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Comparative Pharmacokinetics of Δ9-Tetrahydrocannabinol in Adolescent and Adult Male and Female Rats

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Abstract

Introduction: Studies in rodent models have shown that adolescent exposure to Δ9-THC, the psychotropic constituent of cannabis, produces long-lasting alterations in brain function and behavior. However, our understanding of how age and sex might influence the distribution and metabolism of THC in laboratory rodents is still incomplete. In the present report, we provide a comparative analysis of the pharmacokinetic (PK) properties of THC in adolescent and adult rats of both sexes, and outline several dissimilarities across these groups.

Materials and Methods: A single (acute) or 2-week daily (subchronic) administration of THC (0.5 or 5 mg/kg, acute; 5 mg/kg, subchronic; intraperitoneal) was given to adolescent (33-day-old, acute; 30–44-day-old, subchronic) and young adult (70-day-old, acute only) male and female rats. THC and its first-pass metabolites—11-hydroxy-Δ9-THC (11-OH-THC) and 11-nor-9-carboxy-Δ9-THC (11-COOH-THC)—were quantified in plasma and brain tissue using a selective isotope-dilution liquid chromatography/tandem mass spectrometry assay. Changes in body temperature were measured using abdominally implanted microchips. Biotransformation of THC to its metabolites using freshly prepared liver microsomes was assessed.

Results: At the acute 5 mg/kg dose, maximal plasma concentrations of THC were twice as high in adult than in adolescent rats. Conversely, in adults, brain concentrations and brain-to-plasma ratios for THC were substantially lower (25–50%) than those measured in adolescents. Similarly, plasma and brain concentrations of THC metabolites were higher in adolescent male rats compared with adult males. Interestingly, plasma and brain concentrations of the psychoactive THC metabolite 11-OH-THC were twofold to sevenfold higher in female animals of both ages compared with males. Moreover, liver microsomes from adolescent males and adolescent and adult females converted THC to 11-OH-THC twice as fast as adult male microsomes. A dose-dependent hypothermic response to THC was observed in females with 0.5 and 5 mg/kg THC, whereas only the highest dose elicited a response in males. Finally, subchronic administration of THC during adolescence did not significantly affect the drug's PK profile.

Conclusions: The results reveal the existence of multiple age and sex differences in the distribution and metabolism of THC in rats, which might influence the pharmacological response to the drug.

Keywords: Δ9-tetrahydrocannabinol; cannabis; pharmacokinetics; liquid chromatography/tandem mass spectrometry

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Introduction

During human adolescence, the continuing maturation of prefrontal cortical networks drives profound changes in emotion, cognition, and motivation.[1,2] Because of these transformations, teenage years are a time of great sensitivity to environmental insults and of enhanced vulnerability to the persistent effects of psychoactive substances such as cannabis.[3,4] Evidence suggests that initiation of cannabis use peaks at this time and decreases after the age of 25, and that adolescence-onset use heightens the risk for continued use later in life.[5]

Importantly, there is evidence that high levels of cannabis exposure during adolescence may be associated with higher rates of negative psychiatric, cognitive, and socioeconomic outcomes in adulthood.[6] Supporting a causal link between these events, preclinical studies have shown that treating adolescent mice or rats with the psychotropic constituent of cannabis, Δ^9-tetrahydrocannabinol (THC) or one of its synthetic mimics, causes persistent dysregulations of affect, memory, and reward-seeking behavior.[7]

The influence that age, sex, and species differences exert on the pharmacokinetic (PK) properties of THC is still incompletely understood. To begin to fill this knowledge gap, in a previous report we conducted a comparative PK characterization of THC in adolescent and adult male mice.[8] Two key results emerged from this study. We first found that adolescent male mice preferentially metabolize THC into its inactive metabolite 11-nor-9-carboxy-Δ^9-THC (11-COOH-THC), and second that the brain of adolescent male mice is partly protected against THC by heightened expression of two blood–brain barrier proteins: claudin-5 and ATP-Binding Cassette Subfamily G Member 2 (*Abcg2*).[8]

A reasonable, although unexpected, interpretation of these findings is that male mice might be less sensitive to the psychoactive effects of THC in adolescence than they are in adulthood. Can this result be translated to teenage human cannabis users? Ethical boundaries preclude answering this question, but one achievable step is to assess whether a similar protection might occur in a larger rodent species such as the rat, which is frequently used to evaluate the PK properties of preclinical drug candidates.

In the present study, we determined the PK profile of THC in adolescent (33-day-old) and young adult (70-day-old) male and female rats. We administered the drug by intraperitoneal (IP) injection, a route commonly used in studies conducted on adolescent rodents, and quantified THC and its main cytochrome P_{450} (CYP_{450}) metabolites, 11-hydroxy-Δ^9-THC (11-OH-THC) and 11-COOH-THC, in plasma and brain using a sensitive and selective liquid chromatography/tandem mass spectrometry (LC-MS/MS) assay. The PK results were integrated with pharmacodynamic data (THC-induced hypothermia) and *in vitro* measurements of THC metabolism in liver microsomes. Finally, we asked whether subchronic administration of THC during adolescence might influence the drug's PK profile.

Materials and Methods

Chemicals and solvents

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

Animals

Adolescent (postnatal day [PND], at arrival: 21–22; 30–70 g) and adult (PND 60, 100–250 g) male and female Long-Evans rats were purchased from Charles River (Wilmington, MA, USA). They were housed in same-sex groups of 4 and were allowed to acclimate for at least 7 days before experiments. Housing rooms were maintained on a 12-h light/12-h dark cycle (lights on at 6:30 AM) under controlled conditions of temperature (20°C ± 2°C) and relative humidity (55–60%). Food and water were available *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee at the University of California, Irvine, and carried out in strict accordance with the National Institutes of Health guidelines for the care and use of experimental animals.

PK experiments

PK analyses were performed as described before[8,9] with minor modifications. Briefly, we dissolved THC in a vehicle consisting of Tween80/saline (5:95, v/v)[10] and administered it at a dose of 0.5 or 5 mg/kg by IP injection to adolescent (PND 33) or young adult (PND 70) rats in an injection volume of 0.5 mL/kg. A separate set of adolescent male and female rats received a daily injection of 5 mg/kg THC or vehicle (Tween80/saline, 5:95) from PND 30 to 43 and, on PND 44, were given one additional injection of 5 mg/kg THC.

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The animals were anesthetized with isoflurane at various time points after injection (15, 30, 60, 120, 240, and 480 min; $n = 4$ per time point), blood was collected by cardiac puncture into ethylenediaminetetraacetic acid (EDTA)-rinsed syringes and transferred into 1 mL polypropylene plastic tubes containing spray-coated potassium-EDTA (K_2-EDTA). Plasma was prepared by centrifugation at $1450 \times g$ at 4°C for 15 min, and transferred into polypropylene tubes. The animals were decapitated and their brains were quickly removed. All tissue samples were immediately frozen on dry ice and stored at −80°C until analyses.

Sample preparation

Plasma (0.1 mL) was transferred into 8-mL glass vials (Cat. No.: B7999-3; Thermo Fisher) and proteins were precipitated by addition of 0.5 mL of ice-cold acetonitrile containing 1% formic acid and the following internal standards (ISTD):[^{2} H_3]-THC,[^{2} H_3]-11-OH-THC, and [^{2} H_3]-11-COOH-THC, 50 pmol each. Frozen whole brains were pulverized on dry ice. Aliquots of tissue (20–25 mg) were homogenized using the Precellys CK-14 Soft Tissue Homogenizing Kit (Bertin Corp., Rockville, MD, USA) in a Precellys Evolution apparatus (Bertin) at 4°C on preset setting #4 (6500 RPM × 20 sec × 2) in 0.5 mL of ice-cold acetonitrile containing 1% formic acid and 50 pmol ISTD. Plasma and brain samples were stirred vigorously for 30 sec and centrifuged at $2800 \times g$ 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, USA) and eluted under positive pressure (3–5 mmHg, 1 drop/5 sec; Agilent Technologies). For brain fractionation, EMR cartridges were prewashed 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 $2800 \times g$ 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 pressure was increased gradually to 10 mmHg (1 drop/sec) 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 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). Analytes were separated on an Eclipse XDB C18 column (1.8 μm, 3.0 × 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 until 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: THC 315.2 > 193.0 *m/z*, [^{2} H_3]-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 [^{2} H_3]-11-COOH-THC 348.2 > 302.2 *m/z*. The identity of THC, 11-OH-THC, and 11-COOH-THC was verified by monitoring the transitions 315.2 > 123.0 *m/z*, 331.2 > 105.0 *m/z*, and 345.2 > 299.2 *m/z*, respectively. 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.[11] The MassHunter software (Agilent Technologies) was used for instrument control, data acquisition, and data analysis.

Temperature measurements

We implanted temperature programmable microchips (UCT-2112, 2.1 × 13 mm; Unified Information Devices, Lake Villa, IL, USA) on PND 24 or 63 by IP injection. Three days before testing, we injected 0.5 mL/kg of vehicle (Tween80/saline, 5:95) once daily for 3 days, and measured body temperature before and 15, 30, 60, 120,

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240, and 480 min after injection. The measurements were averaged to establish baseline temperatures for each animal. On PND 33 or 70, animals received an injection of 0.5 or 5 mg/kg THC and temperature was measured before and after injection at the above-listed time points.

Liver microsome preparation

Microsomes were prepared as described[12] with minor modifications from a separate set of naive male and female rats (PND 33 or 70). Briefly, rat livers were weighed and homogenized in extraction buffer (20%, w/v; 10 mM tris pH 7.5, 250 mM sucrose, 1 mM phenylmethylsulfonyl fluoride, protease inhibitor cocktail; Cat No.: 04080-11; Nacalai Tesque, Kyoto, Japan). The homogenates were centrifuged at 3000 × *g* at 4°C for 20 min. Supernatants were collected and centrifuged twice for 20 min at 10,000 × *g* at 4°C. The supernatants from the second centrifugation were centrifuged again for 90 min at 100,000 × *g* at 4°C. The microsome pellets were resuspended in 0.5 mL buffer (50 mM tris pH 7.5, 20% glycerol, 1 mM dithiothreitol, 1 mM EDTA). Protein concentrations were measured using the bicinchoninic acid assay.

THC metabolism in liver microsomes

Microsomes (1 μg protein) were combined in a solution of potassium phosphate (0.1 M, pH 7.4) containing rat CYP_{450} reductase (0.2 μM). After a 5-min 37°C preincubation with THC (40 μM), reactions were initiated by adding 10 mM NADPH (0.1 mL, 1 mM final) and allowed to proceed at 37°C for 30 min, at which point they were quenched with an equal volume of ethyl acetate. Extractions were performed as previously reported.[13]

Briefly, the quenched reactions were vortexed thoroughly, centrifuged for 5 min at 1800 × *g* at 4°C, and the organic layers were transferred into clean tubes. Fresh ethyl acetate was added, and the cycle was repeated twice for a total of three extractions. After drying down the organic layer in a rotary evaporator, extracts were resuspended in acetonitrile (0.1 mL) and 11-OH-THC and 11-COOH-THC were quantified by LC/MS using a 5500 QTRAP LC/MS/MS system (Sciex, Framingham, MA, USA) connected to a 1200 series LC system (Agilent Technologies), which included a degasser, an autosampler, and a binary pump.

The LC separation was performed on an Agilent Eclipse XDB-C18 column (4.6 × 150 mm, 5 μm) with mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile). The flow rate was 0.4 mL/min and the linear gradient was as follows: 0–2 min, 90% A; 10–23 min, 5% A; 24–31 min, 90% A. The autosampler was set at 5°C and injection volume was 10 μL. Mass spectra were acquired under positive (ion spray voltage 5500 V) ESI. The source temperature was 450°C. The curtain gas, ion source gas 1, and ion source gas 2 were 32, 65, and 50 psi, respectively. MRM was used for quantitation: THC 315.2 *m/z* > 193.0 *m/z*; 11-OH-THC 331.2 *m/z* > 313.2 *m/z*; and 11-COOH-THC 345.2 *m/z* > 327.2 *m/z*. ISTD was [$^2H_9$]-THC (324.2 *m/z* > 202.1 *m/z*). Software Analyst 1.6.2 was used for data acquisition and analysis.

PK data analyses

We analyzed PK data[8] using a noncompartmental model.[14] Maximal concentration (C_{max}) and area under the curve (AUC) were measured using GraphPad Prism 8 (La Jolla, CA, USA) and other PK parameters (clearance [CL], volume of distribution [V_D], and half-life time of elimination [$t_{1/2}$]) were determined as described.[14] With regard to CL and V_D calculations, the following equations were used: CL = dose/AUC; V_D = CL/λ_z, where λ_z represents the slope of the terminal half-life. Time at which maximal concentration was reached (T_{max}) was determined by visual inspection of averaged data.

Statistical analyses

Sex and age-dependent differences in PK parameters (C_{max}, AUC) and brain-to-plasma ratios were analyzed by Student's unpaired *t*-test. Microsomal data and temperature measurements were assessed using two-way analysis of variance (ANOVA) with Tukey's *posthoc* test. Outliers (maximum of 1 per time point) were determined using the Grubbs' outlier test. Differences between groups were considered statistically significant at values of $p < 0.05$.

Results

PK profile of THC in rat plasma

Adolescent (PND33) and adult (PND70) rats of both sexes were given a single injection of THC (0.5 or 5 mg/kg, IP) and the PK profiles of the drug and its main first-pass metabolites, 11-OH-THC and 11-COOH-THC, were assessed in plasma and brain tissue using a sensitive and selective LC/MS-MS method [limit of quantification (LOQ) for all the three analytes: 1.0 pmol/mL], which included both quantifier and qualifier MRM transitions.[8,9,11]

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When THC was administered at 0.5 mg/kg, only the parent compound could be accurately quantified in plasma and brain (Fig. 1), whereas its metabolites failed to reach LOQ. Key PK properties (C_{max}, AUC, T_{max}, and $t_{1/2}$) for THC are listed in Supplementary Table S1. Supplementary Table S2 reports the brain-to-plasma ratio, V_d and CL, calculated using a noncompartmental model.[14] In plasma, C_{max} (mean ± SEM) values were slightly but not significantly elevated in females compared with males and in adults compared with adolescents. No difference across groups was observed in the brain, except for the C_{max} of adolescent female rats, which was higher than corresponding values in adult females or in adolescent and adult males (Supplementary Table S1).

The plasma PK profiles for 5 mg/kg THC and its metabolites in adolescent and adult male and female rats are shown in Figure 2. Key parameters for each analyte are listed in Supplementary Tables S3 and S4. Similar to the trend observed at 0.5 mg/kg, C_{max} values for THC were elevated in adult compared with adolescent male rats (Supplementary Table S3). However, AUC values were comparable across all groups (Supplementary Table S3). CL and V_d were higher in adult males than in any other group (Supplementary Table S4), possibly as a result of their larger body mass.[15]

There were two notable differences in the PK profiles of THC metabolites: (1) adolescent males exhibited higher C_{max} and AUC for 11-OH-THC and 11-COOH-THC compared with adult males; and (2) the C_{max} values and AUC for 11-OH-THC were substantially higher in females than in males, irrespective of age. For example, in adolescents, the C_{max} and AUC for 11-OH-THC were 2.5 times higher in females than in males (C_{max} = 28 ± 4 pmol/mL vs. 12 ± 1 pmol/mL, $p = 0.009$; AUC = 10,844 ± 868 pmol/min/mL vs. 4381 ±

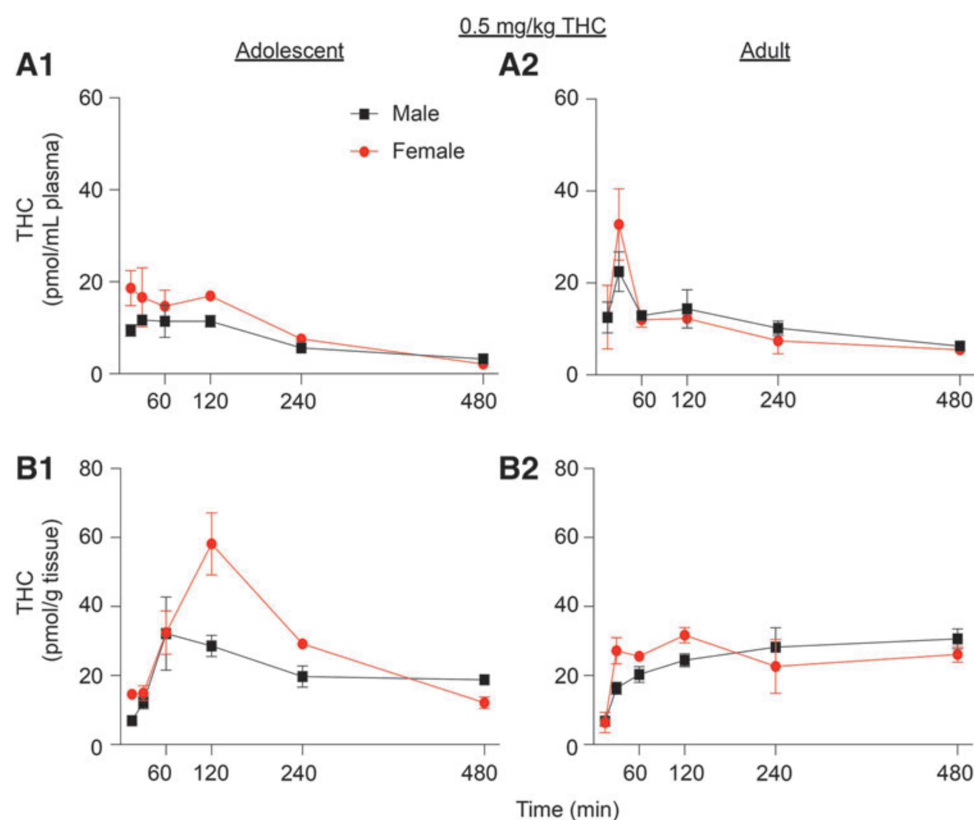

FIG. 1. Concentration of THC in plasma **(A)** and brain **(B)** after IP injection of 0.5 mg/kg THC in adolescent **(A1, B1)** and adult **(A2, B2)** male (black square) or female (red circle) rats. Symbols represent mean ± SEM, $n = 3$ or 4 animals per data point, outliers removed using the Grubb's test. IP, intraperitoneal; THC, Δ^9-tetrahydrocannabinol; SEM, standard error of the mean.

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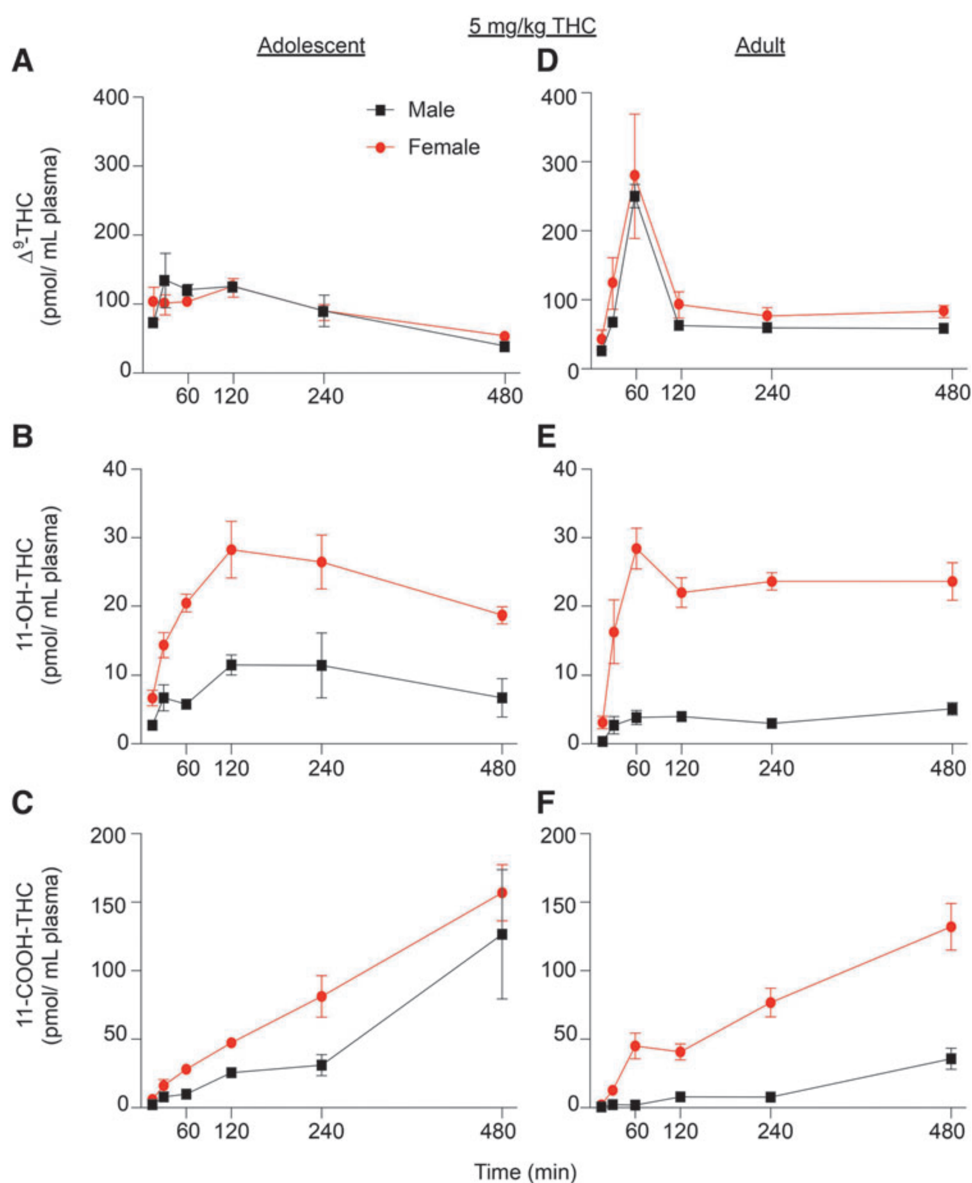

FIG. 2. Plasma concentrations of THC and its first-pass metabolites, 11-OH-THC and 11-COOH-THC, after IP injection of 5 mg/kg THC in adolescent **(A–C)** and adult **(D–F)** male (black square) or female (red circle) rats. Symbols represent mean ± SEM, $n = 3$ or 4 animals per data point, outliers removed using the Grubb's test. 11-COOH-THC = 11-nor-9-carboxy-Δ^9-THC; 11-OH-THC = 11-hydroxy-Δ^9-THC.

1196 pmol/min/mL, $p = 0.01$). In adults, the C_{max} for 11-OH-THC was 7.1 times higher (28 ± 3 pmol/mL vs. 4 ± 1 pmol/mL, $p = 0.001$) and the AUC was 6.1 times higher (10,767 ± 301 pmol/min/mL vs. 2270 ± 337 pmol/min/mL, $p < 0.0001$) in females than in males. Similarly, 11-COOH-THC reached higher C_{max} and greater AUC in adult females compared with males (Supplementary Table S3).

PK profile of THC in brain tissue

The PK profiles of 5 mg/kg THC and its metabolites in the brain of adolescent and adult male and female rats are illustrated in Figure 3. Key PK parameters are reported in Supplementary Table S5. In striking contrast with results previously obtained in mice,[8] the brain C_{max} and AUC for THC were 50–100% higher in adolescent compared with adult males (249 ± 10 pmol/g vs. 121 ± 22 pmol/g,

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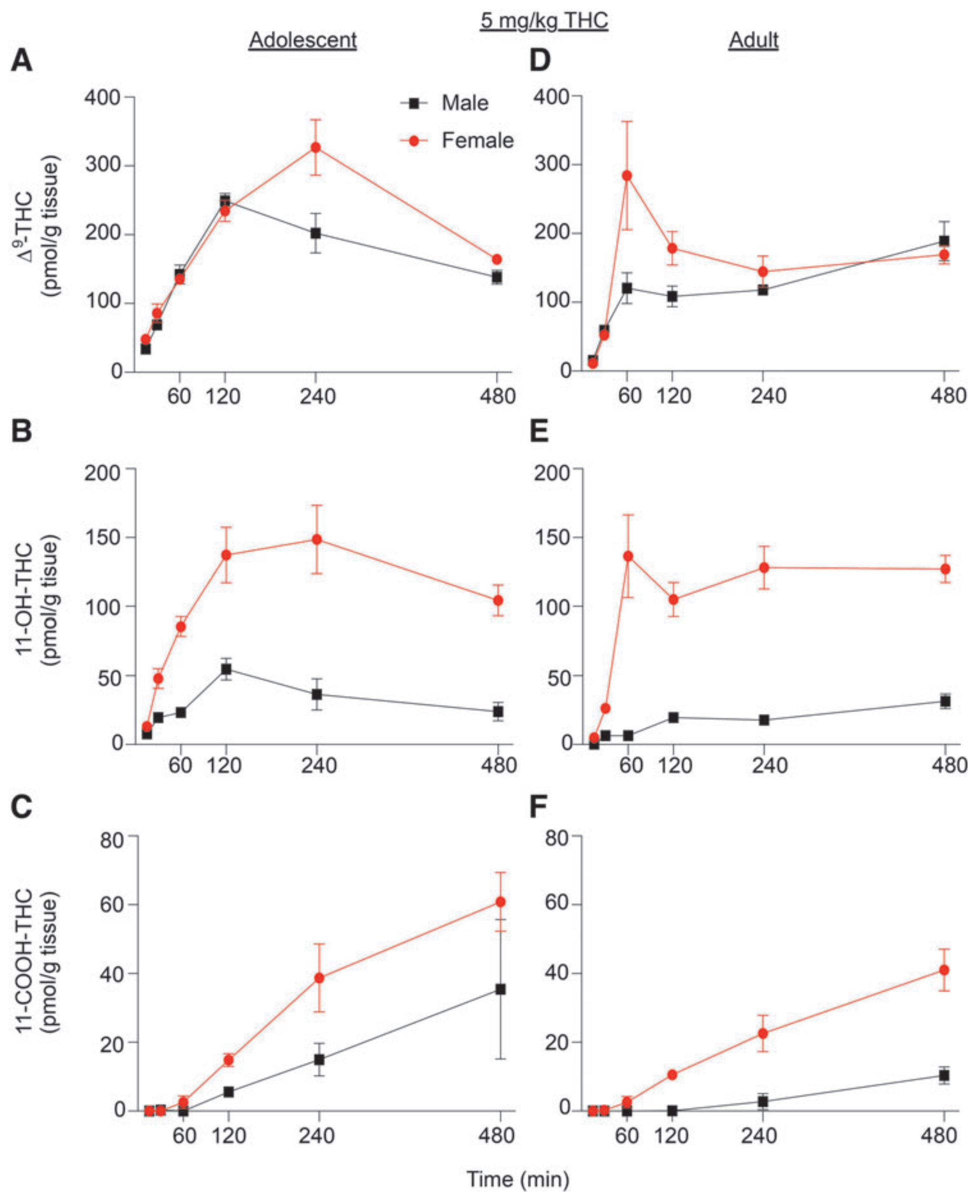

FIG. 3. Brain concentrations of THC and its first-pass metabolites, 11-OH-THC and 11-COOH-THC, after IP injection of 5 mg/kg THC in adolescent **(A–C)** and adult **(D–F)** male (black square) or female (red circle) rats. Symbols represent mean ± SEM, $n = 3$ or 4 animals per data point, outliers removed using the Grubb's test.

$p = 0.002$; 81,185 ± 6507 pmol/g/min vs. 59,413 ± 5399 pmol/g/min, $p = 0.02$). A similar trend in females did not reach statistical significance (Supplementary Table S5).

As seen in plasma, there were differences between levels of 11-OH-THC and 11-COOH-THC in adolescent males compared with adult males and in female rats of both age groups versus male rats (Fig. 3 and Supplementary Table S5). Supplementary Table S6 shows that adolescent males and females exhibited higher brain-to-plasma ratios for THC, compared with adults (2.06 ± 0.50 vs. 1.59 ± 0.06, males, $p = 0.002$; 2.52 ± 0.27 vs. 1.57 ± 0.10, females, $p = 0.0003$), which is again suggestive of greater brain penetration.

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THC metabolism in liver microsomes

As in our previous mouse study,[8] we measured the sequential transformation of THC into 11-OH-THC and 11-COOH-THC by liver microsomes of adolescent and adult rats of both sexes. Microsomal preparations from adolescent male and female animals converted THC into 11-OH-THC at approximately equal rates: 0.38 ± 0.04 in males and 0.42 ± 0.06 pmol/min/mg, $p = 0.9$ in females (Fig. 4A). By contrast, liver microsomes from adult females converted THC to 11-OH-THC at a rate that was approximately thrice as rapid as the rate measured in adult male microsomes (females: 0.40 ± 0.07 pmol/min/mg; males: 0.16 ± 0.03 pmol/min/mg, $p = 0.02$) (Fig. 4A). Irrespective of age, the biotransformation of THC into 11-COOH-THC was similar in males and females (Fig. 4B).

Temperature changes after THC administration

Temperature changes are a sensitive and reliable measure of CB_1 receptor activation in the central nervous system.[16] Figure 5 illustrates the time course of the hypothermic effects of THC (0.5 and 5 mg/kg) in adolescent and adult rats of both sexes. In females, the drug produced comparable dose-dependent hypothermic responses in adolescent and adult animals (Fig. 5A, B). By contrast, in males, only the highest dose of THC was effective (Fig. 5B).

Effect of subchronic administration on the PK profile of THC

Finally, we asked whether repeated administration of THC during adolescence might alter the drug's PK properties (owing, for example, to induction of CYP_{450} enzymes in the liver[17,18]). A schematic of the experiment is illustrated in Figure 6. Adolescent rats received daily injections of THC (5 mg/kg, IP) or its vehicle from PND30 until PND43. On PND44, the PK profile of the same dose of THC was assessed in plasma and brain of both groups, as outlined previously.

Figure 7 shows the plasma PK profiles for THC and its metabolites. C_{max} and AUC values are reported in Supplementary Table S7. The only notable difference among test groups was that the C_{max} for THC was twice as high in male rats subchronically treated with THC compared with females (males: 517 ± 56 pmol/mL; females: 214 ± 43 pmol/mL, $p = 0.01$). In the brain, no statistically detectable differences were seen across groups (Fig. 8; Supplementary Table S8). Brain-to-plasma ratios for THC and its metabolites were also similar (Supplementary Table S9).

Discussion

Understanding the PK properties of THC is a necessary step in the pharmacological evaluation of this psychoactive agent in humans as well as in animal models, but is especially critical to establish whether sex-, age- and

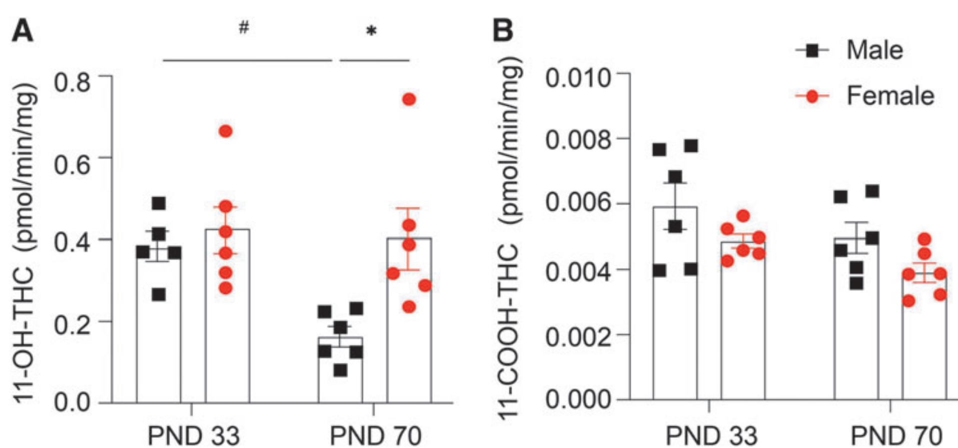

FIG. 4. Rate of formation (pmol/min/mg) of 11-OH-THC **(A)** and 11-COOH-THC **(B)** in liver microsomes of adult and adolescent male (black square) or female (red circle) rats. Bars represent mean ± SEM, $n = 5$ or 6 animals per data point, outliers removed using the Grubb's test. #Age difference, *sex difference, $p < 0.05$, Two-way ANOVA. ANOVA, analysis of variance.

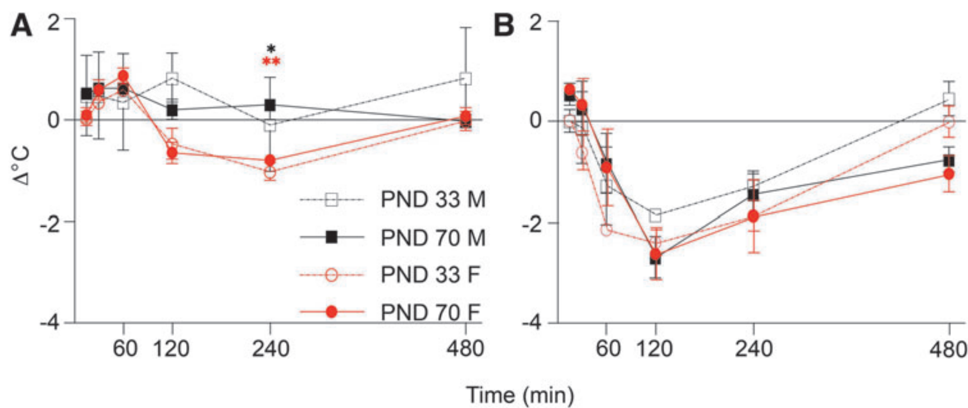

FIG. 5. Effects of THC on abdominal body temperature after IP injection of 0.5 mg/kg **(A)** or 5 mg/kg **(B)** THC on adolescent (broken, unfilled) and adult (solid, filled) male (black square) or female (red circle) rats. Bars represent mean ± SEM, $n = 4$ animals per data point. *$p < 0.05$, adolescent females versus adult males, **$p < 0.01$ adult females versus adult males, Two-way ANOVA.

species-related differences in its effects may result solely from inherent variations in the endocannabinoid system—for example, in the density and localization of cannabinoid receptors—or might also involve alterations in drug distribution, biotransformation and elimination. In a previous report,[8] we determined the PK profiles of THC and its primary first-pass metabolites, 11-OH-THC and 11-COOH-THC, following IP administration in adolescent and young adult male mice. In this study, we extended our investigation to adolescent and young adult rats of both sexes. The results identify several sex- and age-dependent dissimilarities in the distribution and metabolism of THC, as well as marked differences between adolescent rats and mice.

As in our mouse study, we selected the IP route of administration because it is most commonly used in rodent studies and offers a realistic compromise between technical feasibility, reproducibility, and translational relevance. Similar to mice, IP injection of 5 mg/kg THC produced in rats peak plasma drug concentrations that

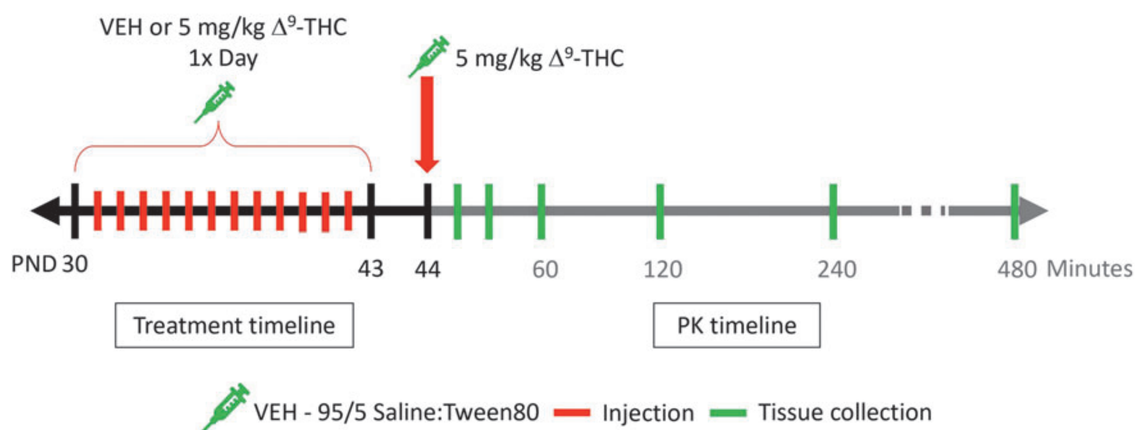

FIG. 6. Schematic for subchronic THC administration. Adolescent male and female rats were given a daily injection of 5 mg/kg THC or vehicle (Tween80/saline, 5:95) from PND 30 to 43 (red marks). On PND 44 all animals were given a single injection of 5 mg/kg THC and the concentrations of THC and its metabolites, 11-OH-THC and 11-COOH-THC, were quantified in the plasma and brain at various time points following injection (green marks). PND, postnatal day; VEH, vehicle.

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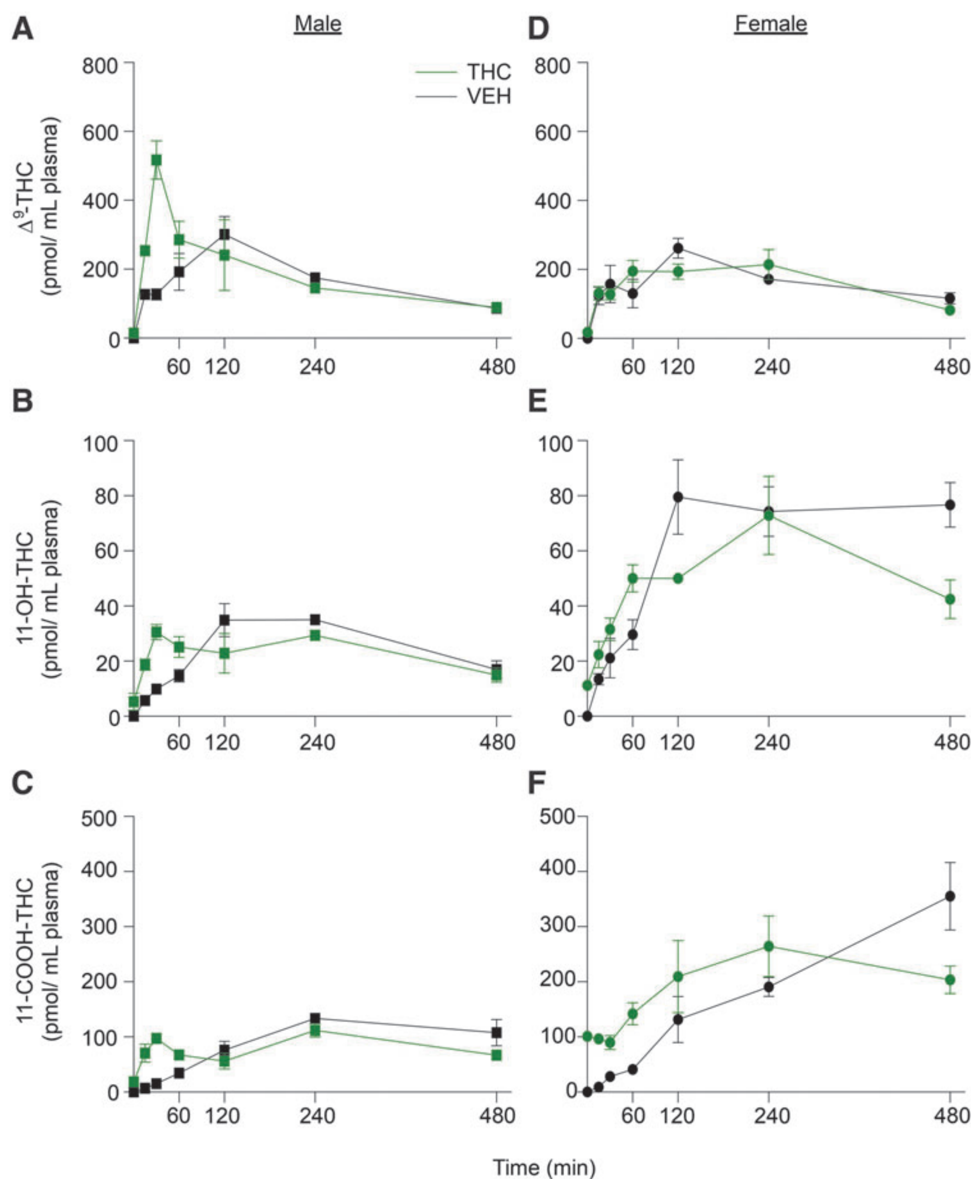

FIG. 7. Plasma concentrations of THC and its first-pass metabolites, 11-OH-THC and 11-COOH-THC, after IP injection of 5 mg/kg THC (PND 44) in adolescent male **(A–C)** or female **(D–F)** rats treated with daily injections of 5 mg/kg THC (THC, green) or vehicle (Tween80/saline, 5:95; VEH, black) from PND 30 to 43. Symbols represent mean ± SEM, *n* = 3 or 4 animals per data point, outliers removed using the Grubb's test. VEH, vehicle.

were comparable to those observed in adult nonmedical cannabis smokers.[19–22] For example, the data reported in Supplementary Table S3 show that the plasma C_{max} values in adult male and female rats (88 ± 14 ng/mL) were similar to plasma C_{max} measured in men and women who had smoked one cannabis cigarette containing ∼30 mg of THC (80–100 ng/mL).[22] These levels of systemic exposure are known to be associated with psychotropic activity in adult humans[19,22] as well as in adolescent rats[9] and adult mice.[8] In the present study, they yielded concentrations of THC in the brain of adolescent (C_{max} ∼250 nM) and, to a lesser extent, adult (C_{max} ∼121 nM) male rats (Supplementary Table S5) that were likely to be fully bioactive.[23] Slightly higher brain concentrations were found in female animals (adolescents: ∼330 nM; adults: ∼280 nM) (Supplementary Table S5).

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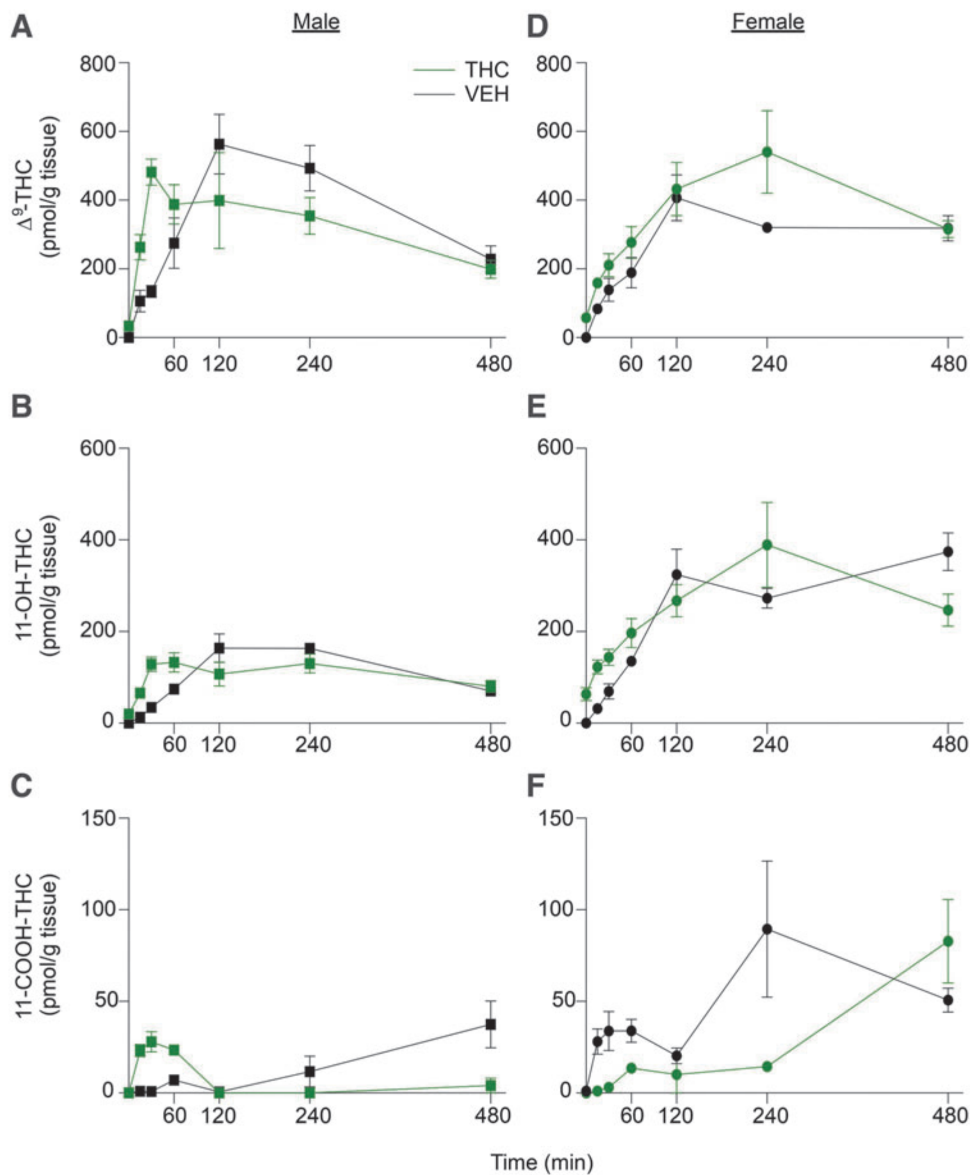

FIG. 8. Brain concentrations of THC and its first-pass metabolites, 11-OH-THC and 11-COOH-THC, after IP injection of 5 mg/kg THC (PND 44) in adolescent male **(A–C)** or female **(D–F)** rats treated with daily injections of 5 mg/kg THC (THC, green) or vehicle (Tween80/saline, 5:95; VEH, black) from PND 30 to 43. Symbols represent mean ± SEM, $n=3$ or 4 animals per data point, outliers removed using the Grubb's test. VEH, vehicle.

Sex-dependent differences in the biotransformation of THC into its pharmacologically active metabolite 11-OH-THC have been previously documented, including in adolescent and adult rats[9,24–29] and in adult humans,[30] with females of both species exhibiting substantially greater conversion of THC into 11-OH-THC relative to males. This dimorphism was confirmed in this study. For example, at the 5-mg/kg dose of THC, the C_{max} values for 11-OH-THC in plasma and brain were ~7 times greater in adult females than in adult males and 2.7 times greater in adolescent females than in adolescent males (Supplementary Tables S3 and S5). Moreover, THC metabolism to 11-OH-THC was twice as fast in liver microsomes from adult females than in microsomes from adult males. This finding is consistent with a previous report showing

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preferential formation of 11-OH-THC by female rat liver microsomes.[31] Given its consistency and size, it is reasonable to suggest that this metabolic dimorphism contributes to the greater sensitivity to THC exhibited by female rodents as well as women.[9,30,32,33]

Adolescent male mice are partially shielded from the psychoactive effects of THC through a mechanism that may involve, at least in part, enhanced brain expression of two blood–brain barrier constituents[8]: the multidrug transporter *Abcg2*, which may extrude Δ^9-THC from brain parenchyma,[33] and claudin-5, which is implicated in gap junction structure and barrier permeability.[34] The present results reveal an unexpected species specificity in the access of THC to the central nervous system. We found that, in contrast with the results obtained in male mice, (1) adolescent male and female rats display higher brain-to-plasma ratios for THC, compared with adults; and (2) C_{max} and AUC values for THC in brain are substantially higher in male adolescent rats relative to adults of the same sex. This discrepancy underscores the need to interpret the pharmacodynamic properties of THC in the context of the drug's species-relevant PK profile.

Two additional findings are noteworthy. First, repeated exposure to THC during adolescence does not ostensibly affect the drug's PK properties, which remain unaltered after a 2-week regimen of once-daily 5 mg/kg THC injections. These results are consistent with previously reported data[35–38] (but see Refs. [17] and [18]) for contrasting findings. Second, the peak concentrations reached by 11-OH-THC in both adolescent and adult animals were $\sim$5 times higher in brain tissue than in plasma. For example, in female adults, the C_{max} for 11-OH-THC was 28 ± 3 pmol/mL in plasma (Supplementary Table S3) versus 149 ± 25 pmol/g in brain (Supplementary Table S5). This result, which closely matches those reported for mice,[8] is consistent with the proposed existence of THC biotransformation in neural tissue.[38] Establishing the molecular bases and functional implications of brain THC metabolism, if any, will require additional experimentation.

The present report has two main limitations. First, in a previous study focused on adolescent rats,[9] we administered THC in 1 mL/kg of vehicle, whereas in the current experiments we used 0.5 mL/kg. This choice, which was motivated by the need to limit the volume of vehicle injected in adult animals, affected PK parameters such as AUC and C_{max}. This discrepancy should be taken into consideration when comparing the two sets of results. Second, the present experiments were conducted in young adult rats (PND70); thus, the results may or may not generalize to older animals. Additionally, cannabis extracts contain a large number of chemical constituents, which may affect the PK properties of THC. For example, CBD may inhibit THC metabolism through CYP3A11,[39] while some terpenoids (e.g., borneol) may alter the blood–brain barrier permeability.[40]

In conclusion, this study provides a systematic overview of the PK properties of THC in adolescent and adult rats of both sexes. Its results confirm that, irrespective of age, female animals transform the drug to its bioactive metabolite 11-OH-THC more effectively than males do. The findings further demonstrate that adolescent rats, unlike adolescent mice,[8] are not protected through PK mechanisms against the psychoactive effects of THC. In fact, the brain concentrations attained by the drug are greater at this age than later in life. These findings have broad implications for the interpretation of studies on THC, and highlight the need for further investigations on absorption, metabolism, and distribution of this drug across the human lifespan.

Authors' Contributions

Participated in research design: D.P., A.T., A.D., and S.V.M.; Conducted experiments: A.T., P.R., L.L., C.V., D.G., V.C.I., J.S.M., and F.A.; Performed data analysis: A.T. and P.R.; Wrote or contributed to the writing of the article: D.P., A.T., A.D., and M.A.H.

Author Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Table S1
Supplementary Table S2
Supplementary Table S3
Supplementary Table S4
Supplementary Table S5
Supplementary Table S6
Supplementary Table S7
Supplementary Table S8
Supplementary Table S9

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Abbreviations Used

11-COOH-THC = 11-nor-9-carboxy-Δ^9-THC
11-OH-THC = 11-hydroxy-Δ^9-THC
Abcg2 = ATP Binding Cassette Subfamily G Member 2
AUC = area under the curve
CBD = cannabidiol
CL = clearance
C_{max} = maximal concentration
CYP_{450} = cytochrome P_{450}
EDTA = ethylenediaminetetraacetic acid
ESI = electrospray ionization
IP = intraperitoneal
ISTD = internal standards
LOQ = limit of quantification
MRM = multiple reaction monitoring
PK = pharmacokinetic
PND = postnatal day
$t_{1/2}$ = half-life time of elimination
THC = Δ^9-tetrahydrocannabinol
T_{max} = time at which maximal concentration
V_D = volume of distribution

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