EFFECTS OF ORAL DELTA-9-TETRAHYDROCANNIBINOL (THC) ADMINISTRATION ON MOTOR ACTIVITY AND ANXIETY-LIKE BEHAVIOR IN ADOLESCENT AND ADULT SPRAGUE-DAWLEY RATS OF BOTH SEXES

BY

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THESIS

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ABSTRACT

Cannabis is one of the most commonly used recreational drugs among adolescents and young adults. Exposure to cannabis and its primary psychoactive component delta-9-tetrahydrocannabinol (THC) may induce modifications in neural circuitry that in turn lead to adverse consequences on behavior. Previous literature has suggested that intraperitoneal, intragastric, or subcutaneous administration of THC may produce an anxiogenic effect and reduce locomotor activity in rats, but it is unclear if this also occurs following volitional, oral intake of THC. Here, we used adolescent and adult rats of both sexes to determine if oral THC would alter locomotor activity in an open-field arena (OFA) and anxiety-like behavior on an elevated plus maze (EPM). Subjects received vehicle- or THC-impregnated crackers (3.0, 5.0, and 10.0 mg/kg) starting on either P35-37 for adolescent-onset groups or P79-81 for adult-onset groups 90 min prior to each behavioral test. Our data suggests that THC induced a dose-dependent decrease in locomotor activity in the OFA. Additionally, drug-treated animals differed in time spent in the open arms in the EPM, however these results were not statistically significant. These locomotor effects appear to be dependent on both age and sex, as adult males exhibited a relatively greater sensitivity to the effects of THC compared to adult females. Furthermore, these results suggest that rats will voluntarily consume THC-laced crackers at doses that influence locomotor activity and anxiety-related behavior.

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INTRODUCTION

Cannabis is one of the most commonly used recreational drugs among adolescents, and its main psychoactive constituent is Δ9-tetrahydrocannabinol (THC). Approximately 47% of adolescents and young adults ranging from ages 12-26 have reported using cannabis at least once in the past year (National Institute of Health [NIH], 2019). Furthermore, state-level recreational legalization of cannabis is becoming more widely accepted in the United States (Hopfer, 2014). Therefore, due to the decreasing stigma of using cannabis, individuals may be more inclined to partake in this drug and engage in different cannabis use practices (Giombi et al., 2018). Oral intake via edibles has become increasingly popular (Barrus et al., 2016), yet knowledge about the potential health risks from this route of administration is limited. There is a clear need to examine the consequences of cannabis consumption in adolescents, as they may be particularly vulnerable to the potential transient and long-term effects.

Compared to adults, adolescents are more likely to initiate drug use and develop substance use disorders (Casey et al. 2008). Current theories have proposed unbalanced neural structures, where an underdeveloped prefrontal cortex (PFC) coupled with mature nucleus accumbens (NAc) underlies adolescent impulsivity in reward seeking (Steinberg et al., 2009). For example, functional magnetic imaging studies (fMRIs) have demonstrated that adolescents tend to have greater activation of limbic regions during reward processing compared to adults. Galvan et al. (2006) assessed behavioral and neural responses to rewards and showed that adolescents had enhanced neural activity in the NAc and greater recruitment of neurons in the orbital frontal cortex (OFC). Evidence from cognitive tasks such as Stroop and Go/NoGo have already shown that adolescents display increased patterns of neuronal activity in brain areas that reflect cognitive control compared to adults; therefore, greater and more dispersed neural activity

in the OFC suggest an underdeveloped structure (Casey et al., 2005). Indeed, fine-tuned neural activity indicates a mature brain structure. These findings reflect a disproportional system where subcortical regions mature much sooner than cortical regions, ensuring an increased motivation for rewards during adolescence.

Rodent models have provided mixed findings regarding adolescent hypersensitivity to reward. In an experiment of cocaine self-administration, adolescent rats lever pressed more for cocaine than their adult counterparts, even during periods of signaled non-availability (Anker et al., 2011). This increased non-reinforced responding may be indicative of increased reward salience and reduced inhibitory control, as subjects are unable to inhibit a previously reinforced response. Some research, however, has suggested that adolescents are not hypersensitive to drug reward. Our lab has shown that adolescent rats are more motivated to lever press for non-drug rewards (e.g., food pellets and sweetened condensed milk) compared to their adult-counterparts (Westbrook et al., 2018). However, results from subsequent experiments showed that adolescents had greater behavioral flexibility and had similar sensitivity to reward value compared to adults. Therefore, results from these latter studies do not support theories of adolescent vulnerability to reward. It is likely that other factors such as pubertal status and motivational states may influence adolescent behavior in rodents (Westbrook et al., 2018).

Research has reported that cannabis use reduces anxiety (Johns et al., 2001). Therefore, it is likely that individuals with a depressive disorder may be more inclined to use cannabis, as they are more prone to develop anxiety compared to the average individual. Schneider et al. (2005) investigated the effects of long-term THC exposure during the juvenile period through mid-adolescence (Postnatal day (P) 15 –P 40) and reported long-lasting changes in anxiety-related behaviors when tested in adulthood. During the adolescent period, rats received 20

intraperitoneal injections (i.p.) that were delivered irregularly (e.g., rats received a single injection of THC on some days, but on other days they did not receive any THC or they received two injections) so as to mimic irregular consumption in humans. In this study, rats that were exposed to THC during adolescence spent significantly less time in the center of an open field arena (OFA) and showed reductions of exploratory behavior, which was considered to be an index of anxiety-related activity.

Human and animal studies have also examined the effects of THC following acute exposure. For example, oral THC (0, 5, 15 and a high dose of either 25 or 30 mg) was administered to 30 healthy female and male participants and physiological measures and symptom ratings were assessed 1 hr post drug administration (Fogel et al., 2017). Participants who consumed THC reported both positive and negative experiences (e.g., a good effect or a bad effect according to the Visual Analog Scale or VAS). In an animal study, male rats were injected with increasing doses of CP 55,940 (0.125, 0.1 and 0.075 mg/kg; i.p.) and were evaluated on an elevated plus maze (EPM) 30 min post drug administration. Drug-treated rats spent less time in the open arms and showed reduced locomotor activity compared to the vehicle group (Arevalo et al. 2001). O'Brien et al. (2013) also administered THC (2.5 mg/kg; i.p.) to rats and reported an anxiogenic response when animals were tested on the light-dark (LD) test. In this experiment, THC-treated animals spent significantly less time in the light box than control animals, indicating an anxiogenic effect. Furthermore, Hložek et al. (2017) reported that forced exposure of THC via oral gavage caused a reduction of locomotor activity in the OFA. Although these experiments have reported similar effects on locomotor activity and anxiety, it is unclear if these behaviors are specific to age and sex. It is important to address this deficiency, as these two factors may contribute to a heightened vulnerability to drug use (Casey et al. 2008).

Several rodent models have already investigated the effects of THC, but most of these studies have used the parenteral route of administration. Indeed, very few studies exist on the behavioral effects of THC following volitional, oral intake. Some research has examined the effects of THC via oral gavage; however, this is not a sufficient model of drug self-administration as subjects are not allowed to administer the drug themselves. Oral gavage is also likely a stressful procedure, thereby further influencing the animal's behavior. For example, rats who experienced negative affective states showed reductions in operant responding for sucrose (Bai et al., 2019). Certainly, this method of drug administration may induce undesirable behaviors that are not attributed to the drug itself.

The purpose of this present study was to show that rats would quickly and voluntarily consume THC-laced crackers that would subsequently produce a psychoactive effect. Nelson et al. (2019) already reported that this method of THC administration induces a behavioral effect in rats, as THC-treated animals increased their food intake. This study, however, investigated the behavioral effects of THC after chronic exposure. Our goal was to provide additional evidence that this model of volitional drug administration could be used to detect the acute, psychoactive effects of THC. Here, we used a rat model to determine if THC-laced crackers would alter locomotor activity in an OFA and anxiety-like behavior on an EPM. We hypothesized that THC would cause significant reductions in locomotor activity in the OFA accompanied by reduced exploration of open arms on the EPM. We also determined that adolescents may be more sensitive to the effects of THC compared to their adult counterparts.

MATERIALS AND METHODS

Subjects

Animals were male (n = 81) and female (n = 72) Sprague-Dawley rats born in-house from male and female breeders originally obtained from Envigo (Indianapolis, IN). Subjects were weaned on P 22 and paired housed with *ad libitum* access to food and water. They remained in a temperature-controlled room on a 12-hr reversed light/dark cycle (lights off at 0900) and were weighed daily beginning on P 25. Pubertal onset was estimated by checking vaginal opening in females and preputial separation in males (Castellano et al., 2011) starting on P 30 and continuing until all rats reached puberty (figure 1). All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Illinois, Urbana- Champaign, and were consistent with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

Apparatus

Locomotor activity was measured in an OFA, which consisted of a clear acrylic box (41 X 41 X 41-cm) and two surrounding photobeam frames (16 beams/dimension; 2.5 cm between beams). The arena was located inside a sound-attenuating cubicle (76 X 80 X 63-cm) that was illuminated by a red ceiling light (4W). Mounted on the ceiling was a video camera that was used to record all behavioral sessions; a speaker was also mounted near the ceiling and it was used to play a tone (~53.5 dB) for the duration of test sessions in order to mask extraneous noise. To analyze these tests, each OFA was linked to a nearby computer running software (Truscan, 2.01, Coulbourn Instruments) that recorded photobeam breaks in the upper and lower planes at a 500 ms sampling rate. Lastly, animals were transferred from their colony to the testing room in

groups of 2 or 4 and they were allowed a 5-min habituation period where they remained undisturbed before testing. Data from 15 adolescents (10 females, 5 males) and 25 adult rats (11 females, 14 males) were excluded from the final analysis due to technical problems or because the rat did not eat its assigned dose of the THC-laced cracker on the testing day, or because they did not have a litter-matched rat in all other groups.

Anxiety-related behavior was measured in an EPM after drug-treated animals received their second exposure to the highest dose of THC (vehicle or 10.0 mg/kg cracker). The apparatus was a wooden, plus-shaped maze that was painted black and elevated 94 cm above the floor. Two closed arms and two open arms that were both 110 cm long formed an unenclosed central square (10.16 × 10.16 cm). The closed arms were supported by three wooden walls that were 42.5 cm high, whereas the open arms remained completely unprotected. Experiments were conducted in a dark room that was dimly illuminated with white light (~40.1 lm) and were analyzed using tracking software (Anymaze, 4.99, Stoelting Company). Data from 22 adolescents (14 females, 8 males) and 29 adult rats (13 females, 16 males) were excluded from the final analysis due to rats not eating their THC-laced cracker on the testing day or because they did not have a litter-matched rat in all other groups.

Drug Treatment and Behavioral Testing

Before drug administration, rats were temporarily separated by Plexiglas cage dividers so that each individual's drug intake could be accurately monitored. The contents of a 10 mg dronabinol liquigel capsule, which are commercially available (Actavis Pharm, Inc.; Parsippany, NJ, USA) and contain a synthetic form of THC, were extracted using a 1 ml syringe. The capsule contents were then suspended in sesame oil at concentrations of .016 mg/µL, .026 mg/

µL, and .051 mg/ µL to prepare the 3-, 5-, and 10.0 mg/kg doses. The remaining stock solution was pipetted onto a cracker (Fudge Brownie Goldfish Grahams, Pepperidge Farm) according to the animal's body weight. At each dose, the concentration was increased so that animals would receive a similar amount of stock solution on their cracker. Sesame oil vehicle was administered isovolumetrically to all corresponding doses for control crackers.

Adolescent and adult rats of both sexes were assigned to one of two treatment groups – control or THC – so that most groups were represented in each litter. Drug-treated animals received intermittent access to THC once every three days, starting at P36 until P45 for adolescents (± 1 day) or P77 – P86 for adults (± 1 day). Vehicle (sesame oil impregnated) crackers were administered for two days after each testing session. Control rats received vehicle crackers throughout the entire experiment. According to Hložek et al. (2017), blood levels of THC administered reach their peak 90-120 min after oral gavage in rats, so behavioral tests were conducted 90 min after cracker self-administration. Before testing, animals were transported individually from their colony to a testing room where they remained for a 5-min acclimation period. For the EPM, rats were placed in the center with their head facing towards an open arm and they were allowed to explore the maze freely for 5 min.

Data Analysis

Horizontal photobeam breaks were used to measure ambulation and vertical photobeam breaks were used to measure rearing, which occurred when rats stood on their hindlimbs. Total distance traveled (m), total number of open arm entries, and % time spent the open arms were evaluated as measures of anxiety-related behaviors. Data for all behavioral tests were analyzed with SAS Statistical Software (SAS Institute Inc., Cary, NC) and are presented as mean ± SEM.

Mixed linear models were utilized for all dependent behavioral measures with litters comprising a random variable. Locomotor activity was analyzed with a three-way repeated measures ANOVA, with treatment, time, and sex as the three factors. Dose response curves for both ambulation and rearing were analyzed with a repeated four-way ANOVA, with testing day, age, sex, and treatment as the four factors. Lastly, anxiety-related behavior was also analyzed with a three-way ANOVA, with treatment, age, and sex as three factors. Significance was determined as $p \leq 0.05$ for all tests.

RESULTS

Puberty

Figure 1 shows average pubertal onset was 36.1 days for female (range: 33-43) and 43.8 days for male rats (range: 40-48). For animals who consumed THC, average pubertal onset was 35.9 days for female (range: 34-37) and 44.2 days for male rats (range: 43-46). Three-way ANOVAs revealed a significant main effect for sex, $F(1, 74) = 362.49$, $p < .0001$.

Locomotor Activity

Shown in Figure 2 are the individual time series data for 20 female (10 THC and 10 vehicle) and 21 male (10 THC and 11 vehicle) adolescent rats during each session. Three-way ANOVAs for the first open field session (vehicle or 3.0 mg/kg cracker) revealed significant main effects for treatment, $F(1, 37) = 6.69$, $p = .0138$; and time, $F(3, 37) = 118.26$, $p < .0001$, as well as a significant sex × time interaction, $F(3, 37) = 3.01$, $p = .0421$ in locomotor activity. There was also a main effect of time for rearing, $F(3, 37) = 53.56$, $p < .0001$, and a significant sex × treatment × time interaction, $F(3, 37) = 3.24$, $p = .0328$. THC-treated rats in the second session (vehicle or 5.0 mg/kg cracker) showed reduced ambulation, $F(1, 37) = 5.25$, $p = .0278$; data also revealed a main effect for time in both ambulation, $F(3, 37) = .31$, $p < .0001$, and in rearing, $F(3, 37) = 29.64$, $p < .0001$. Furthermore, results from the last session (vehicle or 10.0 mg/kg cracker) showed a significant main effect for time, $F(3, 37) = 103.12$, $p < .0001$, as well as a significant treatment × time interaction, $F(3, 37) = 3.12$, $p = .0373$. Post hoc analysis revealed that THC-treated animals significantly reduced ambulation, but only during the first time interval; and, VEH-treated animals significantly reduced ambulation during time intervals 1-3. There was also a main effect of time for rearing, $F(3, 37) = 42.27$, $p < .0001$; and treatment $F(1,$

37) = 7.40, p = .0099, along with a significant treatment × time interaction, $F(3, 37) = 3.68$, p = .0204.

Figure 3 shows the time series data for 19 females (9 THC and 10 vehicle) and 22 male (11 THC and 11 vehicle) adult rats. Three-way ANOVA revealed significant main effects for time, $F(3, 37) = 140.87$, $p < .0001$, as well as a significant sex × time interaction, $F(3, 37) = 4.94$, $p = .0055$ during the first open field session. THC-treated animals reared less than controls, $F(1, 37) = 5.75$, $p = .0217$, and all groups reduced rearing across the four time intervals, $F(3, 111) = 44.72$, $p < .0001$. For ambulation during the second session, there was a main effect for time, $F(3, 37) = 139.28$, $p < .0001$, along with a significant sex × time interaction $F(3, 37) = 4.21$, $p = .0017$ and a significant treatment × time interaction $F(3, 37) = 4.03$, $p = .0141$. Both THC and VEH rats reduced ambulation at time intervals 1-3. Moreover, all groups showed less rearing within the four time intervals $F(3, 36) = 67.51$, $p < .0001$. Results from the last session indicate a main effect for sex in ambulation, $F(1, 37) = 9.73$, $p = .0035$, as well as a significant main effect of time in rearing, $F(3, 111) = 80.90$, $p < .0001$.

Four-way ANOVAs for dose response curves (figure 4) also revealed significant main effects for age, $F(1, 74) = 32.47$, $p < .0001$; and treatment, $F(1, 74) = 13.22$, $p = .0004$, as well as a significant interaction between age and sex, $F(1, 74) = 4.20$, $p = .0440$, and age and session, $F(2, 74) = 6.44$, $p = .0026$. Post hoc analysis indicate that adult females ambulated more than any other group. Adult rats also reared more than adolescents, $F(1, 74) = 49.51$, $p < .0001$, and there was a significant session × age interaction, $F(2, 74) = 6.53$, $p = .0025$.

Elevated Plus Maze

Subjects used to examine anxiety-related behavior in the EPM were adolescent and adult rats (figure 5). Overall, adolescent female rats (10 vehicle and 6 THC) were more active than adolescent males (9 vehicle and 9 THC), F (1, 30) = 6.06, p = .0198. There was also a tendency for adolescent THC-treated rats to have fewer open arm entries, however these results were not significant. Furthermore, adult rats (10 female VEH and 7 female THC; 10 male VEH and 10 male THC) that received THC showed a tendency for reduced open arm entries and less time spent in the open arms, however these results were also not significant.

DISCUSSION

This study used adolescent and adult rats of both sexes to investigate the psychoactive effects of THC following volitional, oral intake. Results from the OFA show that THC-treated adolescent rats significantly reduced ambulation during the first two sessions (3- and 5.0 mg/kg), however this effect was not observed at the highest dose. Instead, rats who consumed 10.0 mg/kg of THC significantly reduced rearing compared to the vehicle group. It is possible that the effects of THC were undetectable during the final session. Here, all groups reduced their exploration of the maze after approximately 10 min of testing, as they were habituated to the apparatus. Our time series graph shows that THC-treated rats significantly reduced ambulation during the first half of the session (figure 2), but this effect was alleviated during the last two time intervals. Given this information, we may need to employ a different method in order to examine the repeated, locomotive effects of this drug. For example, instead of testing subjects three different times using the same test, future research should consider examining this effect in only one dose per group (e.g., a subset of rats gets either 3-, 5-, or 10.0 mg/kg).

Adult drug-treated rats also showed significant reductions in ambulation, but only during the first 15 min of the second session (vehicle or 5.0 mg/kg cracker). Furthermore, these subjects reduced rearing 90 min after consuming the lowest dose of THC (vehicle or 3.0 mg/kg cracker). The dose response curves clearly shows these age-related differences following THC consumption. Therefore, it is likely that adolescents are showing a greater sensitivity to the behavioral effects of THC.

Research has shown that high doses of THC (5–30 mg/kg, i.p.) lead to a reduction of locomotor activity. However, when THC-gelatin was administered to adolescent rats (1.0, 1.5, and 2.0 mg/15ml gelatin), there were no observed differences between control and THC-treated

rats in locomotor activity (Kruse et al., 2019). In this study, rats received 4 hr access to THC and were immediately evaluated in an OFA for 60 min, a time point when rats have habituated to the apparatus. Similar to our final open field session, which is when the 10 mg/kg dose was tested, it is likely that any potential drug effect was obscured due to habituation. Results from the EPM indicated no statistically significant differences in anxiety-related behaviors following THC exposure. However, there was a tendency for both male and female adolescent THC-treated rats to have less open arm entries compared to their vehicle group. Female adolescent rats who consumed 10.0 mg/kg of THC tended to spend less time in the open arms and they had increased exploration of the maze compared to their male counterparts (p = .0198). Furthermore, adult THC-treated rats also tended to have less open arm entries and spend less time in the open arms. Although the effects of THC on anxiety-related behavior were not statistically significant, the tendency for THC-treated animals to have fewer open arm entries and spend less time in the open arms has been previously reported in the literature (0.125, 0.1 and 0.075 mg/kg; i.p.) (Arevalo et al. 2001).

It is possible that we were unable to find any significant differences in anxiety-related behaviors due to limitations of our experiment. In fact, one of the major caveats of this study was that almost half of the animals would not eat the 10.0 mg/kg cracker the second time it was administered (i.e., prior to the EPM test). We determined that this dose of THC was aversive to rats, since they would typically eat their vehicle crackers two days prior. Specifically, rats could have had a negative experience after consuming their first 10.0 mg/kg cracker, or they may have acquired a taste aversion to the high dose of THC. Previous literature has already shown that this drug may be aversive in rats. For example, Han et al. (2017) reported that THC (1, 3, or 5 mg/kg, i.p.) produced dose-dependent conditioned taste and conditioned place aversion in rats.

If our subjects did not consume their THC-laced cracker, they were not included in the analysis; so, all THC groups had a lower sample size relative to their vehicle group. Consequently, it is likely that we were unable to detect any statistical significance in the EPM due to this unbalanced and under-powered design.

We also lost data due to subjects falling off the maze: 6 vehicle rats (2 females and 4 male adolescents) and 2 THC rats (1 female adolescent and 1 male adult) were excluded for this reason. It is possible that a high dose of THC (e.g., 10.0 mg/kg) impaired motor function. Irmia et al. (2015) reported dose-dependent increases in motor impulsivity following THC exposure. In this study, she administered repeated cycles of THC (ten 14-day cycles of Δ9-THC dosing) to rats and found deficits in motor activity via the 5-Choice Serial Reaction Time Task (5-CSRTT). However, it is important to note that this effect was observed after long-term exposure to THC. Furthermore, it could be the case that this response was not mediated at all by THC, as vehicle rats also fell off the EPM.

In the present study, male and female rats in both groups displayed similar effects of locomotor activity and anxiety-related behavior. Previous literature has also suggested a lack of sex differences following THC exposure. For example, Wiley et al. (2007) administered THC (10.0mg/kg; i.p.) to rats 60 min prior to testing and reported that both sexes showed similar reductions of locomotor activity. Javadi-Paydar et al. (2018) also reported similar motoric effects in male and female rats that inhaled THC (12.5, 25, 50, 100, 200 mg/mL) 40 min prior to testing.

Some research, however, has shown the behavioral effects of THC to be greater in females than in males. Harte-Hargrove et al. (2012) reported that females spent significantly less time in the open arms than male rats, indicating a heightened sensitivity to the effects of THC

(15 mg/kg; i.p.). Furthermore, when Craft et al. (2008) examined the behavioral effects of THC (5- or 10 mg/kg; i.p.) in male and female gonadectomized rats, she reported differential effects in rats that were chronically treated with a hormone. Testosterone-treated male rats showed significantly less locomotor suppression than those with no hormone replacement; conversely, estradiol enhanced THC's antinociceptive properties in female rats, but had no change on THC's motoric effects. Therefore, it is likely that sex differences in the effects of THC are specific to the behavioral measure that is being tested. It could also be the case that we did not observe any sex differences due to the potential limitations of our experiment, which includes rats not eating their prior to EPM testing and rats falling off the maze.

Despite these limitations, this study still offers additional support for a useful model of THC self-administration by demonstrating age- and dose-dependent hypolocomotive effects following drug consumption. Edible use is becoming increasingly popular, and this experiment employs a volitional, oral intake model that may be used to examine the potential long-term effects of THC. Future research, however, should avoid extremely high doses of THC in this experimental design.

FIGURES

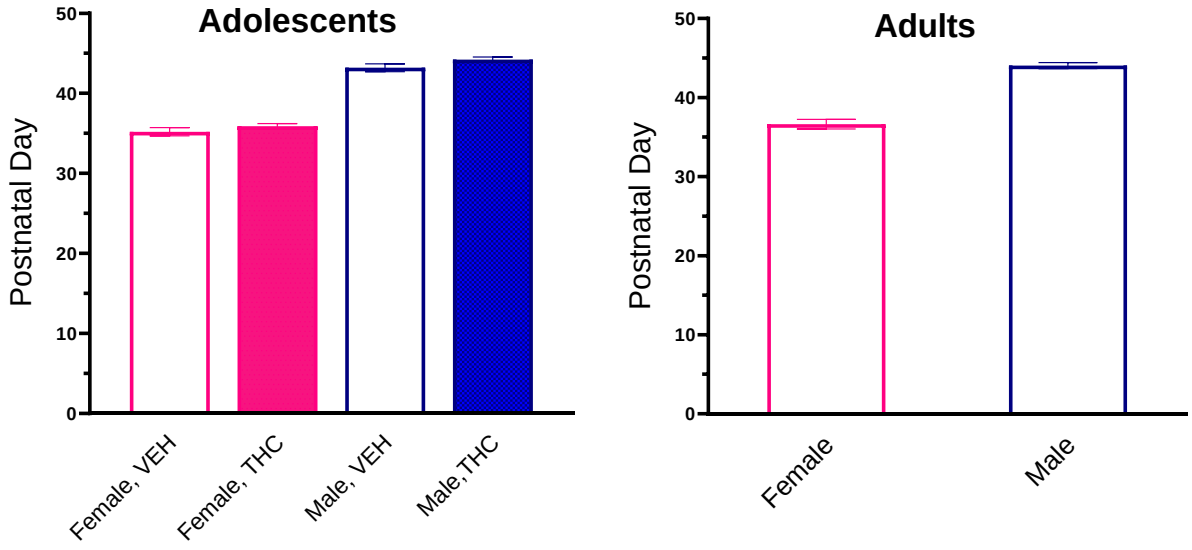

Fig. 1. Average pubertal day for adolescent- and adult- onset rats. Only adolescent rats received THC around pubertal onset, *p <.0001, females vs males collapsed across age and treatment

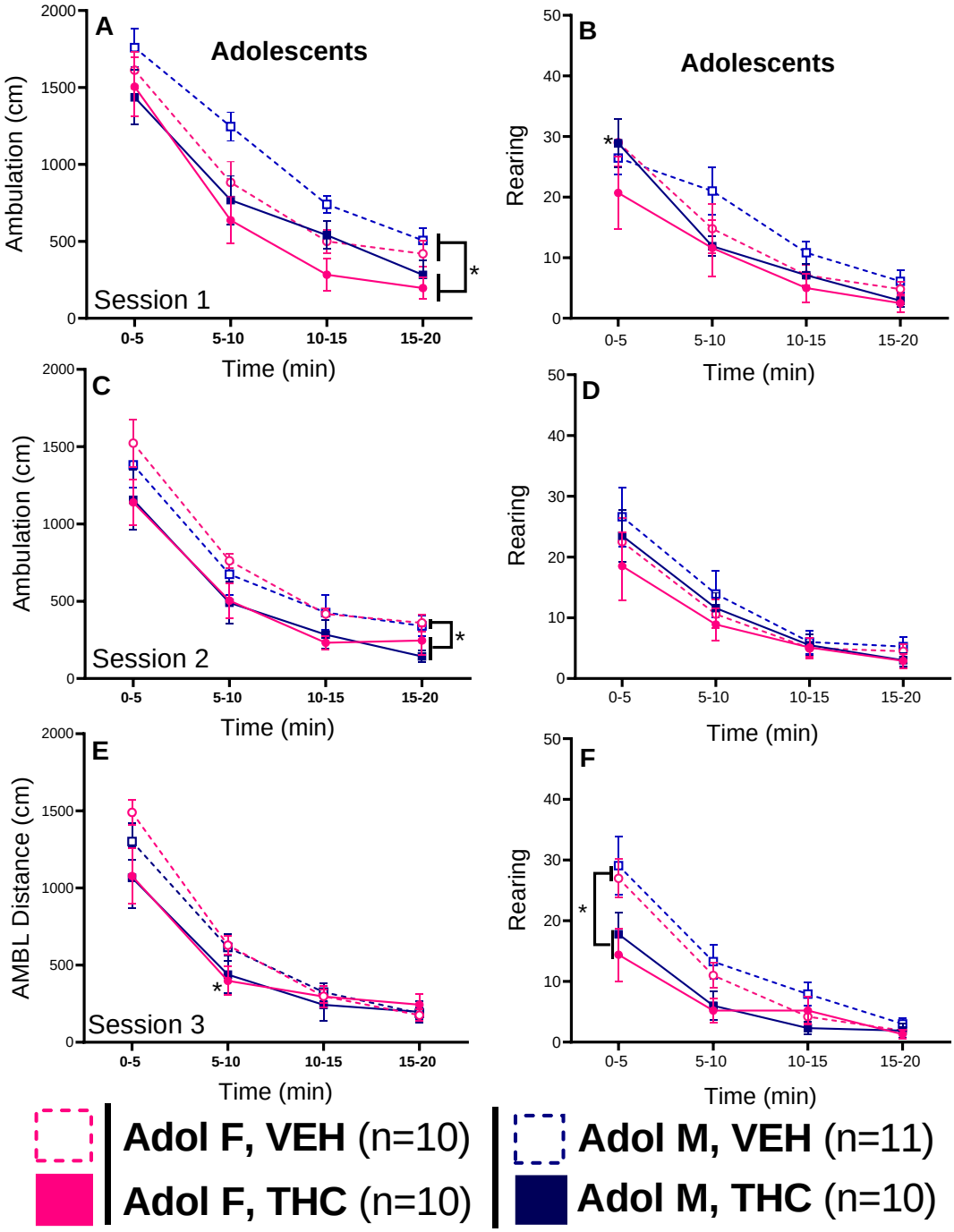

Fig. 2. Effects of THC on behavior in the open-field arena. Shown are the individual time series data for adolescent rats (n=10 -11/group) during each session where THC or vehicle was given 90 min prior to the start of the test. **A,** Total ambulation during the first session (vehicle or 3.0 mg/kg cracker), **p* = .0138, THC vs. VEH collapsed across sex. **B**, Total rearing during the first session (vehicle or 3.0 mg/kg cracker), **p* = .0138, Male THC time interval 1 vs. 2. **C**, Total ambulation in the second session (vehicle or 5.0 mg/kg cracker), **p* = .0278, THC vs. VEH collapsed across sex. **D**, Total rearing during the second session (vehicle or 5.0 mg/kg cracker). E, Total ambulation in the last session (vehicle or 10.0 mg/kg cracker), **p* = .0373, THC-treated rats reduced ambulation vs. the first time interval, collapsed across sex. F, Total rearing in the last session (vehicle or 10.0 mg/kg cracker), **p* = .0099, THC vs. VEH collapsed across sex.

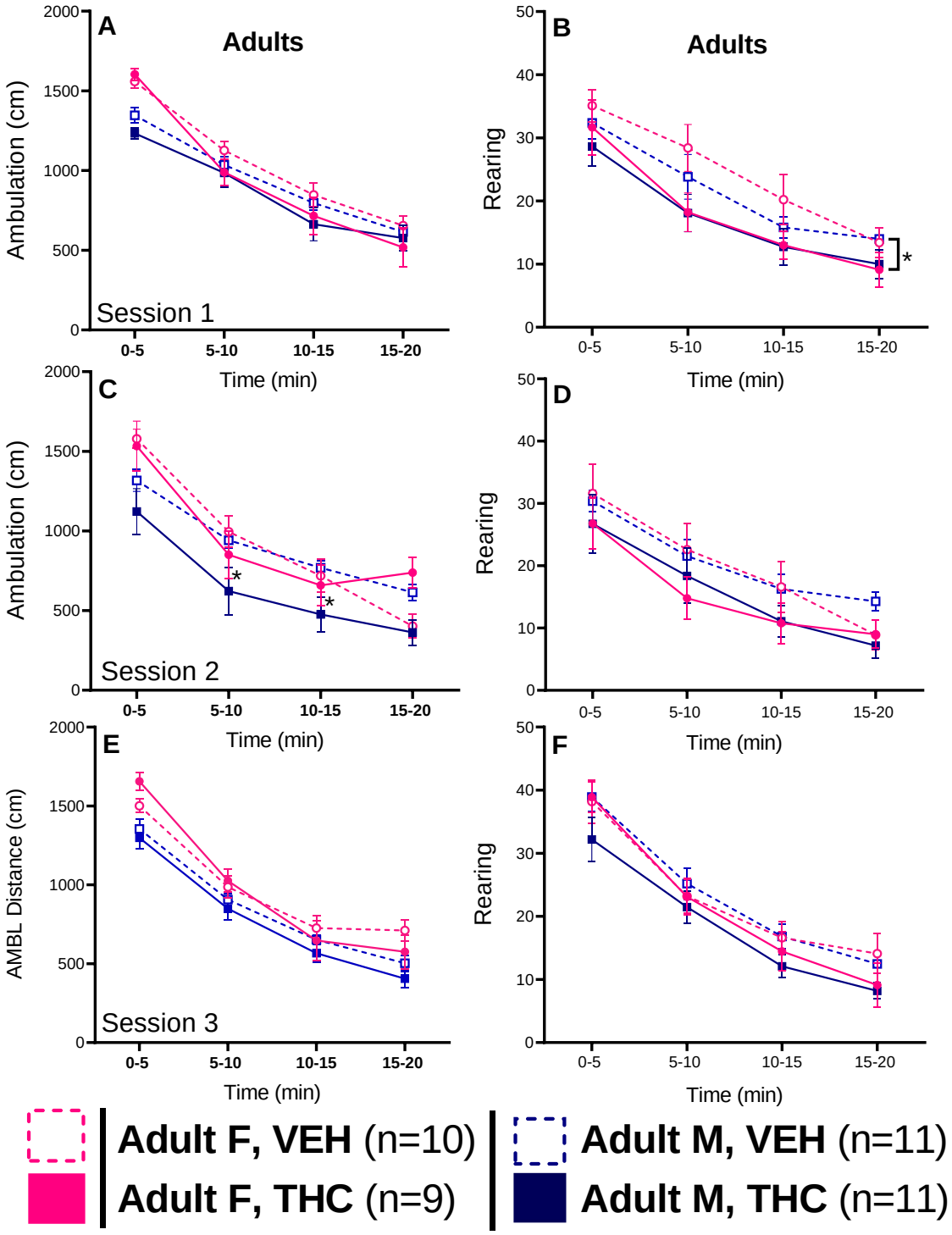

Fig. 3. Effects of THC on behavior in the open-field arena. Shown are the individual time series data for adult rats (n=9 -11/group) during each session where THC or vehicle was given 90 min prior to the start of the test. **A**, Total ambulation during the first session (vehicle or 3.0 mg/kg cracker) **B**, Total rearing during the first session (vehicle or 3.0 mg/kg cracker), **p* = .0217, THC vs. VEH collapsed across sex. **C**, Total ambulation in the second session (vehicle or 5.0 mg/kg cracker), **p* = .0141, THC time interval 1 vs. the 2 and 3, collapsed across sex. **D**, Total rearing during the second session (vehicle or 5.0 mg/kg cracker). **E**, Total ambulation in the last session (vehicle or 10.0 mg/kg cracker). **F**, Total rearing in the last session (vehicle or 10.0 mg/kg cracker).

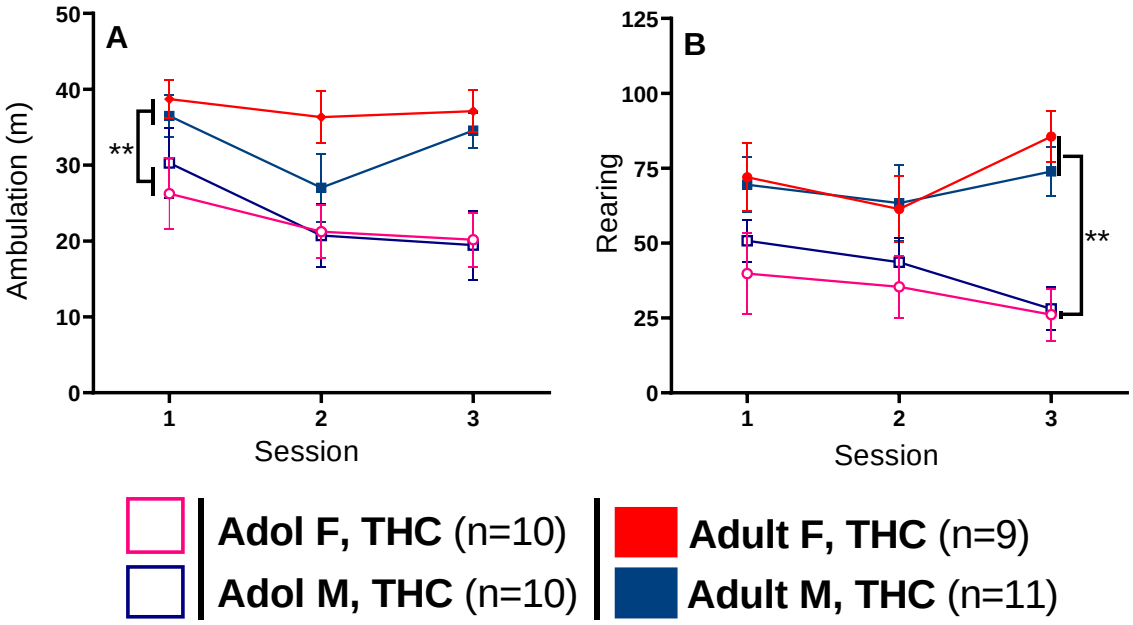

Fig. 4. Cumulative effects of THC on behavior in the open-field arena. Shown are dose response curves for ambulation and rearing (vehicle groups not shown) in both adolescent and adult rats where THC or vehicle was given 90 min prior to the start of the test. **A**, Total ambulation in each session, $^{**}p < .0001$, adolescents vs. adults. **B**, Total rearing in each session, $^{**}p < .0001$, adolescents vs. adults.

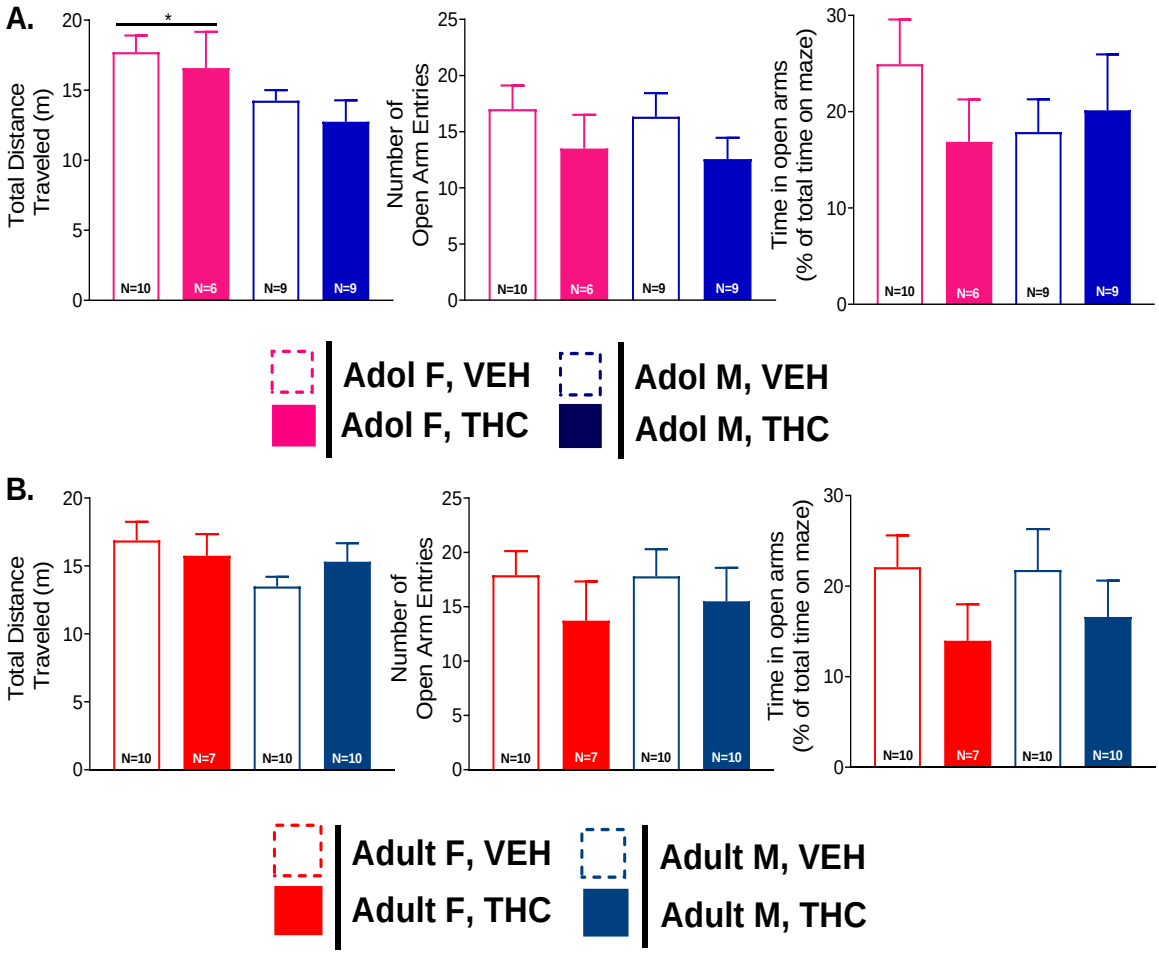

Fig. 5. Anxiety-related behavior in adolescent and adult rats of both sexes. **A**, Total distance traveled, number of open arm entries, and time in open arms for adolescent rats of both sexes, **p* = .0198, adolescent females vs. adolescent males, total distance traveled. **B**, Total distance traveled, number of open arm entries, and time in open arms for adult rats of both sexes.

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