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Pharmacokinetic and pharmacodynamic properties of aerosolized ("vaped") THC in adolescent male and female rats

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Abstract

Rationale Adolescent exposure to Δ^9-tetrahydrocannabinol (THC), the psychotropic constituent of cannabis, might affect brain development, and in rodent models leads to long-term behavioral and physiological alterations. Yet, the basic pharmacology of this drug in adolescent rodents, especially when ingested via ecologically relevant routes like aerosol inhalation, commonly referred to as "vaping," is still poorly characterized. Moreover, sex differences exist in THC metabolism, kinetics, and behavioral effects, but these have not been rigorously examined after vapor dosing in adolescents.

Objectives We investigated the pharmacokinetics and pharmacodynamics of aerosolized THC (30 min inhalation exposure, 25 or 100 mg/ml) in adolescent Wistar rats of both sexes.

Methods Liquid chromatography/mass spectrometry analysis of THC and its major metabolites was conducted on blood plasma and brain tissue at 5, 30, 60, and 120 min following a 30-min aerosol dosing session. Effects on activity in a novel environment for 120 min after aerosol, and temperature, were measured in separate rats.

Results We found sex-dependent differences in the pharmacokinetics of THC and its active (11-OH-THC) and inactive (11-COOH-THC) metabolites in the blood and brain, along with dose- and sex-dependent effects on anxiety-like and exploratory behaviors; namely, greater 11-OH-THC levels accompanied by greater behavioral effects in females at the low dose but similar hypothermic effects in both sexes at the high dose.

Conclusions These results provide a benchmark for dosing adolescent rats with aerosolized (or "vaped") THC, which could facilitate adoption by other labs of this potentially human-relevant THC exposure model to understand cannabis effects on the developing brain.

Keywords THC · Vapor · E-cigarette · Inhalation · Pharmacokinetics · Pharmacodynamics · Anxiety · Locomotion · Blood · Brain

Introduction

The availability of legal cannabis has rapidly spread throughout North America. At the same time, stigma around cannabis use has declined, including in potentially vulnerable populations like adolescents and pregnant women (Bayrampour et al. 2019; Carliner et al. 2017). This is alarming, because human association studies suggest that early-age cannabis use is linked to negative outcomes later in life on cognition, memory, and emotional regulation (Chadwick et al. 2013; Rubino and Parolaro 2015a; Spear 2016; Volkow et al. 2014, 2016). However, it is unclear whether early cannabis use causes later-life deficits or rather if an underlying

Ruiz CM, Torrens A, And Lallai V Denotes equal effort.

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pathology leads to both early cannabis use and negative long-term outcomes.

Animal experiments are thus essential for addressing whether THC might affect the adolescent brain in a persistent manner. Supporting the epidemiological data, these experiments have shown that in adolescent rodents, THC and other cannabinoid receptor agonists, especially when administered at high doses, do in fact produce persistent changes in cognition, emotion, and reward sensitivity, including to other drugs of abuse (Abela et al. 2019; Bruijnzeel et al. 2019; Chadwick et al. 2013; De Gregorio et al. 2020; Friedman et al. 2019; Gibula-Tarlowska et al. 2020; Gomes et al. 2015; Kruse et al. 2019; Lecca et al. 2020, 2019; Miller et al. 2019; Nguyen et al. 2020; Orihuel et al. 2021; Poulia et al. 2020; Renard et al. 2016; Rubino et al. 2012; Schneider 2008; Schoch et al. 2018; Stringfield and Torregrossa 2021). In addition, the persistent effects of THC frequently emerge differentially in male and female rodents (Cooper and Craft 2018; Craft et al. 2013; Dukes et al. 2020; Orihuel et al. 2021; Poulia et al. 2020; Rubino and Parolaro 2011; 2015b; Silva et al. 2016; Stringfield and Torregrossa 2021). In part, this may be due to differences in the biotransformation of THC into its active (11-OH-THC) and inactive (11-COOH-THC) metabolites, with markedly greater persistence of both metabolites in female, relative to male adult (Tseng et al. 2004; Wiley and Burston 2014) and adolescent rats (Ruiz et al. 2021). Importantly, this sex difference in THC metabolism seems also to be present in humans (Nadulski et al. 2005). We therefore contend that such basic pharmacology studies, conducted in both sexes, will be critical for interpreting results of THC administration studies in adolescent rodents and for selecting doses relevant to persons who use cannabis.

Animal models of adolescent THC use are only useful insofar as they realistically model the human condition. In addition to matching overall rodent dosing with THC blood levels achieved by humans after cannabis (Cooper and Haney 2009; Huestis et al. 1992), it is important to consider the role played by the route of administration in THC pharmacology. In rodents, systemic intraperitoneal THC allows precise dosing and yields pharmacokinetic and metabolic profiles that largely, albeit incompletely, mimic those observed in human cannabis smokers (Torrens et al. 2020; Ruiz et al. 2021). However, humans consume legal cannabis products through a variety of routes, including aerosol inhalation (also known as "vaping") and ingestion, but little is known about how these different routes impact pharmacokinetic and pharmacodynamic properties of THC.

In the present study, we adapted previously published whole-body drug aerosol dosing models (Freels et al. 2020; Gilpin et al. 2008, 2014; Glodosky et al. 2020; Hamidullah et al. 2021; Hlozek et al. 2017; Kallupi and George 2017; Manwell and Mallet 2015; Nguyen et al. 2016, 2020, 2019; Smith et al. 2020; Taffe et al. 2014; Weimar et al. 2020) in adolescent male and female Wistar rats, in order to characterize THC aerosol inhalation dosing via blood and brain measurements of THC and its main metabolites over time, as well as effects on behavior and body temperature.

Materials and methods

All procedures were approved by the University of California Irvine IACUC and are in accord with the NIH Guide for the Care and Use of Laboratory Animals.

Subjects Male and female Wistar rats were purchased from Charles River and arrived in our vivarium at postnatal day (PD) 25. Animals were group housed (4/cage) on a 12 h reverse light/dark cycle (testing in the dark phase), with ad libitum food and water in the home cage.

General protocol At PD 31 or PD 32 (day was counterbalanced across testing conditions/ groups), rats received 30 min THC aerosol exposure (general protocol shown in Fig. 1). Rats used for pharmacokinetic analyses ($n = 40$ male, $n = 40$ female) were removed from the chambers ~ 3 min after the 30 min session and placed into their home cages. They were then anesthetized via inhalation of isoflurane at set time points, namely, 5, 15, 60, and 120 min. 2–3 ml of blood was collected from the heart and centrifuged for 10 min at $500 \times g$, and plasma was collected and then centrifuged again at $2000 \times g$ for 10 min to remove any trace of residual cells, which was stored at − 80°C until analysis. Brains were rapidly removed, flash-frozen in methylbutane, and stored at − 80°C until analyses.

Rats used for pharmacodynamic experiments ($n = 30$ male, $n = 33$ female) were implanted on PD 30 with an intraperitoneal temperature programmable microchip (UCT-2112, 2.1×13 mm; Unified Information Devices, Lake Villa, IL), allowing abdominal body temperature measurement before and after vapor exposure. On test

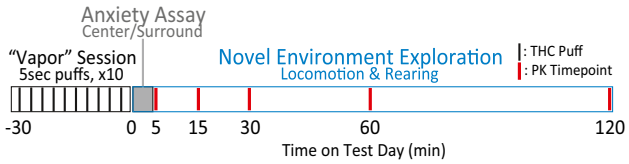

Fig. 1 Aerosol apparatus and experimental timeline. The experimental timeline is shown, with aerosol ("vapor") exposure in occurring over 30 min (ten 5 sec THC puffs at regular intervals). Rats used for pharmacokinetic (PK) experiments were killed at the time points indicated by red lines. Rats used for pharmacodynamic experiments were placed in a novel environment for 2 h following aerosol dosing; the first 5 min was analyzed for center-surround time, and locomotion/rearing was measured for the remainder of the 2 h test

day, male and female rats differed slightly in weight, as expected (m ± SEM grams; males = 106.63 ± 1.88; females = 88.33 ± 1.01). Following 30 min aerosol exposure, rats were immediately placed into 43 × 43 × 30.5 cm novel locomotor testing boxes, in a room with white lighting. Horizontal activity (distance travelled) was automatically tracked for 2 h via ceiling-mounted cameras and EthoVision behavioral tracking software (Vs 15.0). Videos were also scored by an experimenter blind to treatment condition for vertical rearing behavior (number of rears onto the hind legs). Anxiety-like behavior was inferred by analysis of the time spent in the center or surround of the chamber during the first 5 min of the session; the arena was divided into 36 equivalent squares (6 × 6; each 51.37 cm^2): the 16 center squares were designated as a "center zone," and the 20 squares around the edges were designated as the "surround zone." Temperature was measured immediately before vapor exposure and again after the 2 h locomotor test (150 min after the start of vapor exposure).

THC preparation THC in ethanol solvent was generously provided by the NIDA Drug Distribution Program. Ethanol was first evaporated under nitrogen and then dissolved in propylene glycol to dose (25 or 100 mg/ml) while heating to 37°C and sonicating for 15 min. Vaporizer tanks (Smok TFV8 Baby) were filled with 3–4 ml of the THC solution immediately before testing.

Aerosol exposure equipment "Vaping" equipment was designed and manufactured by La Jolla Alcohol Research Inc. (LJARI; San Diego, CA) and were controlled by MedPC hardware and software. LJARI aerosol generators were fourth generation, model 0004–100 W, which rapidly heated the stainless steel coil in the tanks at 61.1 W, 0.4 Ω, to 232.2°C during the 5 sec puff deliveries. Chambers consisted of a 52.7 × 58.4 × 48.8 cm clear, air-tight acrylic box, capable of holding four 22.9 × 21.0 × 43.8 cm clear plastic tub cages with wire tops. Two ports delivered aerosol into the chamber at the upper and lower levels, and four outlet ports allowed a vacuum pump (1.42 psi air compressor) to pull air and aerosol out of the chamber at a steady rate of 1 L/min (achieved via a regulator and flow gauge), resulting in clearing of aerosol from the chamber approximately 3 min after completion of a puff. Exhaust was filtered through a Whatman HEPA-CAP filter and routed to a fume hood for safe clearance.

Aerosol exposure procedure For THC aerosol exposure, rats were placed in pairs (with a cage mate) into tub cages with bedding, on the lower shelf of the larger aerosol containment chambers. They then received aerosolized 25 mg/ml, or 100 mg/ml THC, dissolved in propylene glycol vehicle. Ten, 5 sec puffs were delivered during the 30-min session, one every 175 sec. Approximately 1 ml of each THC solution was aerosolized in the 30 min session using this procedure.

LC–MS analysis of blood and brain Levels of THC

Chemicals and solvents $[^2H_3]$-Δ^9-THC, $[^2H_3]$-11-OH-THC, and $[^2H_3]$-11-COOH-THC were purchased from Cerilliant (Round Rock, TX). All analytical solvents were of the highest grade and were obtained from Honeywell (Muskegon, MI) or Sigma-Aldrich (Saint Louis, MO). Formic acid was from Thermo Fisher (Houston, TX).

Sample preparation Samples were prepared as previously described (Vozella et al. 2019; Ruiz et al. 2021), with minor modifications. Briefly, plasma (0.1 mL) was transferred into 8 mL glass vials (Thermo Fisher), and proteins were precipitated by addition of 0.5 mL ice-cold acetonitrile containing 1% formic acid and the following internal standards (ISTD): $[^2H_3]$-Δ^9-THC, $[^2H_3]$-11-OH-THC, and $[^2H_3]$-11-COOH-THC, 50 pmol each. Half-brains were homogenized in 7 mL ice-cold acetonitrile containing 1% formic acid. The homogenates (0.3 mL) were collected and spiked with 50 pmol ISTD. Plasma and brain samples were stirred vigorously for 30 sec and centrifuged at 2800 × *g* at 4 °C for 15 min. After centrifugation, the supernatants were loaded onto Captiva-Enhanced Matrix Removal (EMR)–Lipid cartridges (Agilent Technologies, Santa Clara, CA) and eluted under vacuum (3–5 mmHg). For brain fractionation, EMR cartridges were pre-washed with water/acetonitrile (1:4, v/v). No pretreatment was necessary for plasma fractionation. Tissue pellets were rinsed with water/acetonitrile (1:4, v/v; 0.2 mL), stirred for 30 sec, and centrifuged at 2800 × *g* at 4°C for 15 min. The supernatants were collected, transferred onto EMR cartridges, eluted, and pooled with the first eluate. The cartridges were washed again with water/acetonitrile (1:4, v/v; 0.2 mL), and vacuum pressure was increased gradually to 10 mmHg to ensure maximal analyte recovery. Eluates were dried under N_2 and reconstituted in 0.1 mL of methanol containing 0.1% formic acid. Samples were transferred to deactivated glass inserts (0.2 mL) placed inside amber glass vials (2 mL; Agilent Technologies).

Liquid chromatography/mass spectrometry (LC–MS/MS) analyses LC separations were carried out using a 1200 series LC system (Agilent Technologies), consisting of a binary pump, degasser, temperature-controlled autosampler, and column compartment coupled to a 6410B triple quadrupole mass spectrometric detector (MSD; Agilent Technologies) (Vozella et al. 2019). Analytes were

separated on an Eclipse XDB C18 column (1.8 µm, 3.0 x 50.0 mm; Agilent Technologies). The mobile phase consisted of water containing 0.1% formic acid as solvent A and methanol containing 0.1% formic acid as solvent B. The flow rate was 1.0 mL/min. The gradient conditions were as follows: starting 75% B to 89% B in 3.0 min, changed to 95% B at 3.01 min, and maintained till 4.5 min to remove any strongly retained materials from the column. Equilibration time was 2.5 min. The column temperature was maintained at 40°C and the autosampler at 9°C. The total analysis time, including re-equilibrium, was 7 min. The injection volume was 5 µL. To prevent carry over, the needle was washed in the autosampler port for 30 sec before each injection using a wash solution consisting of 10% acetone in water/methanol/isopropanol/acetonitrile (1:1:1:1, v/v). The MS was operated in the positive electrospray ionization (ESI) mode, and analytes were quantified by multiple reaction monitoring (MRM) of the following transitions: Δ^9-THC 315.2 > 193.0 m/*z*, [$^2H_3$]-Δ^9-THC 318.2 > 196.1 m/*z*, 11-OH-THC 331.2 > 313.1 m/*z*, [$^2H_3$]-11-OH-THC 334.2 > 316.1 m/*z*, 11-COOH-THC 345.2 > 299.2 m/*z*, and [$^2H_3$]-11-COOH-THC 348.2 > 302.2 m/*z*. In select experiments, we further verified the identity of Δ^9-THC by monitoring the transition 315.2 > 135.0 m/*z*. The capillary voltage was set at 3500 V. The source temperature was 300°C and gas flow was set at 12.0 L/min. Nebulizer pressure was set at 40 psi. Collision energy and fragmentation voltage were set for each analyte as reported (Vozella et al. 2019). The MassHunter software (Agilent Technologies) was used for instrument control, data acquisition, and data analysis.

Statistical analyses Sex-dependent differences in pharmacokinetic parameters (C_{max} and area under the curve; AUC) were assessed using Student's unpaired *t* tests. Behavioral data were analyzed with ANOVAs including THC dose and sex as between-subject variables and time as a within-subject variable when relevant or Student's unpaired *t* tests. One-way ANOVAs in each sex with Tukey post hoc tests were used to determine the nature of main effects and interactions.

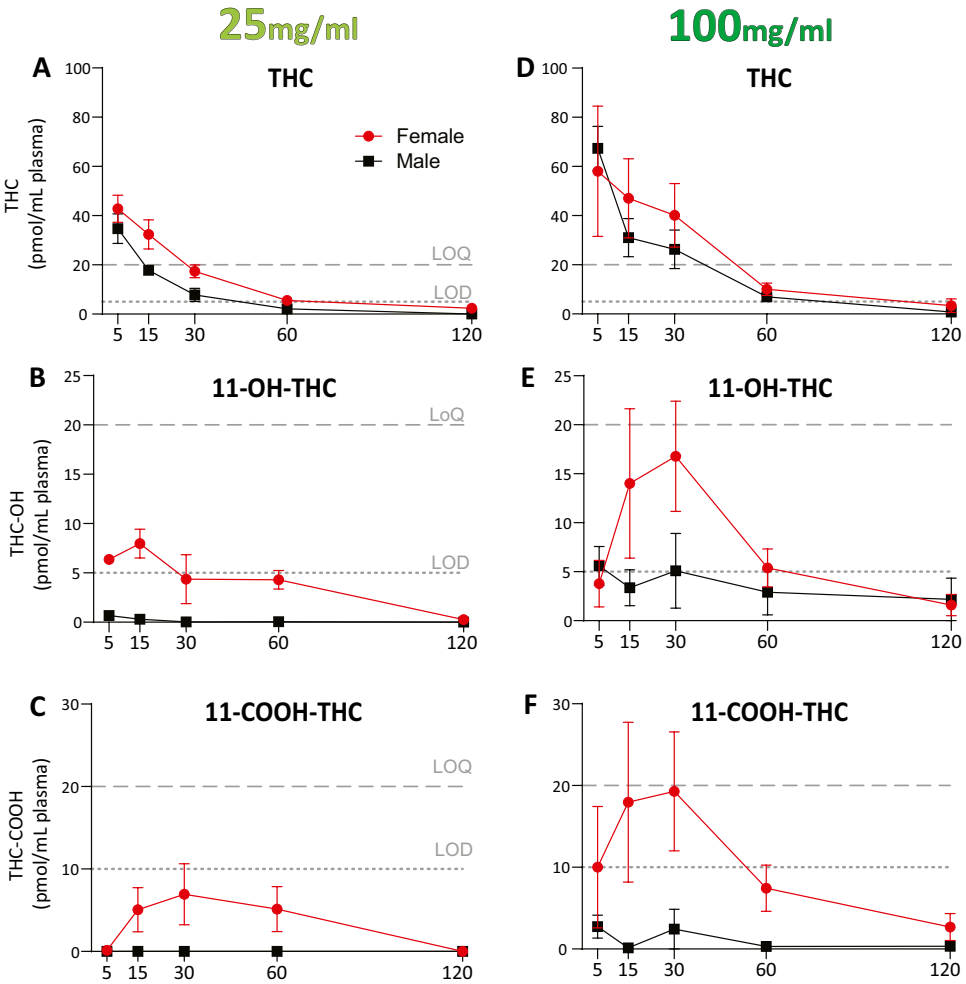

Fig. 2 Plasma concentrations of Δ^9-THC and its first-pass metabolites, 11-OH-THC and 11-COOH-THC, after 30 min (*t* = 0) aerosol exposure of Δ^9-THC. **A**–**C** show the lower 25 mg/mL dose, and **D**–**F** show the higher 100 mg/mL dose in male (black squares) or female (red circles) adolescent rats. Symbols represent the mean ± SEM, *n* = 4–5. Dashed line represents the limit of quantification (LOQ) (20 pmol/mL), and dotted line represents limit of detection (LOD) (5 or 10 pmol/mL) for each analyte, respectively

Results

Pharmacokinetic profile of Δ⁹-THC in rat plasma

Figure 2 shows the plasma pharmacokinetic profiles for THC and its two main first-pass metabolites, 11-OH-THC and 11-COOH-THC, in adolescent (PD 31 or 32) male and female rats after a 30 min aerosol exposure to 25 or 100 mg/mL THC. Table 1 reports the peak concentration values (C_{max}) in plasma, the time at which C_{max} was attained (T_{max}), the half-life time ($t_{1/2}$) of elimination for THC, and the total exposure (area under the curve, AUC) for each of the three analytes.

In male rats, THC reached a C_{max} of 35 and 67 pmol/mL at 5 min for 25 and 100 mg/mL, respectively. The AUC for THC was 667 pmol/min/mL at 25 mg/mL, and 1,653 mg/mL pmol/min/mL at 100 mg/mL C_{max} values for 11-OH-THC were < LOD (5 pmol/mL) at 25 mg/mL and 6 pmol/mL (< LOQ, 20 pmol/mL) at 100 mg/mL. The AUC for 11-OH-THC at 100 mg/mL was 380 pmol/min/mL. For males, 11-COOH-THC values for both doses were below the LOD.

The plasma pharmacokinetic profile of THC was similar in adolescent female rats, compared to males (Fig. 2 and Table 1). At both 25 and 100 mg/mL, the C_{max} values were comparable between the two sexes (Table 1). AUC values for THC were significantly higher or trended to be higher in females for males at both exposures (1329 vs 667 pmol/min/mL, $t_8 = 2.497$; $p = 0.037$ at 25 mg/mL and 2,333 vs 1,653 pmol/min/mL, $t_8 = 0.9581$; $p = 0.37$ at 100 mg/mL). Although not statistically robust, plasma concentrations for THC metabolites trended higher in female than male rats. For example, at 100 mg/mL, the C_{max} for 11-OH-THC was 3 times higher (17 vs 6 pmol/mL, $t_6 = 2.292$; $p = 0.06$), and the AUC was 2.3 times higher (862 vs 380 pmol/min/mL, $t_8 = 1.425$; n.s.). 11-COOH-THC also reached greater C_{max} and AUC at both exposures in females compared to males (Fig. 2C and F, Table 1).

Pharmacokinetic profile of Δ⁹-THC in rat brain

The pharmacokinetic profiles of THC and its metabolites in the brain of adolescent male and female rats are illustrated in Fig. 3. Key pharmacokinetic parameters are reported in Table 2. In male rats at 100 mg/mL, the C_{max} for Δ^9-THC was 170 pmol/g at 5 min, the AUC was 12,366 pmol/min/g, and the brain-to-plasma ratio was 2.6 (Tables 2 and 3). 11-OH-THC reached a C_{max} value of 49 pmol/g at 15 min, and the AUC was 3,222 pmol/min/g with a brain-to-plasma ratio of 7.5 (Tables 2 and 3). 11-COOH-THC was undetectable in the brain at both exposures and in both sexes.

In comparison, at 100 mg/mL, female rats exhibited slightly lower but not significantly different C_{max} values for THC (152 vs 170 pmol/g; $t_6 = 0.50$; n.s.). At 25 mg/mL, C_{max} values for Δ^9-THC were comparable between sexes (129 vs 125 pmol/g at 5 min). The AUC values for THC were also slightly but not significantly higher compared to males at 25 mg/mL and slightly but not significantly lower at 100 mg/mL (7,545 vs 6,834 pmol/min/mg $t_8 = 0.41$ for 25 mg/mL and 10,372 vs 12,366 pmol/min/mg $t_8 = 0.54$ for 100 mg/mL; $ps > 0.5$). However, C_{max} and AUC values for the active metabolite, 11-OH-THC, were significantly higher in females compared to males at both exposures (66 vs 36 pmol/g, $t_6 = 3.64$, $p = 0.01$, and 5931 vs 2849 pmol/min/g, $t_8 = 2.96$, $p = 0.02$ for 25 mg/mL; 90 vs 50 pmol/g, $t_6 = 2.72$, $p = 0.035$, and 8282 vs 3222 pmol/min/g, $t_8 = 2.78$, $p = 0.02$, for 100 mg/mL). Lastly, the brain-to-plasma ratios for THC and 11-OH-THC were similar in females and males for both exposures (Table 3).

Pharmacodynamic profile of THC

Body temperature Prior to aerosol exposure, rats did not differ in body temperature (assigned THC dose, $F_{2,57} = 0.6$, $p = 0.55$; sex, $F_{1,57} = 3.27$, $p = 0.076$). THC lowered

Table 1 Maximal concentration (C_{max}) values in plasma, time at which maximal concentration was reached (T_{max}), half-life of elimination ($t_{1/2}$), and area under the curve (AUC) for Δ^9-THC and its metabolites in adolescent male and female rats after 30 min aerosol exposure of Δ^9-THC (25 or 100 mg/mL). *$p < 0.5$. Student's t test, $n = 4$–5

Analyte	Exposure Δ^9-THC (mg/mL)	Male				Female			
		C_{max} (pmol/mL)	T_{max} (min)	$t_{1/2}$ (min)	AUC (pmol • min/mL)	C_{max} (pmol/mL)	T_{max} (min)	$t_{1/2}$ (min)	AUC (pmol • min/mL)
Δ^9-THC	25	35	5.00	23.9	667	43	5.00	16.7	1329*
	100	58	5.00	18.6	1653	67	5.00	24.2	2333
11-OH-THC	25	< LOD	N/A	-	N/A	6	5.00	-	431
	100	6	5.00	-	380	17*	30.00	-	862
11-COOH-THC	25	< LOD	N/A	-	N/A	7	30.00	-	451
	100	< LOD	N/A	-	N/A	19	30.00	-	1124

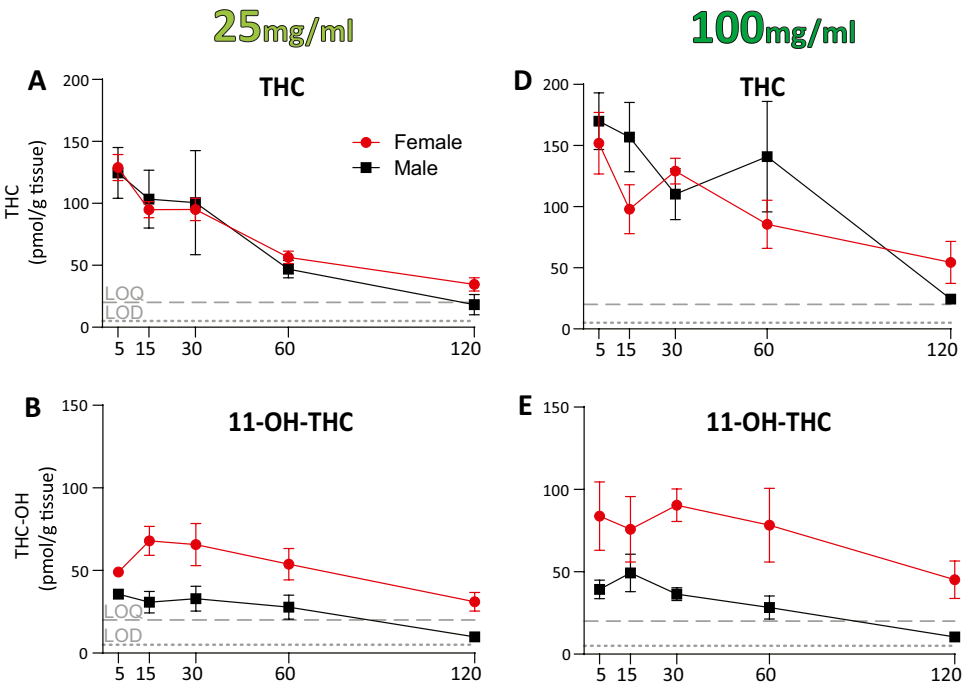

Fig. 3 Brain concentrations of Δ^9-THC and its active first-pass metabolite, 11-OH-THC, after 30 min ($t=0$) aerosol exposure of Δ^9-THC. **A** and **B** show the lower 25 mg/mL dose, and **B** and **C** show the higher 100 mg/mL dose in male (black squares) or female (red circles) adolescents. Symbols represent the mean ± SEM, $n=4$–5. Dashed line represents LOQ (20 pmol/mL), and dotted line represents LOD (5 pmol/mL) for each analyte, respectively. At both exposure levels, 11-COOH-THC was undetectable in brain tissue

temperature in both sexes similarly but did so significantly only at the higher dose [dose (0, 25, 100 mg/ml) × time (pre-vape, 120 min post-aerosol) ANOVA; main effect of dose, $F_{2,57}=4.18$, $p=0.02$; Tukey post hoc on doses versus Veh, 25 mg/ml, $p=0.09$, and 100 mg/ml, $p=0.019$; and no dose × sex interaction, $F_{2,57}=0.43$, $p=0.65$] (Fig. 4A). When change in temperature from pre-aerosol to 120 min post-aerosol measurement was examined, again a main effect of THC ($F_{2,57}=4.55$, $p=0.015$) was seen, without clear sex differences (no sex × dose interaction, $F_{2,57}=0.27$, $p=0.76$) (Fig. 4B).

Anxiety-like behavior THC suppressed the percentage of time spent in the exposed center of a novel environment during the first 5 min of the session (main effect of dose, $F_{2,57}=6.04$, $p=0.004$; 25 mg/ml, $p=0.01$; 100 mg/ml, $p=0.015$), implying a generally anxiogenic effect of aerosolized THC at both doses, with similar effects in both sexes (no main effect of sex, $F_{2,56}=1.84$, $p=0.18$; nor a dose × sex interaction, $F_{2,57}=2.1$, $p=0.13$) (Fig. 4C). This said, when percent center time data were examined separately in each sex, THC suppressed center time in females but only at the low dose ($F_{2,30}=4.16$, $p=0.025$; 25 mg/ml, $p=0.02$; 100 mg/ml, $p=0.49$) (Fig. 4C). In contrast, only high-dose THC significantly suppressed center time in males ($F_{2,27}=3.75$, $p=0.037$; 25 mg/ml, $p=0.15$; 100 mg/ml, $p=0.034$). In this first 5 min period, locomotion was also suppressed in males but not females (sex × dose interaction, $F_{2,56}=3.39$, $p=0.04$; females, $F_{2,30}=2.2$, $p=0.13$; males, $F_{2,27}=3.32$, $p=0.05$), though post hoc comparisons in males showed that neither dose reached statistical significance (25 mg/ml, $p=0.98$; 100 mg/ml, $p=0.068$) (Fig. 4D, top). Rearing in this initial 5 min period was suppressed by low- but

Table 2 Maximal concentration values (C_{max}) in brain, time at which maximal concentration was reached (T_{max}), half-life of elimination ($t_{1/2}$), and area under the curve (AUC) for Δ^9-THC and its active metabolite, 11-OH-THC, in adolescent male and female rats after 30 min aerosol exposure of Δ^9-THC (25 or 100 mg/mL). 11-COOH-THC was undetectable in brain tissue. *$p<0.5$. Student's t test, $n=4$–5

Analyte	Exposure Δ^9-THC (mg/mL)	Male				Female			
		C_{max} (pmol/mL)	T_{max} (min)	$t_{1/2}$ (min)	AUC (pmol • min/mL)	C_{max} (pmol/mL)	T_{max} (min)	$t_{1/2}$ (min)	AUC (pmol • min/mL)
Δ^9-THC	25	125	5.00	17.3	6834	129	5.00	17.9	7545
	100	170	5.00	15.7	12,366*	152	5.00	19.9	10,372
11-OH-THC	25	36	5.00	-	2849	68*	15.00	-	5931*
	100	49	5.00	-	3222	90*	30.00	-	8282*

Table 3 Brain-to-plasma ratio for Δ^9-THC and its metabolite, 11-OH-THC, in adolescent male and female rats after 30 min aerosol exposure of Δ^9-THC (25 or 100 mg/mL). Student's *t* test, $n = 4–5$, no significance detected

Analyte	Exposure Δ^9-THC (mg/mL)	Brain:plasma	
		Male	Female
Δ^9-THC	25	4.02	3.10
	100	2.57	2.09
11-OH-THC	25	N/A	9.12
	100	7.47	7.36

not high-dose THC aerosol (main effect of dose, $F_{2,56} = 3.4$, $p = 0.04$; 25 mg/ml, $p = 0.039$; 100 mg/ml, $p = 0.16$), an effect that was similar in both sexes (no sex × dose interaction, $F_{2,56} = 1.73$, $p = 0.19$) (Fig. 4D, bottom).

Locomotor behavior and rearing High dose (100 mg/ml) THC aerosol suppressed novel environment locomotion overall (main effect of dose, $F_{2,57} = 4.76$, $p = 0.012$; 25 mg/ml, $p = 0.11$; 100 mg/ml, $p = 0.01$) (Fig. 5A). However, these effects again differed by sex (sex × dose interaction, $F_{2,57} = 3.47$, $p = 0.038$), in that only the low dose significantly suppressed locomotion in females ($F_{2,30} = 3.59$, $p = 0.04$; 25 mg/ml, $p = 0.036$; 100 mg/ml, $p = 0.18$), while only the high dose did so in males ($F_{2,27} = 5.65$, $p = 0.009$; 25 mg/ml, $p = 0.98$; 100 mg/ml, $p = 0.025$) (Fig. 5A). This dose-dependent THC-induced locomotor suppression was consistent across the entire 2 h locomotor testing period (no dose × time interaction, $F_{6,171} = 1.01$, $p = 0.42$) (Fig. 5B).

THC effects on vertical exploration (rearing) were similar to those on horizontal locomotion (main effect of dose, $F_{2,57} = 3.6$, $p = 0.03$; 25 mg/ml, $p = 0.11$; 100 mg/ml, $p = 0.035$), again with stronger effects of the low dose in females and the high dose in males (sex × dose interaction, $F_{2,57} = 3.1$, $p = 0.05$; dose main effect in females, $F_{2,30} = 3.32$, $p = 0.05$; 25 mg/ml, $p = 0.04$; 100 mg/ml, $p = 0.33$; dose main effect in males, $F_{2,27} = 3.63$, $p = 0.04$; 25 mg/ml, $p = 1.0$; 100 mg/ml, $p = 0.07$) (Fig. 5C). This dose-dependent suppression of rearing was also consistent over the 2 h session (no dose × time interaction, $F_{6,171} = 1.27$, $p = 0.27$) (Fig. 5D).

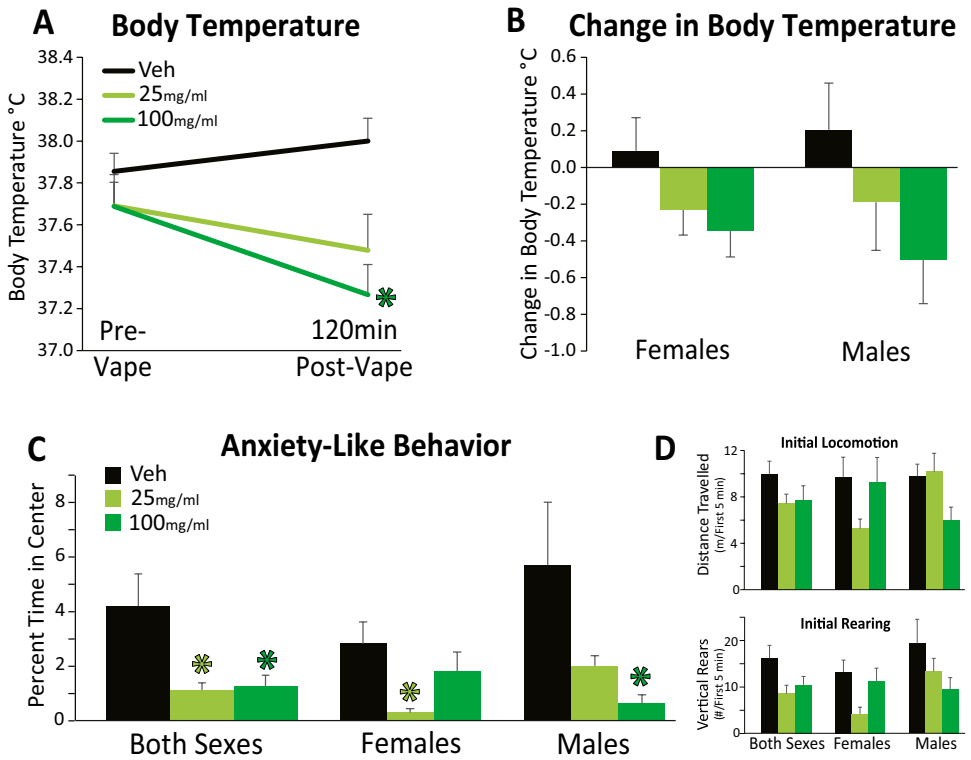

Fig. 4 Aerosolized THC reduces temperature and increases anxiety-like behavior: **A** body temperature prior to aerosol exposure and after the 120 min locomotor session held immediately after aerosol exposure is shown, with significant reductions present at the 100 mg/ml (dark green) but not 25 mg/ml dose. **B** Change in body temperature from pre-aerosol to post-locomotor testing is shown separately in females and males. No significant sex difference in THC vapor-induced hypothermia was observed. **C** Percent time in the first 5 min of exposure to a novel locomotor testing chamber spent in the exposed center of the chamber, an inverse assay of anxiety-like behavior is shown for both sexes and each sex separately. **D** Locomotor behavior (top) and rearing (bottom) during this initial 5 min period in which center-surround behavior was analyzed are depicted. *$p < 0.05$ compared to vehicle

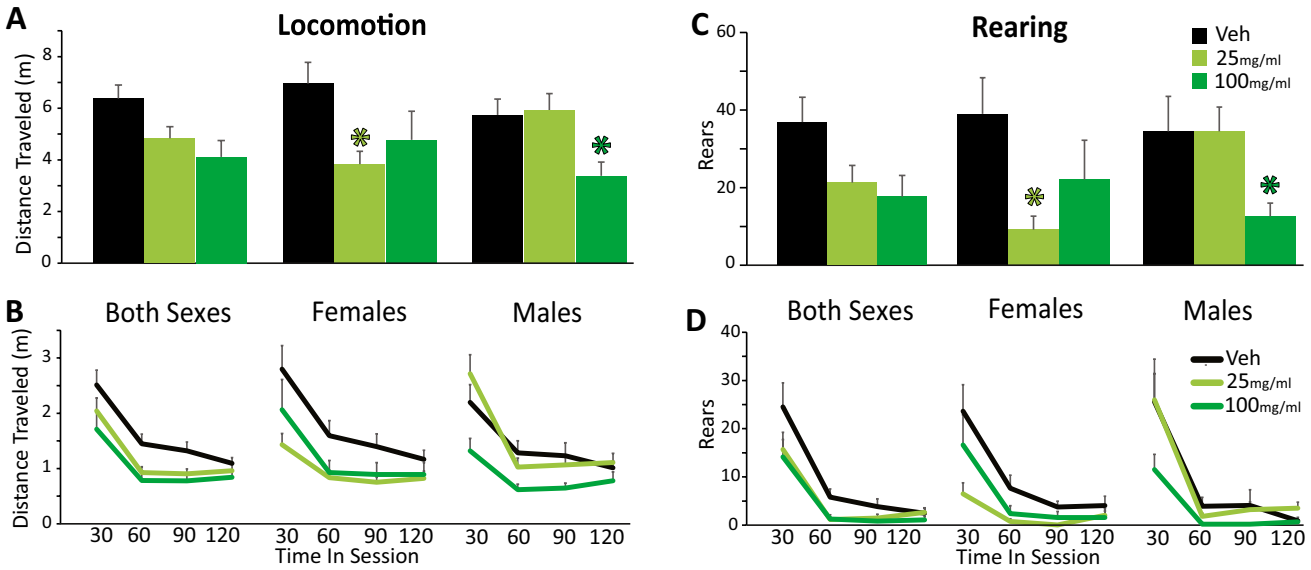

Fig. 5 Aerosolized THC suppresses locomotion and rearing: **A** locomotion, as measured by distance travelled in the 2 h locomotor test, is shown in both sexes, as well as separately for females and males. **B** The time course of locomotion is depicted in 30 min bins across the 2 h session. Vertical rearing is shown using the same scheme, for **C**, the entire 2 h session, and **D** in 30 min bins. $^{*}p < 0.05$ compared to vehicle

Discussion

The present results provide the first thorough characterization of the pharmacokinetics of aerosolized THC (commonly referred to as "vaping") inhaled by adolescent male and female Wistar rats. As seen with i.p. administration (Ruiz et al. 2021; Torrens et al. 2020), females exhibited higher concentrations of the bioactive metabolite 11-OH-THC in the blood and brain than did males, which might explain why the same THC dose inhibits locomotion and induces anxiety-like avoidance of an open space at the lower 25 mg/ml concentration tested here in females but not in males. In males, only the higher 100 mg/ml aerosol suppressed locomotion and produced anxiety-like behavior. At the higher concentration, THC also suppressed body temperature and did so similarly in both sexes—showing that THC's effects are not uniform but instead depend on the outcome being measured as well as on sex and dosage. Together, these findings will serve as a benchmark for aerosol inhalation dosing in adolescent rats, facilitating well-characterized, translationally relevant future studies on the acute and persistent effects of THC on the developing adolescent brain.

The results presented here demonstrate that aerosol inhalation yields significant plasma concentrations of THC and THC metabolites in adolescent rats, comparable to those achieved by low dose i.p. administration of the drug (0.5 mg/kg) (Ruiz et al. 2021; Torrens et al. 2020). As expected, inhalation dosing led to a more rapid rise in THC blood than did i.p. dosing in prior work (Ruiz et al. 2021), with maximum plasma concentrations observed 5 min after the 30 min session in both sexes and at both doses. Also as expected, THC blood concentrations were higher after high-dose, relative to low-dose THC aerosol. No major sex differences were seen in THC blood levels, though females trended toward higher values at the low THC aerosol dose, as previously observed after low-concentration THC aerosol inhalation in adolescent rats (Nguyen et al. 2020). We note that experimenters desiring even higher THC blood levels (e.g., those more similar to a moderate 5 mg/kg i.p. THC dose) (Ruiz et al. 2021) can likely achieve this by adjusting parameters such as the duration or frequency of delivered aerosol puffs or by increasing the duration of the exposure period (Nguyen et al. 2016, 2020; Taffe et al. 2021).

In the brain, THC concentrations were near-maximal 5 min after aerosol exposure in both sexes, and unlike in the blood, brain levels of the drug remained at this high level for at least 30 min, indicating sequestration of the drug to the lipid-rich brain. Again, no sex differences were observed in brain THC levels. Of note, the T_{max} for THC in brain was faster (5 min vs 60 min), and the brain-to-plasma ratio for THC was higher (4.02 vs 2.02 for males; 3.10 vs 2.65 for females) after inhalation than after i.p. administration of a dose (0.5 mg/kg) that produced comparable plasma levels (Ruiz et al. 2021), suggesting that inhalation might afford greater access to the central nervous system than does i.p. injection.

As we and others have previously shown with i.p. THC dosing, there was a major sex difference in blood and brain concentrations of THC metabolites, with females having

greater concentrations of bioactive, behaviorally potent (Craft et al. 2017; Gill and Jones 1972; Tseng et al. 2004) 11-OH-THC in the blood and brain and also of inactive 11-COOH-THC in the blood. Importantly, this sex difference in blood 11-OH-THC is also observed in humans ingesting THC via the oral route (Nadulski et al. 2005). A surprising result was that the C_{max} of 11-OH-THC in the female brain exceeded the C_{max} measured in blood, and it was achieved at a shorter T_{max}. A plausible explanation for this finding, which will require further testing, is that THC may be metabolized in female rat brain tissue, as previously suggested (Watanabe et al. 1988). However, we cannot exclude the possibility that liver-generated 11-OH-THC might enter the CNS more readily than THC does.

Prior data from our labs and others support the notion that sex differences in THC metabolism, and especially the female-specific accumulation of the active THC metabolite 11-OH-THC, may in part explain the drug's generally stronger behavioral effects in females relative to males (Craft et al. 2017; Ruiz et al. 2021; Tseng and Craft 2001; Tseng et al. 2004; Wiley and Burston 2014). The present data also supports this hypothesis, with pronounced effects of low-dose 25 mg/ml THC in females only on locomotion and anxiety-like behaviors. In males, only the higher 100 mg/ml THC dose suppressed locomotion and induced anxiety—potentially suggesting that a higher THC dose is necessary to achieve equivalent behavioral effects in males to those achieved by a considerably lower dose in females. However, high-dose THC aerosol decreased body temperature equivalently in both sexes, suggesting that not all THC effects are equally sensitive to interactions between dose and sex.

Finally, we point out that our studies were conducted in relatively small numbers of adolescent rats, and the pharmacokinetics of THC differs somewhat across rodent species, sexes, and ages (Craft et al. 2019; Rubino and Parolaro 2015b; Ruiz et al. 2021; Schneider 2008; Torrens et al. 2020; Tseng et al. 2004; Wiley et al. 2021). Fortunately, it seems that THC aerosol dosing is likely equivalent across rat strains, as Wistar and Sprague Dawley rats show similar blood THC levels after comparable aerosol dosing using a similar system to ours (Moore et al. 2021; Taffe et al. 2021). We also note that acute "vaping" which often occurs in humans may also yield distinct effects from repeated inhalation over 30 min, as tested here in rats.

In conclusion, the present results provide benchmark validation data for the adolescent THC aerosol administration model in female and male adolescent rats. By providing a rigorous characterization of the pharmacokinetic and pharmacodynamic profiles of aerosolized THC, these findings will help to assess the translational relevance of this exposure model. In doing so, we expect that we will progress toward increasingly translationally relevant animal models of adolescent THC exposure.

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Declarations

Conflict of interest The authors declare no competing interests.

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