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REGULAR RESEARCH ARTICLE

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

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ABSTRACT

Background: Chronic exposure to D^9-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

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Key Words: Cannabis, adolescents, depression

Significance Statement

Several epidemiological studies have shown that chronic exposure to THC during adolescence is linked to depression and suicidality, especially at higher doses. It is still unknown if low doses of THC during adolescence and adulthood may influence emotional behavior and the neurotransmission of monoamines. Here, we have shown that 1 mg/kg per day of THC in adolescence increased anhedonia and anxiety-related behavior paralleled by a decrease in serotonin activity, which is the main neurotransmitter implicated in depression. Similarly, adults exposed to THC presented decreased serotonin activity, suggesting that THC may induce vulnerability in the monoaminergic system, even if the behavioral effects are more pronounced in adolescents.

Introduction

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 D^9-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 (Silins 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 depressive 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 penetrance, 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 D^9-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

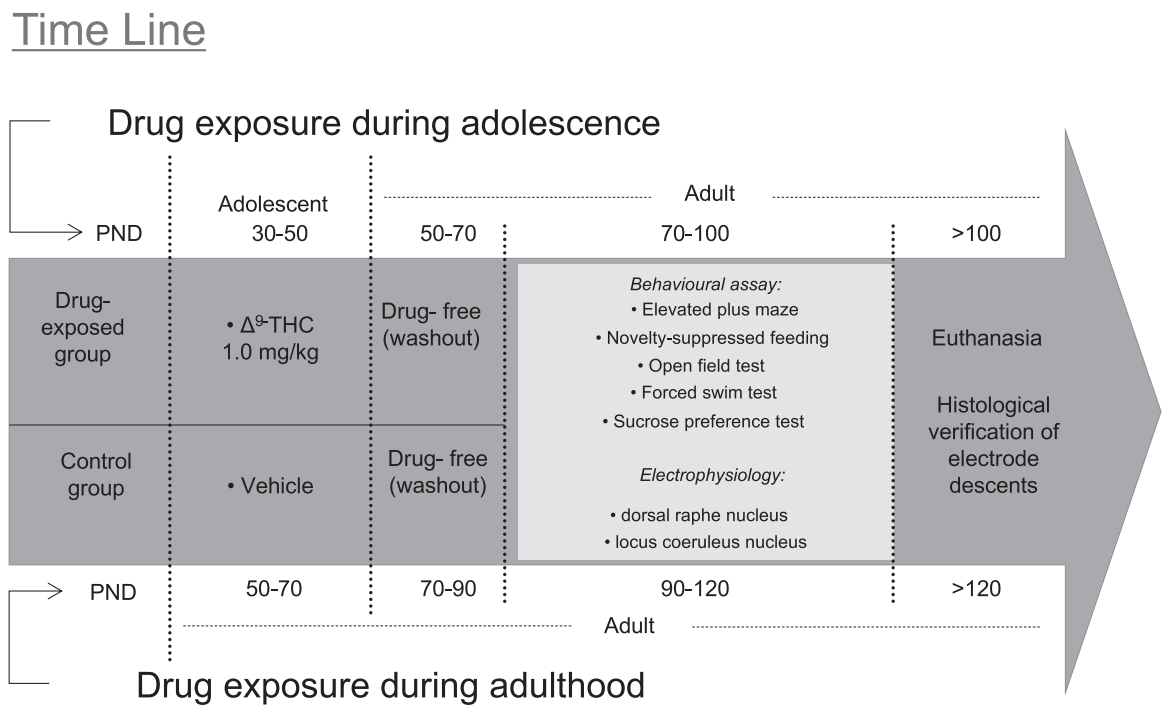

Figure 1. Schematic representation of the design and treatment schedule for each experiment: adolescent drug exposure group (top) and adult drug exposure group (bottom). Numbers on upper and lower margins represent the age of animals. PND, post-natal day.

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, View Point 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^9-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, $F_{(1, 31)}=0.7578$, $P=.3907$, exposure period, $F_{(1, 31)}=1.047$, $P=.3141$, without interaction treatment × exposure period $F_{(1, 31)}=1.332$, $P=.2573$; 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 ($F_{(1, 31)}=0.4915$, $P=.4885$), no significant effect of exposure period ($F_{(1, 31)}=2.267$, $P=.1423$), and no significant interaction ($F_{(1, 31)}=0.2220$, $P=.6408$). 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, $F_{(1, 28)}=3.258$, $P=.0818$; exposure period factor, $F_{(1, 28)}=0.01071$, $P=.9183$; no interaction, $F_{(1, 28)}=1.692$, $P=.2039$; Figure 3C), in the time spent swimming (treatment factor, $F_{(1, 28)}=2.512$, $P=.1242$; exposure period factor, $F_{(1, 28)}=0.4307$, $P=.517$; no interaction, $F_{(1, 28)}=3.185$, $P=.0852$; Figure 3D) and in the time spent climbing (treatment factor, $F_{(1, 28)}=3.060$, $P=.0912$; exposure period factor, $F_{(1, 28)}=6.527$, $P=.0163$; no interaction, $F_{(1, 28)}=0.5538$, $P=.4630$; 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, $F_{(1, 28)}=38.26$, $P<.001$; exposure period factor, $F_{(1, 28)}=155.1$, $P<.001$; interaction, $F_{(1, 28)}=23.66$, $P<.001$, 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

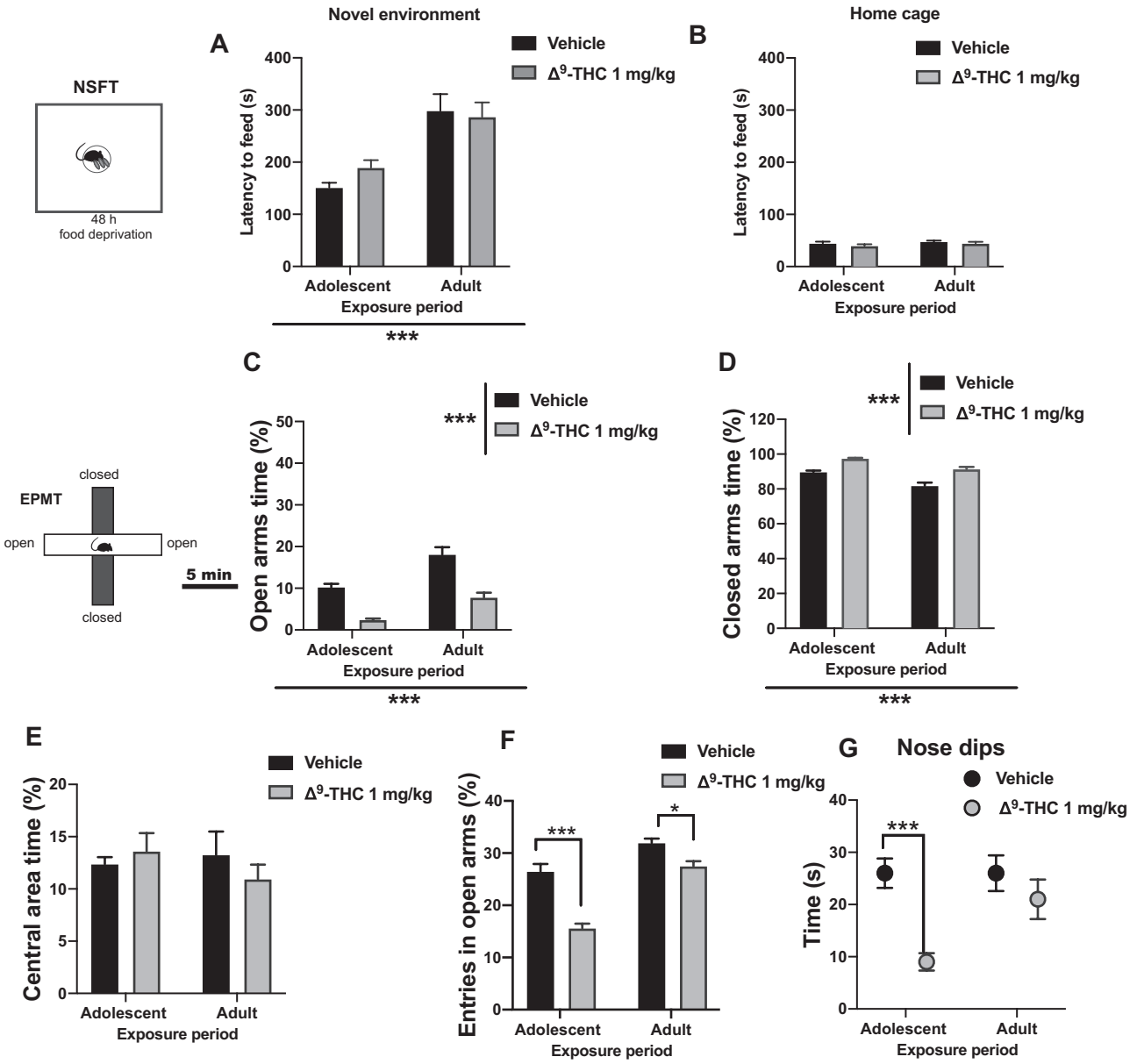

Figure 2. Anxiety behavior in the novelty-induced suppression of feeding test (NSFT) and the elevated plus maze test (EPMT) following chronic administration of D^9-tetrahydrocannabinol (THC; 1.0 mg/kg, i.p.) during adolescence or adulthood. No differences between vehicle and THC was detected after both adolescent and adult exposure in the latency to feed in the novel (A) or familiar (B) environment. Neither adolescent nor adult THC exposure altered the percent time spent in the open arms (C), closed arms (D), or central area (E) and in the percentage of entries in the open arms (F) in the EPMT. However, THC decreased the time of nose dips after adolescent exposure (G). Two-way ANOVA followed by Tukey's post hoc comparisons. Mean ± SEM. $^{**}P < .01$, $^{***}P < .001$. N = 7–10 rats per group.

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, $F_{(1,\ 27)} = 36.34$, $P < .0001$; interaction treatment × 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

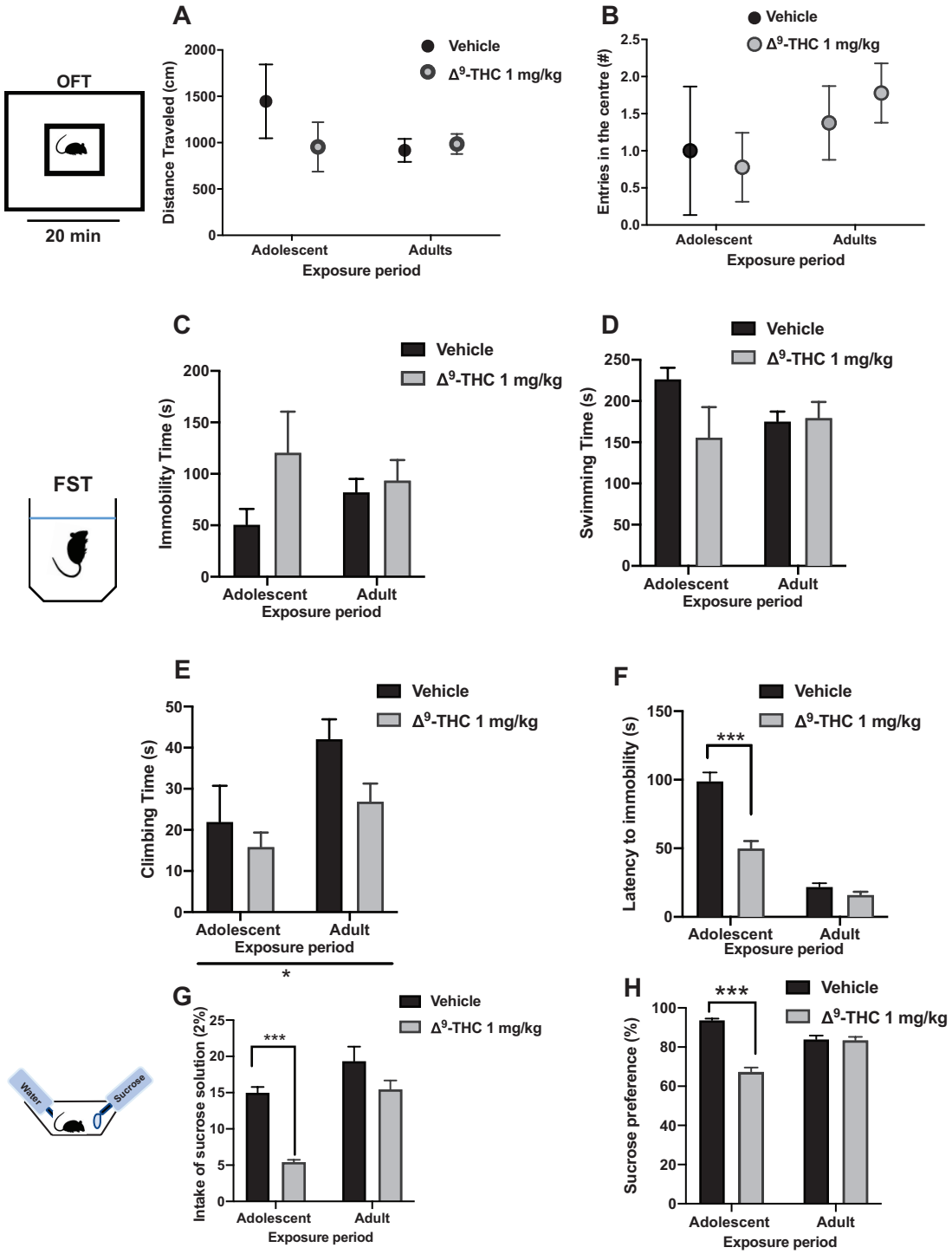

Figure 3. Locomotor activity was assessed in the open field test (OFT) followed by the assessment of depressive behavior in the modified forced swim test (FST) and the sucrose preference test (SPT) after chronic administration of D9-tetrahydrocannabinol (THC; 1.0 mg/kg, i.p.) during adolescence or adulthood. THC did not modify the distance travelled (A) or the number of visits in the central area in the OFT (B). Chronic adolescent THC administration did not produce any changes in immobility time (C), swimming time (D) or climbing time (E) but significantly decreased the latency to the first event of immobility (F). Anhedonic behavior was assessed in the SPT. THC markedly decreased the intake of sucrose solution (2%) (G) and sucrose preference (H) after adolescent but not adult exposure. Two-way ANOVA followed by Tukeys' post-hoc comparison was employed. ***$P < .001$. Each bar or circle represents mean ± SEM. N = 7–10 rats per group.

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, $F_{(1, 29)} = 0.04148$, $P = .8400$;

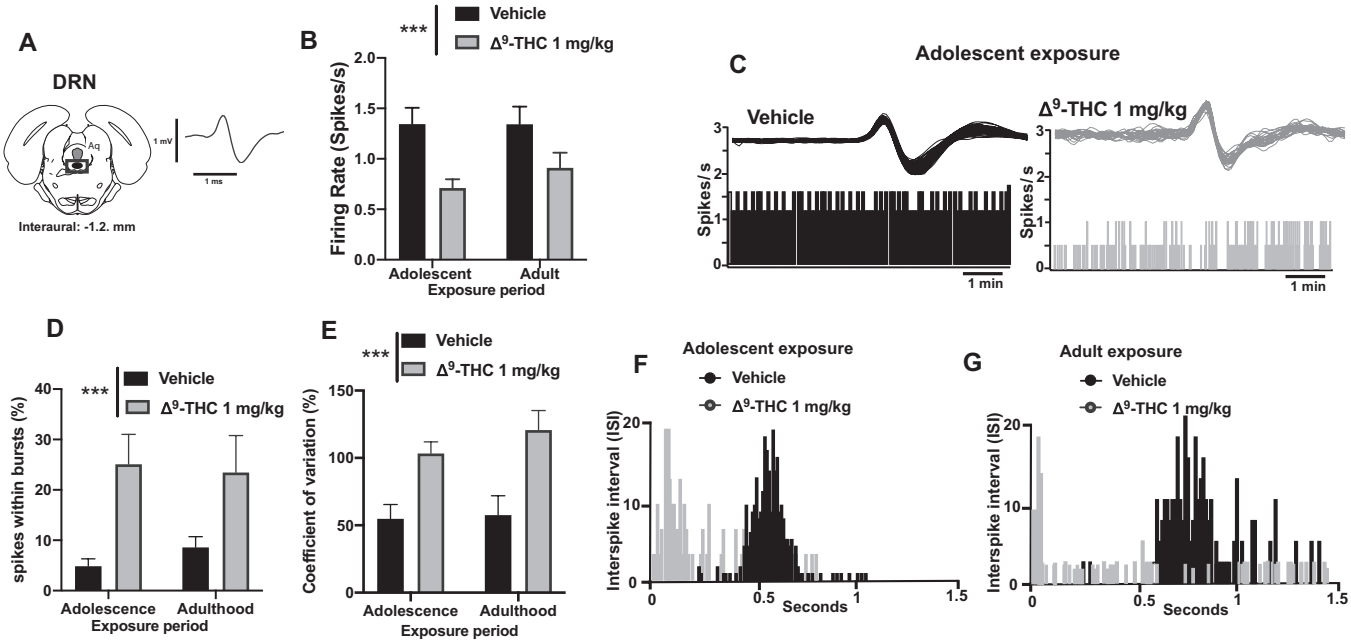

Figure 4. Alteration in serotonergic (5-HT) neurotransmission following chronic administration of D⁹-tetrahydrocannabinol (THC; 1.0 mg/kg, i.p.) during adolescence or adulthood. (A) Illustrative representation of coronal sections of the rat brain (Paxinos and Watson, 2007) showing the location of the dorsal raphe nucleus (DR, arrow). The number at the bottom corresponds to the anterior–posterior coordinate (mm anterior to the interaural line). Aq, Sylvian aqueduct (left). Typical spike characteristics of presumed serotonin (5-HT) neurons (right). Chronic adolescent THC exposure during adolescence decreased 5-HT firing activity to the same extent as chronic adult THC exposure (B). Representative firing rate histograms of 5-HT neurons recorded after adolescent exposure (C). Chronic THC exposure increased burst density of 5-HT neurons (D) and the coefficient of variation after both adolescent and adult exposure (E). Interspike interval histograms of 5-HT neurons after chronic adolescent (F) or adult (G) treatment with vehicle or THC. Two-way ANOVA was employed. ***$P < .001$. Each bar represents mean ± SEM.

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, $F_{(1, 49)} = 1.550$, $P = .2191$; exposure period, $F_{(1, 49)} = 0.7941$, $P = .3772$; interaction, $F_{(1, 49)} = 0.2725$, $P = .6040$; 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

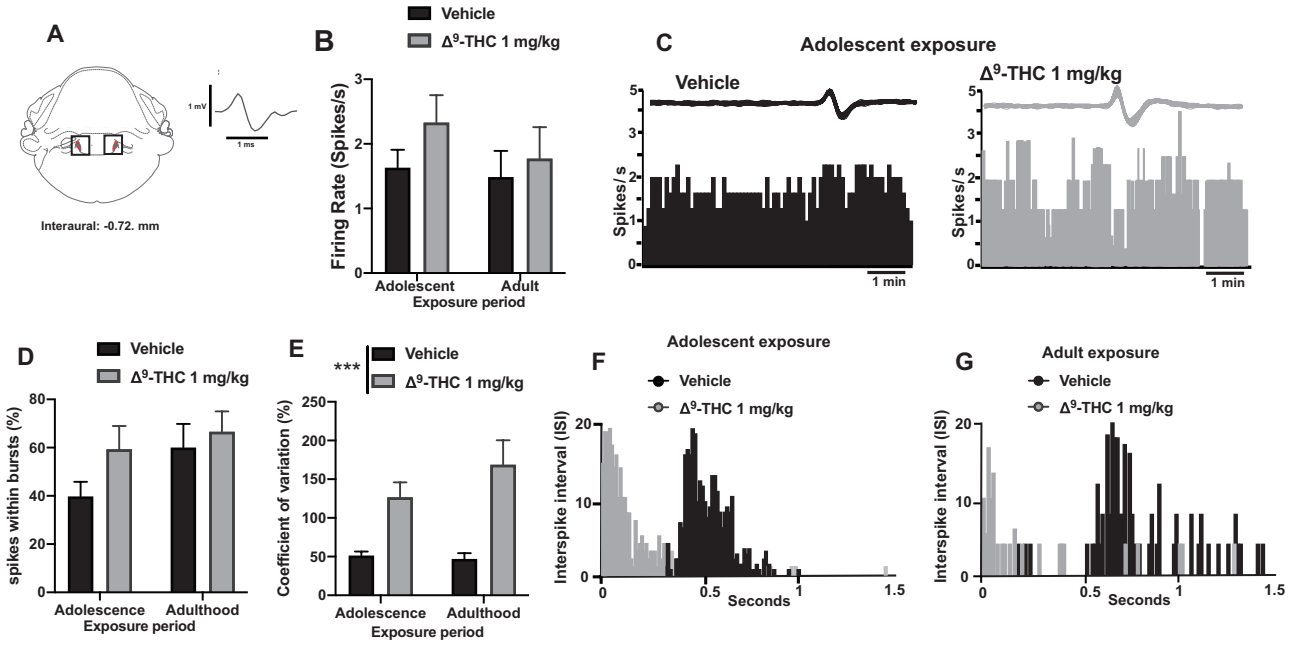

Figure 5. Alteration in noradrenergic (NE) neurotransmission following chronic administration of D9-tetrahydrocannabinol (THC; 1.0 mg/kg, i.p.) during adolescence or adulthood. (A) Illustrative representation of coronal sections of the rat brain showing the location of the locus coeruleus. The number at the bottom corresponds to the anterior–posterior coordinate (mm anterior to the interaural line) (left). Typical spike characteristics of presumed norepinephrine (NE) neurons (right). Chronic THC exposure did not change the basal firing activity after both adolescent and adult exposure (B). Representative firing rate histograms of 5-HT neurons recorded after adolescent exposure (C). THC did not induce a change in burst activity (D) but increased the coefficient of variation after both adolescent and adult exposure (E). Interspike interval histograms of 5-HT neurons after chronic adolescent (F) or adult (G) treatment with vehicle or THC. Two-way ANOVA was employed. ***$P < .001$. Each bar represents mean ± SEM.

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 despair-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 stimuli) 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_{2A} 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 Rodríguez 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_1R 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_1R 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 CB_1R-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 (Higuera-Matas et al., 2012) or glutamateric neurons in the medial prefrontal cortex (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 dopamine 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-HIIA/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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References

Bambico FR, Katz N, Debonnel G, Gobbi G (2007) Cannabinoids elicit antidepressant-like behavior and activate serotonergic neurons through the medial prefrontal cortex. J Neurosci 27:11700–11711.

Bambico FR, Nguyen NT, Gobbi G (2009) Decline in serotonergic firing activity and desensitization of 5-HT1A autoreceptors after chronic unpredictable stress. Eur Neuropsychopharmacol 19:215–228.

Bambico FR, Nguyen NT, Katz N, Gobbi G (2010) Chronic exposure to cannabinoids during adolescence but not during adulthood impairs emotional behaviour and monoaminergic neurotransmission. Neurobiol Dis 37:641–655.

Bambico FR, Hattan PR, Garant JP, Gobbi G (2012) Effect of delta-9-tetrahydrocannabinol on behavioral despair and on pre- and postsynaptic serotonergic transmission. Prog Neuropsychopharmacol Biol Psychiatry 38:88–96.

Berry EM, Mechoulam R (2002) Tetrahydrocannabinol and endocannabinoids in feeding and appetite. Pharmacol Ther 95:185–190.

Buchanan CM, Holmbeck GN (1998) Measuring beliefs about adolescent personality and behavior. J Youth Adolesc 27:607–627.

Castagné V, Moser P, Roux S, Porsolt RD (2010) Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. Curr Protoc Pharmacol 49:5.8. 1–5.8. 14.

Comai S, Gobbi G (2016) Translational research in suicide: is it possible to study suicide in animal models? In: Understanding Suicide, pp 177–188. Cham: Springer International Publishing Switzerland.

Commons KG, Cholanians AB, Babb JA, Ehlinger DG (2017) The Rodent forced swim test measures stress-coping strategy, not depression-like behavior. ACS Chem Neurosci 8:955–960.

Crews F, He J, Hodge C (2007) Adolescent cortical development: a critical period of vulnerability for addiction. Pharmacol Biochem Behav 86:189–199.

Depue RA, Spoont MR (1986) A behavioral dimension of constraint. Ann NY Acad Sci 487:47–62.

Der-Avakian A, Markou A (2012) The neurobiology of anhedonia and other reward-related deficits. Trends Neurosci 35:68–77.

Diana M, Muntoni AL, Pistis M, Melis M, Gessa GL (1999) Lasting reduction in mesolimbic dopamine neuronal activity after morphine withdrawal. Eur J Neurosci 11:1037–1041.

Dillon KA, Gross-Isseroff R, Israeli M, Biegon A (1991) Autoradiographic analysis of serotonin 5-HT1A receptor binding in the human brain postmortem: effects of age and alcohol. Brain Res 554:56–64.

Duman RS, Heninger GR, Nestler EJ (1997) A molecular and cellular theory of depression. Arch Gen Psychiatry 54:597–606.

Ellgren M, Spano SM, Hurd YL (2007) Adolescent cannabis exposure alters opiate intake and opioid limbic neuronal populations in adult rats. Neuropsychopharmacology 32:607–615.

European Monitoring Centre for Drugs and Drug Addiction, EMCDDA, European Drug Report 2019. Trends and Developments, Lisbon, June 2019. https://www.emcdda.europa.eu/publications/edr/trends-developments/2019_en. Accessed February 9, 2020.

Garza JC, Guo M, Zhang W, Lu XY (2012) Leptin restores adult hippocampal neurogenesis in a chronic unpredictable stress model of depression and reverses glucocorticoid-induced inhibition of GSK-3β/β-catenin signaling. Mol Psychiatry 17:790–808.

Geddes SD, Assadzada S, Lemelin D, Sokolovski A, Bergeron R, Haj-Dahmane S, Béïque J-C (2016) Target-specific modulation of the descending prefrontal cortex inputs to the dorsal raphe nucleus by cannabinoids. PNAS 113:5429–5434.

Gobbi G, Atkin T, Zytynski T, Wang S, Askari S, Boruff J, Ware M, Marmorstein N, Cipriani A, Dendukuri N, Mayo N (2019) Association of cannabis use in adolescence and risk of depression, anxiety, and suicidality in young adulthood: a systematic review and meta-analysis. JAMA Psychiatry 76:426–434.

Gobbi G, Blier P (2005) Effect of neurokinin-1 receptor antagonists on serotoninergic, noradrenergic and hippocampal neurons: comparison with antidepressant drugs. Peptides 26:1383–1393.

Greenwood BN, Foley TE, Day HE, Campisi J, Hammack SH, Campeau S, Maier SF, Fleshner M (2003) Freewheel running prevents learned helplessness/behavioral depression: role of dorsal raphe serotonergic neurons. J Neurosci 23:2889–2898.

Hao Z, Wang W, Guo R, Liu H (2019) Faecalibacterium prausnitzii (ATCC 27766) has preventive and therapeutic effects on chronic unpredictable mild stress-induced depression-like and anxiety-like behavior in rats. Psychoneuroendocrinology 104:132–142.

Häring M, Marsicano G, Lutz B, Monory K (2007) Identification of the cannabinoid receptor type 1 in serotonergic cells of raphe nuclei in mice. Neuroscience 146:1212–1219.

Hasin DS, Saha TD, Kerridge BT, Goldstein RB, Chou SP, Zhang H, Jung J, Pickering RP, Ruan WJ, Smith SM, Huang B, Grant BF (2015) Prevalence of marijuana use disorders in the united states between 2001-2002 and 2012-2013. JAMA Psychiatry 72:1235–1242.

Higuera-Matas A, Miguéns M, Coria SM, Assis MA, Borcel E, del Olmo N, Ambrosio E (2012) Sex-specific disturbances of the glutamate/GABA balance in the hippocampus of adult rats subjected to adolescent cannabinoid exposure. Neuropharmacology 62:1975–1984.

Huestis M (2005) Pharmacokinetics and metabolism of the plant cannabinoids, Δ 9-tetrahydrocannibinol, cannabidiol and cannabinol. Handb Exp Pharmacol 168: 657–690.

Huestis MA, Henningfield JE, Cone EJ (1992) Blood cannabinoids. I. Absorption of THC and formation of 11-OH-THC and THCCOOH during and after smoking marijuana. J Anal Toxicol 16:276–282.

Irimia C, Polis IY, Stouffer D, Parsons LH (2015) Persistent effects of chronic Δ9-THC exposure on motor impulsivity in rats. Psychopharmacology (Berl) 232:3033–3043.

Kahn RS, van Praag HM, Wetzler S, Asnis GM, Barr G (1988) Serotonin and anxiety revisited. Biol Psychiatry 23:189–208.

Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M, Watanabe M (2009) Endocannabinoid-mediated control of synaptic transmission. Physiol Rev 89:309–380.

Klein C, Karanges E, Spiro A, Wong A, Spencer J, Huynh T, Gunasekaran N, Karl T, Long LE, Huang XF, Liu K, Arnold JC, McGregor IS (2011) Cannabidiol potentiates Δ⁹-tetrahydrocannabinol (THC) behavioural effects and alters THC pharmacokinetics during acute and chronic treatment in adolescent rats. Psychopharmacology (Berl) 218:443–457.

Kruse LC, Cao JK, Viray K, Stella N, Clark JJ (2019) Voluntary oral consumption of Δ⁹-tetrahydrocannabinol by adolescent rats impairs reward-predictive cue behaviors in adulthood. Neuropsychopharmacology 44:1406–1414.

Lee JY, Brook JS, Finch SJ, Brook DW (2018) Trajectories of cannabis use beginning in adolescence associated with symptoms of posttraumatic stress disorder in the mid-thirties. Subst Abus 39:39–45.

Liu MY, Yin CY, Zhu LJ, Zhu XH, Xu C, Luo CX, Chen H, Zhu DY, Zhou QG (2018) Sucrose preference test for measurement of stress-induced anhedonia in mice. Nat Protoc 13:1686–1698.

Matheson J, Mann RE, Sproule B, Huestis MA, Wickens CM, Stoduto G, George TP, Rehm J, Le Foll B, Brands B (2020) Acute and residual mood and cognitive performance of young adults following smoked cannabis. Pharmacol Biochem Behav 194:172937.

Matthews K, Christmas D, Swan J, Sorrell E (2005) Animal models of depression: navigating through the clinical fog. Neurosci Biobehav Rev 29:503–513.

McCall JG, Siuda ER, Bhatti DL, Lawson LA, McElligott ZA, Stuber GD, Bruchas MR (2017) Locus coeruleus to basolateral amygdala noradrenergic projections promote anxiety-like behavior. Elife 6:e18247.

Molendijk ML, de Kloet ER (2015) Immobility in the forced swim test is adaptive and does not reflect depression. Psychoneuroendocrinology 62:389–391.

Molendijk ML, de Kloet ER (2019) Coping with the forced swim stressor: current state-of-the-art. Behav Brain Res 364:1–10.

Morilak DA, Ciaranello RD (1993) Ontogeny of 5-hydroxytryptamine2 receptor immunoreactivity in the developing rat brain. Neuroscience 55:869–880.

Murphy M, Mills S, Winstone J, Leishman E, Wager-Miller J, Bradshaw H, Mackie K (2017) Chronic adolescent Δ9-tetrahydrocannabinol treatment of male mice leads to long-term cognitive and behavioral dysfunction, which are prevented by concurrent cannabidiol treatment. Cannabis Cannabinoid Res 2:235–246.

Nahas GG, Frick HC, Lattimer JK, Latour C, Harvey D (2002) Pharmacokinetics of THC in brain and testis, male gametotoxicity and premature apoptosis of spermatozoa. Hum Psychopharmacol 17:103–113.

Paus T (2005) Mapping brain maturation and cognitive development during adolescence. Trends Cogn Sci 9:60–68.

Pavcovich LA, Cancela LM, Volosin M, Molina VA, Ramirez OA (1990) Chronic stress-induced changes in locus coeruleus neuronal activity. Brain Res Bull 24:293–296.

Paxinos G, Watson C (2007) The rat brain in stereotaxic coordinates. 6th Edition. San Diego: Academic Press.

Pistis M, Perra S, Pillolla G, Melis M, Muntoni AL, Gessa GL (2004) Adolescent exposure to cannabinoids induces long-lasting changes in the response to drugs of abuse of rat midbrain dopamine neurons. Biol Psychiatry 56:86–94.

Poulia N, Delis F, Brakatselos C, Lekkas P, Kokras N, Dalla C, Antoniou K (2020) Escalating low-dose Δ9 -tetrahydrocannabinol exposure during adolescence induces differential behavioral and neurochemical effects in male and female adult rats. Eur J Neurosci 52:2681–2693.

Prini P, Rusconi F, Zamberletti E, Gabaglio M, Penna F, Fasano M, Battaglioli E, Parolaro D, Rubino T (2018) Adolescent THC exposure in female rats leads to cognitive deficits through a mechanism involving chromatin modifications in the prefrontal cortex. J Psychiatry Neurosci 43:87–101.

Quinn HR, Matsumoto I, Callaghan PD, Long LE, Arnold JC, Gunasekaran N, Thompson MR, Dawson B, Mallet PE, Kashem MA, Matsuda-Matsumoto H, Iwazaki T, McGregor IS (2008) Adolescent rats find repeated delta(9)-THC less aversive than adult rats but display greater residual cognitive deficits and changes in hippocampal protein expression following exposure. Neuropsychopharmacology 33:1113–1126.

Renard J, Rosen LG, Loureiro M, De Oliveira C, Schmid S, Rushlow WJ, Laviolette SR (2017) Adolescent cannabinoid exposure induces a persistent sub-cortical hyper-dopaminergic state and associated molecular adaptations in the prefrontal cortex. Cereb Cortex 27:1297–1310.

Rodríguez de Fonseca F, Ramos JA, Bonnin A, Fernández-Ruiz JJ (1993) Presence of cannabinoid binding sites in the brain from early postnatal ages. Neuroreport 4:135–138.

Rubino T, Vigano' D, Realini N, Guidali C, Braida D, Capurro V, Castiglioni C, Cherubino F, Romualdi P, Candeletti S, Sala M, Parolaro D (2008) Chronic delta 9-tetrahydrocannabinol during adolescence provokes sex-dependent changes in the emotional profile in adult rats: behavioral and biochemical correlates. Neuropsychopharmacology 33:2760–2771.

Schoeler T, Theobald D, Pingault JB, Farrington DP, Coid JW, Bhattacharyya S (2018) Developmental sensitivity to cannabis use patterns and risk for major depressive disorder in mid-life: findings from 40 years of follow-up. Psychol Med 48:2169–2176.

Silins E, Horwood LJ, Patton GC, Fergusson DM, Olsson CA, Hutchinson DM, Spry E, Toumbourou JW, Degenhardt L, Swift W, Coffey C, Tait RJ, Letcher P, Copeland J, Mattick RP; Cannabis Cohorts Research Consortium (2014) Young adult sequelae of adolescent cannabis use: an integrative analysis. Lancet Psychiatry 1:286–293.

Silva L, Black R, Michaelides M, Hurd YL, Dow-Edwards D (2016) Sex and age specific effects of delta-9-tetrahydrocannabinol during the periadolescent period in the rat: the unique susceptibility of the prepubescent animal. Neurotoxicol Teratol 58:88–100.

Spear L (2000) Modeling adolescent development and alcohol use in animals. Alcohol Res Health 24:115–123.

Statistics Canada (2019) National Cannabis Survey, third quarter 2019, released on 2019-10-30. https://www150.statcan.gc.ca/n1/daily-quotidien/191030/dq191030a-eng.htm. Accessed June 2, 2020

Tanaka M, Yoshida M, Emoto H, Ishii H (2000) Noradrenaline systems in the hypothalamus, amygdala and locus coeruleus are involved in the provocation of anxiety: basic studies. Eur J Pharmacol 405:397–406.

Torrens A, Vozella V, Huff H, McNeil B, Ahmed F, Ghidini A, Mahler SV, Huestis MA, Das A, Piomelli D, (2020) Comparative pharmacokinetics of Δ9-tetrahydrocannabinol in adolescent and adult male mice. J Pharmacol Exp Ther.

United Nations Office on Drugs and Crime, UNODC (2017) United Nations Office on Drugs and Crime, World Drug Report 2017. New York, NY: United Nations Publications.

Viveros MP, Llorente R, Suarez J, Llorente-Berzal A, López-Gallardo M, de Fonseca FR (2012) The endocannabinoid system in critical neurodevelopmental periods: sex differences and neuropsychiatric implications. J Psychopharmacol 26:164–176.

Volkow ND, Baler RD, Compton WM, Weiss SR (2014) Adverse health effects of marijuana use. N Engl J Med 370:2219–2227.