Effects of Chronic Exposure to Low-Dose delta-9-Tetrahydrocannabinol in Adolescence and Adulthood on Serotonin/Norepinephrine Neurotransmission and Emotional Behavior

Danilo De Gregorio, Joshua Dean Conway, Martha-Lopez Canul, Luca Posa, Francis Rodriguez Bambico, Gabriella Gobbi

Neurobiological Psychiatry Unit, Department of Psychiatry, McGill University, Montreal, QC, Canada (Drs De Gregorio, Canul, Posa, and Gobbi); Department of Psychology, Memorial University of Newfoundland, St. John’s, NL, Canada (Mr Conway and Dr Bambico); Behavioral Neurobiology Laboratory, Center for Addiction and Mental Health, Toronto, ON, Canada (Dr Bambico).

Correspondence: Francis Rodriguez Bambico, PhD, Department of Psychology, Memorial University of Newfoundland, St. John’s, NL, Canada (fbambico@mun.ca); Gabriella Gobbi, MD, PhD, Department of Psychiatry, McGill University, Montreal, QC, Canada (gabriella.gobbi@mcgill.ca)

D.D.G. and J.D.C. contributed equally to the work.

ABSTRACT

Background: Chronic exposure to D9-tetrahydrocannabinol (THC), the main pharmacological component of cannabis, during adolescence has been shown to be associated with an increased risk of depression and suicidality in humans. Little is known about the impact of the long-term effects of chronic exposure to low doses of THC in adolescent compared with adult rodents.

Methods: THC (1 mg/kg i.p., once per day) or vehicle was administered for 20 days in both adolescent (post-natal day 30–50) and young adult rats (post-natal day 50–70). After a long washout period (20 days), behavioral tests and electrophysiological recordings of serotonin and norepinephrine neurons were carried out.

Results: Adolescent THC exposure resulted in depressive behaviors: decreased latency to first immobility in the forced swim test and increased anhedonia in the sucrose preference test. Decreased entries in the open arms were observed in the elevated plus maze after adolescent and adult exposure, indicating an anxious phenotype. A significant reduction in dorsal raphe serotonergic neural activity without a change in locus coeruleus noradrenergic neural activity was found after adolescent and adult exposure.

Conclusions: Altogether, these findings suggest that chronic low-dose THC exposure during the critical developmental period of adolescence and during adulthood could result in increased vulnerability of the serotonin system accompanied by anxiety symptoms. However, depressive phenotypes occur only after adolescent exposure but not after adult exposure, underscoring the greater vulnerability of young ages to the mental effects of cannabis.

Key Words: Cannabinoids, adolescence, depression, anxiety, serotonin
Cannabis is one of the most used psychotropic drugs among young people globally (UNODC, 2017). The main pharmacologically active ingredient in Cannabis sativa and Cannabis indica plants, which mediates most of its psychoactive and mood-related effects, is D9-tetrahydrocannabinol (THC). Cannabis is prevalently used by adolescents around the world: in the United States (Hasin et al., 2015), Canada (Statistics Canada, 2019), and some countries in Europe (EMCDDA, 2019), about 20% of adolescents have consumed cannabis at least once in the last year or last 3 months. However, in recent years, cannabis use has also been increasing in adults and the elderly (Statistics Canada, 2019), and the long-term consequences are still unknown.

The regular use of cannabis during adolescence is of profound concern as it is associated with an increased likelihood of deleterious consequences, such as diminished scholastic achievement, lower degree attainment and school abandonment, liability to addiction, earlier onset of psychosis, and neuropsychological decline (Volkow et al., 2014).

In a recent meta-analysis and systematic review, Gobbi and colleagues (Gobbi et al., 2019) found that cannabis consumption in adolescence is associated with an increased risk of depression and suicidality in young adulthood, even in individuals that did not have a pre-existent depression or vulnerability to depression before the recreational use. The risk of developing depression in young adulthood for adolescent cannabis users compared with non-users increased by 40%, and the risk of suicidal behavior increased by 50% (Gobbi et al., 2019). The neurobiological mechanism linking cannabis and depression has still not been completely clarified nor has the critical dose that generates mental health consequences. Importantly, a large epidemiological study (Sillins et al., 2014) indicated that even moderate exposure to cannabis in adolescence (i.e., weekly) can increase the risk of suicidality in young people.

Previous work in our laboratory has shown that the administration of the synthetic cannabinoid CB1 receptor agonist WIN55,212 (0.2–1 mg/kg per day) during adolescence produces a dose-response increase in anhedonia and anxiety in adulthood, paralleled by a decrease in the firing rate of serotonin (5-HT, the main neurotransmitter linked to depression) and an increase in norepinephrine (NE, the neurotransmitter linked to anxiety) (Bambico et al., 2010). Similarly, high doses of THC (2.5–10 mg/kg per day) in adolescence induce compulsive-like behavior in females, paralleled by low cAMP response element-binding protein activity in the hippocampal formation and prefrontal cortex, and high activity in the nucleus accumbens, coupled with increased dynorphin expression, while an impaired sensitivity to reward stimuli was observed in males (Rubino et al., 2008). Daily doses of THC (2.5–10 mg/kg daily i.p.) increase compulsive-like behaviors and anxiety as well as impaired attention, object-recognition memory, and short-term spatial memory (Quinn et al., 2008; Irimia et al., 2015; Murphy et al., 2017; Renard et al., 2017; Poulia et al., 2019). However, the effects of relatively low doses of THC in adolescence and adulthood have not yet been explored in animal models. Still, the long half-life of THC and its metabolites, its high brain penetration, and brain half-life (Huestis, 2005) allow us to hypothesize that it could have an impact in the brain even at low doses.

The present study thus aims to investigate the long-term impact of chronic adolescent and adult exposure to low doses of THC by examining the behavioral and electrophysiological consequences throughout adulthood. We therefore examined the effects of chronic adolescent and adult exposure to THC (1 mg/kg per day or the equivalent dose of half/1 smoked joint per day with 12.5% of THC in humans) on anxiety and depressive behavior in adult rats using different behavioral paradigms. We then employed in vivo electrophysiology to ascertain whether these behavioral effects are associated with changes in 5-HT and NE neural activity in the dorsal raphe (DR) and locus coeruleus (LC), respectively. Both these systems are implicated in mood-related and emotional processes as well as in the neurobiological action of antidepressant and anxiolytic drugs (Kahn et al., 1988; Duman et al., 1997; Tanaka et al., 2000).

**MATERIALS AND METHODS**

**Animals**

Twelve pregnant female Sprague-Dawley rats (Charles River, Saint Constant, Quebec, Canada) were used throughout the experiments and housed individually in standard polycarbonate cages. The animals were kept in a well-ventilated and temperature-controlled facility with a constant temperature of 20°C ± 2°C, 50%-60% relative humidity, and a 12-hour-light/-dark cycle (lights on at 7:30 AM). All experiments took place under standard room lighting (350 lux). Stress-free conditions were maintained throughout gestation to minimize the potential for confounding effects on future behavioral tests. At about 3 weeks after parturition, male offspring (post-natal day [PND] 20) were weaned and the litters culled (4–7 pups per cage). The weaned pups were housed 3 animals per cage and were handled by the experimenters every day for 1 week before the beginning of daily drug administration to ensure that they were well habituated to testing conditions. A second group of animals was...
subjected to the same experimental procedures in early adulthood (PND 50–53) at the beginning of the drug treatment. These 2 periods were chosen to minimize, as much as possible, the difference in weight and white adipose tissue that could modify the pharmacokinetics of the highly lipophilic THC (Huestis et al., 1992; Nahas et al., 2016; Torrens et al., 2020). All experimental procedures were approved by local institutional care and use committees and in compliance with the guidelines sanctioned by the Canadian Institutes of Health Research.

Drugs

THC was purchased from Sigma-Aldrich Canada Ltd. THC was dissolved in 5% Tween 80, 5% polyethylene glycol, and 90% saline (0.9% NaCl solution) to a final concentration of 3 mg/mL, as performed in our previous study (Bambico et al., 2012). The same composition was used for the vehicle. Even if there are no comparative studies on THC pharmacokinetics in rats vs humans, studies evaluating the concentration of THC and its metabolites after i.p. injection of THC (1 mg/kg) in rats (Klein et al., 2011) show that the quantity of THC in the plasma after 30 minutes is comparable with the plasma level of THC after a smoked joint of 750 mg of cannabis sativa/indica at 12.5% of THC (Matheson et al., 2020). In both cases, the average plasma THC level is between 20 and 40 ng/mL. Importantly, after daily chronic use of THC in rats, the amount of plasma THC remains stable (Klein et al., 2011). Consequently, 1 mg/kg per day i.p. in a rat corresponds grossly to 1 joint per day with a THC concentration of 12.5%, smoked for about 10 minutes.

Moreover, the dose of 1.0 mg/kg was used because previous electrophysiological and behavioral work has shown it to enhance both 5-HT and antidepressant activity (Bambico et al., 2012).

THC Exposure

Prior to drug exposure, all rats received 1 injection of saline for 3 days. Thirty-gauge needles were used in all injections. This allowed the animals to get used to the procedure, thereby reducing possible drug injection-stress interactions. Upon reaching the onset of adolescence, typically defined as PND 28–30 (Spear, 2000), the adolescent exposure group received a single i.p. injection of either Δ9-THC (1.0 mg/kg) or the vehicle for 20 consecutive days. The adult exposure group was subjected to an identical treatment schedule. The final day of drug treatment was followed by a 20-day drug washout period. The length of the washout duration was selected to assess the long-term effects of drug exposures and to exclude the confounding effects of drug withdrawal. A schematic time-line concerning the exposure period and experimental procedures is represented in Figure 1.

Behavioral Testing

All rats were subjected to a serial testing procedure that has been validated and compared with single testing procedures in our laboratory. The elevated plus maze test (EPMT), open field test (OFT), and novelty-suppressed feeding test (NSFT) were employed to assess anxiety reactivity. The forced swim test (FST) and sucrose preference test (SPT) were used to assess depressive behavior. The experimental groups were divided as follows: rats exposed to the adolescent and adult administration of vehicle (veh) or THC were both divided into different experimental subgroups (n = 7–10 rats per group): a first cohort of 4 groups of rats (treated with veh or THC, during adulthood or adolescence) underwent the OFT, EPMT, and FST on the same day. After an interval of 1–2 days, another cohort of rats of 4 groups (treated with veh or THC, during adulthood or adolescence) underwent the SPT and the NSFT immediately afterwards. In addition, a separate cohort of rats subjected to the same regimen was used

Figure 1. Schematic representation of the design and treatment schedule for each experiment: adolescent drug exposure group (top) and adult drug exposure group (bottom). Numbers on upper and lower margins represent the age of animals. PND, post-natal day.
for the electrophysiological recordings. On the day of testing, the rats were habituated for about 60 minutes in the behavioral room prior to beginning the procedures. The apparatus was cleaned with 70% alcohol after each run. Each batch of rats completed the battery of tests in 3 weeks. The behavioral data were recorded and analyzed offline using an automated behavioral tracking system (Videotrack; ViewPoint Life Sciences, Inc., Canada) equipped with infrared-sensitive cameras or by a rater blind to the experimental manipulations. For details, please refer to the supplemental section.

In Vivo Electrophysiological Recordings of Monoaminergic Neurons

In vivo electrophysiological recordings were conducted following our standardized protocols (Gobbi and Blier, 2005; Bambico et al., 2007, 2009). For details, please see the supplemental section.

Data Analyses and Statistics

All data were analyzed using SPSS version 17 (SPSS Inc., Chicago, IL), Sigma Plot version 12, and GraphPad Prism version 8.2.1 and are presented as mean ± SEM. After testing for assumptions of normality of data distribution and of homogeneity of variance, behavioral and electrophysiology data were accordingly submitted to 2-way ANOVA with treatment (veh vs THC) and exposure period (adolescent vs adult) as factors. Tukey’s honestly significant difference test was used for multiple post hoc comparisons. A probability value of P < .05 was considered to be statistically significant.

RESULTS

Neither Adolescent nor Adult THC Exposure Changes Anxiety-Related Behavior in the NSFT

No anxiety behavior in the NSFT, a paradigm used to assess the hypophagia induced by a novel and stressful environment, was detected. Indeed, we found that the latency to feed was unchanged in all groups of animals both when the rodents were exploring the bright arena and in the home cage. Indeed, chronic D+THC (1.0 mg/kg; i.p.) did not modify the latency to feed in the novel environment (treatment, F(1, 27) = 0.3857, P = .5398; exposure period, F(1, 27) = 32.68, P < .0001; interaction treatment × exposure period, F(1, 27) = 1.350, P = .2554; Figure 2A) and in the familiar environment (treatment, F(1, 27) = 1.872, P = .1825; exposure period, F(1, 27) = 1.743, P = .1978, without significant interaction treatment × exposure period, F(1, 27) = 0.05998, P = .8084; Figure 2B).

Adolescent and Adult THC Exposure Reduces Time Spent and Number of Entries in the Open Arms

In the EPMT, both groups exposed during adolescence and adulthood to THC displayed anxiety. Indeed, these animals spent less time in the anxiogenic open arms of the EPM apparatus, coupled with a decreased number of entries. Indeed, 2-way ANOVA detected a significant effect of exposure period and treatment on percent time spent in the open arms (treatment, F(1, 27) = 66.83, P < .001; exposure period F(1, 27) = 40.09, P < .0001; without interaction treatment × exposure period, F(1, 27) = 1.396, P = .2477; Figure 2C) and in the closed arms (treatment, F(1, 27) = 66.83, P < .001; exposure period, F(1, 27) = 43.14, P < .001; without significant interaction, F(1, 27) = 0.6683, P = .4208; Figure 2D). No effect was found on the percentage of time spent in the central zone (treatment, F(1, 27) = 0.1127, P = .7397; exposure period, F(1, 27) = 0.3061, P = .5846; interaction treatment × exposure period, F(1, 27) = 1.211 P = .2809; Figure 2E) of the maze. In addition, a significant difference was found in the percentage of entries to the maze compartments (treatment, F(1, 27) = 55.81, P < .001; exposure period, F(1, 27) = 72.82, P < .001; with significant interaction treatment × exposure period, F(1, 27) = 9.88, P = .004; Figure 2F). Importantly, Tukey’s post hoc comparisons found that rats treated with THC displayed fewer entries to the open arms after adolescent (P < .001) or adult (P < .05) exposure. On the other hand, 2-way ANOVA revealed a significant difference in the measurement of nose dips (treatment, F(1, 27) = 14.44, P = .0007; exposure period, F(1, 27) = 4.297, P = .0479; interaction treatment × exposure period, F(1, 27) = 4.297, P = .0479; Figure 2G). In particular, Tukey’s post hoc comparisons revealed that rats treated with THC during adolescence displayed decreased nose dip duration compared with veh (P < .001).

Adolescent, But Not Adult, THC Exposure Leads to Despair Behavior in the FST

To rule out influences of non-specific motor activation, locomotor activity in the OFT was also analyzed on the same day as the FST. Importantly, no difference in the locomotion in all the groups of animals was found. Indeed, on the day of the FST, 2-way ANOVA detected no difference between veh and THC treatment during both adolescent and adult exposure on distance travelled in the OFT (treatment, F(1, 27) = 0.7578, P = .3907, exposure period, F(1, 27) = 1.047, P = .3141, without interaction treatment × exposure period F(1, 27) = 1.332, P = .2573; Figure 3A). In addition, when we analyzed the number of entries in the central part of the arena (Figure 3B), we found no statistical effect of treatment (F(1, 27) = 0.4915, P = .4885), no significant effect of exposure period (F(1, 27) = 2.267, P = .1423), and no significant interaction (F(1, 27) = 0.2220, P = .6408). Next, the FST, a behavioral paradigm used to assess despair, which is a symptom of depression in humans, was executed. Two-way ANOVA revealed that compared with veh, chronic THC (1.0 mg/kg; i.p.) induced no significant change after either adolescent or adult exposure in the time spent immobile (treatment factor, F(1, 27) = 3.258, P = .0818; exposure period factor, F(1, 27) = 0.01071, P = .9183; no interaction, F(1, 27) = 1.692, P = .2039; Figure 3C), in the time spent swimming (treatment factor, F(1, 27) = 2.512, P = .1242; exposure period factor, F(1, 27) = 0.4307, P = .517; no interaction, F(1, 27) = 3.185, P = .0852; Figure 3D) and in the time spent climbing (treatment factor, F(1, 27) = 3.060, P = .0912; exposure period factor, F(1, 27) = 6.527, P = .0163; no interaction, F(1, 27) = 0.5538, P = .4630; Figure 3E). By contrast, 2-way ANOVA detected a significant difference in the latency to immobility that is considered a sign of despair behavior (treatment factor, F(1, 27) = 38.26, P < .001; exposure period factor, F(1, 27) = 155.1, P < .001; interaction, F(1, 27) = 23.66, P < .001; Figure 3F). Noteworthy, Tukey’s post hoc comparison found a decreased latency to the first episode of immobility in rats treated with THC during adolescence (P < .001, compared with veh).

Adolescent, But Not Adult, THC Exposure Leads to Anhedonic Behavior

Depressive behavior was further analyzed in the animals exposed to THC during adolescence. The SPT was performed. The SPT is a paradigm employed to assess the consumption of sucrose from which anhedonia, another core symptom of depression (Comai and Gobbi, 2016), can be inferred. Animals treated
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with THC during adolescence but not during adulthood showed less preference in drinking a pleasant sucrose solution compared with animals treated with veh. More specifically, significant decreases in absolute sucrose intake (effect of treatment, \( F_{(1, 27)} = 35.26, P < .0001; \) exposure period, \( F_{(1, 27)} = 40.24, P < .0001; \) interaction treatment × exposure period, \( F_{(1, 27)} = 6.203, P = .0192; \) Figure 3G), sucrose preference (effect of treatment, \( F_{(1, 27)} = 61.64, P < .0001; \) exposure period, \( F_{(1, 27)} = 3.348, P < .0783; \) interaction treatment × exposure period, \( F_{(1, 27)} = 57.05, P < .001; \) Figure 3H) and relative sucrose intake (effect of treatment, \( F_{(1, 27)} = 36.34, P < .0001; \) exposure period, \( F_{(1, 27)} = 11.22, P = .0024; \) supplementary Figure 1) were found. On the contrary, no significant changes were observed in animals exposed during adulthood. Indeed, Tukey’s post-hoc comparison detected that only rats exposed to THC during adolescence, compared with rats treated with veh, displayed reduced absolute sucrose intake (\( P < .001; \) Figure 3G), diminished relative sucrose intake (\( P < .001; \) Figure 3H), and decreased sucrose preference (\( P < .001; \) Figure 3I).

### Adolescent and Adult THC Exposure Decreases Serotonergic But Not Noradrenergic Firing Activity

Next, we investigated if the adolescent exposure to THC leads to a change in the neuronal transmission of 5HT in the DRN and NE neurotransmissions in the LC, 2 brain regions strongly involved in the development of both depression and anxiety (Greenwood et al., 2003; McCall et al., 2017). Comparisons of the...
mean spontaneous 5-HT neuronal firing rate showed profound decrement induced by chronic THC after both adolescent and adult exposure to a similar extent. Indeed, 2-way ANOVA revealed a main effect of treatment (F(1, 77) = 14.62, P = .0003) without any effect of exposure period (F (1, 77) = 0.5167, P = .4744) and with no significant treatment × exposure period interaction (F(1, 77) = 0.5206, P = .4728) (Figure 5B-C). These effects were coupled with an increased burst density detected in both adolescent and adult exposure (main effect of treatment, F (1, 29)  = 11.72, P = .0019; no effect of exposure period, F (1, 29) = 0.04148, P = .8400;
interaction, $F_{(1, 29)} = 0.2705, P = .6070$) (Figure 5D). In addition, THC produced an increased coefficient of variation percentage in both adolescent and adult exposure (main effect of treatment, $F_{(1, 77)} = 21.86, P < .0001$; no effect of exposure period $F_{(1, 77)} = 0.7136, P = .4009$; interaction, $F_{(1, 77)} = 0.3748, P = .50422$) (Figure 5E). Indeed, interspike interval (ISI) histograms generated from the mean 5-HT neural activity showed a skewed ISI profile of rats treated with THC compared with the normally distributed ISI profile of rats treated with veh in both adolescent (Figure 4F) and adult exposure (Figure 4G). These data suggest that THC administration triggers an irregular firing activity as opposed to the regular or rhythmic firing activity in rats treated with veh. Comparisons in the NE neuronal activity conversely did not show a significant change induced by chronic THC after adolescent or adult exposures in either the basal firing rate (treatment, $F_{(1, 43)} = 1.550, P = .2191$; exposure period, $F_{(1, 43)} = 0.7941, P = .3772$; interaction, $F_{(1, 43)} = 0.2725, P = .6040$; Figure 5B–C) or burst density (treatment, $F_{(1, 44)} = 2.464, P = .1237$; exposure period, $F_{(1, 44)} = 2.733, P = .1054$; interaction, $F_{(1, 44)} = 0.6127, P = .4380$; Figure 5D). However, we found irregular NE neuronal activity in rats treated with THC after both adolescent and adult exposure, similar to the 5-HT firing activity. Indeed, an increased coefficient of variation (main effect of treatment, $F_{(1, 49)} = 32.04, P < .0001$; exposure period, $F_{(1, 49)} = 1.166, P = .2854$; interaction, $F_{(1, 49)} = 1.787, P = .1874$; Figure 5E) and a skewed ISI histogram profile of rats treated with THC, compared with veh during both adolescence (Figure 5F) and adulthood (Figure 5G), was found.

**Discussion**

The main finding of the present study is that chronic adolescent exposure to low-dose THC leads to persistent behavioral abnormalities related to some but not all aspects of depressive reactivity. In particular, THC induces a decrease in sucrose consumption and a decreased latency to the first immobility in the FST. THC also induces some anxious behavior in the EPMT and a decrease in nose dips (exploratory behavior). In addition, we found altered 5-HT firing rate activity in rats treated with THC after adolescent exposure, but there was no change in NE firing activity, thus suggesting that the THC effects are quite specific for areas implicated in mood regulation. Importantly, these depressive phenotypes were not observed in adult exposure, suggesting that the emotional liabilities during adolescence were linked to an age-dependent vulnerability of the developing serotonergic system.

However, we found that serotonergic activity was also decreased in rats exposed during adulthood, even if it was not paralleled by significant changes in depressive/anxiety behaviors, except for an anxious phenotype in some parameters of the EPMT. Depression is a complex disease and several core symptoms, such as feelings of hopelessness and low self-esteem are hardly reproduced in animals (Matthews et al., 2005). The FST is mostly used to assess one of the main "symptoms" of depression, which is "behavioral despair," characterized by an increased immobility time or change in the latency to the first immobility episode; it mostly has been used to screen the effect of antidepressant drugs and not a depression model per se (Castagné et al., 2010). However, several studies have shown that depressogenic manipulations (e.g., chronic unpredictable stress, genetic deletion) may increase the immobility time in the FST, thus suggesting that the test can measure a depressive phenotype (Garza et al., 2012; Hao et al., 2019). Nevertheless, one cannot rule out the possibility that the immobility duration or the decreased latency of immobility may represent a resilient behavior or coping strategy, as previously reported (Molendijk and de Kloet, 2015, 2019; Commons et al., 2017). Following these recent...
interpretations, the decreased latency to swim observed after adolescent THC exposure can be interpreted as a coping strategy or resilience, even if further studies are required to better characterize the behavioral effects of THC on desirous-like behaviors.

Anhedonia is another core symptom of depression, which is characterized by the inability to experience pleasure from rewarding or enjoyable activities (Der-Avakian and Markou, 2012). We used the SPT, which does not directly measure anhedonia but rather the reduction in the preference for sucrose (a pleasant and rewarding stimulus) over water (neutral stimuli) (Liu et al., 2018). Compared with exposure during adulthood, THC exposure during adolescence decreased the consumption of sucrose, which is interpreted as increased anhedonic behavior. Since SPT experiments were performed in animals after food deprivation for 24 hours and considering the involvement of the cannabinoid system in appetite and food intake (Berry and Mechoulam, 2002), this behavioral condition might have masked the anhedonia outcome in the SPT. Nevertheless, NSFT revealed no changes in the latency to feed in the home cage between animals treated with vehicle or THC during adolescence or adulthood, suggesting that food deprivation had minimal impact on the anhedonia behavior. Adolescence is marked by typical neurobehavioral characteristics that include distinct cognitive and social behavior (e.g., increased play and interaction), emotional volatility (e.g., anxiety and self-consciousness), disproportionately extensive recklessness, novelty- or sensation-seeking, and risk-taking behaviors (Buchanan and Holmbeck, 1998). Mounting evidence also suggests that the adolescent nervous system undergoes continuous structural modification in gray and white matter as well as progressive and regressive refinements in network connectivity, migration and pattern formation, differentiation, and cytogenesis (Paus, 2005). Age-dependent differences in the transmission of key neuromodulators and transmitters involved in emotional processing, most notably monoamines and endocannabinoids, have also been reviewed (Crews et al., 2007). These differences include low levels of 5-HT activity (Depue and Spoont, 1986) and 5-HT$_{1A}$ receptor binding (Dillon et al., 1991) in adolescence and high levels of 5-HT$_{1A}$ expression (Morilak and Ciaranello, 1993). The constitution of cannabinoid receptors, particularly the CB1Rs, mature slowly during the post-natal period, and receptor binding in the hippocampus reaches higher levels in adolescence than in adults (de Fonseca Rodriguez et al., 1993). Importantly, CB1Rs play a critical role in neuronal development due to their ability to modulate the release of neurotransmitters and expression, establishment of synaptic connection (Kano et al., 2009), and epigenetic regulation (Prini et al., 2018). The pharmacological exogenous manipulation of the endocannabinoid system by THC led to changes in neuronal development and homeostasis, pointing out the role of the endogenous cannabinoid system in neurodevelopment (Viveros et al., 2012).

Several animal studies have highlighted that adolescent cannabinoid exposure persistently impairs cognition, social behavior, motivation and anxiety, and produces long-lasting cross-tolerance to psychostimulants and other cannabinoid agonists (Pistis et al., 2004). Our previous results using the potent CB$_R$ full agonist WIN55,212-2, found instead a depressive behavior and anxious behaviors after adolescent exposure, but not after adult exposure. This difference could account for the fact that THC is a partial agonist at CB$_R$ with a longer half-life compared with WIN55,212-2, gating stronger adverse anxiety-related effects in adults (Bambico et al., 2010). Our findings are in agreement with Silva and collaborators (Silva et al., 2016) showing that chronic THC treatment (3 mg/kg per day) during adolescence...
produced anxiolytic and antidepressant behavior as measured by the EPMT and FST. In contrast, Rubino and colleagues observed that chronic administration of THC (2.5–10 mg/kg per day during adolescence) increased anhedonia but not despair in males (Rubino et al., 2008) without anxiety in both sexes. More recently, it was reported that THC in adolescent male rodents produced impaired Pavlovian reward-predictive cue behaviors, paralleled by a loss of CB1-R-expressing vGlut-1 synaptic terminals in the ventral tegmental area in males during adulthood (Kruse et al., 2019).

Our behavioral data were coupled with a dysfunction in serotonergic neuronal activity in the DR and noradrenergic activity in the LC, both implicated in depression and anxiety. In particular, we found a parallel between a significantly decreased 5-HT activity and a depressive phenotype after THC adolescent exposure, as also reported in our meta-analysis on humans (Gobbi et al., 2019), pointing out a preferential role of cannabis in the dysregulation of mood. The significant alteration induced by THC on 5-HT firing activity may be due to the activation of CB1 receptors expressed on GABAergic neurons of the DR (Häring et al., 2007) or via an increased activity of GABAergic neurons in the hippocampus (Bambico et al., 2007; Geddes et al., 2016) that regulate the synaptic excitatory/inhibitory balance governing the excitability of 5-HT neurons. The attenuation of 5-HT neurotransmission could also occur via a dopaminergic hyperactivity in the prefrontal cortex (Renard et al., 2017) or indirectly through a decreased activation of dopaminergic neurons in the ventral tegmental area (Diana et al., 1999; Pistis et al., 2004) or via an indirect opioid receptor activation (Ellgren et al., 2007).

Recently, it was observed that increasing doses of THC in adolescence (0.3–3 mg/kg twice per day from PND 35 to 45) 5-HIAV/5-HT rate in the hippocampus (Poulia et al., 2019), which could be interpreted as a compensation for the low 5-HT rate. More studies are needed to establish this direct correlation between cannabinoid receptors and 5-HT firing activity.

Interestingly, when THC was administered during adulthood, we found a decreased 5-HT firing activity that was not associated with any reduction of sucrose consumption or with a decreased latency to the first FST immobility, but only with an increased time and exploration of the open arms in the EPMT. This low 5-HT could be a neurobiological hallmark of a particular susceptibility to stress or trauma, as observed by (Lee et al., 2018), who found that people consuming cannabis have an elevated risk of developing post-traumatic stress disorder after exposure to trauma. For instance, several studies have demonstrated that animal models of chronic stress are correlated with a decrease in the firing activity of monoamine neurons, including 5-HT (Pavcovich et al., 1990; Bambico et al., 2009). Nevertheless, further studies must be conducted to determine the link between cannabis exposure, vulnerability to mental health problems in adulthood, and the 5-HT system.

While in previous investigations with WIN55,212-2 we found that an increase in spontaneous LC NE activity was associated with enhanced anxiety response (Bambico et al., 2010), here this dose of THC also did not have the capacity to modify NE firing, even if the coefficient of variation in NE neurons was changed in both adolescent and adult exposure; this indicates a drastic change in firing pattern regularity also in NE neurons.

We cannot rule out that this NE activity pattern disturbance might have conveyed some degree of vulnerability indicated by the observed changes in EPMT behaviors after both adolescent and adulthood exposure.

Pertaining to the generally inert effect of chronic THC exposure during adulthood, it is worth mentioning that we have published that an identical dose given sub-chronically (4 days) during adulthood (PND 70) instead yielded potent antidepressant activity when assayed immediately after the treatment (Bambico et al., 2012). In the current study, the FST was done after a long period of washout (20 days). This suggests that adult exposure to cannabis does not produce any significant long-term effects on mood, thus indicating that the period from adolescence to young adulthood is critical for developing depression after THC consumption. Remarkably, this age difference on mood by THC was also reported in humans (Schoeler et al., 2018), indicating that only after 27 years of age could chronic cannabis use produce positive effects on mood, while before 27 years of age, cannabis is associated with depression.

In summary, both chronic adolescent and adult exposure to a low dose of THC impairs monoaminergic transmission, with mild anxiogenic effects, but a more profound anhedonia is produced in rats treated during adolescence. A limitation of our study is the lack of data on female rodents, and further studies should be aimed at establishing whether these neurobiological THC-induced differences are also detectable in females or if other dysfunctions are present, as already observed by (Rubino et al., 2008; Poulia et al., 2019). This study in rodents shows that the daily administration of THC alters emotional behavior and impacts monoaminergic neurotransmission. More preclinical and clinical investigations are needed to better understand the impact of the daily and weekly doses of cannabinoids on the brain and their potential risk of increasing suicidality and depression (Silins et al., 2014).

Supplementary Materials

Supplementary data are available at International Journal of Neuropsychopharmacology (IJNPPY) online.

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