) and O'Tuathaigh et al. (2010) demonstrated anxiolytic effects of repeated adolescent administration on later adult behavior.

Although previous findings have been inconclusive on how THC affects behavior, an adolescent history of THC has reliably led to protracted deficits in object discrimination in the NOR task and anxiolytic behavior in the EPM. However, these studies have not consistently included the assessment of adult groups, which is necessary to conclude whether adolescents are differentially susceptible to the effects of THC. To observe how acute and repeated treatment with THC may differentially affect behavior when administered during adolescence or adulthood we used inbred C57Bl/6J (B6) and DBA/2J (D2) mice from Jackson Laboratories. These strains have been previously demonstrated to exhibit strain- and age-specific differences in NOR, EPM, and open field behaviors at the time of acute adolescent exposure used in the current study (Moore et al., 2011; Balsevich et al., 2014). Identification of strain-specific differences may help to identify genetic markers of THC-related susceptibility. Based on previous research, we hypothesized that acute treatment would be ineffective in altering object recognition in both ages and strains, but would be anxiogenic in the EPM and would elicit a sedative response in the open field. Following acute assessment, mice received repeated injections of THC or vehicle and were tested again following a period of no drug exposure to assess whether an adolescent history of THC resulted in different behavioral consequences than exposure occurring during adulthood. We hypothesized that a repeated history of adolescent injections would impair later adult object discrimination but be anxiolytic in the EPM compared to vehicle groups, whereas an adult history would have no effect on later behavior. Finally, CB1R binding increases across the brain during the transition from adolescence to adulthood (Verdurand et al., 2011), but repeated treatment may result in receptor downregulation (Breivogel et al., 1999). Therefore we hypothesized that adolescent treatment with THC would cause long-term changes in CB1R receptor expression compared to adult treated mice.

2. Method

2.1. Animals

Eighty B6 and D2 mice were purchased from Jackson Laboratories and arrived at age 3 weeks (20 per genotype) or 8 weeks (20 per genotype). Mice were singly housed upon arrival and maintained on a 12:12 light cycle in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Single-housing was chosen to avoid detrimental effects of subordinate/dominant hierarchies which could affect outcomes of the behavioral tasks in an uncontrolled manner (Blanchard et al., 2001; Singewald et al., 2009). Food and water was available at all times apart from during behavioral tests. Testing began at PND27 and PND68 for adolescent and adult mice, respectively. All procedures were approved by the IUPUI School of Science Institutional Animal Care and Use Committee and conformed to the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

2.2. Drugs

THC was obtained from the National Institutes of Health/National Institute on Drug Abuse (Bethesda, MD) at a concentration of 1 mg per 50 µl of 200 proof ethanol. For the work describe herein, the THC was then diluted to a concentration of 10 mg in vehicle comprised of 0.9% saline, Tween 80 (Sigma Aldrich, St. Louis, MO), and 200 proof ethanol (Pharmco, Inc., Brookefield, CT). Vehicle was similarly composed of 90% saline, 5% Tween 80, and 5% ethanol. Animals received 6 injections of THC or vehicle throughout the course of the study. THC or vehicle was delivered via intraperitoneal injection in a volume of 0.1 ml per 10 g of body weight. Although previous studies finding object memory deficits employed a twice/day injection paradigm (Realini et al., 2011; Zamberletti et al., 2012), even the heaviest adolescent users do not use cannabinoids on a daily basis (Scalco and Colder, 2016). Therefore, we chose to administer injections every 72 h. A 10 mg/kg injection was chosen for its ability to produce anxiogenic activity in the EPM following acute administration (Onaivi et al., 1990). Weights were recorded for every injection day and on the day of brain extraction. Table 1 outlines the general experimental procedure and mouse age (PND) at each test. All behavioral assays were run under red light conditions (approximately 8 lx) from PND27-29 or PND68-70.

Table 1
Indicates mouse age (PND) at each behavioral test, as well as test order throughout the course of the study. Ado = adolescent.

	NOR1 habituation	NOR1 training injection 1	NOR1 test	Injection 2 EPM1 OF1	Injections 3–6 (every 72 h)
Acute ado	27	28	29	32	35–44
Acute adult	68	69	70	73	76–85
	NOR2 habituation	NOR2 training	NOR2 test	EPM2 OF2	Brain extraction
Ado history	71	72	73	76	77
Adult history	117	118	119	122	123