



Effects in rats of adolescent exposure to cannabis smoke or THC on emotional behavior and cognitive function in adulthood

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Abstract

Rationale Cannabis use is common among adolescents and some research suggests that adolescent cannabis use increases the risk for depression, anxiety, and cognitive impairments in adulthood. In human studies, however, confounds may affect the association between cannabis use and the development of brain disorders.

Objectives These experiments investigated the effects of adolescent exposure to either cannabis smoke or THC on anxiety- and depressive-like behavior and cognitive performance in adulthood in Long-Evans rats.

Methods Adolescent rats of both sexes were exposed to either cannabis smoke from postnatal days (P) 29–49 or ascending doses of THC from P35–45. When the rats reached adulthood (P70), anxiety-like behavior was investigated in the large open field and elevated plus maze, depressive-like behavior in the sucrose preference and forced swim tests, and cognitive function in the novel object recognition test.

Results Despite sex differences on some measures in the open field, elevated plus maze, forced swim, and novel object recognition tests, there were no effects of either adolescent cannabis smoke or THC exposure, and only relatively subtle interactions between exposure conditions and sex, such that sex differences on some performance measures were slightly attenuated.

Conclusion Neither cannabis smoke nor THC exposure during adolescence produced robust alterations in adult behavior after a period of abstinence, suggesting that adverse effects associated with adolescent cannabis use might be due to non-cannabinoid concomitants of cannabis use.

Keywords Cannabis · THC · Depression · Anxiety · Cognition · Behavior · Adolescents · Adult · Sex differences · Rats

Introduction

Cannabis use is common among adolescents and young adults, but the long-term consequences of such use are a topic of debate. Cannabis use typically starts during early adolescence and peaks

when users are in their mid-20s (Hasin et al. 2015). In a large US survey, 7.4% of adolescents reported cannabis use during the past month and 13.1% during the past year (Azofeifa 2016). Cannabis use can have adverse health effects, including increased risks for lung, cardiovascular, and periodontal diseases (Gordon et al. 2013; Jouanjus et al. 2017). Its effects on development of cognitive and affective dysfunction, however, have been less conclusive. An initial study reported that cannabis use, particularly during adolescence, contributes to a lasting neurocognitive decline including an 8-point drop in IQ from childhood to adulthood (Meier et al. 2012). More recent studies, however, do not support this conclusion. For example, cannabis users perform worse on cognitive tests than non-users, but the performance of users is comparable with their non-using twins (Jackson et al. 2016; Meier et al. 2018). Such data suggest that the relatively poor cognitive performance of cannabis users may be due more to familial factors than to cannabis use. On a similar note, earlier studies suggested that adolescent cannabis use can play a causal role in anxiety and mood disorders (Patton et al.

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2002; Rey et al. 2002). More recent work suggests that depressed individuals are more likely to use cannabis, but evidence for a causal relationship between cannabis use and depression is lacking (Feingold et al. 2015; Horwood et al. 2012; Moore et al. 2007; Quinn et al. 2008).

These recent data casting doubt on the causal relationship between adolescent cannabis use and long-term cognitive/affective dysfunction stand in contrast to a large body of research in animals showing that adolescent exposure to Δ 9-tetrahydrocannabinol (THC) or other cannabinoid receptor agonists does produce adverse effects in adulthood. Many animal studies suggest that exposure to THC during adolescence causes anxiety- and depressive-like behavior in adulthood, as well as impairments in spatial and non-spatial learning and working memory (Cha et al. 2006; O'Shea et al. 2004; Quinn et al. 2008; Realini et al. 2011; Rubino et al. 2009b; Rubino et al. 2008). One possible explanation for the disparity between findings in humans and animals is that subjects in animal studies (e.g., mice and rats) typically receive high doses of cannabinoids, whereas humans inhale cannabis smoke in which THC levels are likely lower. Thus, animals may be exposed to higher doses of cannabinoids than humans would typically consume, which could potentially explain the differences between human and animal studies (Huestis 2007; Rubino et al. 2009a; Zgair et al. 2015). Furthermore, cannabis smoke contains numerous cannabinoids aside from THC, which might counteract potentially deleterious effects of THC (Curran et al. 2016; Niesink and van Laar 2013).

The data reviewed above suggest that experimental designs typically employed in animal studies of adolescent cannabinoid exposure may fail to model potentially critical aspects of human cannabis use. To address this issue, we used a rat model of cannabis smoke exposure developed in our laboratory (Bruijnzeel et al. 2016). In previous work with this model, we showed that in adult rats, acute cannabis smoke exposure alters exploratory behavior and working memory, and that chronic exposure leads to dependence (Blaes et al. 2018; Bruijnzeel et al. 2016). In the present studies, we determined how smoke exposure during adolescence affects anxiety-like behavior, depressive-like behavior, and cognitive function. Male and female rats were exposed to cannabis smoke from postnatal day (P) 29–49, which corresponds to the period of early and mid-adolescence (Tirelli et al. 2003). For comparison, a similar set of experiments was conducted with rats that received THC injections during adolescence (P35–45), using a protocol shown previously to produce adult affective/cognitive dysfunction (Rubino et al. 2008).

Materials and methods

Subjects

Long-Evans rats were obtained from Charles River at P22. In experiment 1 (cannabis smoke exposure), the rats were housed

in single sex groups with 3 per cage throughout the smoke exposure period. Shortly, after the smoke sessions were completed, several rats engaged in fighting with their cagemates. To address this, all rats were single housed for the remainder of this experiment. In experiment 2 (THC administration), the rats were housed in single sex groups with 2 per cage for the first part of the study. The rats were single housed before the sucrose preference test and remained single housed afterwards. All rats were housed in the vivarium in the McKnight Brain Institute at the University of Florida on a 12 h light/dark cycle (lights off at 8 AM), with food and water available ad libitum except as noted below. Seventy-two rats (36 male, 36 female) were used for experiment 1 (cannabis smoke exposure) and 40 rats (20 male, 20 female) were used for experiment 2 (THC exposure). Body weights were recorded for each rat on a daily basis during the period of smoke or THC exposure. Estrous phase was not assessed. All animal procedures were performed in accordance with the University of Florida Institutional Animal Care and Use Committee as well as National Institutes of Health guidelines.

Drugs

Cannabis cigarettes, placebo cigarettes, and THC were obtained from the NIDA Drug Supply Program. The cannabis cigarettes were the size of standard tobacco cigarettes (approximately 700 mg each), and contained approximately 5.6% THC, 0% cannabidiol (CBD), and 0.4% cannabinol (CBN) as per the analytical data accompanying the cigarettes. The placebo cigarettes were composed of cannabis plant material from which cannabinoids were extracted, and contained 0.002% THC, 0.001% CBD, and 0.004% CBN. THC was obtained in 100% ethanol. The ethanol was evaporated under a light stream of nitrogen gas applied indirectly to the solution-containing vial inside a fume hood, and the remaining THC was dissolved in a vehicle containing 5% kollophor, 5% ethanol, and 90% saline (Blaes et al. 2018). In experiment 2, the rats received THC (or an equal volume of vehicle) from P35–45. During this period, the rats received injections (i.p.) twice a day according to the following schedule: P35–37, 2.5 mg/kg; P38–41, 5 mg/kg; P42–45, 10 mg/kg (Rubino et al. 2008).

Smoke exposure

Freely moving rats were exposed to smoke from burning cannabis or placebo cigarettes in two separate cohorts (with equivalent numbers of males and females) as described in our previous work (Blaes et al. 2018; Bruijnzeel et al. 2016; Ravula et al. 2018). Briefly, smoke exposure was conducted in a microprocessor-controlled cigarette smoking-machine (model TE-10, Teague Enterprises, Davis, CA) that burned cigarettes using a standardized smoking procedure (35 cm³

puff volume, 1 puff per minute, 2 s per puff)(Teague et al. 1994). Mainstream and sidestream smoke were transported to a mixing and diluting chamber, where the smoke was diluted with air to a concentration of about 250 mg of total suspended particles (TSP) per cubic meter before being introduced into the exposure chamber. Just prior to the exposure sessions, rats were moved from their home cages and placed into clean, standard polycarbonate rodent cages (38 × 28 × 20 cm; L × W × H) with corncob bedding, wire tops, and a water bottle (rats from the same home cage were moved into the same exposure cage). This whole-body exposure regimen allowed for free movement (i.e., not restrained) and social housing (3 rats/cage) during the exposure sessions. During the sessions, exposure conditions were monitored for carbon monoxide (CO) and TSP levels. CO levels were assessed using a continuous CO analyzer that measures CO levels between 0 and 2000 ppm (Monoxor III, Bacharach, New Kensington, PA USA). In order to measure TSP levels, smoke was pumped out of the chamber through a pre-weighed filter for 5 min (Pallflex Emfab Filter, Pall Corporation, Port Washington, NY USA). The TSP level was calculated by dividing the weight increase of the filter by the volume of airflow through the filter. Rats in the smoke exposure groups were exposed to smoke for 1 h per day. During this period, 5 cannabis cigarettes were burned (10 min per cigarette with a 2 min break between cigarettes). TSP and CO levels are reported in Table S1. Rats in the clean air condition were placed in the smoke exposure chamber as described above with the machine on to allow continuous airflow, but no cigarettes were loaded and no smoke was delivered. Exposure sessions were conducted daily from P29 to P49 for a total of 21 days.

Behavioral testing

Behavioral testing began after P70 and was conducted at the following ages for rats in both the smoke exposure (experiment 1) and THC (experiment 2) studies: large open field, P77; elevated plus maze, P85; sucrose preference, P93; forced swim, P98; novel object recognition, P129. Each behavioral test was completed in 1–2 days.

Large open field test

The large open field test is used to assess anxiety-like behavior (Liebsch et al. 1998). The test was conducted in a dimly lit room (75 lx) as described previously (Bruijnzeel et al. 2016; Qi et al. 2016). The open field apparatus consisted of a large arena measuring 120 × 120 × 60 cm (L × W × H). The arena was made of black high-density polyethylene panels that were fastened together and placed on a plastic bottom plate (Faulkner Plastics, Miami, FL). The rats' behavior was recorded with a camera mounted above the arena and analyzed with EthoVision XT 11.5 software (Noldus Information

Technology, Leesburg, VA). The open field was divided into two zones: a border zone (20-cm wide) and a center zone (80 × 80 cm; L × W). The following behaviors were analyzed: total distance traveled, distance traveled in the border and center zone, latency to enter the center zone, number of center zone entries, and duration in the center zone. The open field was cleaned with a Nolvasan solution between rats.

Elevated plus maze test

The elevated plus maze test is used to assess anxiety-like behavior and was conducted as described previously (Qi et al. 2016; Rylkova et al. 2009). The test apparatus consisted of four black polypropylene arms (Coulbourn Instruments, Whitehall, PA). The two “open” arms had 0.5-cm ledges and the two “closed” arms had 30-cm walls. The open arms were placed opposite of each other. The arms were 10-cm wide, 50-cm long, and were placed on 55-cm tall acrylic legs. Testing occurred in a quiet, dimly lit (75 lx) room. At the beginning of each test, the rats were placed in the center of the apparatus facing an open arm. Rats were allowed to explore the apparatus for 5 min, and their behavior was recorded with a camera mounted above the maze. The elevated plus maze was divided into 5 zones (two open arms, two closed arms, and center). Behavior was scored manually using EthoVision XT 11.5 software by an experienced observer who was blind to the treatment conditions. The following behaviors were analyzed: duration in the open and closed arms and the number of open and closed arm entries. An arm entry was counted when the rat had all four paws on that arm. The apparatus was cleaned with a Nolvasan solution between rats.

Sucrose preference test

The sucrose preference test was conducted in the rats' home cages in the vivarium (Coelho et al. 2014). Before testing began, the rats were acclimated to the bottles containing the 2% sucrose solution for 48 h in the absence of tap water. The sucrose solution was provided in an identical pair of 6-oz. glass bottles with stainless steel drip-resistant sipper tubes (Kaytee Chew Proof Bottle), which were fastened to the home cage lid to minimize movement during liquid ingestion. During the 24-h two-bottle choice test, one bottle was filled with a 2% sucrose solution and the other with tap water (the left/right position of the bottles was randomly counterbalanced across rat cages to minimize effects of cage side preference). Bottles were weighed before and after the 24-h choice test. The difference in bottle weight was used as an indicator of sucrose and water intake during the 24-h test and was converted to a sucrose preference index (Coelho et al. 2014). A reduced preference for the sucrose solution over water is considered indicative of anhedonia (Willner et al. 1987).

Forced swim test

The forced swim test was conducted as described previously (Detke et al. 1995; Slattery and Cryan 2012) and consisted of two stages: a 15-min pre-test followed by a 5-min test 24 h later. Immediately before each stage, a large Pyrex cylinder (21 × 46 cm; Fisher Scientific) was filled with tap water (23–25 °C) to a depth of 30 cm. The rats' behavior was recorded with a digital camcorder that was placed 1 m above the cylinder. An experienced observer who was blind to the treatment conditions scored the behavior from the DVD recordings. Three behaviors were scored continuously: immobility, swimming, and climbing. Swimming was defined as horizontal movements across the cylinder. Climbing was defined as upward-directed movement of the forepaws against the cylinder wall. The rats were considered immobile if they were floating in the water and made only the movements necessary to keep their heads above the water. After each test phase was completed, the rats were removed from the water and dried with clean towels.

Novel object recognition test

The novel object recognition test was conducted in the large open field apparatus (described above). This test was conducted in three stages: habituation, familiarization, and novel object recognition testing (Bevins and Besheer 2006; Rojas et al. 2016). During the habituation phase, the rats explored the empty open field for 5 min. After the habituation session was completed, 2 sample objects were placed in the open field (20 cm apart and 10 cm from the wall) and the rats were placed in the open field for the 5-min familiarization stage. The novel object recognition test was conducted 2 h after the familiarization stage. This test session was identical to the familiarization stage except that one of the sample objects was replaced by a novel object. During the familiarization stage, all the rats were exposed to the same two sample objects, which were almost identical but had a slightly different shape and color. In the recognition test session, for half the rats the left sample object was replaced with a novel object and for the other half the right sample object was replaced with a novel object. The novel objects were of a similar size as the sample objects but had a different shape and color (Felix-Ortiz and Febo 2012). The floor of the arena and the objects were cleaned with a Nolvasan solution between rats. The rats' behavior was recorded during the novel object recognition test with a digital camcorder suspended above the arena. The time spent with the sample and novel objects was scored manually from the DVD recordings using Ethovision XT 11.5 by an observer who was blind to the treatment conditions. A rat was considered to be exploring an object when its head was pointed towards the object and its nose was within 1 cm of the object (sniffing and interacting with the object). A discrimination index (time

with novel object – time with familiar object) / total time with objects) was calculated on the basis of the time spent interacting with the two objects.

Statistics

Data were analyzed with multi-factor ANOVA appropriate for each experimental design, using IBM SPSS Statistics version 25 or GraphPad Prism version 7. Body weight data were analyzed using a three-factor ANOVA, with exposure condition (clean air, placebo, and cannabis in experiment 1; THC and vehicle in experiment 2) and sex as between-subjects factors, and day as a within-subjects factor. Behavioral test data were analyzed using two-factor ANOVA, with exposure condition and sex as between-subjects factors. For all statistical analyses, significant interactions in the ANOVA were followed by Bonferroni's post hoc tests to determine which groups differed from each other. Partial eta-squared (η^2_p) values are provided as a measure of effect size (Cohen 1973). *P* values less or equal to 0.05 were considered significant. Significant main effects, interactions, and post hoc comparisons are reported in the “Results” section.

Results

Experiment 1: effects of adolescent cannabis smoke exposure

Cannabis smoke and body weights

Across all groups of rats, males weighed more than the females ($F_{1,66} = 39.38$, $P < .0001$, $\eta^2_p = 0.374$, Figure S1a). There was a significant increase in body weight across smoke exposure days (day, $F_{20,1320} = 3883.95$; $P < 0.0001$; $\eta^2_p = 0.983$), and the magnitude of this increase was significantly greater in males than females (day × sex, $F_{20,1320} = 145.23$; $P < .0001$; $\eta^2_p = 0.688$; Figure S1a). There were, however, no main effects or interactions involving exposure condition. Males continued to weigh more than females at P70 (sex, $F_{1,66} = 261.45$; $P < 0.0001$; $\eta^2_p = 0.798$), but there was no effect of exposure condition (Figure S1b).

Cannabis smoke and the large open field test

In the large open field test, there were no main effects or interactions involving smoke exposure condition on total distance traveled, distance traveled in the border zone, distance traveled in the center zone, number of center zone entries, or time spent in the center zone (Figs. 1 and S2). There was a significant interaction between exposure condition and sex on latency to enter the center zone (exposure condition × sex, $F_{2,66} = 5.246$; $P < 0.01$; $\eta^2_p = 0.137$; Figure S2a). Post hoc

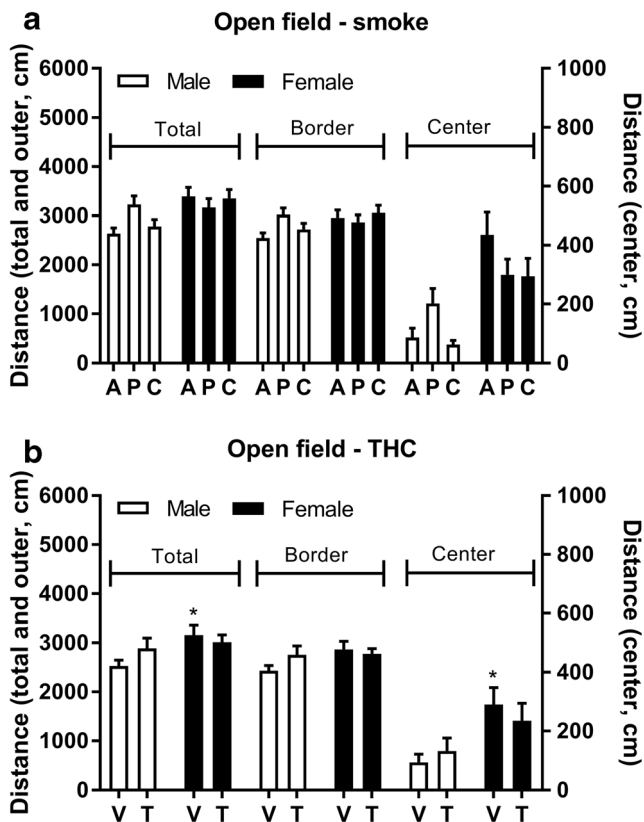


Fig. 1 Exposure to cannabis smoke or THC during adolescence does not affect behavior in the large open field test. Male and female rats were exposed to cannabis smoke (a) or THC (b) and tested in the large open field in adulthood. Asterisks ($*P < 0.05$) indicate a greater distance traveled in the females compared with the corresponding male group. Cannabis smoke, placebo smoke, and air: $n = 12/\text{group}/\text{sex}$. THC and vehicle: $n = 10/\text{group}/\text{sex}$. A, air; C, cannabis smoke; P, placebo smoke; T, THC; V, vehicle. Data are expressed as means \pm SEM

analyses indicated that the sex difference observed under clean air control conditions was significantly attenuated in the placebo and cannabis smoke conditions. In addition, there were robust sex differences in total distance traveled ($F_{1,66} = 9.028$, $P < 0.01$, $\eta^2_p = 0.120$, Fig. 1a), distance traveled in the center zone ($F_{1,66} = 25.9$, $P < 0.0001$, $\eta^2_p = 0.282$, Fig. 1a), number of entries into the center zone ($F_{1,66} = 19.99$, $P < 0.0001$, $\eta^2_p = 0.232$, Figure S2b), and total time in the center zone ($F_{1,66} = 32.71$, $P < 0.0001$, $\eta^2_p = 0.331$, Figure S2c). In all instances, these sex differences were in the direction of greater locomotor activity and/or less anxiety-like behavior in females compared with males.

Cannabis smoke and the elevated plus maze test

Consistent with the data from the open field test, there were no main effects or interactions involving exposure condition on any of the measures in the elevated plus maze test, including percentage of open arm entries and percentage of time spent on the open arms (Fig. 2) and the number of open arm entries or closed arm entries (Figure S3). There were, however, robust

sex differences, such that female rats had a greater percentage ($F_{1,66} = 8.676$, $P < 0.01$, $\eta^2_p = 0.116$, Fig. 2a) and number ($F_{1,66} = 5.044$, $P < 0.05$, $\eta^2_p = 0.071$, Figure S3a) of open arm entries compared with male rats and spent a greater percentage of time on the open arms ($F_{1,66} = 10.29$, $P < 0.01$, $\eta^2_p = 0.135$, Fig. 2b). There were no effects of sex on the number of closed arm entries (Figure S3b), suggesting that the greater number of open arm entries in females compared with males was not due to greater locomotor activity, but instead due to less anxiety-like behavior.

Cannabis smoke, sucrose preference, and the forced swim test

There was no effect of exposure condition or sex on sucrose preference (Table 1). However, total fluid intake ($F_{1,66} = 5.351$, $P < 0.05$, $\eta^2_p = 0.075$, Table S2) and water intake ($F_{1,66} = 8.687$, $P < 0.05$, $\eta^2_p = 0.116$) were greater in the males than the females and there was a trend towards greater sucrose intake in the males ($F_{1,66} = 3.297$, $P = 0.07$, $\eta^2_p = 0.048$). There were no effects of exposure condition or sex in the forced swim test (Fig. 3a).

Cannabis smoke and the novel object recognition test

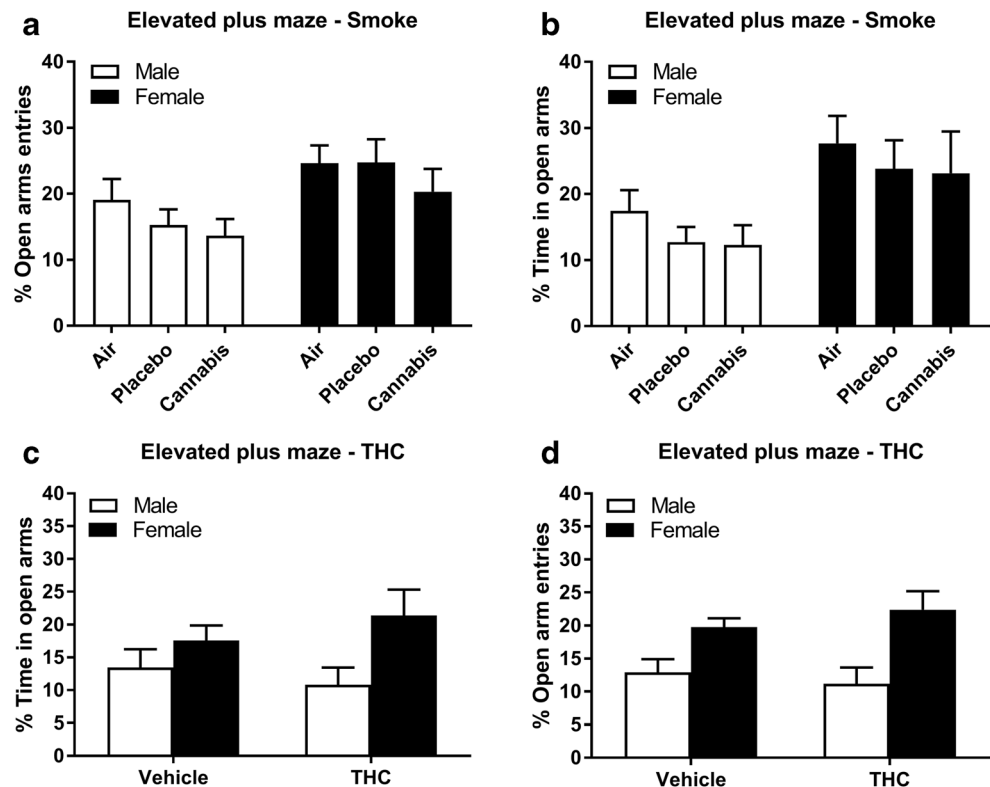
There were no effects of exposure condition or sex on the novel object recognition task either on the discrimination index measure (Fig. 4a) or on any of the measures of time spent interacting with the objects (Figure S4).

Experiment 2: effects of adolescent THC exposure

THC and body weights

Across both groups of rats, there was a significant increase in body weight across the days of THC or vehicle administration (day, $F_{10,360} = 1038.47$; $P < 0.0001$; $\eta^2_p = 0.966$), and the magnitude of this increase was significantly greater in males than females (day \times sex, $F_{10,360} = 102.55$; $P < 0.0001$; $\eta^2_p = 0.74$; Figure S5). Against this background, THC administration caused a significant reduction in body weight gain ($F_{1,36} = 4.68$, $P < 0.05$, $\eta^2_p = 0.115$), which became larger across days of drug administration (day \times drug condition, $F_{10,360} = 33.37$; $P < 0.0001$; $\eta^2_p = 0.481$) and differed by sex (day \times sex \times drug condition, $F_{10,360} = 2.59$; $P < 0.01$; $\eta^2_p = 0.067$). Post hoc analyses indicate that at treatment day 6 and later, the body weights of the male-vehicle rats were greater than those of the female-vehicle rats, and at day 9 and later, the body weights of the male-vehicle rats were greater than those of the male-THC rats. Follow-up two-factor ANOVAs comparing drug conditions separately in each sex revealed a significant attenuation in body weight gain in both males (day, $F_{10,180} = 661.2$; $P < 0.0001$; $\eta^2_p = 0.973$; day \times drug condition, $F_{10,180} =$

Fig. 2 Exposure to cannabis smoke or THC during adolescence does not affect behavior in the elevated plus maze test. Male and female rats were exposed to cannabis smoke (a, b) or THC (c, d) and tested in the large open field in adulthood. Cannabis smoke, placebo smoke, and air: $n = 12/\text{group}/\text{sex}$. THC and vehicle: $n = 10/\text{group}/\text{sex}$. Data are expressed as means \pm SEM



19.34; $P < 0.0001$; $\eta^2_p = 0.518$) and females (day, $F_{10,180} = 380.5$; $P < 0.0001$; $\eta^2_p = 0.955$; day \times drug condition, $F_{10,180} = 15.12$; $P < 0.0001$; $\eta^2_p = 0.457$), indicating that despite overall sex differences in body weight, THC robustly attenuated normal growth in both males and females.

THC and the large open field test

In the large open field test, there were no main effects or interactions involving drug condition on distance traveled in the border zone, distance traveled in the center zone, number of center zone entries, or time spent in the center zone (Figs. 1b and S6). There was a significant interaction between drug

condition and sex on total distance traveled in the open field (sex \times drug condition, $F_{1,36} = 4.745$; $P < 0.05$; $\eta^2_p = 0.116$), and post hoc comparisons revealed that the sex difference in total distance traveled in vehicle-treated rats (females greater than males) was diminished in THC-treated rats (Fig. 1b). In addition, the overall ANOVA revealed significant main effects of sex on distance traveled in the center of the open field ($F_{1,36} = 9.421$, $P < 0.01$, $\eta^2_p = 0.207$, Fig. 1b), number of entries into the center zone ($F_{1,36} = 10.18$, $P < 0.01$, $\eta^2_p = 0.22$, Fig. S6b), and time in the center ($F_{1,36} = 6.811$, $P < 0.05$, $\eta^2_p = 0.159$, Figure S6c). As in experiment 1, these sex differences were all in the direction of greater locomotor activity and/or less anxiety-like behavior in females compared with males.

Table 1 Exposure to cannabis smoke or THC during adolescence does not affect sucrose preference

Treatments	Sucrose preference (%)	
	Male	Female
Adolescent cannabis smoke exposure		
Air	84.0 \pm 7.3	87.4 \pm 7.0
Placebo	80.4 \pm 7.3	86.2 \pm 7.3
Cannabis	89.3 \pm 3.2	92.5 \pm 4.1
Adolescent THC exposure		
Vehicle	93.8 \pm 1.1	87.0 \pm 6.4
THC	88.3 \pm 5.2	92.9 \pm 3.0

THC and the elevated plus maze test

As was the case with cannabis smoke in experiment 1, THC had no effect (no main effects or interactions) on any of the measures in the elevated plus maze. There were, however, robust sex differences on several of the measures. Compared with males, females spent a greater percentage of time on the open arms ($F_{1,36} = 6.105$, $P < 0.05$, $\eta^2_p = 0.145$, Fig. 2c) and had a higher percentage of open arm entries ($F_{1,36} = 16.64$, $P < 0.001$, $\eta^2_p = 0.316$, Fig. 2d). There was a trend towards a greater number of open arm entries in females compared with the males ($F_{1,36} = 3.086$, $P = 0.088$, $\eta^2_p = 0.079$, Figure S7a),

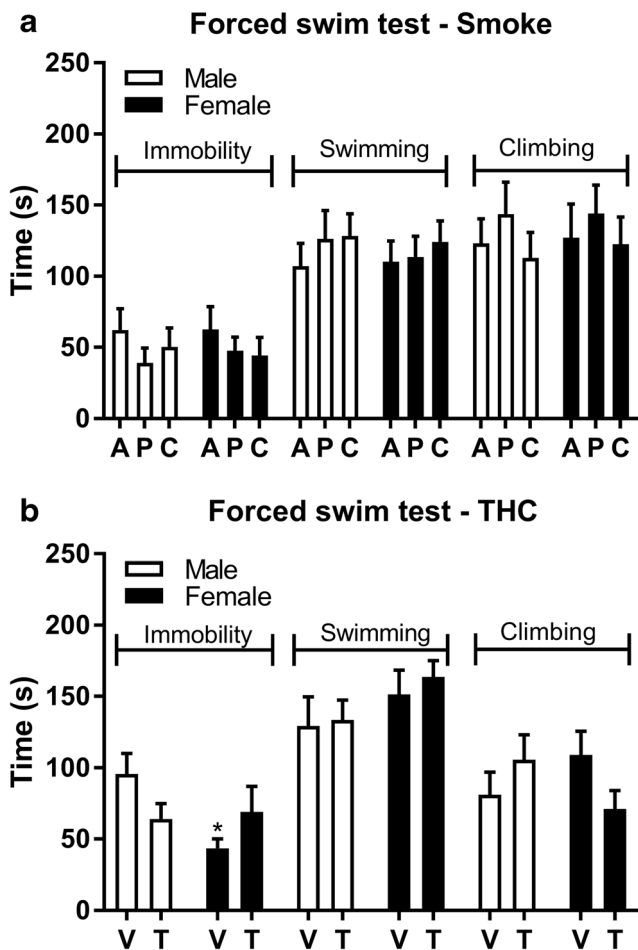


Fig. 3 Exposure to cannabis smoke or THC during adolescence does not affect behavior in the forced swim test. Male and female rats were exposed to cannabis smoke (a) or THC (b) and tested in the forced swim test in adulthood. Cannabis smoke, placebo smoke, and air: $n = 12/\text{group}/\text{sex}$. THC and vehicle: $n = 10/\text{group}/\text{sex}$. A, air; C, cannabis smoke; P, placebo smoke; T, THC; V, vehicle. Data are expressed as means \pm SEM

whereas males had a greater number of closed arm entries ($F_{1,36} = 18.29$, $P < 0.0001$, $\eta^2_p = 0.337$, Figure S7b).

THC, sucrose preference, and the forced swim test

Neither THC nor sex produced significant effects on sucrose preference (Table 1) or total fluid intake (Table S2). In the forced swim test, there were no main effects of either drug condition or sex on swimming or climbing (Fig. 3b), but there was a sex \times drug condition interaction for immobility ($F_{1,36} = 4.304$, $P < 0.05$, $\eta^2_p = 0.107$). Post hoc tests indicated that the sex difference in immobility in the vehicle-treated rats was diminished in THC-treated rats. There was also a trend towards a sex \times drug condition interaction for climbing behavior ($F_{1,36} = 3.519$, $P = 0.069$, $\eta^2_p = 0.089$). In the vehicle condition, the females spent more time climbing than the males but in the THC condition the males spent more time climbing.

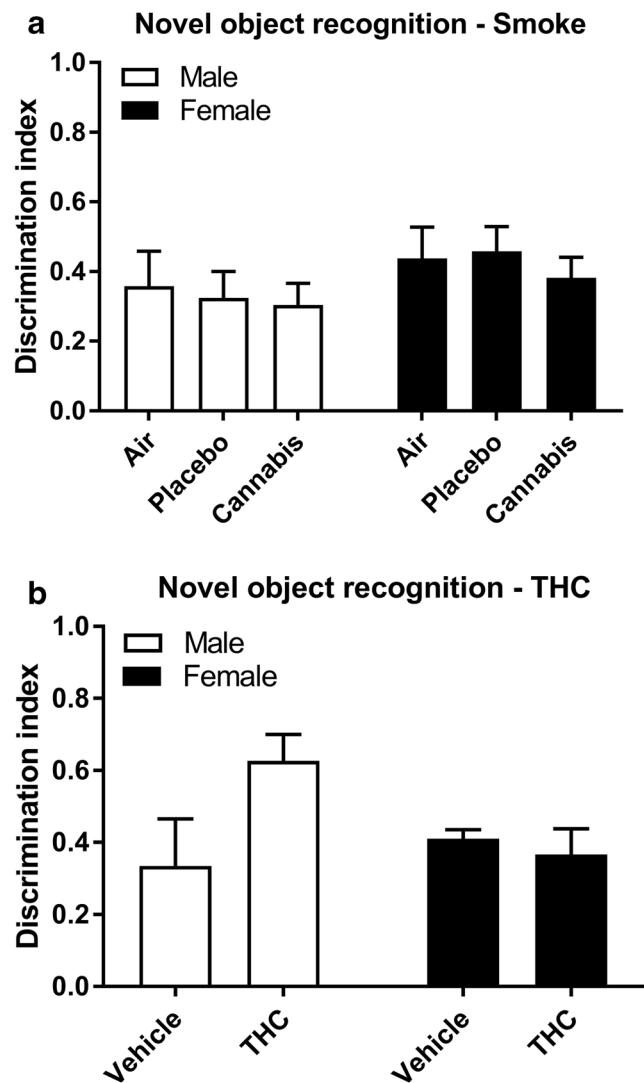


Fig. 4 Exposure to cannabis smoke or THC during adolescence does not affect the discrimination index in the novel object recognition test. Male and female rats were exposed to cannabis smoke (a) or THC (b) and tested in the novel object recognition test in adulthood. Cannabis smoke, placebo smoke, and air: $n = 12/\text{group}/\text{sex}$. THC and vehicle: $n = 10/\text{group}/\text{sex}$. Data are expressed as means \pm SEM

THC and the novel object recognition test

In the novel object recognition test, there were no main effects of either drug condition or sex on the discrimination index (Fig. 4b). There was a trend towards an interaction between sex and drug condition on the discrimination index measure ($F_{1,36} = 4.029$, $P = 0.052$, $\eta^2_p = 0.101$), with a trend towards a larger discrimination index (indicative of better retention) in THC- compared with vehicle-treated males. Analyses of the other performance measures during the novel object recognition test showed that females spent more time with the objects than males ($F_{1,36} = 33.99$, $P < 0.0001$, $\eta^2_p = 0.486$, Figure S8a), and that this difference was evident in both time spent with the novel objects ($F_{1,36} = 19.64$, $P < 0.0001$, $\eta^2_p =$

0.353, Figure S8b) and time spent with the familiar objects ($F_{1,36} = 31.94$, $P < 0.0001$, $\eta^2_p = 0.47$, Figure S8c). In addition, there was a significant interaction between sex and drug condition on time spent with the familiar objects (sex \times THC, $F_{1,36} = 4.15$; $P < 0.05$; $\eta^2_p = 0.103$; Figure S8c). Post hoc comparisons revealed that the female-THC rats spent more time with the familiar object than the male-THC rats.

Discussion

In the present studies, we evaluated the effects of adolescent exposure to cannabis smoke or THC on anxiety-like behavior, depressive-like behavior, and cognition in adulthood. In contrast to previous work on adult outcomes of adolescent cannabinoid exposure in rodent models, we observed few and only small effects of either cannabis smoke or THC exposure on any of the behavioral tests. These data suggest that adverse adult outcomes are not inevitable consequences of even relatively extensive regimens of adolescent cannabinoid exposure.

Numerous prior studies have shown that dysregulated affect and impaired cognition in adulthood can be caused by a variety of adolescent cannabinoid exposure regimens in rats, using both THC and synthetic cannabinoids such as WIN 55,212-2 or CP 55,940 (Cha et al. 2006; O'Shea et al. 2004; Quinn et al. 2008; Realini et al. 2011; Rubino et al. 2009b; Rubino et al. 2008; Schneider and Koch 2003). For example, adolescent THC exposure decreased sucrose preference in adult male and female rats and led to increased immobility in the forced swim test in adult female rats (Rubino et al. 2008). Findings of adverse effects of adolescent cannabinoid exposure are, however, not consistent in the published literature. For example, adolescent treatment with THC did not affect spatial and non-spatial learning in the water maze in adult male rats (Cha et al. 2006). In addition, even in studies in which adverse effects of cannabinoid exposure are reported on some measures, there are often no effects on other measures in the same animals, suggesting that cannabinoid effects on affect and cognition are by no means universal (Rubino et al. 2008).

Given that there was no evidence for adverse effects on affective/cognitive outcomes in the present experiments, it is important to consider potential explanations. One possibility is that the THC doses to which the adolescent rats were exposed were too low to produce lasting behavioral alterations. The smoke exposure study was designed to model the most frequent route of human cannabis ingestion (inhalation of smoked cannabis), and exposure to smoke from 5 cannabis or placebo cigarettes/day (across 1 h/day) took place over 21 days. Recent work from our labs using a highly sensitive and selective liquid chromatography-tandem mass spectrometry method showed that these same cannabis smoke exposure conditions yielded plasma THC levels of 12 ng/ml, 10–30 min after exposure (Ravula et al. 2018). As plasma THC levels

drop rapidly after cessation of cannabis smoke inhalation (Heuberger et al. 2015), it is likely that peak levels are even higher. Indeed, non-compartmental analysis of our prior pharmacokinetic data suggests that the maximum THC concentration (C_{max}) after exposure to 5 cannabis cigarettes is approximately 18 ng/ml (Gabrielsson and Weiner 2012). The maximum THC concentration in humans after smoking one 5.9% THC cannabis cigarette varies between 1.9 and 43.6 ng/ml (average 28.3 ng/ml) (Lee et al. 2015). Thus, the maximum THC level in our cannabis smoke study is in the low to middle range of what has been observed after cannabis smoking in humans. In addition, we showed in previous work in adult rats that the cannabis smoke exposure regimen used in the present study (5 consecutively smoked cannabis cigarettes) produces both acute (locomotor activity changes, alterations in working memory) and chronic (dependence) effects on behavior. Hence, irrespective of plasma THC levels, the exposure regimen is sufficient to yield behaviorally active THC levels (Blaes et al. 2018; Bruijnzeel et al. 2016).

In order to address issues of potentially insufficient dosing following smoke exposure, however, experiment 2 employed an adolescent THC exposure regimen that was identical to that used in a series of experiments by Rubino and colleagues that caused depressive-like behavior and cognitive impairments in adulthood (Rubino et al. 2009b; Rubino et al. 2008). As with cannabis smoke, this THC regimen did not affect adult behavior in this present study; it is important to consider, however, that the doses in this regimen (2.5–10.0 mg/kg) are higher than those that would be consumed voluntarily. Place conditioning and taste aversion experiments in rats indicate that THC doses higher than 1–3 mg/kg produce aversive effects (Braida et al. 2004; Elsmore and Fletcher 1972; Parker and Gillies 1995). Furthermore, rats that self-administer THC consume 0.5 mg/kg or less in 1.5–2-h sessions (Spencer et al. 2018; Wakeford et al. 2017). In addition, THC doses in the range employed here and by others yield plasma THC levels that are 10–100 times greater than those observed in humans smoking cannabis. For example, plasma THC levels are roughly 9000 ng/ml after an i.v. bolus of 5 mg/kg THC in rats, whereas they are roughly 80 ng/ml after ad libitum cannabis smoking in humans (Lee et al. 2015; Rubino et al. 2008). Even accounting for differences in species and routes of administration, it seems likely that the THC doses employed in the present study more closely model conditions of cannabis toxicity or overdose than recreational use. The fact that THC significantly attenuated normal increases in body weight during the exposure period (as it did in previous work with this regimen; Rubino et al. 2008) is consistent with this interpretation.

Aside from doses, another potential account for the absence of behavioral consequences of the adolescent exposure regimens in the present study concerns the rat strains used. Previous studies assessing the effects of adolescent cannabinoid exposure (including the work of Rubino and colleagues)

have employed Sprague-Dawley rats, whereas the present study employed Long-Evans rats. There is some evidence for strain differences in responses to cannabinoids (Deiana et al. 2007; Lepore et al. 1996). In addition, Sprague-Dawley and Long-Evans rats exhibit significant differences in their response to stressors, such that chronic mild stress diminishes stress-induced corticosterone release in Long-Evans rats but not in Sprague-Dawley rats (Bielajew et al. 2002). As both smoke inhalation and THC administration are likely to be perceived as stressful, it is possible that Long-Evans rats are more protected from the effects of developmental stressors (Zuardi et al. 1984). Such differences might account for the failure to observe adverse effects of smoke or THC in the present study. It is also unlikely that age differences at the time of testing could account for differences between our study and those previous. In the present study, the sucrose preference test and forced swim test were conducted from P93–P98, while Rubino and colleagues conducted these tests from P75–P100 (Realini et al. 2011; Rubino et al. 2008).

Although there were no main effects of cannabis smoke or THC on any of the behaviors tested, a close look at the findings revealed that exposure to either cannabis smoke or THC attenuated sex differences on several of the task performance measures. In both experiments 1 and 2, rats in the control conditions showed large sex differences in performance in the open field, such that females had more center zone entries and spent more time in the center zone than males, potentially indicative of less anxiety-like behavior in females. This sex difference was diminished after exposure to cannabis smoke or THC. Exposure to cannabis smoke also attenuated the sex difference in latency to enter the center zone of the open field, and exposure to THC attenuated the sex difference in immobility in the forced swim test. Notably, similar sex-specific behavioral changes have been observed in mice after exposure to estrogenic endocrine disruptors during pregnancy and the early postpartum period, such that males exposed to endocrine disruptors (e.g., the plastic derivative bisphenol A and the insecticide methoxychlor) display more female-like behavior, and females exposed to endocrine disruptors display more male-like behavior (Gioiosa et al. 2007). Rubino and colleagues measured plasma estradiol levels immediately following the THC regimen used in the present study and found no changes in estradiol levels (Rubino et al. 2015). It is possible, however, that THC and other cannabinoids affect estrogen receptor (ER) signaling in the brain. ER-deficient male mice exhibit female-type behavior in the open field test (e.g., increase in center entries) (Ogawa et al. 1997). Furthermore, there is evidence that THC may cause anti-estrogenic effects by upregulating ER β receptor expression and the formation of ER α /ER β dimers (Takeda 2014). Considering these similarities in the effects of endocrine disruptors and cannabinoids, it is possible that some of the effects of cannabis smoke and THC exposure in the present study are due to disruption of ER signaling mechanisms.

Arguing against this possibility, however, is the fact that the effects of placebo smoke in the open field test were somewhat similar to those of cannabis smoke (e.g., Figure S2). This suggests that, at least in the smoke exposure study, factors aside from THC might affect the expression of sex differences. Burning cannabis plant material generates high levels of polycyclic aromatic hydrocarbons, which are also potent endocrine disruptors (Moir et al. 2007; Santodonato 1997). Therefore, smoking cannabis might lead to inhalation of compounds that could disrupt endocrine signaling and ultimately the expression of sex-specific behaviors later in life.

Aside from effects of cannabinoid exposure, the present studies revealed the presence of robust sex differences on several of the behavioral tests. In the cannabis smoke experiment, compared with males, females traveled a greater distance in the center of the open field, had shorter latencies to enter the center zone, spent more time in the center zone, and made more entries into the center zone. A similar pattern of results was observed in the vehicle-control males and females in the THC experiment. This finding is in line with previous studies of open field locomotion showing that female rats travel a greater distance in the open field than male rats (Brotto et al. 2000; Masur et al. 1980; Romero and Chen 2004; Slob et al. 1986). As anxiolytic drugs such as chlordiazepoxide and diazepam increase entries into the center zone of the open field and time spent in the center of the open field (Britton and Britton 1981; Gentsch et al. 1987; Prut and Belzung 2003), these data could indicate that females are less anxious than males in this test. A similar pattern of results was observed in the elevated plus maze test, in which females spent a greater percentage of time on the open arms and had a higher percentage of open arm entries. Anxiolytic drugs increase open arm time and open arm entries, and therefore this pattern of results suggest that the females are less anxious than the males. The results of the present study are in line with a previous study in which adult female rats made a greater percentage of entries into the open arms and showed a trend towards an increase in the percentage of time spent on the open arms (Johnston and File 1991).

In the forced swim test, there were no main effects of sex or exposure condition on immobility, swimming, or climbing, although in experiment 2 there was a sex \times drug condition interaction. Follow-up analyses indicated that vehicle females displayed less immobility than the vehicle males, and that this effect was diminished after THC treatment. Immobility in the forced swim test has traditionally been interpreted as a state of “behavioral despair” and resignation (Castagné et al. 2009), although more recent studies have questioned the validity of this interpretation (De Pablo et al. 1989; Molendijk and de Kloet 2015). It has also been suggested that increasing immobility is an adaptive coping strategy that helps to save energy and increase chance of survival. Several studies have compared sex differences in the forced swim test. Interestingly, some of these studies reported that females display less

immobility than males (Alonso et al. 1991a; Alonso et al. 1991b) although other studies have found no sex differences (Armario et al. 1995; Frye and Walf 2002).

In conclusion, these studies found no evidence that repeated exposure to either cannabis smoke or THC during adolescence in rats produces adverse affective or cognitive outcomes during adulthood, although cannabinoid exposure appeared to attenuate sex differences in some aspects of anxiety- and depressive-like behavior. Although the failure here to observe such adverse effects may have been due to the particular rat strain employed, it was unlikely due to insufficient dosing, particularly because the THC regimen was identical to one used previously to produce such adverse effects. In addition, it is important to note that although rats underwent a fairly extensive battery of affective testing, cognitive function was tested with only a single measure (novel object recognition, which is sensitive to some forms of hippocampal and prefrontal cortical disruption) (Cohen and Stackman Jr 2015). Finally, although adolescent cannabinoid exposure did not robustly affect adult behavior in the current studies, it is important to appreciate the limitations of rodents with respect to modeling human development and substance use, including the duration of substance intake during adolescence, the self-selected nature of the human cannabis-using population, and frequent concomitants of human cannabis use including psychiatric disorders, environmental stressors, and polysubstance use (Liu et al. 2018). Going forward, it will be important to address such limitations experimentally (e.g., by using cannabinoid self-administration models and studying the interactions between cannabis use and comorbidities), in order to better model the impact of cannabis on the developing brain and behavior.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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