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ARTICLE

Pharmacokinetic and pharmacodynamic profile of high-dose D^9-tetrahydrocannabinol in male and female rats

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ABSTRACT

Cannabis products with elevated Δ^9-tetrahydrocannabinol (THC) content are prevalent in parts of North America, yet their pharmacokinetic (PK) and pharmacodynamic (PD) properties remain incompletely characterized. We investigated the effects of a single administration of high-dose THC (10 and 30 mg/kg, intraperitoneal, IP) compared with a lower dose (3 mg/kg, IP) in male and female Long Evans rats. Plasma, brain, and white adipose tissue concentrations of THC and its metabolites 11-hydroxy-THC and 11-carboxy-THC were quantified over acute (up to 24 h) and prolonged (14 days) periods. Behavioral endpoints included ingestive behavior, pica, locomotor activity, anxiety-like and stress-coping responses, and nociception. Plasma concentrations of multiple cytokines were also assessed. PK results showed nonlinear disposition with disproportionate increases in maximal concentration at higher doses, greater systemic and tissue exposure in females, and extensive sequestration in adipose tissue. PD effects were also dose- and sex-dependent: suppression of food/water intake and body weight, transient locomotor stimulation in females at low dose and suppression at high doses, increased pica in females at 30 mg/kg, modest anxiety-like shifts, bidirectional stress-coping changes in males, and antinociception primarily in males at 1 hour and 24 hours postdosing. No meaningful changes in proinflammatory or anti-inflammatory cytokines were observed in either sex. The findings indicate that high-dose THC produces nonlinear sex-dependent PK with prolonged central and peripheral retention, alongside distinct acute PD profiles compared with lower doses.

Significance Statement: High-potency cannabis products are common, yet their impact on health remains poorly understood. This study shows that high-dose THC produces nonlinear, sex-dependent distribution and metabolism in rats, with females showing markedly greater systemic exposure, prolonged brain retention, and extensive adipose sequestration, alongside distinct acute pharmacological profiles. These findings show that high-dose THC generates disproportionate systemic and central exposure and distinct acute pharmacological profiles compared with lower doses.

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1. Introduction

Cannabis is consumed, either legally or illegally, by millions of people worldwide for its euphoric, relaxing, and therapeutic effects, though its use is also associated with risks including dependence, cognitive impairment, and psychiatric symptoms in vulnerable individuals.[1–3] The psychotropic constituent of cannabis, Δ^9-tetrahydrocannabinol (THC), is a highly hydrophobic terpenoid molecule that exerts its intoxicating effects through

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partial agonism at cannabinoid CB_1 receptors in the central nervous system.[3] Activation of CB_1 receptors modulates the release of excitatory, inhibitory, and modulatory neurotransmitters, shaping THC's unique pharmacological profile.[4,5] This includes antiemesis, appetite stimulation, and analgesia—which are currently the object of renewed therapeutic interest[6–8]—along with non-therapeutic effects such as euphoria, sedation, impairment of memory and motor coordination, and behavioral reinforcement[8] Whether therapeutic or not, most if not all THC's actions are mediated by its CB_1-mediated interaction with the endocannabinoid signaling system, whose endogenous lipid-derived ligands THC functionally mimics.

Over the last decades, the THC content in cannabis, colloquially referred to as 'cannabis potency', has progressively increased.[8] In the 1960s–1970s, typical cannabis preparations contained approximately 1%–4% THC. By the 1990s, average levels rose to around 4%, and analyses of seized samples and market products show a continued upward trend, with mean THC concentrations in herbal cannabis reaching 12% to 15% by the mid-2010s and often exceeding 20% in flower cultivars currently available in legal markets across North America. Concentrates and other high-potency formulations frequently contain up to 95% THC, representing increases of 5- to 20-fold or more compared with historical levels.

This marked escalation in THC content raises the possibility that highly concentrated products exhibit distinct pharmacokinetic (PK) and pharmacodynamic (PD) properties relative to lower-potency cannabis used in the past. Administration of high doses of THC may produce nonlinear PK, including disproportionate increases in systemic exposure, altered formation of first-pass metabolites—bioactive 11-hydroxy-THC (11-OH-THC) and inactive 11-carboxy-THC (11-COOH-THC)—prolonged retention in lipid-rich compartments such as brain and adipose, and sex-dependent differences in disposition. From a PD perspective, elevated exposures may not only amplify canonical effects but also alter their qualitative profile, potentially shifting dose–response relationships toward increased adverse outcomes. For example, cannabis hyperemesis syndrome—a condition characterized by recurring nausea and vomiting after cannabis consumption—is thought to have emerged only after the advent of concentrated THC products and to result from frequent exposure to such products.[9]

Despite the increasing prevalence of high-potency cannabis products, most published studies examine the behavioral and physiological effects of THC doses in the low-to-moderate range, typically between 1 and 5 mg/kg, with a few studies including 10 mg/kg. Consequently, the PK and PD consequences of higher systemic THC exposures remain insufficiently characterized. This knowledge gap limits our ability to interpret the biological effects of contemporary high-potency cannabis preparations and to model the consequences of excessive THC exposure in experimental animals. The objective of the present study was to address this gap by characterizing the PK and PD profile of high-dose or very-high-dose THC (10 and 30 mg/kg, respectively) in male and female rats after intraperitoneal (IP) administration, in comparison to a moderate dose (3 mg/kg, IP). Building on our previous work using low-to-moderate THC doses (0.3-3 mg/kg) in mice and rats of both sexes,[10–14] we measured plasma, brain, and white adipose tissue (WAT) concentrations of THC and its first-pass metabolites over acute and prolonged periods (up to 14 days) following a single exposure. We further assessed key behavioral and physiological endpoints, including food intake, water intake, fluid balance, pica, locomotor activity, spontaneous behaviors, anxiety-like responses, stress-coping, autonomic output, and nociception. Finally, we measured circulating cytokines to assess THC's impact on systemic inflammation.

2. Materials and methods

2.1. Animals

Young adult male and female Long-Evans rats (postnatal day [PND] at arrival: 70–80, 250–300 g) were purchased from Charles River. All experiments were conducted on animals of the same age and weight range. Animals were housed in groups of 2 in individually ventilated cages with corncob bedding and shredded paper enrichment. Housing rooms were maintained on a 12-h light/dark cycle (lights on at 6:30 AM) under controlled conditions of temperature (20 ± 2 °C) and relative humidity (55%–60%). Food and water were available ad libitum. Animals were allowed to acclimate for at least 7 days before experiments. All procedures were approved by the Institutional Animal Care and Use Committee at the University of California, Irvine (Animal Use Protocol 24-151) and carried out in strict accordance with the National Institutes of Health guidelines for the care and use of experimental animals.

2.2. Solvent and chemicals

Liquid chromatography/mass spectrometry (LC/MS)-grade water, methanol, acetonitrile, and isopropanol were from Honeywell (Muskegon, Michigan). LC/MS-grade formic acid was from Thermo Fisher (Houston, Texas). THC, 11-OH-THC, 11-COOH-THC along with internal standards (ISTD), [$^2H_3$]-THC, [$^2H_3$]-11-OH-THC, and [$^2H_3$]-11-COOH-THC were from Cayman Chemicals (Ann Arbor, Michigan).

2.3. Drug preparation and administration

THC was prepared shortly before experiments by evaporating the commercial acetonitrile solution under a light stream of N_2 and dissolving the residue in Tween 80/sterile saline (5:95, v/v). All cage mates received the same treatment.

2.4. Pharmacokinetic analyses

PK experiments were performed as described previously,[15] with modification for dosage. THC was administered by IP injection at doses of 3, 10, or 30 mg/kg in a volume of 1 mL/kg. The rats were anesthetized with isoflurane at various time points after injection (15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 1 day, 3 days, 7 days, and 14 days; n = 4 per time point). Blood was collected into EDTA-rinsed syringes by cardiac puncture and transferred into polypropylene tubes containing spray-coated potassium-EDTA (Greiner Bio-One, Kremsmünster, Austria). Samples were centrifuged at 1500 × *g* at 4°C for 15 min, plasma was transferred into glass vials and stored at −80 °C until analysis. Brain and WAT samples were collected, flash-frozen in dry ice, and stored at −80 °C. Stock standard solutions (1.0 μg/mL) of THC, 11-OH-THC, and 11-COOH-THC were prepared in methanol. Deuterium-containing internal standards were added to final concentrations of: [$^2H_3$]-THC, 4 ng/mL; [$^2H_3$]-11-OH-THC, 20 ng/mL; and [$^2H_3$]-11-COOH-THC, 20 ng/mL. Serial dilutions of stock solutions (from 1.0 μg/mL to 0.01 ng/mL) were used to generate calibration curves, as previously described.[16] PK parameters were determined using a noncompartmental model[17] implemented in GraphPad Prism v10.1. Statistical differences in C_{max} and AUC values were determined using Student's unpaired *t test* or two-way ANOVA with Bonferroni post hoc analysis using all data points up to 24 hours.

2.5. Sample preparation

Sample extraction was performed as described,[17] with minor modifications. Plasma samples (50 mL) were transferred into 8-mL glass vials (Cat. No.: B7999-3; Thermo-Fisher), and proteins were precipitated by the addition of ice-cold acetonitrile (450 μL) containing 1% formic acid along with [$^2H_3$]-THC, [$^2H_3$]-11-OH-THC, and [$^2H_3$]-11-COOH-THC. WAT and brain samples were homogenized using Precellys CK-14 kits in Precellys Evolution bead mills (Bertin) at 4 °C on preset setting #4 (6500 RPM rotational speed × 20 seconds × 2 cycles).

WAT samples (5 mg) were transferred into 2-mL bead mills (Bertin; Cat. No.: P000912-LYSK1-A.0) and were homogenized with the addition of ice-cold acetonitrile containing 1% formic acid (450 μL) along with [$^2H_3$]-THC, [$^2H_3$]-11-OH-THC, and [$^2H_3$]-11-COOH-THC. Brain samples were transferred into 7-mL bead mills (Bertin; Cat. No.: P000940-LYSK0-A.0) and were homogenized with the addition of ice-cold acetonitrile containing 1% formic acid (450 μL) along with [$^2H_3$]-THC, [$^2H_3$]-11-OH-THC, and [$^2H_3$]-11-COOH-THC. Supernatants were loaded onto Captiva-Enhanced Matrix Removal (EMR)-Lipid cartridges (Agilent Technologies) and eluted under positive pressure (3-5 torr, 1 drop/5 sec). Pellets were rinsed with water/acetonitrile (1:4, v/v; 200 μL), vortexed for 30 s, and centrifuged at 3000 × *g* at 4°C for 15 min. The supernatants were transferred onto EMR cartridges and eluted. The cartridges were then washed with water/acetonitrile (1:4, v/v, 200 μL), and pressure was increased to 10 Torr (1 drop/s) to maximize analyte recovery. Recoveries were as follows: plasma, 89%; brain, 96%; WAT, 79%. Eluates were dried under a light N_2 stream and reconstituted in 100 μL of methanol. Samples were transferred to deactivated glass inserts and placed inside amber glass vials (2 mL, Agilent Technologies).

2.6. LC-MS/MS analyses

Chromatographic separations were conducted as described[17] using an Agilent 1260 Infinity II LC system consisting of a binary pump, degasser, temperature-controlled autosampler, and column compartment coupled to an Agilent 6460 triple quadrupole mass spectrometric detector. Analytes were separated on an Eclipse XDB C_{18} 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 0.3 mL/min. The gradient conditions were as follows: starting from 75% B to 80% B in 5.0 min, changing to 95% B at 5.01 min, and maintained until 6 min to remove any strongly retained materials from the column. Equilibration time was 9 min. The column was maintained at 40 °C, and the autosampler at 9 °C. The total analysis time, including reequilibrium, was 15 min. The injection volume was 2 μL. To prevent carryover, the needle was washed in the autosampler port for 10 sec before each injection using a wash solution consisting of water/methanol/isopropanol/acetonitrile (1:1:1:1, v/v). The MS was operated in positive Agilent Jetstream electrospray ionization (AJS ESI) mode, and analytes were quantified by multiple reaction monitoring (MRM) of the following transitions: THC *m/z* 315.2 > 193.0, [$^2H_3$]-THC *m/z* 318.2 > 196.1, 11-OH-THC *m/z* 331.2 > 313.1, [$^2H_3$]-11-OH-THC *m/z* 334.2>316.1, 11-COOH-THC *m/z* 345.2 > 299.2, and [$^2H_3$]-11-COOH-THC *m/z* 348.2 > 302.2. Lowest limits of quantification (LLOQ) were as follows: THC, 0.636 pmol/mL; 11-OH-THC, 0.303 pmol/mL; 11-COOH-THC, 0.290 pmol/mL. Each analytical batch included an 11-point calibration curve (0.05 ng to 1000 ng on column, $R^2 > 0.98$) along with quality controls and blank samples to monitor carryover, background contamination, and instrument performance. The capillary voltage was set at 3500 V. The source temperature was 300°C, and gas flow was 12.0 L/min. Nebulizer pressure was 40 psi. Collision energy and fragmentation voltage were set for each analyte as previously reported. The Mass Hunter software (Agilent Technologies) was used for instrument control, data acquisition, and data analysis.

2.7. Physiological parameters

Baseline measures of food intake, water intake, body weight, and number of fecal bolis were taken. Animals were weighed immediately before and 24 h after drug or vehicle administration, which was done at approximately 8:00 AM. Food and water intake was measured by weighing and subtracting preweighed food hoppers and water bottles before values after access, also considering spillage/uneaten remnants. Number of fecal bolis were counted manually in the home cages.

2.7.1. Pica intake

Laboratory-grade kaolin clay (K1004; Spectrum Chemical MFG Corporation) was mixed in a ratio of 5:1.5 with water, shaped into a dough, cut into pellets, and allowed to air-dry for 48 h in a chemical fume hood. Rats were habituated to the pica diet for 3 days prior to injections.

2.8. Biochemical measurements

Blood was drawn by cardiac puncture into anticoagulated test tubes and plasma was immediately prepared by centrifugation (2600 × *g*, 4 °C, 10 min) and stored at −20 °C until analysis. Brains were stored at −80 °C until analysis. Plasma concentrations of antidiuretic hormone (ADH), copeptin, and angiotensin II were measured by solid-phase sandwich ELISA kits for rat tissue lysates following the manufacturer's guidelines (ADH: catalog reference: E-EL-R0522, Elabscience; copeptin: catalog reference: NBP3-42433, Novus Biologicals; Angiotensin II: catalog reference: EIA-ANGII-1, Ray Biotech). Plate absorbance was read at 450 nm, employing a Tecan Spark 10 M Multimode Plate Reader (Tecan). Plasma concentrations of kidney function biomarkers (blood urea nitrogen [BUN], creatinine, creatinine kinase, and BUN-to-creatinine ratio); liver function (alanine transaminase [ALT], alkaline phosphatase [ALP], aspartate transaminase [AST], protein [globulin, albumin, total protein, and albumin-to-globulin ratio]); electrolytes (sodium, chloride, phosphorus, potassium, and bicarbonate); and cytokine concentrations (IL-4, IL-6, Il-10, IL-1α, IL-1β, IL-12, GM-CSF, and INF-γ) were measured at IDEXX Bio Analytics using the standard comprehensive chemistry panel code-6006.

2.9. Behavioral Tests

2.9.1. Open field

Changes in locomotor activity were evaluated in an open field, as previously described by Ishola et al.[18] All animals were randomized, and observers were blinded to treatment groups. Measures were made 10 min and 1 h after THC or vehicle injection in the animals' home cage. One day after injection, rats were placed in an open field box (100 cm × 100 cm × 45 cm). Total distance traveled, average speed, number of rotations, and number of grooming, climbing, and freezing episodes were recorded using the ANY-maze Video Tracking Software (Stoelting Co) for a 10 min duration after 1 min of acclimatization. At the end of each trial, the box was thoroughly cleaned with an odor eliminator.

2.9.2. Light-dark (L/D) box

The L/D box apparatus consisted of a 30 cm × 40 cm light chamber; a 20 cm × 40 cm dark chamber; and a 10 cm door with

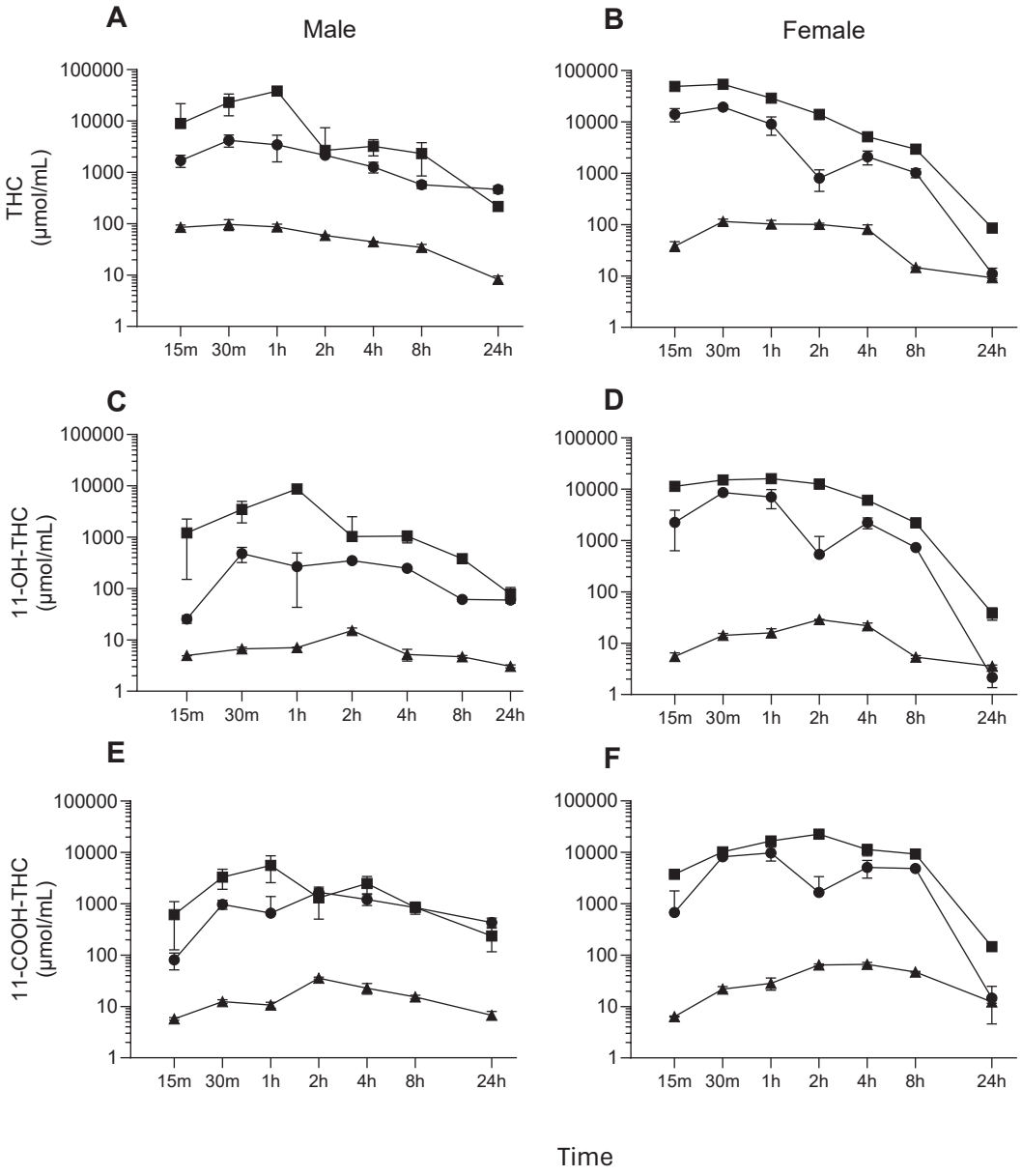


Fig. 1. Pharmacokinetic profile of high-dose THC in plasma. THC (A, B), 11-OH-THC (C, D), and 11-COOH-THC (E, F) after IP administration of 3 mg/kg (insets, triangles), 10 mg/kg (open squares), and 30 mg/kg (closed squares) of THC in adult male (A, C, E) and female (B, D, F) rats. Symbols represent mean ± SEM ($n = 3–4$ animals per group). Data were analyzed using one-way ANOVA. *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$.

an infrared translucent lid for the dark chamber top box with 2 compartments, one of which was illuminated with white light (~400 lux), while the other remained dark. Each animal was placed at the junction of the light/dark, facing the illuminated compartment. The time spent in the light compartment was recorded by observers blinded to treatment condition.[19] After each test, the box was cleaned with an odor eliminator.

2.9.3. Elevated plus maze (EPM)

The EPM apparatus was elevated to 75 cm above the floor and consisted of two open arms (50 × 10 cm) crossed at a central platform (right angles) with two opposed arms of the same size enclosed by walls (40 cm high), with squares drawn on the floor. Each rat was placed on the central platform of the apparatus facing an open arm and was allowed to explore the maze for 5 min.[20] Behavior was recorded using the ANY-maze Video Tracking Software with a camera placed above the center of the apparatus. After 5 min, the animals returned to their home cages. After each test, the maze was cleaned with an odor eliminator.

2.9.4. The forced swim test (FST)

The FST was conducted 1 day postinjection in a transparent Plexiglas cylinder (25 cm in diameter, 50 cm height).[21] Rats were allowed to swim for 5 min in a cylinder filled with 25 ± 1°C water to a 35 cm depth to prevent the animals' tails from touching the bottom of the tank.[22] Time spent in immobility, center positioning,

total distance, average speed, and number of fecal bolis were measured. Testing occurred under dim lighting (~20 lux) with white noise (30 dB) in the background. After the sessions, rats were removed and wiped with towels until dry.

2.9.5. Tail immersion

Baseline nociception was measured by tail immersion, as reported by Fotio et al.[23] Briefly, rats were gently held in a soft tissue pocket. The distal half of their tail was submerged in a water bath maintained at 54 °C, and the latency to tail withdrawal was measured in duplicate with a cutoff time set at 10 seconds. The average tail withdrawal latencies were measured 10 min, 1 h, and 1 day after injections.

2.10. Statistical analysis

Results are presented as means ± S.E.M., and statistical differences between groups were determined using unpaired *t* test or one-way ANOVA, as appropriate. The significance level was set as *$P < .05$, **$P < .01$, ***$P < .001$, and ****$P < .0001$.

3. Results

3.1. Pharmacokinetics of high-dose THC in plasma

Administration of THC (3, 10, and 30 mg/kg, IP) produced rapid, dose-dependent increases in plasma concentrations of the drug in both male and female rats, reaching C_{max} values well over 50 mM (Fig. 1A, B, and Table 1; nontransformed data shown in Supplemental Fig. S1A, D). At 10 and 30 mg/kg, both C_{max} and AUC were markedly greater in females than in males. Unpaired *t*-test between C_{max} values showed significant differences at 10 mg/kg THC ($P = 0.0006$) and 30 mg/kg THC ($P = 0.006$), indicating sex-dependent differences. Moreover, C_{max} increased more than proportionally with dose, based on a power model applied to log-transformed data (men: $\beta = 2.6$, 95% CI: 2.3−3.0; women: $\beta = 2.7$, 95% CI: 2.0−3.4) (Supplementary Fig. 2A). Visual inspection of the time courses indicated rapid attainment of peak concentrations followed by a decline over 2 to 4 hours and only partial clearance (Fig. 1A, B, Supplemental Fig. S1A, D).

Plasma concentrations of the bioactive THC metabolite 11-OH-THC rose rapidly after THC administration, with peak concentrations occurring in both sexes at 30−60 min (Fig. 1C, D; nonlog-transformed data shown in Supplemental Fig. 1B, E). Unpaired *t*-test of C_{max} values in males versus females showed significant differences at 3 mg/kg THC ($P = .004$), 10 mg/kg THC ($P = .0002$), and 30 mg/kg THC ($P = .007$). As for parent THC, C_{max} increased more than proportionally with dose (men: $\beta = 2.8$; 95% CI, 2.5−3.1; women: $\beta = 2.7$; 95% CI, 1.7−3.6) (Supplemental Fig. 2B). At all THC doses and in both sexes, 11-OH-THC remained detectable in plasma for the entire duration of the experiment (Fig. 1C, D; Supplemental Fig. S1B, E).

The inactive metabolite 11-COOH-THC displayed a delayed time course relative to its precursors, with T_{max} values ranging from 60 to 240 min depending on dose and sex (Fig. 1E, f; nonlog-transformed data shown in Supplemental Fig. S1C, F). T_{max} occurred later in females (up to 240 min at 3 mg/kg THC), consistent with sustained formation and/or slower clearance. Unpaired *t*-test of C_{max} values in males versus females showed significant differences at 3 mg/kg THC ($P = .01$), 10 mg/kg THC ($P = .05$), and 30 mg/kg THC ($P = .01$). C_{max} increased more than proportionally with dose (men: $\beta = 2.2$; 95 % CI: 1.5−2.9; women: $\beta = 2.6$; 95 % CI: 1.8−3.3) (Supplemental Fig. 2C). Plasma 11-COOH-THC remained detectable at 24 h for all doses and in both sexes (Fig. 1E, F; Supplemental Fig. S1C, F).

In sum, across all analytes, the relationship between dose and exposure deviated from linearity such that increasing the THC dose from 3 to 30 mg/kg resulted in disproportionately large increases in C_{max} for both THC and its metabolites. Moreover, women consistently exhibited higher C_{max} values than men at equivalent doses, with the magnitude of the difference increasing with dose. These patterns indicate that dose- and sex-dependent factors influence both systemic exposure and metabolic conversion.

3.2. Brain distribution of high-dose THC and metabolites

THC accumulated in the brain with a delayed T_{max} relative to plasma and sustained tissue exposure (Fig. 2A, B, and Table 2; nonlog-transformed data shown in Supplemental Fig. S3A, D). At the 3 and 10 mg/kg doses, brain THC concentrations started declining after the 8-hour time point. At 30 mg/kg, concentrations were still in the micromolar range at 24 hours. Exposure increased approximately in proportion to dose based on the point estimates; however, formal statistical criteria for dose proportionality were not met because the confidence intervals around the slope estimate were too wide (Supplemental Fig. 2D). Unpaired *t*-test of C_{max} values in males versus females showed significant differences at 10 mg/kg THC ($P = .001$), confirming the existence of sex-dependent differences in THC disposition.

Peak concentrations of 11-OH-THC occurred between 1 and 4 hours postinjection, depending on dose and sex (Fig. 2C, D; nontransformed data shown in Supplemental Fig. 3B, E). At 10 mg/kg THC, they were higher in women than in men, with an unpaired

Table 1
Pharmacokinetics and biotransformation of THC in plasma
Statistical comparisons of Cmax values between males and females were performed using unpaired *t*-test ($n = 3/4$ rats per group) after 24 hours. *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$.

Analyte	Dose (mg/kg)	Male				Female			
		Cmax (pmol/mL)	AUC (pmol•min/mL)	AUC Metabolite / AUC THC	Tmax (min)	Cmax (pmol/mL)	AUC (pmol•min/mL)	AUC Metabolite / AUC THC	Tmax (min)
D9-THC	3	104 ± 23	45,823 ± 5,242	-	30	117 ± 13	45,685 ± 4,926	-	30
	10	4,617 ± 1,829***	1,343,780 ± 186,377	-	30	19,650 ± 1,672	2,216,287 ± 365,199	-	30
	30	38,322 ± 2,400**	6,155,862 ± 1,667,435	-	60	53,856 ± 2,064	7,104,797 ± 711,519	-	30
11-OH-THC	3	16 ± 2**	7,250 ± 557	0.158	120	29 ± 0	12,674 ± 881	0.277	120
	10	551 ± 158***	176,092 ± 17,331	0.131	30	8,665 ± 491	1,639,459 ± 258,190	0.740	30
	30	8,739 ± 460**	1,072,584 ± 125,505	0.174	60	16,125 ± 1,270	4,879,980 ± 548,606	0.687	60
11-COOH-THC	3	36 ± 1**	21,109 ± 2,218	0.461	120	67 ± 6	53,876 ± 2,409	1.179	240
	10	1746 ± 276*	1,214,120 ± 140,101	0.904	120	10,901 ± 2935	5,120,325 ± 599,166	2.31	60
	30	8,848 ± 3026**	1,871,482 ± 399,057	0.304	60	22,909 ± 2279	11,056,331 ± 973,548	1.56	120

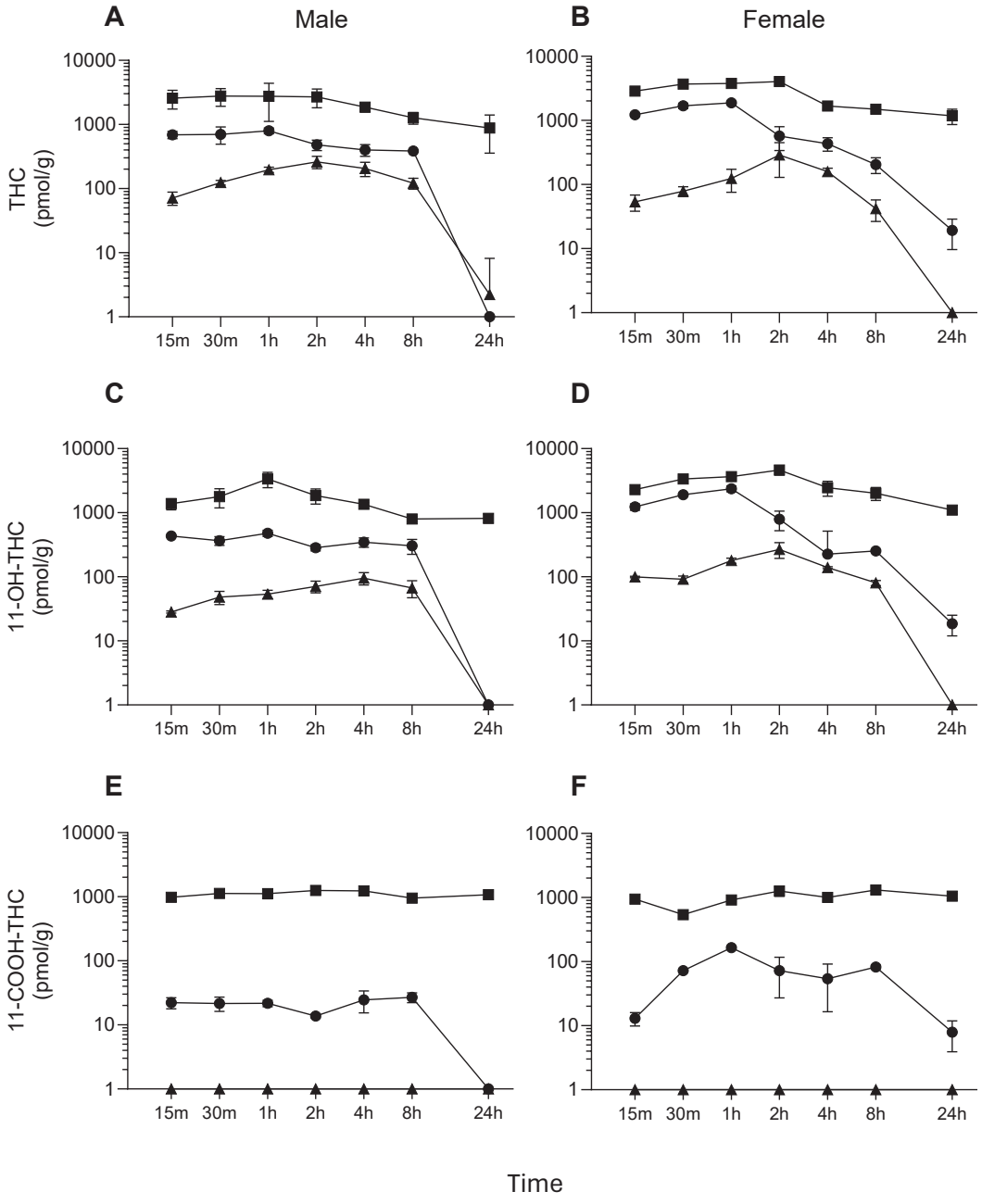


Fig. 2. Pharmacokinetic profile of high-dose THC in brain. THC (A, B), 11-OH-THC (C, D), and 11-COOH-THC (E, F) after IP administration of 3 mg/kg (insets, triangles), 10 mg/kg (open squares), and 30 mg/kg (closed squares) of THC in adult male (A, C, E) and female (B, D, F) rats. Symbols represent mean ± SEM ($n = 3–4$ animals per group) after 24 hours. Data were analyzed using one-way ANOVA. *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$.

t-test of C_{max} values showing a significant difference between the 2 sexes ($P = .003$). As in plasma, the increase in exposure to dose was nonlinear. Dose proportionality was not met for C_{max} in either men ($\beta = 1.5$; 95% CI: 1.2–1.9) or women ($\beta = 1.3$; 95% CI: 0.9–1.6) (Supplemental Fig. 2E).

Unlike THC and 11-OH-THC, 11-COOH-THC showed relatively low accumulation in the brain (Fig. 2E, F; nontransformed data shown in Supplemental Fig. 3C, F). At 3 mg/kg THC, the metabolite was not reliably detected in brains of either sex. At 10 mg/kg THC, it remained detectable until the 8-hour time point and declined thereafter, while at 30 mg/kg THC, its concentrations remained low micromolar for the entire duration of the experiment. Unpaired *t*-test of C_{max} values in males versus females showed significant differences at 10 mg/kg THC ($P = .0008$).

In sum, across all analytes, the delayed T_{max} and prolonged retention of THC and 11-OH-THC in the brain relative to plasma indicates slow equilibration between the 2 compartments and sustained central exposure. Moreover, for 11-OH-THC, the relationship between dose and exposure deviated from linearity, and women exhibited higher brain concentrations than males at

Table 2
PK and biotransformation of THC in brain. Statistical comparisons of Cmax values between males and females were performed using unpaired *t*-test ($n = 3/4$ rats per group) after 24 hours. *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$

Analyte	Dose (mg/kg)	Male					Female				
		Cmax (pmol/g)	AUC (pmol•min/g)	AUC Metabolite / AUC THC	Tmax (min)	AUC Tissue / AUC Plasma	Cmax (pmol/g)	AUC (pmol•min/g)	AUC Metabolite / AUC THC	Tmax (min)	AUC Tissue / AUC Plasma
D9-THC	3	284 ± 57	157,866 ± 28,735	-	120	3.445	410 ± 160	103,125 ± 25,822	-	120	2.257
	10	809 ± 82***	410,265 ± 23,633	-	60	0.305	1,892 ± 117	409,121 ± 74,161	-	60	0.185
	30	3,632 ± 1,633	2,284,790 ± 591,352	-	60	0.371	4,132 ± 334	2,480,094 ± 332,573	-	120	0.349
11-OH-THC	3	100 ± 21	74,091 ± 20,180	0.469	240	10.219	294 ± 73	111,071 ± 11,710	1.077	120	8.764
	10	484 ± 54**	311,430 ± 57,147	0.759	60	1.768	2,408 ± 246	523,832 ± 88,091	1.278	60	0.113
	30	3,626 ± 909	1,536,001 ± 164,218	0.672	60	1.432	4,831 ± 791	3,006,321 ± 523,500	1.212	120	0.616
11-COOH-THC	3	-	-	-	-		-	-	-	-	-
	10	28 ± 9***	24,873 ± 5,147	0.061	480	0.020	165 ± 9	91,094 ± 13,714	0.223	60	0.018
	30	1,257 ± 19	1,520,159 ± 136,466	0.665	120	0.812	1,312 ± 217	1,684,611 ± 203,933	0.679	120	0.152

equivalent doses, consistent with enhanced metabolic conversion and/or distributions.

3.3. Extensive accumulation of high-dose THC in white adipose tissue

As anticipated from previous work[24], THC accumulated at very high concentrations in WAT (exceeding 100 mM in both sexes at T_{max}) and displayed delayed and prolonged kinetics (Fig. 3A, B and Table 3; nontransformed data shown in Supplemental Fig. S4A, D). C_{max} increased more than proportionally with dose in both men ($\beta = 1.5$; 95% CI: 0.2−3.2) and women ($\beta = 2.1$; 95% CI: 0.4−3.9) although the wide CIs (which include values <1) prevent formal demonstration of superproportionality. At 10 mg/kg, THC concentrations in WAT were higher in women than in men ($P = .003$).

11-OH-THC also accumulated in WAT, with slower kinetics than observed in plasma or brain (Fig. 3C, D; nontransformed data shown in Supplemental Fig. S4B, E). Unpaired *t*-test of C_{max} values in males versus females showed no significant differences at all doses in 11-OH-THC. T_{max} values were delayed relative to plasma and brain, reaching 240 min at 3 and 10 mg/kg and 480 min at 30 mg/kg. Dose linearity was not met for C_{max} in either men ($\beta = 2.1$; 95 % CI: 1.6−2.5) or women ($\beta = 2.1$; 95 % CI: 1.6−2.6).

11-COOH-THC was present in WAT primarily after higher doses (10 and 30 mg/kg) and at substantially lower concentrations than THC and 11-OH-THC (Fig. 3E, F; nontransformed data shown in Supplemental Fig. S4c, F). Irrespective of sex, at 3 mg/kg THC, the metabolite was not quantifiable in WAT.

Across analytes, the delayed T_{max} and sustained concentrations observed in WAT indicate slow uptake and prolonged retention, consistent with partitioning of THC into lipid-rich compartments. The relationship between dose and exposure was nonlinear, with disproportionate increases in C_{max} at 10 and 30 mg/kg. At these doses, females exhibited greater adipose exposure than males.

3.4. Persistence of high-dose THC and metabolites in plasma, brain, and WAT.

To assess longer-term retention, the concentrations of THC, 11-OH-THC, and 11-COOH-THC were measured in plasma, brain, and WAT over a 14-day period after a single administration of 10 and 30 mg/kg THC (Fig. 4). In plasma of both male and female rats, parent THC was measurable until day 7 after dosing (Fig. 4A, D). In males, 11-OH-THC and 11-COOH-THC concentrations were lower than the LLOQ values at all time points (Fig. 4B, C), whereas, in females, they were quantifiable and higher than those of parent THC until postdosing day 7 (Fig. 4E, F), suggesting redistribution from storage sites and metabolism. In the brain, concentrations of THC and its metabolites decreased from day 3 to 7 after dosing but remained measurable for the entire duration of the study. In WAT, micromolar concentrations of THC were measurable until postdosing day 14 (Fig. 4M, P). In men, 11-OH-THC was measurable only at postdosing day 3 and at the highest dose, whereas 11-COOH-THC was undetectable at all time points (Fig. 4N). In females, both metabolites were measurable until day 7, and 11-COOH-THC remained measurable until day 14 (Fig. 4Q, R).

3.5. Acute effects of high-dose THC on ingestive behavior and body weight

Administration of THC variably affected 24-h food intake in male and female rats (Fig. 5A, B). In males, 30 mg/kg THC suppressed intake to near-minimal concentrations in at least one animal (Fig. 5A). Lower doses produced either a more modest nonsignificant effect (10 mg/kg) or no effect (3 mg/kg). Females also exhibited a reduction in food intake following THC administration. This was noticeable at all doses but reached significance only at 30 mg/kg (Fig. 5b). Differential changes are shown in Figure 5C.

Water intake was also variably affected. Administration of 30 mg/kg THC reduced 24-h intake in both male and female rats (Fig. 5D, E). More modest effects were produced at lower doses, with only 3 mg/kg THC producing a significant reduction in water intake in females only (Fig. 5d, e). Differential changes are reported in Figure 5f.

Consistent with its overall impact on ingestive behavior, 30 mg/kg THC caused a significant decrease in body weight, assessed 24 h after injection (Fig. 5G, H). Differential changes are shown in Figure 5I. Together, the findings indicate that high-dose THC acutely disrupts both food intake and water intake.

3.6. High-dose THC acutely alters fluid balance−related parameters and pica behavior

To probe potential mechanisms underlying THC's effects on ingestive behavior, we assessed circulating markers of fluid homeostasis along with pica, a well established indicator of nausea[25], at 24-h following THC or vehicle injection. Plasma concentrations of ADH (Fig. 6A) and copeptin (Fig. 6B), a 39-amino acid glycopeptide derived from the same precursor as ADH, were not significantly altered by THC in rats of either sex. Angiotensin II concentrations were unchanged in males but were significantly decreased in females at 30 mg/kg THC (Fig. 6C). The BUN/creatinine ratio showed a significant increase in both males and females

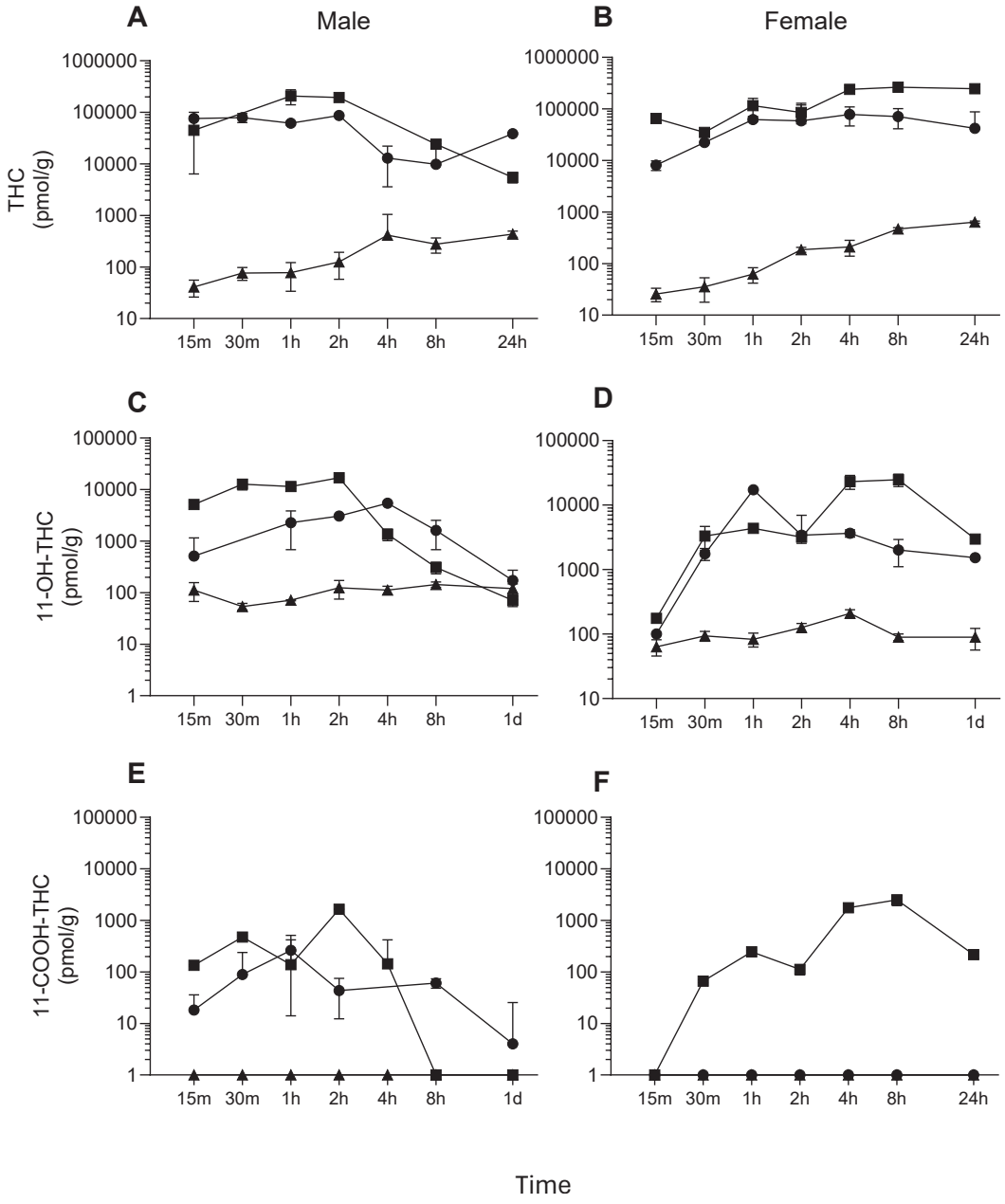


Fig. 3. Pharmacokinetic profile of high-dose THC in white adipose tissue. THC (A, B), 11-OH-THC (C, D), and 11-COOH-THC (E, F) after IP administration of 3 mg/kg (insets, triangles), 10 mg/kg (open squares), and 30 mg/kg (closed squares) of THC in adult male (A, C, E) and female (B, D, F) rats. Symbols represent mean ± SEM ($n = 3$-4 animals per group) after 24 hours. Data were analyzed using one-way ANOVA. *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$.

at 30 mg/kg THC (Figure 6D), while plasma phosphorus concentrations were decreased in females at 10 mg/kg THC only (Figure 6E). Bicarbonate (TCO_2) concentrations were unaffected in males and modestly affected in females (Fig. 6). Albumin concentrations were significantly increased in females at 10 mg/kg THC (Fig. 6G), with no change in males. Liver enzyme activities were not significantly altered by THC in either sex, including alanine transaminase (Fig. 6H), alkaline phosphatase (Fig. 6I), and aspartate transaminase (Fig. 6J).

Pica behavior was affected in a sex-dependent manner. In male rats, THC produced no significant change in pica intake (Fig. 6K). In women, both 3 and 30 mg/kg THC significantly increased pica intake, whereas 10 mg/kg had no such effect (Fig. 6I).

Additional biochemical analyses, conducted on plasma collected 24 hours after THC or vehicle injection, revealed limited effects of THC on systemic metabolic and electrolyte parameters (Table 4). Absolute BUN concentrations were not significantly altered by THC in either males or females, whereas creatinine concentrations were modestly reduced in men at 10 mg/kg. Creatine kinase showed no consistent changes in males but was modestly increased in females at 10 and 30 mg/kg. Total protein and globulin concentrations exhibited small but statistically

Table 3
PK and biotransformation of THC in WAT. Statistical comparisons of Cmax values between males and females were performed using unpaired *t*-test ($n = 3/4$ rats per group) after 24 hours. *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$.

Analyte	Dose (mg/kg)	Male					Female				
		Cmax (pmol/g)	AUC (pmol•min/g)	AUC Metab olite / AUC THC	Tmax (min)	AUC Tissue / AUC Plasma	Cmax (pmol/g)	AUC (pmol•min/g)	AUC Metaboli te / AUC THC	Tmax (min)	AUC Tissue / AUC Plasma
D9-THC	3	817 ± 630	580,963 ± 181,012	-	240	12.678	531 ± 63	564,150 ± 42,137	-	240	12.348
	10	86,634 ± 799**	52,736,398 ± 14,859,466	-	120	39.245	143,287 ± 69,441	86,372,229 ± 38,932,736	-	120	38.972
	30	232,364 ± 66,490	80,730,614 ± 19,437,096	-	60	13.114	269,894 ± 34,424	337,286,611 ± 39,718,929	-	480	47.473
11-OH-THC	3	147 ± 17	188,485 ± 33,156	0.324	480	25.998	215 ± 28	161,775 ± 34,231	0.287	240	12.764
	10	4,059 ± 1,971	2,379,421 ± 1,061,356	0.045	30	13.512	5,541 ± 2,968	3,858,620 ± 1,398,627	0.045	240	2.354
	30	17,091 ± 981	2,890,340 ± 195,852	0.036	120	2.695	26,066 ± 5,393	21,838,323 ± 4,808,759	0.065	480	4.475
11-COOH-THC	3	-	-	-	-	-	-	-	-	-	-
	10	735 ± 238	119,147 ± 31,505	0.002	60	0.098	-	-	-	-	-
	30	1,648 ± 22	298,694 ± 76,766	0.004	120	0.160	2,618 ± 558	2,597,764 ± 477,962	0.008	480	0.235

significant increases at intermediate doses in both sexes, without consistent changes in the albumin-to-globulin ratio. Electrolytes, including sodium, chloride, and potassium, remained largely stable across doses, except for a modest decrease in potassium at the highest dose in women.

These findings indicate that administration of high-dose THC variably alters indices of fluid balance in rats of both sexes and increases pica behavior in women, in the absence of major disruption of systemic metabolic or electrolyte homeostasis. Thus, the observed reductions in food and water intake may reflect a broad suppression of ingestive behavior, accompanied in females by a malaise-like state.

3.7. Acute effects of high-dose THC on locomotor activity and climbing behavior

To characterize behavioral responses to high-dose THC, we assessed locomotor activity and climbing behavior at 10 min and 1 h after administration in the animals' home cage and at 24 h in an unfamiliar open field (Fig. 7).

At 10 min post-THC, locomotor activity was differentially affected across doses and sexes (Fig. 7A–C). In male rats, total distance, average speed, and climbing behavior were not significantly altered across doses (Figure 7A–C). In women, 3 mg/kg THC increased total distance traveled, average speed, and climbing behavior, with no effect seen at the higher doses (Fig. 7A–C).

At 1 h after administration, locomotor activity and climbing behavior were comparable across treatment groups in males (Fig. 7D–F). In females, THC increased average speed at 10 mg/kg relative to vehicle (Fig. 7E), while total distance traveled and climbing behavior were unaffected (Fig. 7D and F).

At 24 h post-THC, locomotor activity in the open field did not differ significantly across treatment groups in either sex (Fig. 7G, H). Climbing behavior was also largely similar across groups, except for a significant reduction observed in males at the highest dose (Fig. 7I).

The results suggest that high-dose THC produces transient, sex-dependent alterations in locomotor activity, as assessed in a familiar environment, with females showing enhanced activity at the 3 mg/kg dose only.

3.8. Acute effects of high-dose THC on spontaneous behavioral indices of activity, self-directed behavior, and immobility

Using the same experimental protocol as in Figure 7, we quantified rotations (an index of spontaneous locomotor/exploratory activity), grooming episodes (an index of self-directed behavior often linked to stress or arousal), and freezing episodes (an index of immobility and defensive-like behavior) at 10 min and 1 h after THC or vehicle administration in the home cage and at 24 h in an unfamiliar open field (Fig. 8).

At 10 min post-THC, behavioral responses varied with sex and dose. In men, the number of rotations was markedly increased at 3 mg/kg relative to controls but was unchanged at 10 and 30 mg/kg (Fig. 8A). Grooming episodes were significantly decreased at 3 mg/kg compared with vehicle or higher doses (Fig. 8B). The number of freezing episodes was robustly increased at 10 and 30 mg/kg (Fig. 8C). In females, rotations were not significantly affected across doses, although a trend increase was observed at 3 mg/kg (Fig. 8A). Grooming showed a trend decrease at 3 mg/kg and no significant changes at higher doses (Fig. 8B), whereas freezing significantly increased at 30 mg/kg (Fig. 8C).

At 1 h after THC administration, the behavioral response profile changed. In men, rotations were not significantly altered across

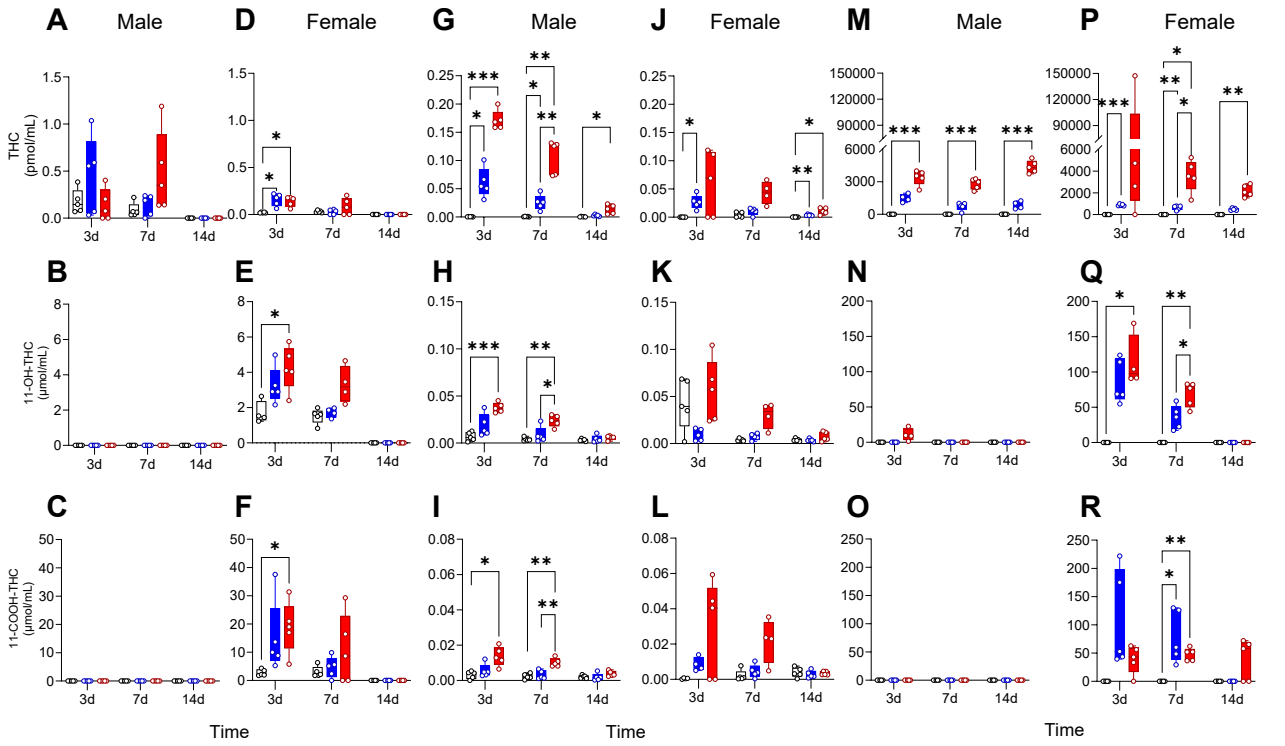


Fig. 4. Persistence of high-dose THC and metabolites in plasma, brain, and WAT. Plasma (A–F), brain (G–L), and WAT (M–R) concentrations of THC (A, D, G, J), 11-OH-THC (B, E, H, K), and 11-COOH-THC (C, F, I, L) were measured at 3, 7, and 14 days after a single IP administration of vehicle (open bars), 10 mg/kg THC (blue bars), or 30 mg/kg (red bars) in male (A–C, G–I) or female (D–F, J–L) rats. Data are expressed as mean ± SEM ($n = 5$ per group) and were analyzed by one-way ANOVA. *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$.

doses (Fig. 8D). Grooming was dose-dependently reduced at 3, 10, and 30 mg/kg (Fig. 8E), while freezing was increased at 10 and 30 mg/kg (Fig. 8F). In women, rotations were significantly increased at 10 and 30 mg/kg (Fig. 8D). Grooming was strongly suppressed at these doses, with near-complete blockades in several animals (Fig. 8E). Freezing episodes were increased at 30 mg/kg, with no detectable effect at 3 and 10 mg/kg (Fig. 8F).

At 24 h post-THC, most behavioral measures had returned to normal. In both sexes, rotations did not differ across treatment groups (Fig. 8G), indicating recovery of spontaneous locomotor activity. In male rats, grooming showed a trend decrease at the highest dose relative to control, which did not reach statistical significance (Fig. 8H). In females, grooming was significantly increased at 3 mg/kg but decreased at 30 mg/kg (Fig. 8H). There were only a few freezing episodes, and their numbers did not differ consistently across groups in either sex (Fig. 8I).

Overall, high-dose THC produced dose- and sex-dependent changes across behavioral domain indexing activity, self-directed behavior, and immobility. By 24 h, locomotor and freezing behaviors had normalized, whereas grooming remained altered, particularly at the highest dose.

3.9. Acute effects of high-dose THC on anxiety-like behavior

Anxiety-like behavior was assessed in the EPM and light/dark box tests at 24 hours after THC or vehicle administration.

3.9.1. EPM

Representative movement traces in the EPM are shown to illustrate exploration patterns across treatment groups (Fig. 9A, B). Quantitative measures were conducted 24 h after THC or vehicle administration and included the number of entries in and time spent in open and closed arms (Figure 9c-f). In men, THC did not significantly affect EPM responses at any dose level. Open-arm and closed-arm entries and time in open and closed arms were not altered (Figure 9c-f). In women, THC produced more pronounced effects. Open-arm entries significantly increased at 3 mg/kg and decreased at 30 mg/kg (Fig. 9C), while closed-arm entries remained stable (Fig. 9d). The effects on the time spent in the open and closed arms were variable and largely nonsignificant (Fig. 9E, F).

3.9.2. Light-dark box

In this test, both sexes exhibited a significant reduction in time spent in the light compartment at 30 mg/kg THC in males and at all doses in females (Fig. 9G). Time spent in the dark compartment was increased in males at 30 mg/kg but not at lower doses, with no significant effect seen in females (Fig. 9H). Total distance travelled and average speed were measured to control for potential motor confounds. In males, total distance traveled and average speed showed a variable, non-significant increase at 3 mg/kg, with no changes at higher doses (Fig. 9I, J). In females, locomotor parameters were not significantly altered across doses (Fig. 9I, J).

Overall, THC produced dose-dependent changes in the EPM and light/dark tests, which were consistent with increased avoidance of open and illuminated areas, particularly at the highest dose (30 mg/kg). These effects were more pronounced in females than in males and occurred in the absence of consistent reductions in locomotor activity, indicating that the behavioral changes are unlikely to be attributable to motor impairment.

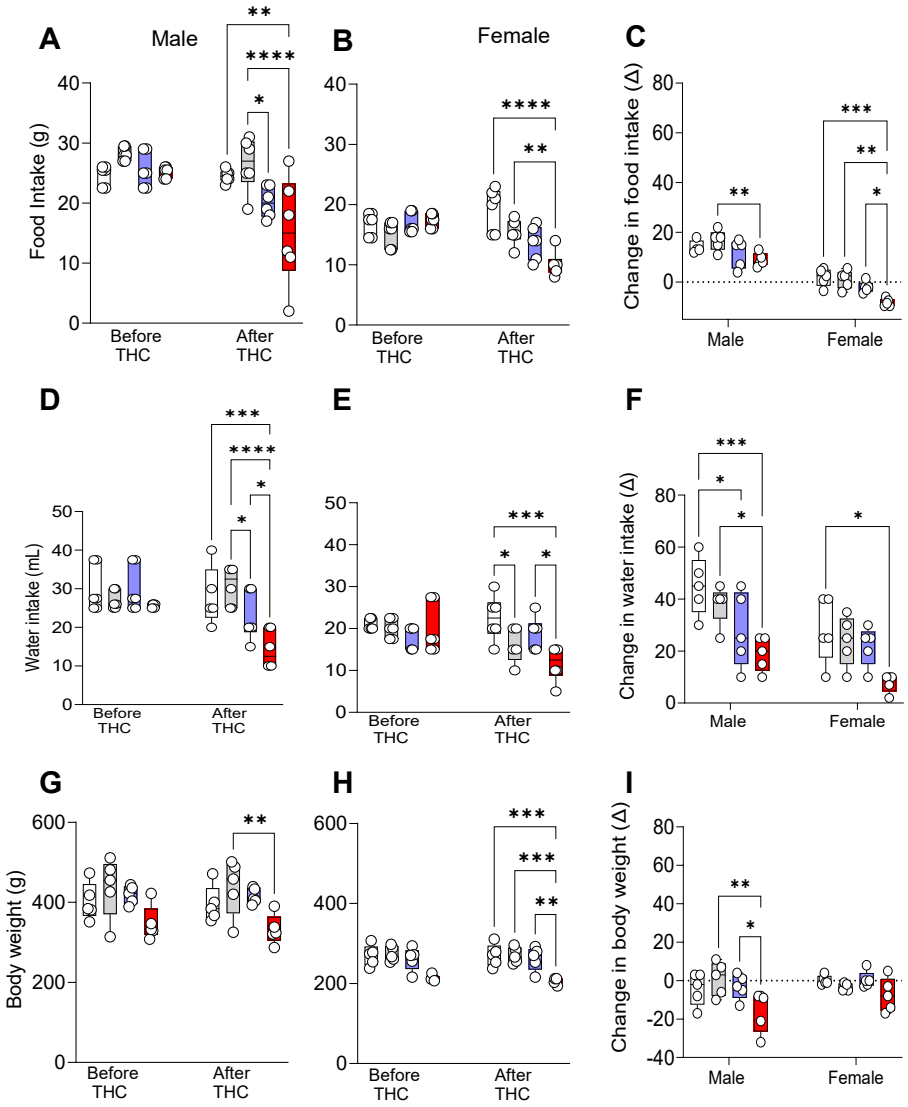


Fig. 5. Effects of high-dose THC on ingestive behavior. Rats received a single IP administration of vehicle or THC (3, 10, or 30 mg/kg) and were monitored for 1 day after injection. (A, B) Total food intake (g/kg). (C) Change in food intake. (D, E) Total water intake (mL/kg). (F) Change in water intake. (G, H) Body weight. (I) Change in body weight. Data are expressed as mean ± SEM ($n = 5$ per group) and were analyzed by one-way ANOVA. *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$.

3.10. Acute effects of high-dose THC on depression-related behavior, autonomic output, and nociception

To further assess behavioral and physiological responses to high-dose THC, rats were evaluated in the open field, and fecal boli output was quantified as an index of autonomic activation 24 hours after injection. Nociceptive responses were measured using the tail-immersion test at 10 min, 1 h, and 24 h after THC (Fig. 10).

3.10.1. Forced swim test

In males, THC significantly decreased mobility time and increased immobility time at 3 mg/kg but not at higher doses (Fig. 10A, B. In females, THC had minimal effects on FST performance: neither mobility nor immobility time differed significantly across treatment groups (Fig. 10A, B). Locomotor-related measures in the FST, including total distance traveled and average speed, were not consistently altered across doses in either sex (Figure 10c, d), indicating that changes in immobility were not attributable to gross motor impairment.

3.10.2. Fecal boli output

Autonomic output, as measured by fecal boli counts, was not consistently altered by THC in either sex (Figure 10f), with substantial variability across groups.

3.10.3. Tail-immersion test

At 10 min, THC did not significantly alter tail withdrawal latency in males, whereas in females a modest increase was observed at the highest dose (Fig. 10g). At 1 h, THC increased latency in both sexes, with more pronounced effects at the 30 mg/kg dose (Fig. 10h). In male rats, the effect persisted until 24 h, with significant increases in latency at 10 and 30 mg/kg (Fig. 10i). In females, 30 mg/kg THC prolonged latency both in 10 min and 1 hour.

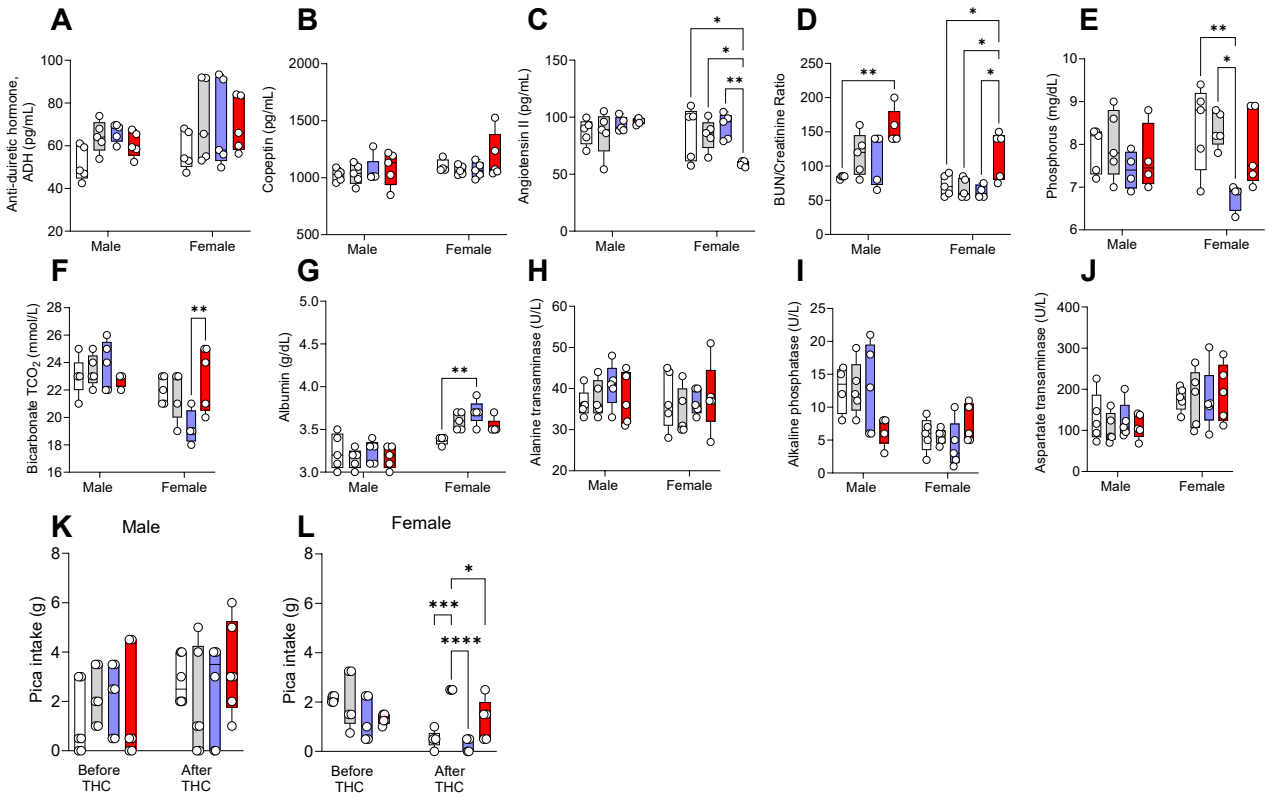


Fig. 6. Effects of high-dose THC on hormonal, pica, and clinical chemistry parameters. Rats received a single IP administration of vehicle or THC (3, 10, or 30 mg/kg) and were monitored for 1 day after injection. (A–C) Circulating markers related to vascular or renal function, including antidiuretic hormone (ADH) (A), copeptin (B), and angiotensin II (C). (D–J) Standard clinical chemistry parameters, including blood urea nitrogen (BUN)/creatinine ratio (D), phosphorus (mg/dL) (E), bicarbonate (total CO_2, mmol) (F), albumin (g/dL) (G), alanine aminotransferase (ALT, U/L) (H), alkaline phosphatase (ALP, U/L) (I), and aspartate aminotransferase (AST, U/L) (J). (K, L) Pica intake in male (K) and (K) and female (L) rats. Data are expressed as mean ± SEM ($n = 5$ per group) and were analyzed by one-way ANOVA. *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$.

Overall, THC produced dose- and sex-dependent effects across the behavioral domains surveyed in these tests. In the FST, males showed bidirectional changes in mobility and immobility, whereas women were largely unaffected. In contrast, THC produced clear antinociceptive effects in the tail-immersion test, particularly at 1 h in both sexes and 24 h in men.

3.11. Acute effects of high-dose THC on circulating cytokines

Plasma concentrations of proinflammatory (IL-1α, IL-1β, IL-6, IL-12, IFN-γ, GM-CSF) and anti-inflammatory (IL-4, IL-10) cytokines remained unchanged 24 h after administration of THC at 3, 10, or 30 mg/kg (Fig. 11).

3.12. Residual effects of single high-dose THC administration

3.12.1. Physiological parameters

To identify possible residual effects of THC[26], we extended testing to include 3, 7, and 14 days after THC or vehicle injection. Over the 14-day observation period, a single administration of THC (10 or 30 mg/kg) produced limited persistent effects on body weight, thermoregulation, and nociceptive behavior,

Table 4
Effects of high-dose THC on blood clinical parameters. Rats received a single IP administration of vehicle or THC (3, 10, or 30 mg/kg) and clinical parameters were assessed in plasma 24 hours post injection
Data are expressed as mean ± SEM ($n = 5$ per group) and were analyzed by one-way ANOVA. *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$.

Biochemical markers	Male				Female			
	Dose (mg/kg)				Dose (mg/kg)			
	0	3	10	30	0	3	10	30
BUN (mg/dL)	17.6 ± 1.94	15.2 ± 2.77	14.2 ± 1.09	15.6 ± 2.61	14.4 ± 3.05	13.4 ± 2.88	12.4 ± 1.67	15.00 ± 1.22
Creatinine (mg/dL)	0.20 ± 0.00	0.14 ± 0.05	0.14 ± 0.05***	0.10 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.18 ± 0.04	0.14 ± 0.05
Creatine kinase (U/L)	840 ± 676	355 ± 216	463 ± 289	532 ± 241	871 ± 274	902 ± 552	844 ± 516*	931 ± 507*
Total Protein (g/dL)	5.90 ± 0.30	5.88 ± 0.29	6.08 ± 0.36	5.82 ± 0.39	5.83 ± 0.10	6.42 ± 0.21*	6.40 ± 0.22*	6.22 ± 0.16
Globulin (g/dL)	2.66 ± 0.18	2.83 ± 0.10	2.84 ± 0.23*	2.53 ± 0.13*	2.55 ± 0.06	2.82 ± 0.13*	2.70 ± 0.10	2.68 ± 0.08
ALB/GLOB ratio	1.18 ± 0.05	1.14 ± 0.11	1.14 ± 0.05	1.22 ± 0.08	1.30 ± 0.00	1.28 ± 0.04	1.36 ± 0.05	1.30 ± 0.00
Sodium (mmol/L)	162.6 ± 4.03	166.25 ± 5.91	166.8 ± 6.61	160.8 ± 10.83	169.4 ± 7.67	165.8 ± 3.56	168 ± 7.17	163 ± 7.64
Chloride (mmol/L)	85.75 ± 0.96	87.80 ± 4.97	89.40 ± 4.03	85.00 ± 3.24	91.00 ± 3.39	90.8 ± 0.45	91.4 ± 1.67	89.8 ± 3.03
Potassium (mmol/L)	19.83 ± 0.21	18.38 ± 1.31	19.80 ± 0.40	20.00 ± 0.00	18.68 ± 1.30	19.45 ± 0.48	20.00 ± 0.00	18.14 ± 1.79*

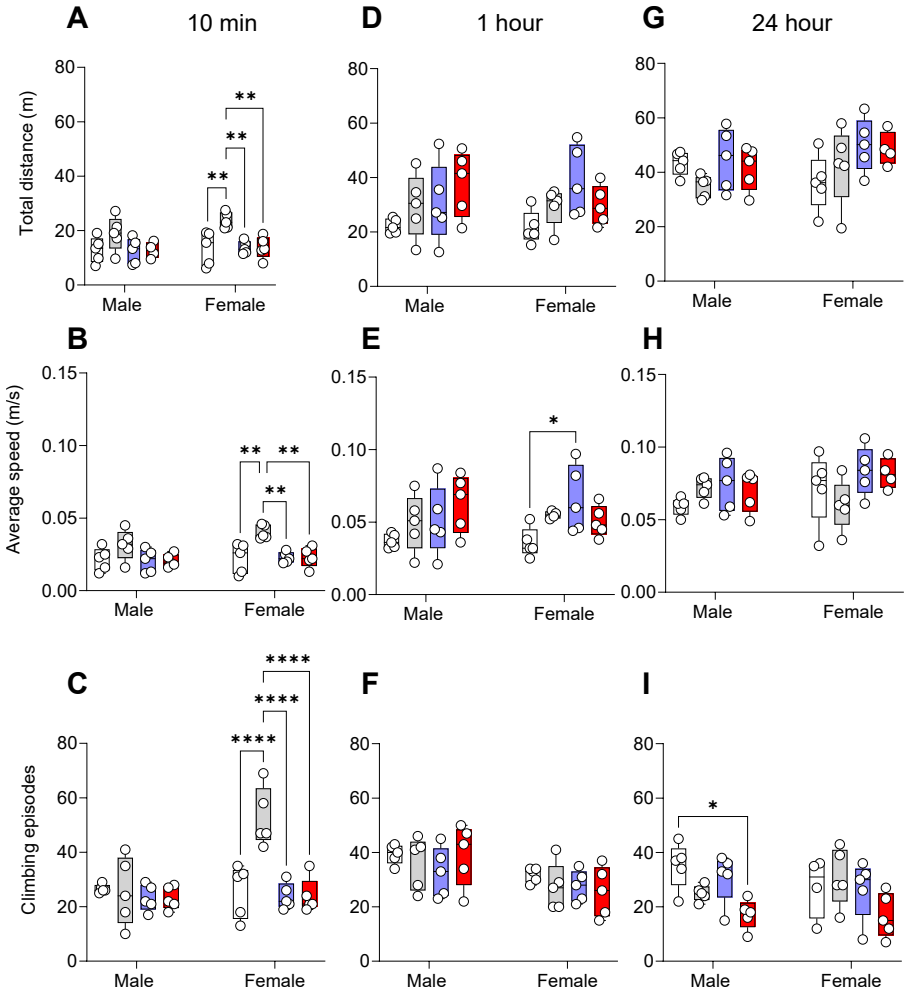


Fig. 7. Effects of high-dose THC on locomotor behavior. Rats received a single IP administration of vehicle or THC (3, 10, or 30 mg/kg) and behaviors were assessed for 10 minutes in their home cages at 10-minute (A − C) and 1 hour (D − F) post injection. (G − I) At 24 hour, they were placed in an open field arena and behavior was monitored for 10 minutes. (A, D, G) Total distance traveled. (B, E, H) Average speed. (C, F, I) Climbing episodes. Data are expressed as mean ± SEM ($n = 5$ per group) and were analyzed by one-way ANOVA. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

compared with vehicle (Fig. 12). In male rats, THC did not affect body weight at any of the doses tested (Fig. 12aA). At the 10 mg/kg dose, body temperature was still significantly lower than control at day-3 posttreatment but normalized at later time points (Fig. 12B); a similar trend was observed after administration of 30 mg/kg THC. At both 10 and 30 mg/kg THC, withdrawal latency was still higher than controls at day 3, with effects resolving by day 7 (Fig. 12C). In women (Fig. 12D–F), 30 mg/kg THC reduced body weight on day 3, while other doses were ineffective (Fig. 12D). Changes in body temperature were modest and variable, with no clear persistent hypothermia (Fig. 12E).

3.12.2. Circulating cytokines

Over the same 14-day observation period as Fig. S5, little or no change was observed in either proinflammatory or anti-inflammatory circulating cytokine concentrations at the three dose concentrations (Supplementary Fig. S5), except for a modest increase in IL-10 in males at 30 mg/kg THC on day 3 postdosing (Supplementary Fig. S5k).

4. Discussion

The present study provides a comprehensive characterization of the short-term and residual PK and PD properties of high-dose THC in male and female Long-Evans rats—which are often preferred to albino strains to conduct behavioral studies.[27] At doses ranging from 3 mg/kg (comparable to moderate human use) to 30 mg/kg (modeling acute substantial overconsumption), we observed nonlinear PK with disproportionate increases in systemic exposure, markedly higher plasma, brain, and adipose tissue concentrations in females, extensive and prolonged sequestration in adipose tissue, sustained brain and adipose retention of THC and the bioactive metabolite 11-OH-THC up to 14 days. Pharmacodynamically, high-dose THC produced dose- and sex-dependent effects, including robust suppression of ingestive behavior and decreased body weight, transient and bidirectional locomotor/exploratory changes (eg, stimulation at low doses in females), increased pica suggestive of malaise in females, modest alterations in anxiety-like behavior, and bidirectional effects on stress-coping and nociception in males. These findings build directly on our

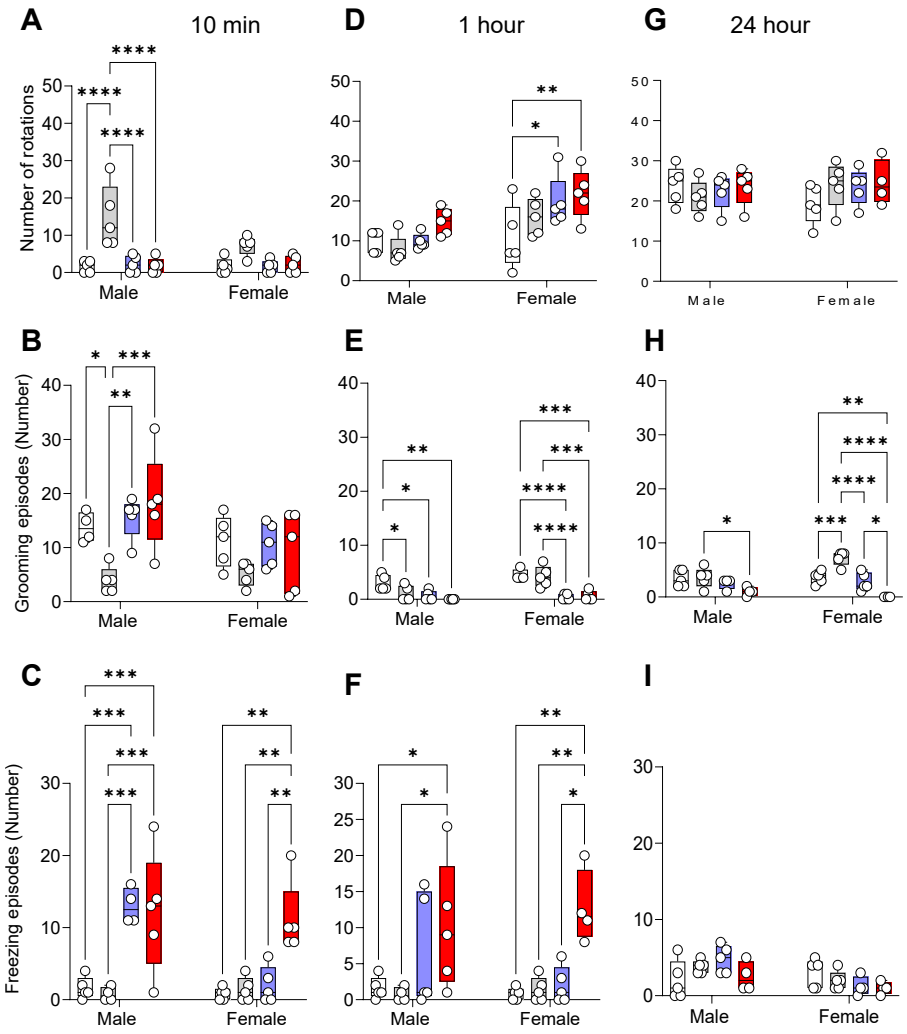


Fig. 8. Effects of high-dose THC on exploratory and stress-related behaviors. Rats received a single IP administration of vehicle or THC (3, 10, or 30 mg/kg) and behaviors were assessed for 10 minutes in their home cages at 10-minute (A–C) and 1 hour (D–F) post injection. (G-I) At 24-hour, they were placed in an open field arena, and behavior was monitored for 10 minutes. (A, D, G) Number of rotations. (B, E, H) Grooming episodes. (C, F, I) Freezing episodes. Data are expressed as mean ± SEM ($n = 5$ per group) and were analyzed by one-way ANOVA. *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$.

laboratory's earlier investigations in mice and rats, where low-to-moderate THC doses (0.3–5 mg/kg, IP) yielded linear or near-linear PK profiles, more modest sex differences (often favoring higher metabolite exposure in females), shorter tissue retention, and less pronounced or qualitatively different behavioral responses.[10–14]

In humans, cannabis is consumed primarily by smoking, vaporization, or oral ingestion. These routes produce markedly different PK profiles and substantial interindividual variability arising from differences in inhalation topography, gastrointestinal absorption, first-pass metabolism, and formulation. For this reason, cross-species comparisons based solely on dose are of limited value; plasma concentrations (Cmax, AUC) and, secondarily, body-surface-area–scaled human-equivalent doses provide a more appropriate frame of reference. Using the standard FDA conversion (rat dose divided by 6.2), the 3, 10, and 30 mg/kg doses used here correspond to approximate human-equivalent doses of 0.5, 1.6, and 4.8 mg/kg (roughly 30–35, 100–110, and 290–340 mg in a 60–70 kg adult). Controlled human studies have documented wide ranges of plasma THC concentrations after smoked or oral cannabis.[28,29] However, most of those investigations used preparations with substantially lower THC content than products now widely available in North American legal markets. Whereas cannabis flower in prior studies typically contained <10% THC, many current commercial products exceed 20%–30% THC, and concentrated extracts frequently reach 50–95%. This increase in potency has raised the likelihood of systemic exposures that exceed those examined in the available human PK literature, so a direct numerical match between the plasma concentrations measured here and "typical" human values is not possible. The primary objective of the present study was to address this translational gap by characterizing the PK and PD properties of high-dose THC in rats under controlled and reproducible conditions.

We selected the IP route because it provides rapid and reliable systemic exposure while minimizing the variability inherent to oral or inhalational dosing. Indeed, despite recent advances,[30] oral administration of THC in rodents remains technically challenging owing to poor palatability, inconsistent voluntary consumption, and variable gastric emptying. The inhalation route, while more

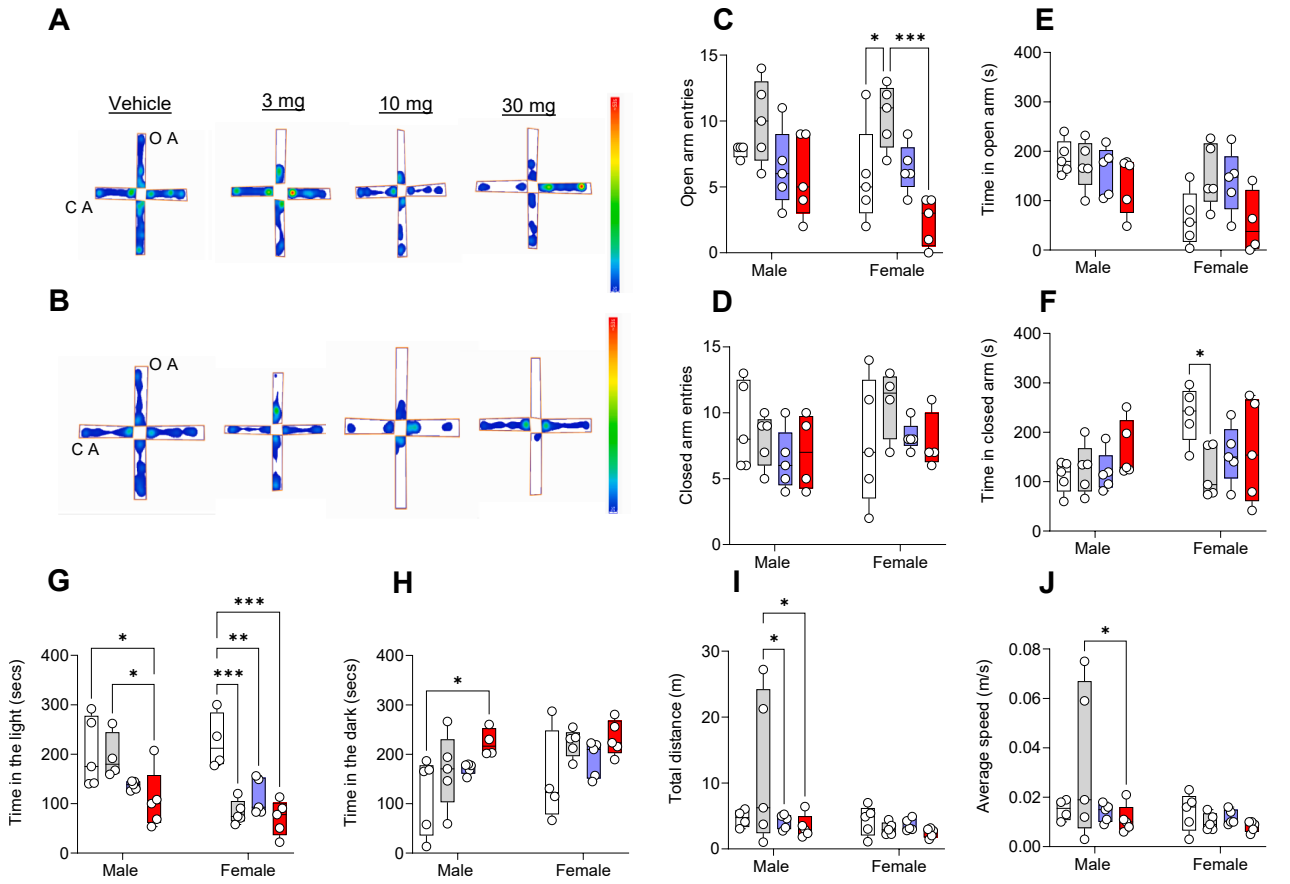


Fig. 9. Effects of high-dose THC on anxiety-like behaviors. Rats received a single IP administration of vehicle or THC (3, 10, or 30 mg/kg) and were monitored for 1 day post injection. (A, B) Representative heat maps of exploratory activity in the EPM for male (A) and female (B) rats treated with vehicle or THC (3, 10, or 30 mg/kg). Warmer colors indicate greater occupancy. OA and CA are indicated. (C–F) Quantification of EPM behavior, including number of open arm entries (C), closed arm entries (D), time spent in open arms (E), and time spent in closed arms (F). (G–J) Light–dark box test measures, including time spent in the light compartment (G), time spent in the dark compartment (H), total distance traveled (I), and average speed (J). Data are expressed as mean ± SEM ($n = 5$ per group) and were analyzed by one-way ANOVA. *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$.

closely mimicking the predominant human modalities of cannabis use (smoking and 'vaping'), introduces other experimental confounds related to differences in aerosol generation, chamber exposure, and delivered dose. Moreover, because rodents are obligate nasal breathers, inhalational paradigms result in substantial THC deposition in the nasal cavity and olfactory mucosa, creating local reservoirs that can directly access the brain.[31] By circumventing these route-dependent sources of variability, IP administration enabled us to define more accurately the relationship between administered dose and systemic THC exposure. Our dose selection (3, 10, and 30 mg/kg) was aimed at comparing moderate, high, and very high exposure concentrations. The selected doses include the range commonly used in rodent pharmacological studies of THC (typically ≤5 mg/kg) while extending into higher exposures relevant to contemporary high-potency cannabis products. This design enabled detection of nonlinear PK behavior—including sex-dependent differences and prolonged tissue retention—that would not have been evident over the narrower dose ranges evaluated previously.

While prior lower-dose studies reported roughly linear PK in plasma,[11,14] the present results revealed disproportionately large increases in C_{max} for both unchanged THC and its metabolites when moving from 3 to 10 and 30 mg/kg THC in both sexes. Saturation of metabolic and/or elimination pathways at higher exposures is unlikely to be the primary driver of this super-proportional nonlinearity ($\beta > 1$), as saturation would typically produce sub-proportional or plateauing metabolite exposure at higher doses.[32] An alternative possibility is that the observed PK profile could be driven by saturable tissue distribution, particularly extensive sequestration into WAT—which was indeed observed in our study as in previous ones. At higher doses, adipose storage capacity may become partially saturated, leading to greater partitioning into plasma and other compartments, thereby elevating systemic exposure for both parent and metabolites.

The disposition of high-dose was markedly influenced by sex, with female rats displaying overall higher concentrations of THC conversion to bioactive 11-OH-THC than males. This finding aligns with and amplifies prior observations of sex differences in THC handling at doses up to 10 mg/kg,[13,33,34] in which female rats metabolize THC more readily to 11-OH-THC via hepatic CYP450 enzymes, resulting in elevated plasma and brain concentrations relative to males.[32] This sex-dependent divergence became more pronounced at the higher doses tested here, as evidenced by markedly greater 11-OH-THC exposure in females at 10 and 30 mg/kg compared with 3 mg/kg. Greater accumulation of the inactive

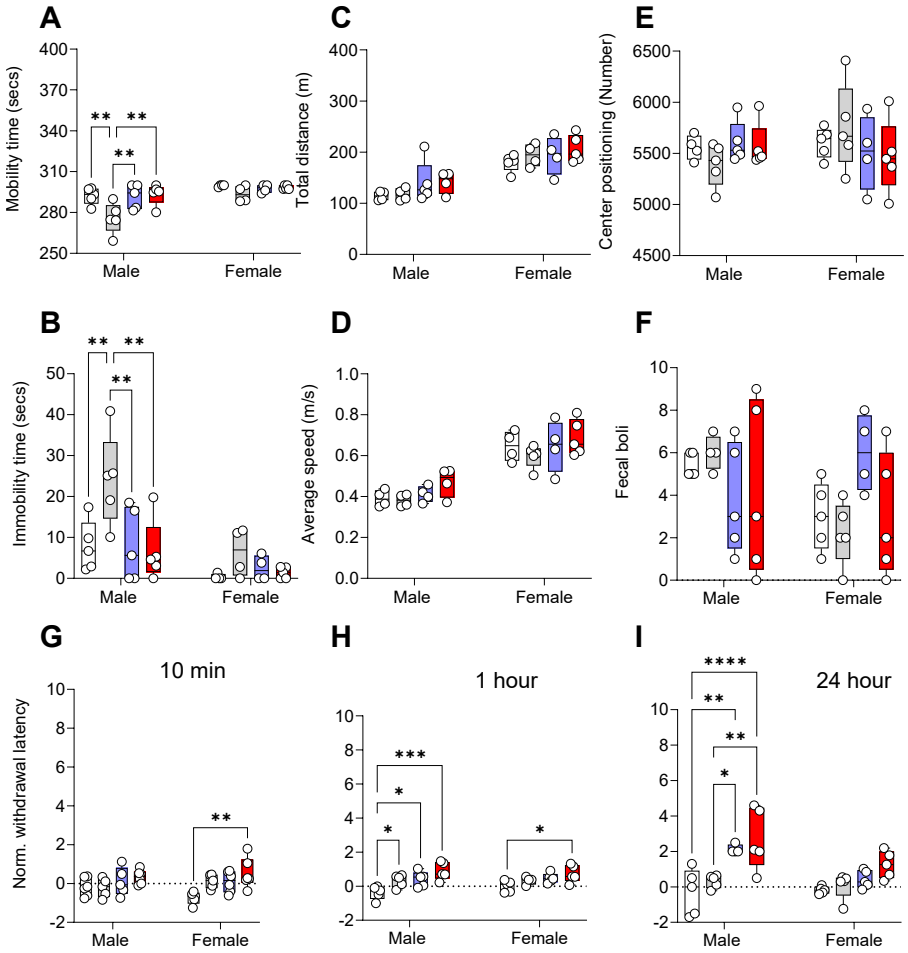


Fig. 10. Effects of high-dose THC on depression-like behaviors and nociceptive responses. Rats received a single IP administration of vehicle or THC (3, 10, or 30 mg/kg) and were monitored for 1 day post injection. (A–F) forced swim test measures, including mobility time (A), immobility time (B), total distance traveled (C), average speed (D), center positioning (E), and fecal boli count (F). (G–I) Nociceptive responses assessed as normalized withdrawal latency (tail immersion test) at 10 minute (G), 1 hour (H), and 24 hour (I) after treatment. Data are expressed as mean ± SEM ($n = 5$ per group) and were analyzed by one-way ANOVA. *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$.

metabolite 11-COOH-THC in females further suggests enhanced overall metabolic throughput.

Brain distribution revealed important compartment-specific kinetics. THC and 11-OH-THC showed delayed T_{max} (typically 60–240 min) and more prolonged retention compared with plasma, indicating slow equilibration across the blood-brain barrier and sustained central exposure even as plasma concentrations waned. This is consistent with prior reports of delayed brain peaks relative to plasma.[34] Females again displayed higher brain concentrations, especially of 11-OH-THC, reinforcing the notion that sex-dependent metabolism influences central exposure. In contrast, the inactive 11-COOH-THC showed limited brain penetration, as expected from its polarity, and was generally undetectable at 3 mg/kg.

The results confirmed WAT's role as a major depot for THC,[14,31] with concentrations orders of magnitude higher than in plasma or brain, delayed T_{max}, and prolonged retention—patterns previously documented at lower doses and attributable to THC's high lipophilicity.[10–15] Our high-dose data extend this by showing nonlinear accumulation and pronounced sex differences, with females exhibiting substantially greater WAT exposure (particularly for THC at 30 mg/kg). This suggests that females have a larger reservoir for THC redistribution over time. The persistence data over 14 days support this: THC remained detectable in plasma up to day 7 and in brain/WAT through day 14 post injection, with evidence of metabolite redistribution (especially in females), consistent with slow release from adipose stores.

Overall, the PK findings demonstrate that high-dose THC produces not only quantitatively greater but qualitatively different exposure profiles—nonlinear escalation, amplified sex differences, and prolonged tissue retention—compared with the lower doses that dominate the existing preclinical literature. These characteristics have important implications for interpreting PD effects at high doses, potential accumulation with repeated exposure, and translational relevance to scenarios involving high THC intake in humans. The pronounced female bias in exposure may contribute to reported sex differences in THC sensitivity and warrants consideration in future dosing regimens and risk assessments.

The PD profile of high-dose THC was dominated by suppression of ingestive behavior (food and water intake), transient alterations in locomotor activity, increased anxiety-like behavior at the highest dose, and antinociception, with minimal effects on systemic inflammation. These outcomes extend prior findings obtained predominantly at lower doses (≤3–5 mg/kg)[33,34] by

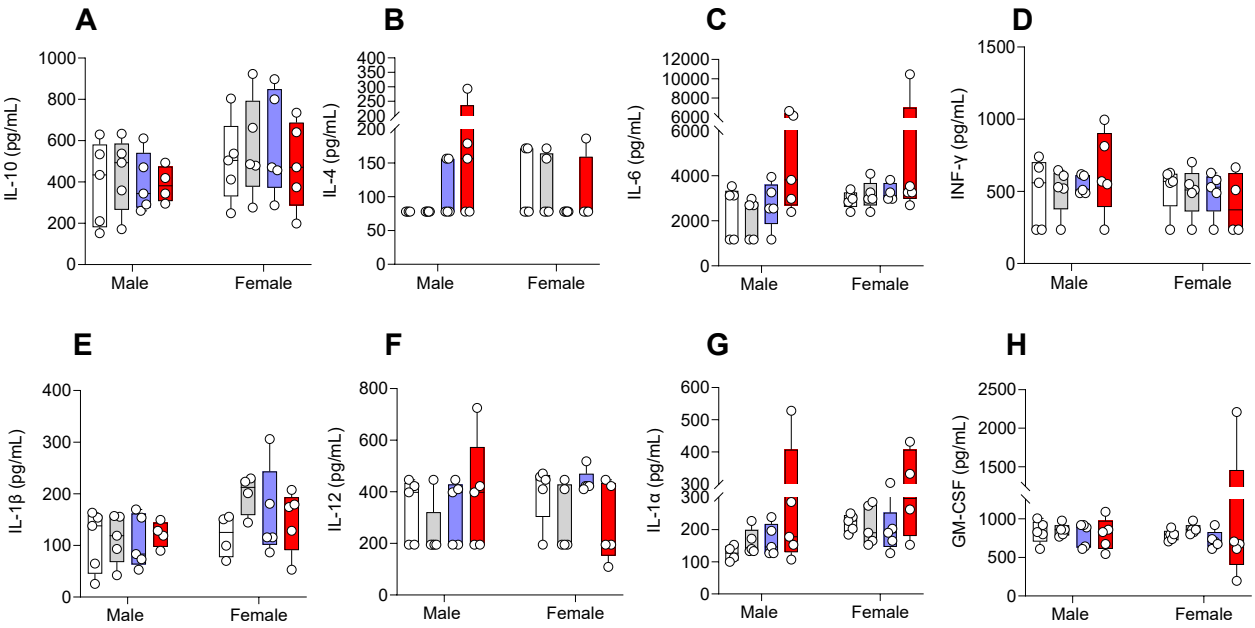


Fig. 11. Effects of high-dose THC on circulating cytokine concentrations. Rats received a single IP administration of vehicle or THC (3, 10, or 30 mg/kg) and cytokines were measured in plasma at day 1 after injection. (A) Interleukin-6 (IL-6), (B) interferon-gamma (IFN-γ), (C) interleukin-1β (IL-1β), (D) interleukin-12 (E) interleukin-1α (IL-1α), (F) low granulocyte-macrophage colony-stimulating factor (GM-CSF), (G) interleukin-10 (IL-10), (H) and interleukin-4 (IL-4) male (open blue circles) and female (closed red squares) rats. Data are expressed as mean ± SEM ($n = 5$ per group) and were analyzed by one-way ANOVA. *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$.

demonstrating that higher exposures elicit more pronounced and, in some domains, qualitatively distinct effects.

The anorectic effect of 30 mg/kg THC was evident in both sexes but generally more robust in females. It is possible, though remains to be ascertained, that this effect was mediated by excessive activation of hypothalamic feeding circuits expressing CB_1 receptors.[35–38] The accompanying reduction in water intake appeared to reflect a broad suppression of ingestive behavior rather than a specific disruption of fluid homeostasis, as circulating ADH, copeptin, and most electrolyte parameters remained stable. The increase in BUN/creatinine ratio at 30 mg/kg further supports mild, transient dehydration secondary to reduced water intake rather than direct renal toxicity.[39] Notably, females displayed increased pica behavior at both low and high doses, suggestive of a malaise-like state, which may contribute to the observed sex differences in ingestive suppression. These findings, which contrast with the well-known anti-nausea and orexigenic effects of low-dose THC in rodents and humans,[7,9,38,39] may hold relevance for cannabis hyperemesis syndrome, a condition thought to result from frequent exposure to concentrated THC products.[40,41]

Locomotor and spontaneous behaviors showed rapid, biphasic, and sex-dependent responses. Females exhibited transient hyperlocomotion and increased rotations at lower doses (3—10 mg/kg) in the home cage, whereas males showed dose-dependent increases in freezing (immobility) at higher doses. Grooming was generally suppressed in a dose-dependent manner, particularly at 1 hour postadministration. By 24 h, most locomotor and freezing measures had normalized, indicating that the behavioral effects of a single high-dose exposure are largely transient despite sustained THC and metabolite concentrations in the brain and adipose tissue. These findings align with the known biphasic dose-response of cannabinoids on motor activity—stimulatory at low doses and suppressive or cataleptic at high doses[35] while highlighting greater sensitivity of females to the stimulatory effects at lower exposures.

In anxiety-related assays conducted 24 hours after administration, 30 mg/kg THC increased avoidance of open areas in both the EPM and L/D box, with effects more pronounced in females. This anxiogenic-like profile, which contrasts the anxiolytic-like responses observed at low THC doses in rodent[42,43] and human studies—occurred without consistent locomotor deficits, suggesting a specific modulation of emotional behavior. THC also produced clear, time-dependent antinociceptive effects in the tail-immersion test that were more sustained in males. Collectively, these data indicate that high systemic and central exposures to THC engage multiple behavioral domains, including suppression of ingestive behavior, emotional reactivity, and pain modulation, with patterns that partially diverge from those reported at lower doses.

Most pharmacological effects of high-dose THC administration were transient. The acute reduction in body weight largely recovered within days, with only a modest decrease remaining in females on day 3 after 30 mg/kg. Minor hypothermia was observed in males at day 3 (significant at 10 mg/kg) but resolved thereafter. Antinociceptive effects persisted modestly until day 3 in males but normalized by day 7 in both sexes. Females showed minimal prolonged changes. Systemic cytokine concentrations remained largely unchanged over 14 days.

Overall, high-dose THC elicits nonlinear, sex-dependent pharmacokinetics with prolonged systemic and central exposure, alongside qualitatively distinct acute behavioral effects compared with lower doses. These findings underscore the importance of potency in determining the quantity and quality of cannabis outcomes and highlight the need for further studies on high-concentration products in diverse models to inform public health risks associated with current cannabis use.

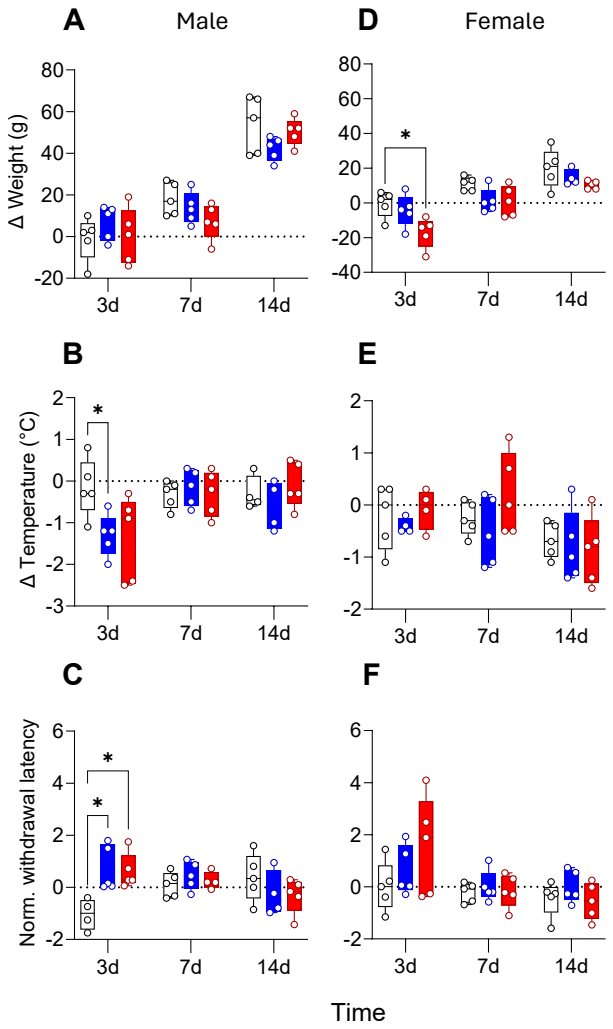


Fig. 12. Residual effects of high-dose THC on physiological parameters and nociceptive responses. Rats received a single IP administration of vehicle or THC (3, 10, or 30 mg/kg) and responses were assessed on day 3, 7, and 14 post injection. (A, D) Body weight changes in males (A) and females (D). (B, E) Body temperature changes in males (B) and females (E). (C, F) Nociceptive responses assessed as normalized withdrawal latency (tail immersion test) in males (C) and females (F) rats. Data are expressed as mean ± SEM ($n = 5$ per group) and were analyzed by one-way ANOVA. *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$.

Abbreviations

ADH, antidiuretic hormone; ALB, albumin; ALP, alkaline phosphatase; AST, aspartate transaminase; IL, interleukin; IFN-γ, interferon-γ; AUC, area under the curve; BUN, blood urea nitrogen; C_{max}, maximal concentration; GLOB, globulin; 11-COOH-THC, 11-carboxy-Δ^9-tetrahydrocannabinol; 11-OH-THC, 11-hydroxy-Δ^9-tetrahydrocannabinol; T_{max}, time to reach maximum concentrations; EMR, enhanced matrix removal; EPM, elevated plus maze; FST, forced swim test; IP, intraperitoneal; L/D, light and dark box; OA, open arms; CA, closed arms; PND, postnatal day; WAT, white adipose tissue; LLOQ, lowest limit of quantification; PD, pharmacodynamics; PK, pharmacokinetics; THC, Δ^9-tetrahydrocannabinol; CB_1, cannabinoid receptor type 1; CL, clearance.

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Conflict of interest

The authors declare no conflicts of interest.

Data availability

The authors declare that all the data supporting the findings of this study are contained within the article and its supplementary material.

CRediT authorship contribution statement

Olufunke Florence Ajeigbe: Methodology, Investigation, Formal analyses, Data Curation, Project administration, Software, Funding acquisition, Writing—Original draft preparation, Writing—Review and editing. **Adren Tran**: Methodology, Funding acquisition, Software, Investigation, Data Curation, Project administration, Writing—Review & Editing. **Rodrigo Klein:** Methodology, Project administration, Investigation, Data Curation, Writing – Review & Editing. **Alex Mabou Tagne**: Methodology, Funding acquisition, Investigation, Data Curation, Writing—Review & Editing. **Heidi C. Avalos**: Investigation, data curation, writing—review and editing. **Faizy Ahmed**: Funding acquisition, resources, writing—review and editing, supervision. **Daniele Piomelli**: Conceptualization, Funding acquisition, Writing—Review & Editing, Project administration, Resources, Supervision.

Supplemental material

This article has supplemental material available at jpet.aspetjournals.org.

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