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Authors

Moore, Catherine F
Davis, Catherine M
Harvey, Eric L
et al.

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Appetitive, antinociceptive, and hypothermic effects of vaped and injected Δ−9-tetrahydrocannabinol (THC) in rats: exposure and dose-effect comparisons by strain and sex

Catherine F. Moore[1,*], **Catherine M. Davis**[1,†], **Eric L. Harvey**[2], **Michael A. Taffe**[2], **Elise M. Weerts**[1]

[1]Division of Behavioral Biology, Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD

[2]Department of Psychiatry, University of California San Diego, La Jolla, CA

Abstract

Advances in drug vapor exposure systems have enabled evaluation of Δ−9-tetrahydrocannabinol (THC) vapor effects in laboratory animals. The purpose of this study was to 1) establish a range of parameters of THC vapor exposure in rats sufficient to produce a behavioral dose-effect curve in a battery of tasks sensitive to THC; and 2) to investigate sex differences in the effects of THC vapor exposure and THC injection (intraperitoneal, IP) on these behaviors in two strains of outbred rats. Male and female Sprague Dawley and Wistar rats (N=22, 5–6/sex per group) received THC via passive vapor exposure (200 mg/ml; 5 conditions) and IP injection (1–20 mg/kg) in a within subject design. The effects of vaped and injected THC on appetite was determined using progressive ratio responding for food pellets. THC effects on nociception, measured using the tail withdrawal assay, and body temperature were also assessed during a 5-hr test period for evaluation of time course of effects. Plasma THC concentrations were assessed after THC vapor and 10 mg/kg IP THC. THC vapor produced exposure-related increases and decreases in motivation to obtain food under the progressive ratio schedule. IP THC (3–20 mg/kg) reduced breakpoints. Vaped and injected THC produced exposure and dose-dependent antinociception and hypothermia. Sex and strain differences in THC effects were also observed. Plasma THC concentrations were higher after 10 mg/kg IP THC (152 ng/mL) compared to the highest vapor exposure condition tested (38 ng/mL), but magnitude of behavioral effects were comparable. THC vapor exposure produced reliable, dose orderly effects on food-maintained behavior, nociception, and body temperature that are comparable to effects of IP THC, although there were differences in the time course of behavioral outcomes.

[*]Corresponding author at: Johns Hopkins Bayview Research Campus, Behavioral Biology Research Center, 5510 Nathan Shock Drive, Suite 3000, Baltimore, MD 21224, USA. cfmoore@jhmi.edu (C.F. Moore).

[†]Current affiliation: Department of Pharmacology and Molecular Therapeutics, Uniformed Services University of the Health Sciences, Bethesda, MD

Keywords

THC; Vapor Exposure; Cannabinoids; Appetite; Nociception; Hypothermia

1. Introduction

Cannabis is one of the most widely used drugs in the world, including in the United States. It is most often used by inhalation, and more recently, vaping of cannabis and cannabis extracts containing Δ−9-tetrahydrocannabinol (THC, the primary psychoactive constituent of cannabis) is on the rise (Giroud et al., 2015; Lee et al., 2016; Trivers et al., 2019). Rates of cannabis vaping are estimated to be between 20%−37% (past 30 days) and 60% (lifetime) for cannabis users (Goodman et al., 2020; Lee et al., 2016; Schauer et al., 2020). Preclinical vapor exposure models have been developed using methods for aerosolizing THC (Lichtman et al., 2000; Wilson et al., 2002), use of desktop vaporizers (i.e. Volcano© vaporizer) (Manwell et al., 2014a), and e-vape technology (Nguyen et al., 2016); (for review, see Miliano et al., 2020; Moore et al., 2020). Route of administration is important from a translational perspective and for interpretation of the pharmacokinetic and pharmacodynamic effects of cannabinoids.

The main objective of this study was to develop a full range of THC vapor exposure conditions to characterize a behavioral dose-effect curve (i.e., range from no effect to response inhibition). To provide context with the broader literature, the behavioral and physiological effects of intraperitoneally (IP) injected THC were also determined. The studies were conducted to establish the vapor exposure model in our laboratory at Johns Hopkins University. To validate our procedures, we examined sex and strain differences for comparison with findings in other laboratories. Vapor exposure parameters that were manipulated included puff duration, puff frequency, and total exposure time in order to generate a range of vaporized e-liquid volumes. A behavioral test battery was conducted for both routes and included food-maintained operant responding, a tail withdrawal (TW) assay for nociception, and rectal measurements of body temperature. These behaviors were chosen based on the established effects of THC on appetite, antinociception, and hypothermia (Compton et al., 1993; Higgs et al., 2005; Metna-Laurent et al., 2017). Sprague Dawley and Wistar rats, the two most commonly used rat strains in cannabinoid vapor exposure models (Moore et al., 2020), were used to assess potential sex and strain differences in THC effects. We hypothesized that we would observe differences in behavioral outcomes with respect to time course based on known pharmacokinetic differences between these two routes of administration.

2. Methods

2.1 Subjects

Adult male and female Sprague Dawley rats (N=12, 6 per sex) and Wistar rats (N=12, 6 per sex)(Charles River, Wilmington, MA) weighing 250–350 grams at the start of experiments were single housed in wire-topped, plastic cages (27 × 48 × 20 cm) with standard enrichment. The vivarium was on a 12 hr reverse light cycle (lights off at 9:00 a.m.) and was

humidity and temperature controlled. Since food-maintained behavior was a primary outcome, rats were maintained at 90% of their free feeding weight throughout the experiments. Diet was a corn-based chow (Teklad Diet 2018; Harlan, Indianapolis, IN) and rats had free access to water except during test procedures. All procedures used in this study were approved by the Johns Hopkins Institutional Animal Care and Use Committee. The facilities adhered to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were AAALAC-approved. Two rats were euthanized for health reasons unrelated to the treatments; therefore, final group sizes were Sprague Dawley (N=6 males, N=5 females) and Wistar (N=6 males, N=5 females).

2.2 Drugs

THC stock solution (200 mg/ml in 95% ethanol) was provided by the U.S. National Institute on Drug Abuse Drug Supply Program. The 200 mg/ml ethanol-based THC stock solution was mixed in 100% propylene glycol and the ethanol was evaporated using nitrogen to yield a 200 mg/ml THC solution for vaporization. While e-liquids can contain different concentrations of propylene glycol or vegetable glycerin, we used a 100% propylene glycol vehicle for vapor exposure conditions based on its use in prior vapor studies (Javadi-Paydar et al., 2018; Nguyen et al., 2016), and its efficiency for drug delivery (Baassiri et al., 2017; Spindle et al., 2018b). For intraperitoneal (IP) injections, the THC stock was dissolved in a vehicle solution of 0.9% sterile saline, ethanol, and Cremophor EL (18:1:1 ratio) for final doses of 1, 3, 5.6, 10, and 20 mg/ml, administered at 1 ml/kg. Composition of i.p. vehicle solutions and initial test doses were selected based on the literature (Craft et al., 2019; Solinas and Goldberg, 2005; Taffe et al., 2015).

2.3 Vapor exposure system

A commercial vapor chamber system (La Jolla Alcohol Research Institute, La Jolla CA) was utilized, which contained four sealed polycarbonate rat cages (35 × 28 × 26 cm; 25L) adapted for the delivery of vaporized drug, and an electronic vapor device (Smok Baby Beast Brother TFV8 Sub- Ohm Tank with the V8 X- Baby M2 0.25- Ω coil; SMOKTech, Shenzhen, China) connected to an air pump to regulate airflow. The chamber air was vacuum controlled by pump which pulls room ambient air into the chamber through an intake valve and out through the exhaust valve at a constant rate (2–3 L/min). The e-vape controller was set to a maximum temperature of 400°F (~30W), which is the same temperature used for vaporization in our clinical research (Spindle et al., 2020).

2.4 Vapor Delivery System Evaluation

Vapor delivery systems were tested without animals in the chambers to determine volumes delivered under different puff durations and frequencies. The amount of e-liquid (mL) vaporized per delivery and condition was determined by weighing the tank before and after deliveries of the propylene glycol vehicle. Each condition was tested in triplicate in randomized order to assess consistency for calibration of puff and volume parameters. Such testing for reliability permits cross comparison and reproducibility across laboratories and vapor systems.

2.5 Study design

A randomized, within subject design was utilized for the study. One vehicle test and one drug test were conducted each week, with a minimum of 7 days between each THC dose. Weekly vehicle tests were included to assess carry-over drug effects and control for baseline shifts over time. Each week, half of the rats received vapor and half received IP injections, and the route of THC administration alternated weekly until all doses/exposure conditions were completed. THC injections were administered in a blinded, within subject Latin square design (0–20 mg/kg). For the vapor exposure testing, the number of puffs, duration of puffs, and inter-puff intervals were systematically increased to produce 5 vapor conditions as detailed in Table 1, tested in ascending order. Testing began at time 0 with IP injection or initiation of the 30 min exposure to THC vapor (conditions 2–5). Since vapor condition 1 was only 12 min in duration, it was completed in the last 12 min of the same 30 min period. Vapor Condition 1 was repeated in the middle of the testing period (week 8) to assess any changes in response to THC vapor exposure. The total testing period was approximately 14 weeks.

2.6 Behavioral Test Battery

2.6.1 Food-maintained Operant Responding

Training.: Operant sessions ran in dedicated experimental chambers interfaced with a computer with Med-PC hardware and software for experimental control of behavior (Med Associates, St. Albans, VT). Each chamber was equipped with a nose-poke key, a cue light, and a food cup connected to an automated pellet feeder, all positioned inside a sound-attenuating enclosure with exhaust fans. Rats were initially trained to press a nose poke key for delivery of a 45 mg food pellet (grain-based chow #F0165, BioServ, Flemington, NJ) under a fixed ratio 1 (FR1) schedule of reinforcement in daily 30 min sessions (Mon-Fri). After establishing responding and stability of food pellets earned under an FR1 schedule (responses ±10%), the FR requirement was increased by 1 to a final FR10 over the course of 2–5 days; FR was increased during the session based on individual performance (i.e., responding was maintained and did not decrease over consecutive reinforcers).

Progressive ratio.: After stability of responding under the FR10 schedule, a progressive ratio (PR) procedure with a 10 min limited hold was used. The number of responses required to produce a food pellet was progressively increased (1, 2, 4, 6, 9, 12, 15, etc.)(Richardson and Roberts, 1996). The last ratio requirement completed before the animal stops responding, or before the maximum session time was reached, was defined as the 'breakpoint' (Richardson and Roberts, 1996). Breakpoints for food pellets were assessed immediately after vapor exposure and 30 min after IP administration. Response rates were calculated as total responses/total responding time (min) and used as an indicator of motor impairment. Non-drug PR sessions were run on subsequent weekdays to ensure stable responding for food was maintained.

2.6.2 Antinociception (thermal pain sensitivity)—Thermal pain sensitivity was assessed using the tail withdrawal (TW) assay. In this test, the distal end of the rat's tail (~50 mm from the tip) is exposed to radiant heat from a precise photobeam (Harvard Apparatus,

Cambridge, MA, USA) and latency (s) to respond to the heat stimulus by flexion of the tail is recorded. The maximum duration was limited to 10s. Prior to testing, the radiant heat setting was calibrated to achieve a group average baseline latency of 4s. Baseline TW latencies were obtained 24 hrs prior to drug administration. Antinociception was calculated as percent of maximum possible effect (% MPE= [(test TW latency− baseline latency)/ (maximum TW latency − baseline TW latency)] × 100). Rats were tested at 3 time points post-drug administration: 15 min (IP only), 60 min, 120 min (vapor only), and 300 min. The 15 min time point was not conducted for the vapor condition, as animals were being exposed to vapor during that period. The 60 min time points in both groups occurred immediately after the completion of the PR session.

2.6.3 Body temperature—Body temperature was determined with a digital rectal thermometer with a lubricated flexible probe across 5 time points on the test day (30, 60, 90, 150, 300 min).

2.7 Blood Collection Procedures

Vapor condition 5 and 10 mg/kg IP injection were repeated for blood sampling and analysis of plasma THC levels. Rats were extensively handled to acclimatize to handling and holds needed for blood sampling. Blood sampling was completed at the end of the study, 3 weeks after all behavioral data was collected as a study endpoint. All staff conducting blood draws had extensive training in restraint and blood sampling techniques to reduce discomfort. Sampling was completed as follows: The leg area was shaved the day before. On the collection day, the shaved area was wiped with alcohol followed by application of sterile lubricating gel. A 25-gauge needle was used to puncture the saphenous vein so that a drop of blood forms immediately at the puncture site and beads on the lubricant. Blood (~250 μl) was collected in an EDTA-coated microcentrifuge tube, then gentle pressure over the puncture site stopped blood flow. Each animal had two blood draws, 3 weeks apart. While blood sampling for all vapor conditions and IP doses were planned, these could not be completed due to discontinuation of research at Johns Hopkins University because of the COVID-19 pandemic. Blood samples were collected immediately (approx. 10 min) following the end of the vapor exposure or 30 min after IP injection. Blood samples were placed immediately on wet ice for 30 min, and then centrifuged at 3000G for 10 min. Plasma supernatant was transferred to low protein binding microcentrifuge tubes and stored at −80°C until shipment on dry ice for analysis.

2.8 Plasma THC analysis.

Plasma THC concentrations were quantified using fast liquid chromatography/mass spectrometry (LC/MS) adapted from (Irimia et al., 2015; Lacroix and Saussereau, 2012; Nguyen et al., 2018). 50 μL of plasma were mixed with 50 μL of deuterated internal standard (100 ng/mL CBD-d3 and THC-d3; Cerilliant), and cannabinoids were extracted into 300 μL acetonitrile and 800 μL of chloroform and then dried. Samples were reconstituted in 100 μL of a methanol/water (2:1) mixture. Separation was performed on an Agilent LC1100 using a Poroshell 120 EC-C18 column (4.0μm, 2.1mm × 100mm) using isocratic elution with water and methanol, both with 0.2 % formic acid (250 μL/min; 81% MeOH). THC was quantified using an Agilent 6140 single quadrupole MSD using

electrospray ionization and selected ion monitoring [THC (m/z=315.2) and THC-d3 (m/z=318.2)]. Calibration curves were conducted daily for each assay at a concentration range of 0–200 ng/mL and observed correlation coefficients were 0.999.

2.9 Data Analysis

Outcome measures analyzed included: breakpoints, response rate (responses/min), %MPE, body temperature (°C), and plasma THC concentration (ng/mL). For each route of administration (vapor and IP), three- or four-way repeated measures ANOVAs were conducted with strain and sex as between subjects variables and vapor condition/dose and time (when applicable) as within subject variables. Based on the study aims, strains were then disaggregated and two- or three-way ANOVAs were conducted, with sex as a between subjects variable and vapor condition/dose and time (when applicable) as within subject variables. Dunnett's post hoc tests were used to analyze differences in outcomes between THC dose/condition and vehicle. Comparisons between sex and strain were planned *a priori* and were determined with Sidak's post hoc tests. For plasma THC levels, a two-way ANOVA was conducted (strain, sex as between subjects variables) in each route of administration, followed by two-sided unpaired t-tests as post hoc tests to evaluate sex differences. Statistics were performed in Statistica 11 (Stat Soft, Inc.) and GraphPad Prism with $P \leq 0.05$ for significance. Only the highest order effect/interactions are reported in the text.

3. Results

3.1 Vapor Delivery System Evaluation

The vapor delivery system produced linearly increasing volumes of vapor from the e-liquid (mL) according to the puff duration and volumes delivered were consistent over repeated tests (see Supplemental Figure S1). The amount of e-liquid vaporized ranged from 0.1– 2.1 mL for conditions 1–5 (see Table 1).

3.2 THC effects on breakpoints for food

Breakpoints remained stable over time, as demonstrated by no effect of repeated vehicle vapor tests ($F(4, 80) = 0.29$, *n.s.*) or IP vehicle injections ($F(6, 120) = 1.04$, *n.s.*). Breakpoints were altered by THC vapor in a condition and strain dependent manner (Fig. 1A), confirmed by a vapor condition × strain interaction ($F(5, 90) = 2.53$, $p<0.05$). THC vapor produced biphasic effects on breakpoints in Sprague Dawley rats ($F(5, 45) = 20.20$; $p<0.001$; Fig. 1B). Breakpoints increased after the lower vapor exposure conditions (1 and 2) and decreased after the two highest vapor exposure conditions (4 and 5; p's<0.05). The increased breakpoints seen after vapor condition 1 appear to be driven by females ($p<0.05$), while males showed increased breakpoints at vapor condition 2 ($p=0.05$). In Wistar rats, THC vapor exposure only reduced breakpoints ($F(5, 45) = 20.57$; $p<0.001$; Fig. 1C); with significant reductions observed after conditions 4 and 5 (p's<0.05). THC vapor condition 1 was repeated in the middle of the testing period (week 8) to assess any changes in effects after repeated exposure to THC, and breakpoints were equivalent when tested in week 1 and week 8 ($F(1, 19) = 2.476$, *n.s.*). An average of these two weeks was used in the above described analysis.

In both Sprague Dawley and Wistar rats, breakpoints decreased after 3–20 mg/kg IP THC (p's<0.05; Fig. 1D). Overall, there was a significant main effect of strain ($F(1, 18) = 16.34$, $p<0.001$) on breakpoints, and Sprague-Dawley rats had a higher breakpoint than Wistar rats after 1 mg/kg IP THC ($p<0.05$). When disaggregated by strain, there were significant main effects of dose in Sprague Dawley ($F(5, 45) = 8.39$, $p<0.001$; Fig. 1E) and Wistar rats ($F(5, 45) = 13.20$, $p<0.001$; Fig. 1F). In Sprague Dawley rats, there was also a significant main effect of sex ($F(1, 45) = 6.63$, $p<0.05$), but effects were not isolated in post hoc tests.

THC exposure also altered response rates under the PR schedule of reinforcement ($F(5,90) = 28.87$, $p<0.001$; Fig. 2A). In Sprague Dawley rats, THC vapor exposure increased response rates for condition 1 and decreased response rates for conditions 3–5 (p's<0.05; Fig. 2B). In Wistar rats, THC vapor exposure only decreased response rates in conditions 4–5 (p's<0.05; Fig. 2C), though there was a trend for an increase in breakpoints after vapor condition 2 ($p=0.06$). There were no sex differences observed in THC vapor effects on response rates in either strain. Following IP injection, there was a main effect of THC dose ($F(5,90) = 7.40$, $p<0.001$) and sex ($F(1,18) =4.81$, $p<0.05$) on response rates (Fig. 2D). When disaggregated by strain, this main effect of sex was only present in Sprague Dawley rats ($F(1,9) =6.63$, $p<0.05$; Fig. 2E), though there were no sex differences isolated by post-hoc tests. In Sprague Dawley rats, IP THC reduced response rates ($F(5,45) =5.86$, $p<0.001$), with significant decreases after 10 and 20 mg/kg (p's<0.05). In Wistar rats, there was an effect of THC dose on response rates ($F(5,45) =2.75$, $p<0.05$), with significant decreases after 20 mg/kg (Fig. 2F). These reductions in response rates after THC at the highest exposure conditions/doses indicate a motor suppressive effect of THC on PR responding.

3.3 THC effects on TW latency

THC vapor exposure increased %MPE values in the TW test (Fig. 3A) as confirmed by a significant interaction of vapor condition × time ($F(10, 180) =6.43$, $p<0.001$). There was also an interaction of time × strain ($F(2, 36) =3.30$, $p<0.05$). When disaggregated by strain, there were significant main effects of vapor condition ($F(5, 45) =20.37$, $p<0.001$) and time ($F(2, 18) =3.64$, $p<0.05$) in Sprague Dawley rats (Fig. 3B). In Wistar rats, there were significant interactions between time × sex ($F(2, 18) =3.94$, $p<0.05$) and vapor condition × time ($F(10, 90) =6.24$, $p<0.001$; Fig. 3C). In both Sprague Dawley and Wistar rats, the highest THC vapor conditions (4–5) increased antinociception during one or more time points when compared to vehicle vapor. After vapor condition 4, %MPE was only significantly greater than vehicle at the first time point tested (60 min) in both strains (p's<0.05). After the highest vapor condition (5), some antinociceptive effects lasted the duration of the testing period, though peak effects occurred at 60 min for Wistar rats and at 120 min for Sprague Dawley rats. No sex differences were observed in post hoc tests for either strain.

IP THC increased %MPE values (Fig. 3D). There were significant interactions of time × strain ($F(2, 36) =3.78$, $p<0.05$) and dose × time × sex ($F(10, 180) =2.08$, $p<0.05$). When disaggregated by strain, there was a significant interaction of dose × time × sex ($F(10, 90) =2.29$, $p<0.05$) in Sprague Dawley rats (Fig. 3E). Female Sprague Dawley rats had higher %MPE compared to males after 10 mg/kg THC at 300 min ($p<0.05$). In Wistar rats, there were significant main effects of dose ($F(5, 45) =21.77$, $p<0.001$) and time ($F(2, 18) =6.57$,

p<0.01) on TW latency (Fig. 3F). Female Wistar rats had higher %MPE compared to males after 3 mg/kg THC at 300 min (p<0.05). In both Wistar and Sprague Dawley rats, almost all THC doses tested (3–20 mg/kg) increased TW latency during one or more time points when compared to IP vehicle (p's<0.05), although peak effects occurred at 60 min for Wistar rats and at 300 min for Sprague Dawley rats.

3.4 THC effects on body temperature

THC vapor exposure decreased body temperature (Fig. 4A), confirmed by significant 3-way interactions of time × strain × sex ($F(4, 72) = 4.20$, $p<0.01$), vapor condition × time × strain ($F(20, 360) = 2.76$, $p<0.001$), and vapor condition × time × sex ($F(20, 360) = 1.66$, $p<0.05$) on body temperature. Wistar rats had lower body temperatures than Sprague Dawley rats after vapor condition 4 at 90 min (p<0.05). When data were disaggregated by strain, there were significant interactions between time × sex ($F(4, 36) = 2.67$, $p<0.05$) and vapor condition × time ($F(20, 180) = 6.30$, $p<0.001$) in Sprague Dawley rats (Fig. 4B). Male Sprague Dawley rats had lower body temperatures than females after vapor condition 2 at 300 min (p<0.05). In Sprague Dawley rats, all THC vapor conditions (1–5) reduced body temperature during one or more time points when compared to vehicle vapor (p's<0.05). At 300 min, body temperatures had mostly returned to control levels except for vapor conditions 2 and 5. In Wistar rats, there was a significant 3-way interaction between vapor condition × time × sex ($F(16, 144) = 2.09$, $p<0.05$) on body temperature (Fig. 4C). In Wistar rats, THC vapor conditions 3–5, but not 1–2, reduced body temperature during one or more time points when compared to vehicle vapor, all returning to control levels by 300 min. Wistar males had lower body temperatures than females after vapor condition 3 at 300 min (p<0.05). For most exposure conditions, the temperature nadir occurred 30–90 min post vapor initiation in all strains and sexes.

IP THC also decreased body temperature (Fig. 4D) as confirmed by a significant 3-way interaction of dose × time × strain ($F(20, 360) = 2.86$, $p<0.001$). Wistar rats had a lower body temperature than Sprague Dawley rats after 5.6 mg/kg (300 min) and 10 mg/kg THC (60 and 150 min; p's<0.05). When disaggregated by strain, there were interactions between dose × sex ($F(5, 45) = 2.78$, $p<0.001$) and dose × time ($F(20, 180) = 7.89$, $p<0.001$) in Sprague Dawley rats (Fig. 4E); all THC doses (1–20 mg/kg) reduced body temperature during one or more time points when compared to vehicle (p's<0.05). There were no significant sex differences isolated by post hoc tests. In Wistar rats, there was a significant 3-way interaction between dose × time × sex ($F(20, 180) = 2.39$, $p<0.05$; Fig. 4F). In Wistar rats, all THC doses (1–20 mg/kg) also reduced body temperature during one or more time points when compared to vehicle (p's<0.05). Male Wistar rats had lower body temperatures than females after 10 mg/kg THC (60 min; p<0.05). In all groups, the hypothermic effects of IP injected THC continued to increase over the testing period, with the temperature nadir observed at the last time point tested (300 min).

3.5 Plasma THC following injection and vapor exposure

One plasma sample was determined to be an outlier (>15 standard deviations from jackknifed group mean) and was removed (1 Sprague Dawley female, 10 mg/kg IP). Analysis of plasma THC after vapor condition 5 demonstrated no effects of strain or sex

(Fig. 5A). Analysis of plasma THC after 10 mg/kg IP showed an effect of sex ($F(1, 17)=5.34$, $p<0.05$) on plasma THC concentrations, with Sprague Dawley females showing higher plasma THC compared to males of the same strain (Fig. 5B). Plasma THC concentrations following 10 mg/kg IP THC (30 min after injection) were 4-fold higher than observed after THC vapor exposure (10 min following removal from vapor chamber).

4. Discussion

This series of experiments aimed to characterize exposure/dose-response relationships for THC vapor and compare with IP injection in male and females of two rat strains to validate a passive THC vapor exposure model in our laboratory. Our vapor exposure model produced consistent, reliable, and puff-orderly amounts of vapor, and plasma THC concentrations detected after THC vapor exposure were comparable to those seen in humans. THC vapor exposure and IP THC produced effects on appetite, antinociception, and hypothermia that differed with respect to time course. Some strain and sex differences were also observed, though these effects were often limited to one or two doses/exposure conditions per outcome. These data provide information on sex differences to explore further and inform group effect sizes needed to power future studies. Below, we discuss each finding, including strain and sex differences in THC effects to put them in the context of the field.

The present study is the first to evaluate the effects of THC vapor on progressive ratio performance in male and female rats. Low THC vapor exposure conditions increased breakpoints and high THC vapor exposure conditions decreased breakpoints. The observed increases in breakpoints for food are consistent with the appetite stimulating effects of THC in humans (Badowski and Perez, 2016) and hyperphagic effects of THC vapor observed in studies using free feeding paradigms (Brutman et al., 2019; Manwell et al., 2014b; Nguyen et al., 2020b). The decreases in breakpoints at high THC vapor exposure conditions were accompanied by reductions in response rates indicating motor suppressive effects. In our study, IP THC (3–20 mg/kg) produced dose dependent decreases in breakpoints, and at the highest doses this was accompanied by reduced response rates (i.e., motor suppressive effects). Reduced breakpoints for food at doses >5 mg/kg IP THC have been observed previously (Higgs et al., 2005; Solinas and Goldberg, 2005). Prior studies testing lower IP doses of THC, reported either increased breakpoints (1–3 mg/kg)(Higgs et al., 2005; Solinas and Goldberg, 2005) or no effects (0.3–3 mg/kg)(Olarte-Sanchez et al., 2015). Doses of IP THC below 1 mg/kg were not tested in the current study and may have increased breakpoints. The lack of an increase in breakpoints in the current study is most likely due to differences in food pellet reinforcers between studies (e.g. chow vs sucrose). The ability of THC and other CB1 agonists to increase appetite is particularly evident in more palatable food (i.e. energy dense, high in fat and/or sugar) (Berry and Mechoulam, 2002; Jager and Witkamp, 2014; Koch, 2001). The appetite-enhancing effects of THC vapor exposure were observed in Sprague Dawley rats, although at different vapor conditions for males and females. Overall, sex differences in the appetite-stimulating effects of THC vapor are underexplored in preclinical models and may be important for translation of the clinical use of cannabis and THC for stimulation of appetite.

In the present study, we observed antinociceptive effects of THC that were exposure/dose-orderly. To avoid disruption of PR performance, measurement of TW latency was determined after the 30 min operant session. Antinociceptive effects of THC vapor were highest at this time point (60 min), and return towards control levels by 300 min. We acknowledge that antinociceptive effects of THC vapor likely also occurred earlier as reported by other laboratories utilizing vapor exposure models (Javadi-Paydar et al., 2018; Nguyen et al., 2018). Following IP THC, antinociceptive effects continued to increase in magnitude, with peak antinociceptive effects occurring at the last time point tested (300 min after injection). These data concur with prior studies testing IP THC (Craft et al., 2019; Tseng and Craft, 2001), although peak effects of IP THC have been reported to occur earlier in the testing period (Tseng and Craft, 2001). There were some strain effects observed on antinociceptive effects of THC, which may be attributable to subtle differences in the time course of effects (e.g. Wistars showed peak effects at earlier time points after both vapor and IP THC).

There was no significant difference in the magnitude of antinociceptive effects of THC vapor between males and females, though this may be due to statistical power related to group sizes and ceiling effects in this procedure. We observed almost 100% MPE for THC vapor in Wistar females compared to ~50% MPE in Wistar males. Other studies have reported Wistar females may show greater antinociceptive effects to THC vapor Javadi-Paydar et al. (2018), but cf. (Nguyen et al., 2018). Sex differences in antinociception to IP THC was observed in our study. Low to moderate IP THC doses that did not produce antinociception in males significantly increased MPE, and by a greater magnitude, in female rats. These data are in agreement with findings by Craft and colleagues, in which IP THC produced greater antinociceptive effects (Craft et al., 2019; Tseng and Craft, 2001), and antinociceptive effects lasted longer in Sprague Dawley female rats compared to male rats (Tseng and Craft, 2001). To our knowledge, this is the first study to assess the time course of THC vapor-induced antinociception in female Sprague Dawley rats.

In the current study, THC produced exposure and dose-dependent hypothermia which followed a similar time course as observed for antinociception. Following THC vapor exposure, maximal temperature decreases were observed at earlier time points and returned to near control levels by 300 min. IP THC produced progressively greater hypothermia across the 5 hr testing period, which is in stark contrast to the time course of hypothermic effects observed after THC vapor exposure. These data are in agreement with prior studies demonstrating hypothermic effects of THC vapor exposure and IP THC (Javadi-Paydar et al., 2018; Nguyen et al., 2016; Nguyen et al., 2020b). In contrast to one study that reported that Sprague Dawley rats showed greater hypothermic effects of THC vapor and IP THC (Taffe et al., 2020), the magnitude of hypothermic effects in the current study was greater in Wistar rats after higher exposure/dose conditions (vapor condition 4, 5.6–10 mg/kg). These contrasting effects may be due to different vendors for Sprague-Dawley rats (Charles River vs. Harlan); comparisons of the degree of hypothermia observed in Wistar rats of both studies (both from Charles River) appear similar. Making direct strain comparisons is important for generalizability across studies, particularly with novel methodologies such as vapor exposure to cannabinoids. The results here suggest that there may be some quantitative differences (i.e. magnitude of effects) depending on the behavioral outcome

assessed, though both Wistar and Sprague-Dawley rats showed similar qualitative outcomes (e.g. presence or absence of effect) in response to THC vapor and IP administration.

In the current study, there were some effects of sex observed on hypothermia. Temperatures in male Sprague Dawley and Wistar rats were slower to recover to control levels after THC vapor compared to females. The magnitude of hypothermia was greater in Wistar males compared to females after 10 mg/kg IP THC. Prior studies have also observed subtle sex differences in hypothermic response to THC vapor, primarily manifesting in the time course of effects (e.g. earlier onset, faster offset for males)(Javadi-Paydar et al., 2018); Nguyen et al. (2016). Studies in Long Evans rats have observed greater magnitude of hypothermia in females compared to males at very high IP doses (100–176 mg/kg), but equivalent effects at low to moderate doses (1–30 mg/kg)(Wiley et al., 2007). In Wistar rats, equivalent hypothermic effects of 1 mg/kg intravenous THC were observed in both sexes, however a lower dose (0.3 mg/kg) immediately reduced body temperature in male rats, but not female rats (Nguyen et al., 2020a). Taken together, these data suggest sex differences in THC-induced hypothermia can differ depending on the strain of rats used.

In the current study, plasma THC levels assessed after exposure to the highest vapor exposure condition were ~38 ng/ml immediately following the 30 min vapor exposure period. To put this in context, human laboratory studies report plasma levels of THC peaked at 15 ng/ml after vaping cannabis containing 25 mg of THC, a dose that produces significant subjective drug effects and cardiovascular effects (increased heart rate)(Spindle et al., 2018a). In other preclinical THC vapor studies, 30 min exposure to 100 mg/ml and 200 mg/ml THC produced plasma THC concentrations of 67 ng/ml (Nguyen et al., 2019) and 150–360 ng/ml (Nguyen et al., 2020b; Nguyen et al., 2018). There are some parameter differences across labs such as exposure chamber size (9 vs. 25L), e-vape system used, and air flow (continuous circulation vs. closed system) (Nguyen et al., 2016; Nguyen et al., 2020b; Nguyen et al., 2018). Plasma THC following an injection of 10 mg/kg THC was ~150 ng/ml 30 min after injection, which is consistent with other studies (Nguyen et al., 2016). In the present study, female Sprague Dawley rats had higher plasma THC after IP THC than male rats. Evidence for sex differences in THC metabolism is mixed. THC vapor exposure studies reported similar plasma THC levels in male and female rats (Javadi-Paydar et al., 2018) or higher THC levels in female rats (Nguyen et al., 2020b). Studies examining IP administration of THC have reported no sex differences in blood THC levels (Britch et al., 2017; Tseng et al., 2004), but higher levels of the active THC metabolite 11-OHTHC in female rats (Britch et al., 2017). Interestingly, though plasma THC concentrations following vapor exposure in the current study were considerably lower compared to 10 mg/kg IP THC, behavioral outcomes were similar. Future studies will investigate the time course of plasma THC concentrations, as well as critical metabolites such as 11-OH-THC, after vapor exposure.

A main objective of this study was to establish a range of dose effects using a behavioral battery and a within-subjects design. While chronic (daily) administration can cause desensitization and downregulation of cannabinoid CB-1 receptor (Burston et al., 2010; Hirvonen et al., 2012), such downregulation appears to be transient with rapid recovery towards baseline within days (D'Souza et al., 2016). Using a regimen of 7 days in between

active doses, we did not observe behavioral tolerance to the effects of THC vapor: repeated testing of THC vapor condition 1 in week 1 and 8 produced similar effects on PR breakpoints for food. Furthermore, THC administered IP was done in a randomized design, while every other week THC vapor exposure was administered in an ascending order, and dose-orderly behaviors were observed in both designs. A within-subjects design is valuable as it permits cross comparison of drug effects across routes and outcome measures to aid dose selection and interpretation of results.

In summary, the present study demonstrates a reliable and orderly exposure-effect curve of THC vapor on behavior. The time course of behavioral outcomes produced by vapor exposure was more rapid in onset and shorter in duration when compared to the IP route of administration. Optimal parameters for puff length and duration of vaporized drug may vary from lab to lab as a function of equipment used (e.g. e-cigarette device, size of chambers, air flow). These parameters will then need to be adjusted to reach a desired 'dose' based on the behavioral outcome of interest, for example, non-sedating doses for operant studies investigating drug reinforcement (Freels et al., 2020), studies of pain relief, cognitive effects, etc. Continued validation of vapor exposure methods across laboratories and comparison of effects to standard routes of administration are important to infer dose response relationships and increase reproducibility between labs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Abbreviations:

THC	Δ9-tetrahydrocannabinol
IP	intraperitoneal
TW	tail withdrawal
FR	fixed ratio
PR	progressive ratio
MPE	Maximum possible effect

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Highlights

- This study characterized THC vapor effects in two strains of male and female rats
- A range of THC vapor conditions were used to characterize a dose-effect curve
- THC produced orderly effects on appetitive behavior, nociception, and temperature
- Time course of effects varied with respect to route of administration (vapor, IP)
- Sex and strain differences were observed in response to THC effects

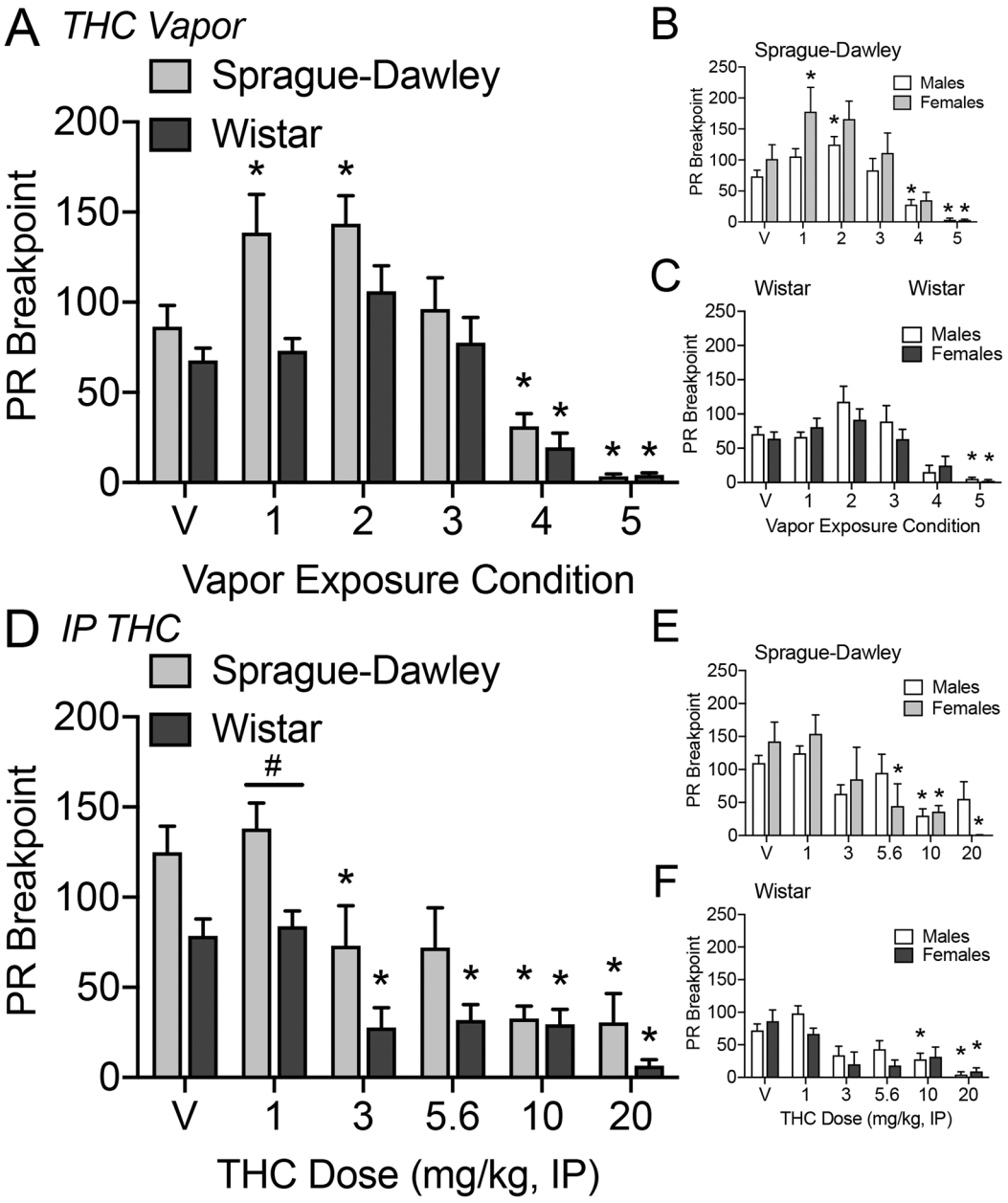

Figure 1.

[A-C] Mean breakpoints after exposure to THC (200 mg/ml; conditions 1–5) or the propylene glycol vehicle (V) vapor. [D-F] Mean breakpoints after IP THC (1–20 mg/kg) or vehicle (V). Bars represent the group mean ± SEM for all Sprague Dawley and Wistar rats (A and D) and when data for each strain are disaggregated by sex (right panel). Asterisks (*) denote difference from Vehicle within the respective group; pound sign (#) denotes a difference between strains ($p<0.05$).

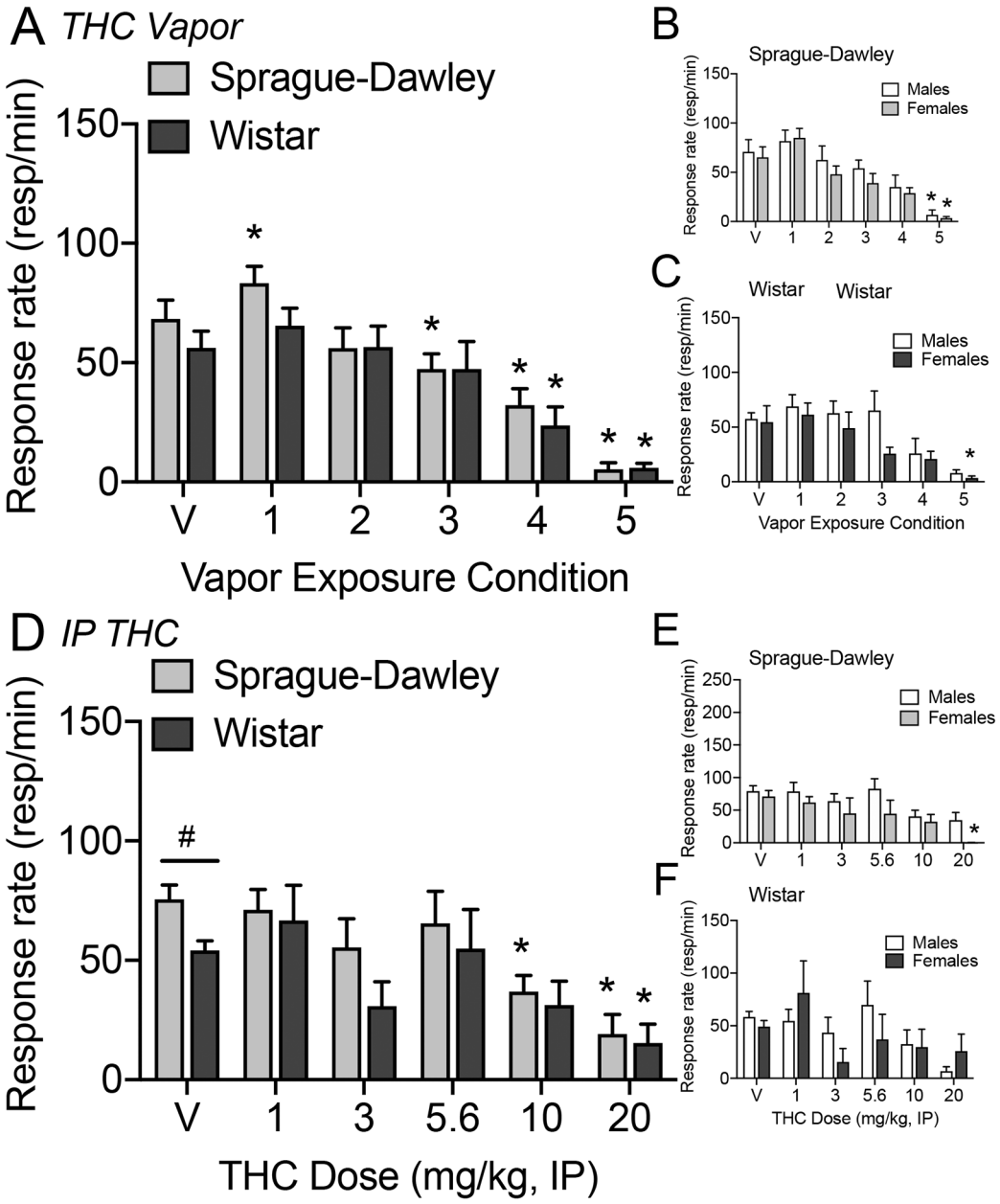

Figure 2.

[A-C] Mean PR response rates after exposure to THC (200 mg/ml; conditions 1–5) or the propylene glycol vehicle (V) vapor. [D-F] Mean PR response rates after IP THC (1–20 mg/kg) or vehicle (V). Bars represent the group mean ± SEM for all Sprague Dawley and Wistar rats (A and D) and when data for each strain are disaggregated by sex (right panel). Asterisks (*) denote difference from Vehicle within the respective group; pound sign (#) denotes a difference between strains ($p<0.05$).

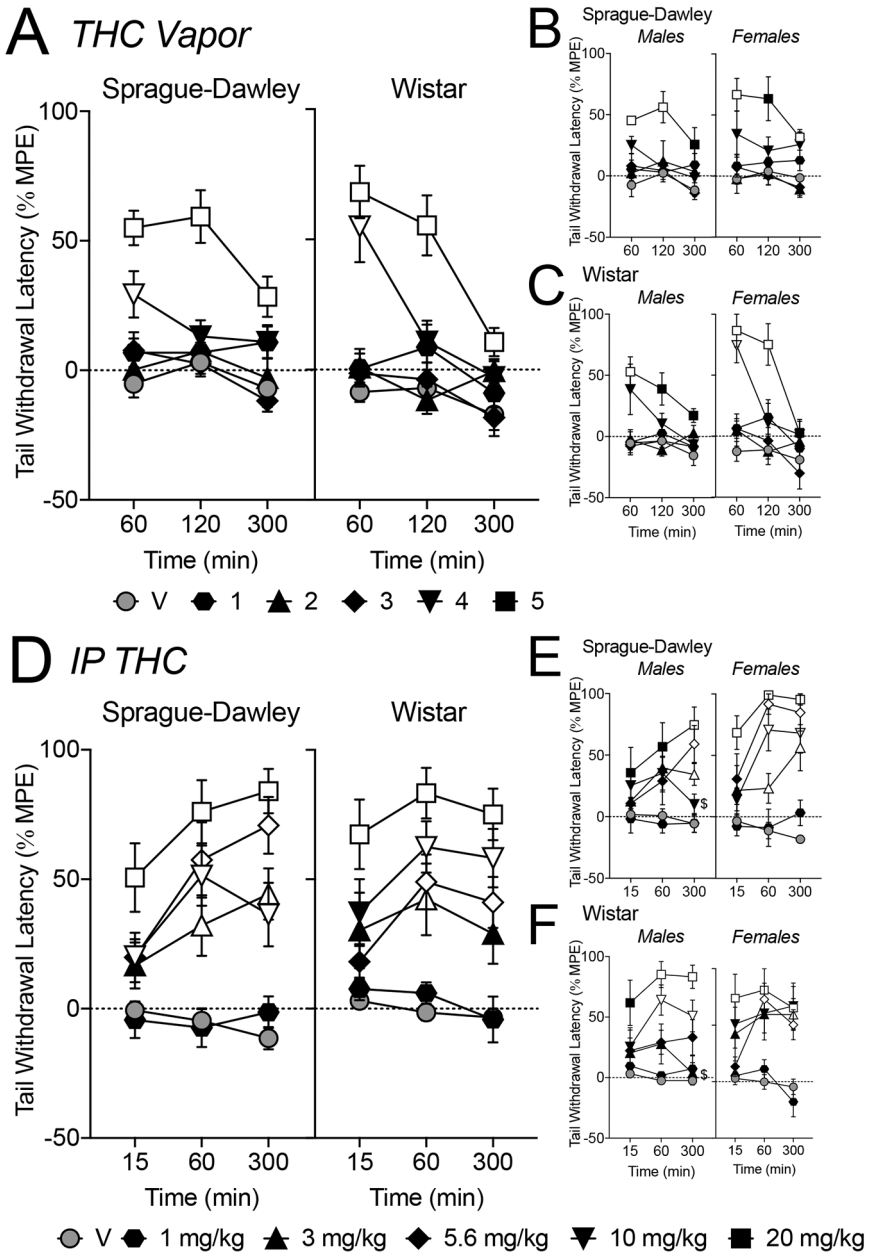

Figure 3.

[A-C] Tail withdrawal latencies, expressed as % of maximum possible effect (%MPE) after exposure to THC (200 mg/ml; conditions 1–5) or the propylene glycol vehicle (V) vapor. Data for each strain are disaggregated by sex in panels B-C. [D-F] Tail withdrawal latencies (%MPE) after IP THC (1–20 mg/kg) or vehicle (V). Data for each strain are disaggregated by sex in panels E-F. Data are Mean ± SEM. Symbol colors: Vehicle symbols are dark gray; other symbols are drug, and unfilled symbols denote a difference from Vehicle ($p<0.05$). Pound sign (#) indicates strain differences, ($) indicates sex differences ($p<0.05$).

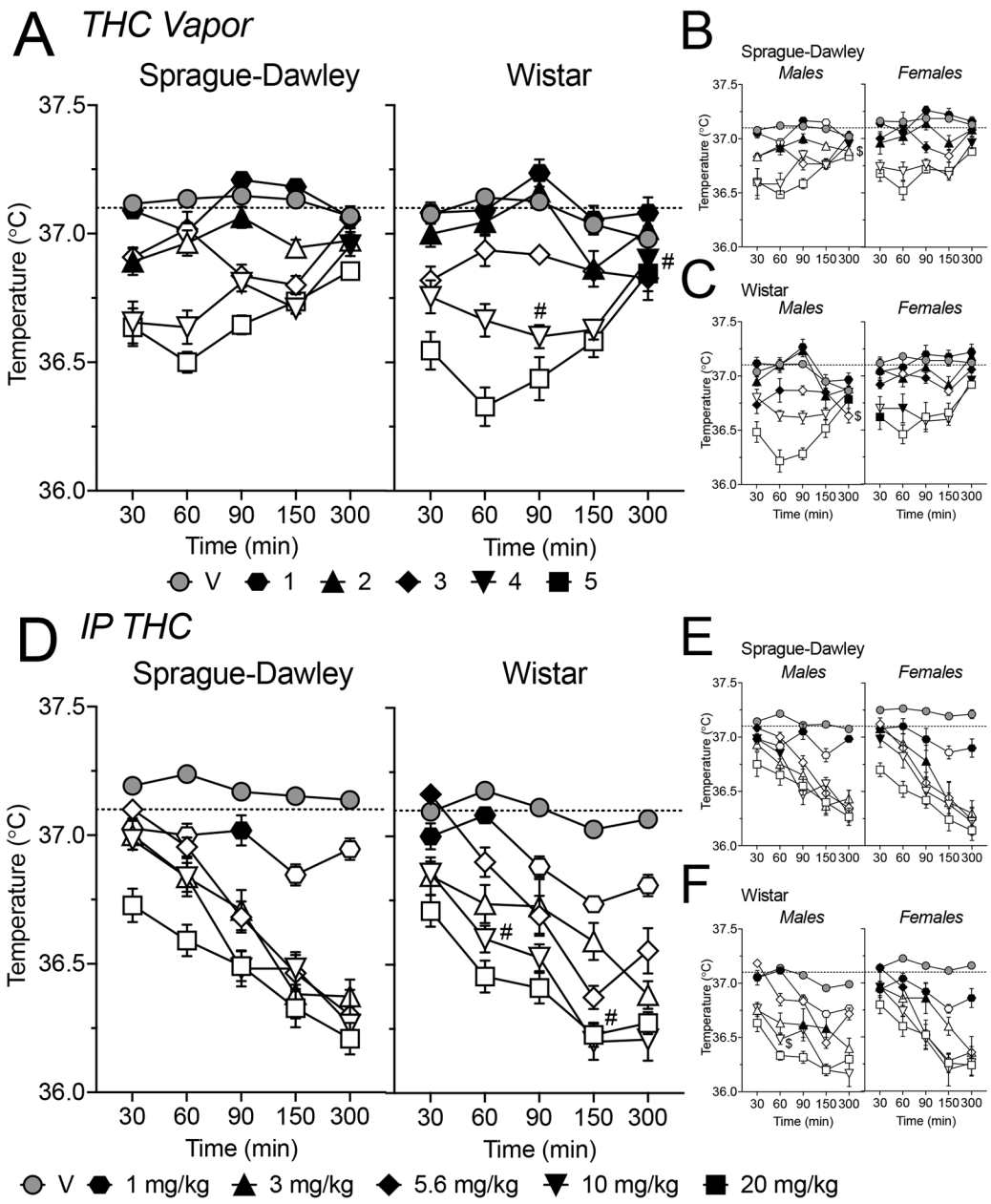

Figure 4.

[A-C] Body temperature (°C) after exposure to THC (200 mg/ml; conditions 1–5) or the propylene glycol vehicle (V) vapor. Data for each strain are disaggregated by sex in panels B-C. [D-F] Body temperature (°C) after IP THC (1–20 mg/kg) or vehicle (V). Data for each strain are disaggregated by sex in panels E-F. Reference line reflects the overall average temperature under vehicle conditions (37.1°C). Data are Mean ± SEM. Symbol colors: Dark gray symbols are Vehicle; other symbols are drug, and unfilled symbols denote a difference from Vehicle ($p<0.05$). Pound sign (#) indicates strain differences, ($) indicates sex differences ($p<0.05$).

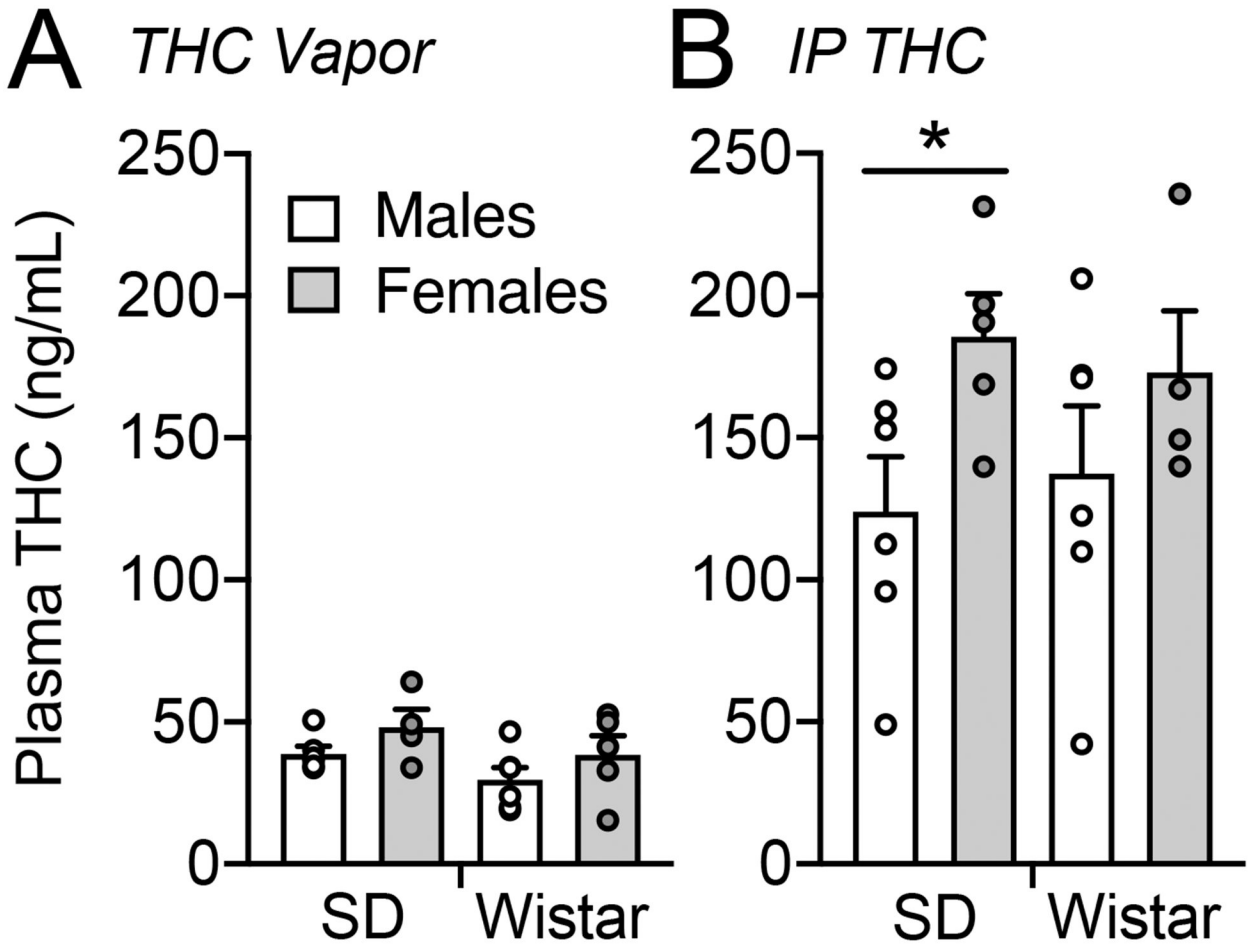

Figure 5.

Plasma THC concentrations (ng/mL) following THC vapor exposure in Condition 5, 20 × 9s puffs of 200 mg/ml THC (A) or 10 mg/kg IP THC in male and female Sprague-Dawley (SD) and Wistar rats. Blood collection occurred 30 min from injection or immediately after a 30 min THC vapor exposure session. Data are Mean ± SEM. Asterisks (*) denote a sex difference ($p<0.05$).

Table 1.

Summary of parameters used in vapor exposure conditions. For conditions 4 and 5 a series of 2 puffs was delivered in rapid succession with 2s in between to mitigate overheating of the vapor coil. Following the last vapor delivery of each condition, rats remained in the cage to allow enough time (e.g., 4–10 min) for vapor exposure and clearance of vapor from the chamber prior to testing. *Based on 200 mg/ml concentration used in these studies; this amount of vaporized drug is divided evenly between 4 chambers.

Vapor Condition	Puff Parameters: (# × duration [s]) × #	Exposure Length (min)	Inter-puff-interval (min)	mL e-liquid used	Calculated THC amount* (mg)
1	(1 × 3s) × 5	12	2	0.1 + 0.0	25.8 + 4.0
2	(1 × 6s) × 5	30	5	0.3 + 0.0	61.5 + 4.7
3	(1 × 6s) × 10	30	2.5	0.6 + 0.0	114.0 + 6.2
4	(2 × 6s) × 10	30	2.5	1.3 + 0.1	258.7 + 8.7
5	(2 × 9s) × 10	30	2.5	2.1 + 0.1	413.2 + 11.5