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Δ^9-Tetrahydrocannabinol discrimination: Effects of route of administration in rats

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ABSTRACT

Cannabis users typically smoke or vape cannabis or ingest it in edibles, whereas cannabinoids are typically administered via injection in rodent research. The present study examined the effects of route of administration (ROA) of Δ^9-tetrahydrocannabinol (THC), the primary psychoactive constituent of cannabis. Adult female and male Long Evans rats were trained to discriminate intraperitoneal (i.p.) THC from vehicle in a drug discrimination procedure. Following acquisition, dose-effect curves were determined with THC using i.p., oral (p.o.), and subcutaneous (s.c.) injection in both sexes and aerosol exposure in males only, followed by a time course with one dose for each ROA. Both sexes acquired THC discrimination in a similar number of sessions, although baseline response rates were significantly lower in females than males. THC fully substituted for the 3 mg/kg i.p. training dose across all ROA. While potencies were similar for ROA involving first-pass metabolism (i.p. and p.o.), THC potency was lower with s.c. administration. During the time course analysis, aerosol administration had the shortest latency to onset of discriminative stimulus effects and the shortest duration of effect, whereas s.c. administration had the longest duration. The results of this examination of the effects of ROA on an abuse-related effect of THC provide an empirical foundation to facilitate choice of ROA for mechanistic investigation of THC's pharmacology. Further, animal models using translationally relevant ROA may facilitate more accurate predictions of their effects in humans.

1. Introduction

Δ^9-Tetrahydrocannabinol (THC), the primary psychoactive constituent of *Cannabis sativa/indica*, produces its characteristic cannabinoid effects through activation of the endocannabinoid system, one of several lipid signaling systems in the brain. Verified components of this system include two G-protein coupled receptors, their signaling pathways, two predominant endogenous ligands (anandamide and 2-arachidonoyl glycerol), and synthetic and metabolic pathways. Of the two identified receptors, one type (CB_1) is found in largest concentrations in the brain (Herkenham et al., 1990), and is responsible for the psychoactive effects of THC, whereas the other type (CB_2) is primarily, but not exclusively (Van Sickle et al., 2005; Xi et al., 2011), located in the periphery (Galiègue et al., 1995). THC binds to and activates CB_1 and CB_2 receptors with approximately equal affinity (Showalter et al., 1996). Discovery of the mechanisms underlying cannabinoid action and their effects on physiology and behavior was due, in part, to research conducted in animals, and specifically, in rodent models (e.g., Cravatt et al., 2001; Rinaldi-Carmona et al., 1994; Zimmer et al., 1999). Despite the notable advances facilitated by preclinical research, however, efforts to overcome challenges and increase translational relevance of animal models continue (e.g., Moore et al., 2020).

Route of administration is an important pharmacokinetic variable that is often overlooked in this quest for better predictive models. For example, smoking cannabis in the form of a cigarette (joint) or in a pipe remains the most common method of use in human users (Schauer et al., 2016), although adaptation of electronic cigarette (e-cigarette) devices to vape e-liquids infused with cannabis extracts has been gaining popularity, particularly among youth and young adults (Fataar and Hammond, 2019). In addition, a substantial proportion of users report consumption of cannabis in the form of edibles (e.g., reviewed in Barrus et al., 2016). Yet, most behavioral studies of the acute effects of cannabinoids in rodents have used intraperitoneal (i.p.) injections of THC or other cannabinoids (e.g., Järbe et al., 2006; McMahon et al., 2008; Wiley et al., 2014), with only a few exceptions (e.g., Bruijnzeel et al., 2016; Manwell et al., 2014a, b; Marshell et al., 2014; Nguyen et al., 2016a). This different route of administration for animals versus humans raises a couple of issues in translational relevance. First, first-pass metabolism of

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THC results in at least one metabolite [11-hydroxy-tetrahydrocannabinol (11−OH-THC)] that is psychoactive in its own right (Browne and Weissman, 1981; Wiley et al., 2021). Second, heating or burning THC and other cannabinoids through vaping or smoking may change their chemical composition, as can degradation during storage (Bell and Nida, 2015; Eichler et al., 2012; Thomas et al., 2017). Both of these issues could affect the magnitude and/or timing of the resulting pharmacological effects. Hence, first-pass metabolism and delayed onset may play a greater role in animal models than in human users of smoked cannabis (Huestis, 2007; Turner et al., 2011).

The primary goal of the present study was to determine the effects of route of administration on the magnitude and duration of THC's psychoactivity in a rodent model related to THC's abuse liability. While non-medicinal use of cannabis in humans is driven by the reinforcing effects of THC (Lupica et al., 2004), reliable i.v. self-administration [i.e., the most robust animal model of an abused drug's reinforcing effects (O'Connor et al., 2011)] has not been achieved reliably with THC in rodents, as it has with many other classes of abused drugs such as stimulants and opioids. However, the interoceptive effects of an abused drug such as THC play a contributory role in its use (Andrade et al., 2019; Bevins and Besheer, 2014). Because THC's reinforcing effects cannot be reliably assessed in animals directly, THC drug discrimination, an animal model of the subjective effects of THC intoxication in human cannabis users, has been recommended as a primary method for preclinical evaluation of cannabinoid abuse liability by U.S. federal agencies such as the FDA and DEA (Food and Drug Administration, 2010). Unlike some other common rodent models of cannabimimetic activity such as the cannabinoid tetrad (Martin et al., 1991; Wiley and Martin, 2003), THC discrimination has an advantage of pharmacological selectivity, in that it detects cannabimimetic psychoactivity of various classes of cannabinoids, including phytocannabinoids, synthetic cannabinoids and endocannabinoids whereas non-cannabinoid drugs do not produce a positive THC-like signal in the assay (Balster and Prescott, 1992; Wiley et al., 2018). Determination of the effects of route of administration on the magnitude and duration of THC's psychoactivity (as modeled by its discriminative stimulus effects) is foundational for design of further preclinical mechanistic research on factors underlying non-medical cannabis use.

2. Materials and methods

2.1. Subjects

Adult male and female drug naïve and experimentally naïve Long Evans rats (250−274 g for males and 175−199 g for females at the beginning of the experiment; Envigo, Indianapolis, IN) were individually housed upon arrival in polycarbonate cages with hardwood bedding in a temperature-controlled (20−22 °C) environment with a 12 h light-dark cycle (lights on at 7am). Rats were maintained at 85–90 % of free-feeding body weights by restricting their daily ration of rodent chow (Purina® Certified 5002 Rodent Chow, Barnes Supply, Durham, NC, USA). Water was available *ad libitum* in their home cages. All studies were carried out in accordance with guidelines published in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011) and were approved by our Institutional Animal Care and Use Committee. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available.

2.2. Apparatus

Standard rat operant chambers (Habitest Modular System, Coulbourn Instruments, Whitehall, PA, USA) were enclosed in light- and sound-attenuating isolation cubicles equipped with exhaust fans. Each operant chamber contained a house light near the ceiling, 2 retractable levers, a multicolored three-light array above each lever, and a food cup with a light located between the levers. A pellet dispenser, located outside of the chamber, delivered 45 mg pellets (Bioserv Inc., Frenchtown, NJ, USA) into the food cup accompanied by illumination of the food cup light. During sessions, ~80 db of white noise was delivered via a speaker located inside the isolation cubicle. Illumination of lights, delivery of food pellets, and recording of lever presses were controlled by a computer-based system (Coulbourn Instruments, Graphic State Software, v 3.03).

For the aerosol route of administration, THC aerosol was delivered to rat-sized chambers (10cm × 23cm X 10 cm; EZ-178 Sure-Seal, E-*Z*-Anesthesia, Palmer, PA) via a commercially available vaporizer (Model SVS-200, Scientific Vapor, Bend, OR) connected to an e-vape controller (LJARI, La Jolla, CA), as described previously (Wiley et al., 2019). Airflow was constant (1 L/min) and aerosol was dispensed from an e-cigarette tank (Innokin Zenith, Element Vape, South El Monte, CA) via Tygon tubing (Fisher Scientific, Pittsburgh, PA, USA). The system was configured at 10 W using a 1.6 Ω atomizer (Innokin Z-Coil 1.6 Ω, Element Vape, South El Monte, CA).

2.3. Chemicals

Δ^9-Tetrahydrocannabinol (National Institute on Drug Abuse, NIDA, Rockville, MD) was suspended in a 7.8 % polysorbate 80 (Fisher Scientific, Hampton, NH) and 92.2 % saline (Patterson Vet Supply, Charlotte, NC) mixture for systemic administration. Intraperitoneal (i.p.), subcutaneous (s.c.), and oral (p.o.) injections of THC or vehicle were given at a volume of 1 mL/kg. For aerosolization, THC was mixed in propylene glycol (PG) (Fisher Scientific, Fair Lawn, NJ). Concentrations for aerosol administration are expressed as mg/mL in the e-cigarette tank and may not be representative of the actual amount of drug administered.

2.4. Procedure

Rats of both sexes (n = 8 of each sex at start of study) were trained to press one lever following administration of 3 mg/kg THC and to press another lever after injection with vehicle according to a fixed ratio 10 (FR10) schedule of food reinforcement, under which 10 consecutive responses on the correct (injection-appropriate) lever resulted in delivery of a food pellet. During training, THC and vehicle were administered i.p. 30 min prior to the start of the training session. Responses on the incorrect lever reset the ratio requirement on the correct lever. Prior to each daily training session, rats received a single injection of THC or vehicle in a double alternation schedule (e.g., two sessions with THC pre-injection followed by two sessions with vehicle pre-injection). These single daily 15 min training sessions were held on weekdays until the rats consistently met three criteria: (1) the first completed FR10 was on the correct lever, (2) ≥ 80 % of the total responding occurred on the correct lever, and (3) response rate was ≥ 0.1 responses/s. When these criteria had been met for the most recent THC training dose and vehicle sessions and 8 of the 10 most recent sessions, reliable discrimination had been established and stimulus substitution testing began.

Following successful acquisition of the discrimination, stimulus substitution tests were typically conducted on Tuesdays and Fridays during 15-min test sessions. Training continued on Mondays, Wednesdays, and Thursdays. During test sessions, responses on either lever delivered reinforcement according to a FR-10 schedule. In order to be tested, rats must have completed the first FR on the injection-appropriate lever, made at least 80 % of all responses on the injection-appropriate lever, and had a response rate ≥ 0.1 responses/s during the preceding day's training session. In addition, the rat must have met these same criteria during the most recent training session with the alternate training compound (i.e., THC training dose or vehicle). After passing stimulus substitution tests for the training drug and vehicle, an initial substitution dose-response curve was determined for intraperitoneal (i.p.) THC in each sex. Subsequently, a dose-response curve was

determined for THC administered via oral gavage (p.o.) followed by time course assessments of 3 mg/kg THC delivered orally and via i.p. injection at different pre-session times. After completion of the i.p. and oral time course tests, a subcutaneous (s.c.) THC dose-effect curve was conducted, followed by a single dose (10 mg/kg) s.c. time course assessment. The study was competed with a concentration-effect curve for THC and single concentration (560 mg/mL) time course evaluation with aerosolized THC. Time course examinations administered THC at 5, 15, 30, 60, 120, 180, and 240 min pre-session. The dose/concentration chosen for the time course tests for each route of administration was one that produced full (average of $\geq$ 80 % THC-lever responding) in both sexes for the given route of administration.

For the i.p., p.o. and s.c. dose-effect curves, THC was injected 30 min prior to the start of the test session. For the aerosol concentration-effect curve, exposures occurred in the aerosol chambers prior to placement in the drug discrimination chambers. Rats were exposed to each THC concentration for ten 3-s infusions, with a 10-s inter-infusion interval. Hence, total time in the aerosol chamber was 130 s. After the exposure session, rats were placed in their home cage to await placement in the operant chamber for the drug discrimination session. Pre-session wait time was 15 min for the concentration-effect curve and varied from 5 min to 4 h for the time course determination.

2.5. Data analysis

For each test session, mean (±SEM) percent responding on the drug lever and rate of responding (responses/s) were calculated for the entire session. ED_{50}s (and 95 % confidence limits) were calculated separately for each sex and route of administration using least-squares linear regression on the linear part of the dose-effect curves for percent drug-lever responding, plotted against $\log_{10}$ transformation of the dose. Because rats that responded less than 10 times during a test session did not press either lever a sufficient number of times to earn a reinforcer, their lever selection data were excluded from data analysis, but their data were included in response rate calculations. A two-sample *t*-test was used to compare number of days until acquisition across sex. Acquisition was defined as the number of sessions until the training criteria were met for 8 of 10 sessions, and for the most recent THC training dose and vehicle sessions. For i.p., s.c., and p.o. administration, percent responding on the drug lever and response-rate data were analyzed using separate mixed factorial analysis of variance (ANOVA) across dose (repeated factor) and sex (between-subjects factor). For aerosol administration, these measures were analyzed by separate repeated measures ANOVAs across concentration because aerosolized THC was not evaluated in females. Significant ANOVAs were followed by Tukey post hoc tests ($\alpha = 0.05$) to determine differences between means. NCSS 11 Statistical Software (2016; NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/ncss) was used for all analyses.

3. Results

Of the eight rats per sex that began training, seven females and six males successfully acquired the discrimination and completed their initial THC dose-effect curve. Significant sex differences in the rate of acquisition [$t(11) = 0.13$, $p > 0.05$] were not observed, with an average (± SEM) of 28 (± 8.1) and 27 (± 6.1) discrimination sessions for females and males, respectively. Over the course of the study, the performance accuracy of one male and two female rats began to deteriorate; hence, they were dropped from further testing, but their data were retained for all routes of administration that they completed. Another female rat developed health problems and was sacrificed. Due to the low number of remaining female rats ($n = 3$), evaluation of aerosolized THC was conducted only in males.

Examination of the split-plot ANOVAs for the percent of THC-lever responding variable for the i.p., p.o., and s.c. dose-effect curves and time courses showed that power estimates for the sex factor were too low (i.e., range 0.07 – 0.45) for adequate reliability in determining sex differences for any of the routes of administration. Hence, major conclusions are based upon the effects of dose or time, respectively, across routes of administration, although the data are presented in a manner that allows for visual inspection of trends across sex. Accordingly, in Figs. 1 and 2, percent of THC-lever responding is shown as individual subject data for male and female rats, with overlay of the group means for all rats and for males and females separately. In contrast, examination of the ANOVAs for response rates revealed consistently higher power estimates for the sex factor for this dependent variable (i.e., range = 0.73 – 0.94). Hence, response rates are presented as separate means for each sex at each dose (Fig. 1) or time point (Fig. 2).

Fig. 1 shows the results of tests with different doses of THC delivered i.p. (top panels), p.o. (middle panels), and s.c (bottom panels) on percent of THC-lever responding (left panels) and response rate (right panels). As expected, i.p. THC produced full, dose-dependent substitution for the 3 mg/kg training dose in both female and male rats. While near maximal substitution was observed in both sexes at higher 3 and 10 mg/kg doses [Fig. 1, panel A; main effect of dose: $F(6,66) = 42.37$, $p < 0.00001$], greater separation was seen between females and males in THC-lever at a lower 1 mg/kg dose. ED_{50} values of 0.3 mg/kg (females) and 0.75 mg/kg (males) were obtained for i.p. THC's discriminative stimulus effects (Table 1), with slightly overlapping 95 % confidence intervals. In contrast with the one-dose difference in the pattern of percent THC-lever responding across sex, females showed consistently and significantly lower rates of responding compared to males across all THC doses except 10 mg/kg [Fig. 1, panel B; significant sex X dose interaction: $F(6,66) = 5.07$, $p = 0.0003$]. At the 10 mg/kg dose, response rates for males were significantly attenuated compared to their responding after receiving vehicle. Females did not show a similar decrease in rates; however, their baseline response rates were already as low as those of the males after 10 mg/kg THC.

As shown in the middle panels of Fig. 1, orally administered THC also produced full and dose-dependent substitution for i.p. 3 mg/kg THC and did so over the same dose range in both female and male rats, with significant increases in THC-like responding at 1−10 mg/kg [Fig. 1, panel C; main effect of dose: $F(5,55) = 31.89$, $p < 0.00001$]. Potencies for producing THC-like effects were similar across sex (ED_{50}s = 0.45 and 0.42, respectively, for female and male rats; Table 1). In contrast, response rates were notably lower in females than males after vehicle administration and across all THC doses [Fig. 1, panel D; main effect of sex: $F(1,55) = 10.64$, $p = 0.008$]. Oral THC did not affect response rates compared to vehicle in either sex.

THC administered s.c. also fully and dose-dependently substituted for i.p. 3 mg/kg THC (Fig. 1, panel E) at similar potencies across sex (ED_{50}s = 1.80 and 1.46 mg/kg in female and male rats, respectively; Table 1). Notably, in both sexes, THC was less potent when administered s.c. than when administered i.p. or p.o. Significant substitution occurred over a dose range of 3−56 mg/kg, s.c. [Fig. 1, panel E; main effect of dose: $F(6,60) = 13.93$, $p < 0.00001$]. Response rates were generally unaffected by THC in either sex, with exception of isolated decreases at 30 mg/kg and at the 3 mg/kg THC control point in male rats. As with other routes of administration, however, response rates in females were significantly lower than those observed in males following vehicle administration and across the THC dose-effect curve [Fig. 1, panel F; dose X sex interaction: $F(6,60) = 2.70$, $p = 0.02$].

Fig. 2 shows the time course of active doses of THC across route of administration. A 3 mg/kg i.p. dose of THC increased the magnitude of THC-like responding significantly above vehicle levels at 5 min following injection in both sexes [Fig. 2, panel A; main effect of time: $F(8,80) = 8.16$, $p < 0.00001$]. THC-like responding remained significantly above vehicle levels across sex (and not significantly different from the THC control point) until at least 240 min post-injection (i.e., furthest time point tested). As seen for the dose-effect curve, response rates were consistently lower in females than males across all time points [Fig. 2, panel B; main effect of sex: $F(1,80) = 15.24$, $p = 0.003$].

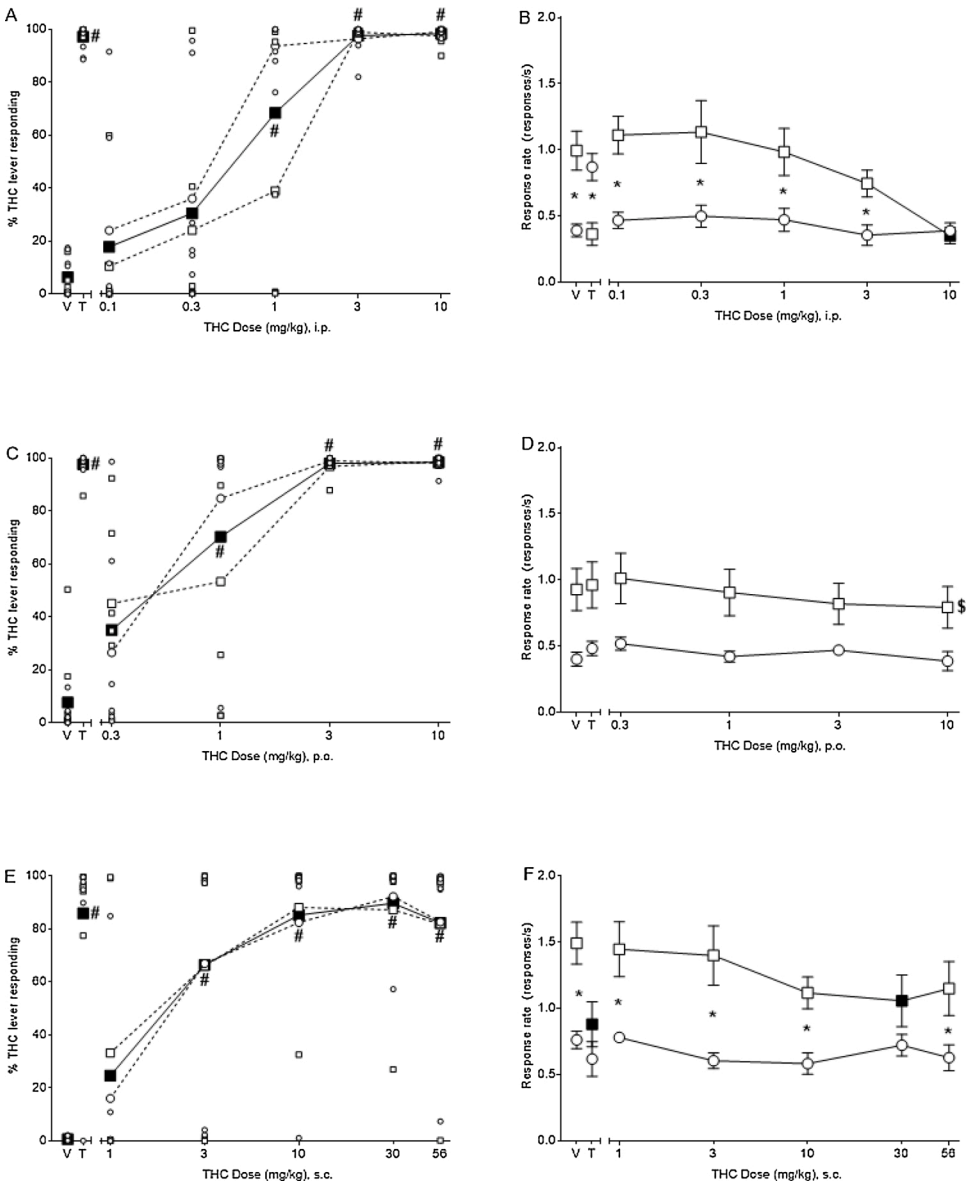

Fig. 1. Effects of THC administered intraperitoneally (i.p.; panels A and B), via oral gavage (p.o.; panels C and D), or subcutaneously (s.c.; panels E and F) on percentage of responses that occurred on the THC-associated lever (left panels) and response rates (right panels) in adult female (circles) and male (squares) Long-Evans rats trained to discriminate 3 mg/kg THC (i.p.) from vehicle in a two-lever drug discrimination procedure. Note different dose ranges on x-axes across route of administration. Control tests with vehicle (V; administered via same route of administration as in the THC dose-effect curve) and 3 mg/kg THC (T; administered i.p.) were conducted prior to each dose-effect curve, with results shown at the left side of the panels. Percentage of THC-associated lever responding is graphed as individual subject data (small circles and squares for female and male rats, respectively), separate means for female and male rats (circles and squares, respectively, connected with dotted line for each sex), and grand means for all rats (filled squares). For response rate data, each point represents the mean (± SEM) of data for female (n = 7 for i.p. and p.o.; n = 6 for s.c.) and male (n = 6) rats. For both dependent variables, pound sign (#) indicates a significant main effect of dose, with a significant post-hoc difference ($p < 0.05$) from vehicle for the indicated dose. For the response rate data, black filled symbols indicate a significant difference from vehicle for the indicated sex and dose (sex X dose interaction) ($p < 0.05$). Asterisks (*) indicate a significant difference between the sexes at a given dose (sex X dose interaction) ($p < 0.05$). Dollar sign ($) indicates a significant main effect for sex across all doses ($p < 0.05$) for the specific dose-effect curve.

The early temporal pattern observed with oral gavage was similar to that observed after i.p. injection, as the magnitude of the THC-like discriminative stimulus effects of 3 mg/kg p.o. also increased sharply over the first 30 min of the session. At 5 min, responding on the THC lever was more similar to vehicle whereas at 15 min, responding on the THC lever was significantly different from vehicle and from the THC control point across sex [Fig. 2, panel C; main effect of time: $F(8,80) = 15.68$, $p < 0.00001$]. Responding on the THC lever was maximal at 30 min post-administration and showed steady decreases after 60 min, with visibly faster decrease in males than females (although the sex X time interaction was not statistically significant). Response rates were consistently lower in females than males across all times [Fig. 2, panel D; main effect of sex: $F(1,80) = 13.96$, $p = 0.004$].

As shown in Fig. 2 (panel E), the early effects of 10 mg/kg s.c. THC exhibited a similar pattern to i.p. and p.o. THC, in that a sharp rise in THC-lever responding occurred between 5 and 30 min post-injection. Maximal THC-like responding was attained at 30 min for both sexes [main effect of time: $F(8,72) = 16.82$, $p < 0.00001$], with no decreases occurring over the ensuing 210 min. Response rates were significantly lower in females than males following vehicle administration and during the first 30 min after s.c. injection of 10 mg/kg THC [sex X time interaction: $F(8,72) = 3.46$, $p = 0.002$]; however, rates for males significantly declined and were at similar magnitude as females from 60 to 240 min post-injection.

Fig. 3 shows the results of aerosolized THC on percent THC lever responding (left panels) and response rates (right panels) in male rats. As seen in panel A, responding on the THC lever increased in a concentration-dependent manner, with full substitution occurring at 560 mg/mL [Fig. 3, panel A; $F(5,25) = 6.48$, $p = 0.0005$]. Response rates showed a concomitant small, but statistically significant, decrease at this concentration [Fig. 3, panel B; $F(5,25) = 4.70$, $p = 0.004$]. When 560 mg/mL aerosolized THC was tested at different time points after administration, significant substitution for 3 mg/kg i.p. THC was observed from 5 to 120 min, followed by a sharp decrease in responding on the THC lever and return to vehicle baseline levels by 240 min [Fig. 3, panel C; $F(7,28) = 31.03$, $p < 0.00001$]. Response rates exhibited few significant changes compared to vehicle over the 240-min time course, with a significant decrease observed only at the 15-min post-administration time point [Fig. 3, panel D; $F(7,28) = 7.21$, $p = 0.00006$].

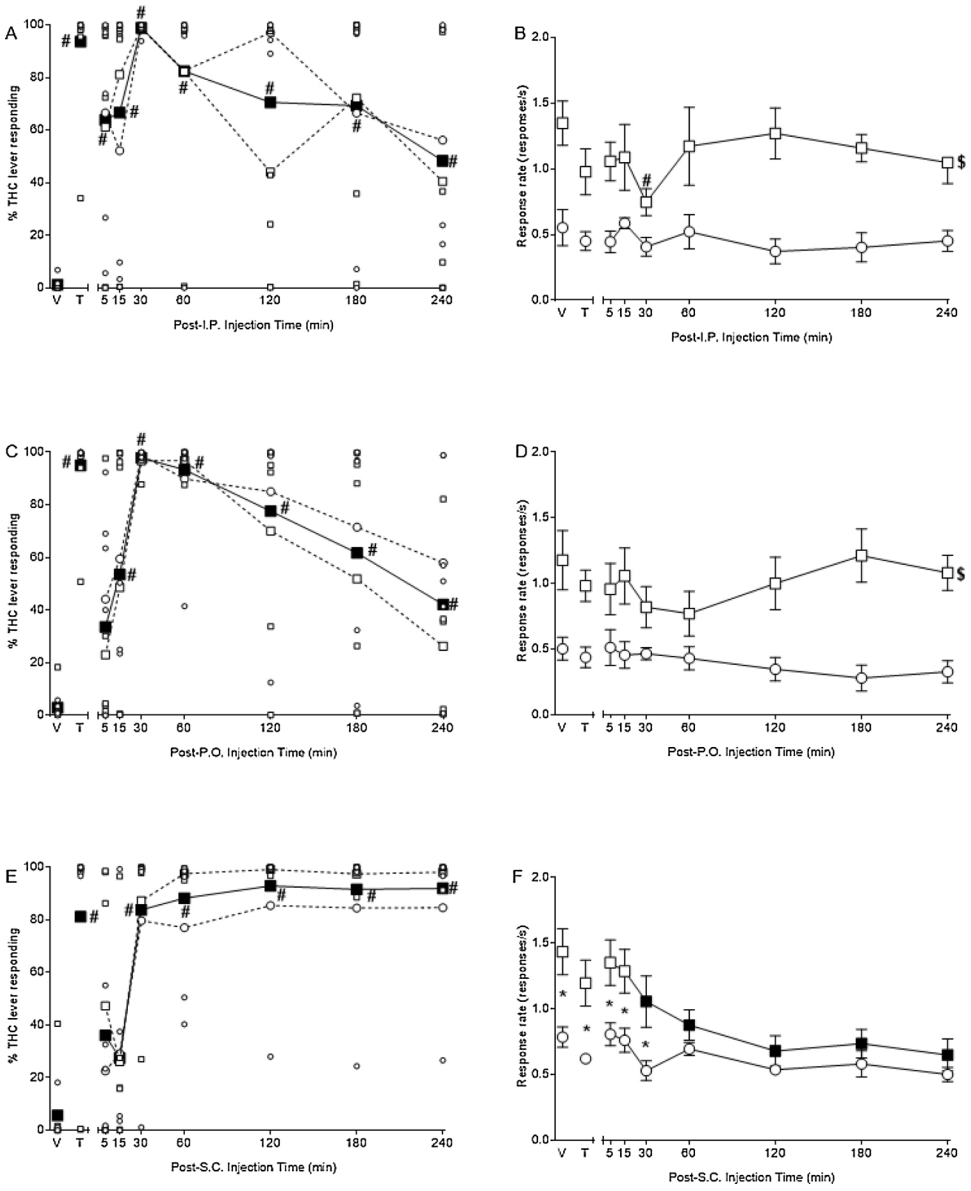

Fig. 2. Effects of THC as a function of time on percentage of responses that occurred on the THC-associated lever (left panels) and response rates (right panels) in adult female (circles) and male (squares) Long-Evans rats trained to discriminate 3 mg/kg THC (i.p.) from vehicle in a two-lever drug discrimination procedure. THC dose was 3 mg/kg for the i.p. (panels A and B) and p.o. (panels C and D) time courses and 10 mg/kg for the s.c. time course (panels E and F). Control tests with vehicle (V; administered 30 min pre-session via same route of administration as in the THC dose-effect curve) and 3 mg/kg THC (T; administered i.p. 30 min pre-session) were conducted prior to each dose-effect curve, with results shown at the left side of the panels. Percentage of THC-associated lever responding is graphed as individual subject data (small circles and squares for female and male rats, respectively), separate means for female and male rats (circles and squares, respectively, connected with dotted line for each sex), and grand means for all rats (filled squares). For response rate data, each point represents the mean (± SEM) of data for female (n = 6 for i.p. and p.o.; n = 5 for s.c.) and male (n = 6) rats. For both dependent variables, pound sign (#) indicates a significant main effect of dose, with a significant post-hoc difference ($p < 0.05$) from vehicle for the indicated dose. For the response rate data, black filled symbols indicate a significant difference from vehicle for the indicated sex and dose (sex X dose interaction) ($p < 0.05$). Asterisks (*) indicate a significant difference between the sexes at a given dose (sex X dose interaction) ($p < 0.05$). Dollar sign ($) indicates a significant main effect for sex across all doses ($p < 0.05$) for the specific dose-effect curve.

Table 1
THC potency in drug discrimination across route of administration in female and male Long Evans rats.

Route of Administration	Females ED_{50} (± 95% CI)	Males ED_{50} (± 95% CI)
Intraperitoneal (i.p.)	0.30 mg/kg (0.18–0.48)	0.75 mg/kg (0.43–1.33)
Oral gavage (p.o.)	0.45 mg/kg (0.34–0.60)	0.42 mg/kg (0.26–0.69)
Subcutaneous (s.c.)	1.80 mg/kg (0.54–6.06)	1.46 mg/kg (0.61–3.47)
Aerosol	Not tested	61.95 mg/ml (35.38–108.49)

4. Discussion

With a few exceptions (e.g., Marshell et al., 2014; Wiley et al., 2019), most prior studies of the discriminative stimulus effects of cannabinoids that have been conducted in rodents administered cannabinoids via i.p. injection to male rodents. While i.p. injections also were used for training Long-Evans rats to discriminate THC (3 mg/kg) from vehicle in the present study, THC was tested across several routes of administration. In addition, rats of both sexes were evaluated. The present results showed that female and male Long-Evans rats readily acquired this discrimination with similar acquisition durations; however, successful acquisition of the THC discrimination using a 3 mg/kg training dose in females is in partial contrast with previous results. For example, female rats of different strains (i.e., Sprague-Dawley and Lister Hooded) required lower doses (1–1.7 mg/kg) for acquisition of THC discrimination in previous acute and cumulative dosing procedures (Rowton et al., 2020; Wiley et al., 2021, 2017). Further, Winsauer et al. (2012) reported within-group variability in the training dose required for acquisition of THC discrimination in female Long-Evans rats, with an even wider range from 0.32 to 3.2 mg/kg. In male rats, we (and others) routinely use 3 mg/kg THC as a training dose (Gatch and Forster, 2018; Järbe et al., 2011; Wiley et al., 2004), albeit several studies have used other doses to examine the role of training dose in THC discrimination (Järbe et al., 2000, 1998; Järbe et al., 2006). In contrast, C57Bl/6 mice did not exhibit a sex difference in training dose required for acquisition of THC discrimination (Wiley et al., 2021). Together, these results emphasize the importance of considering sex and rodent species/strain in choice of THC training dose to maximize acquisition.

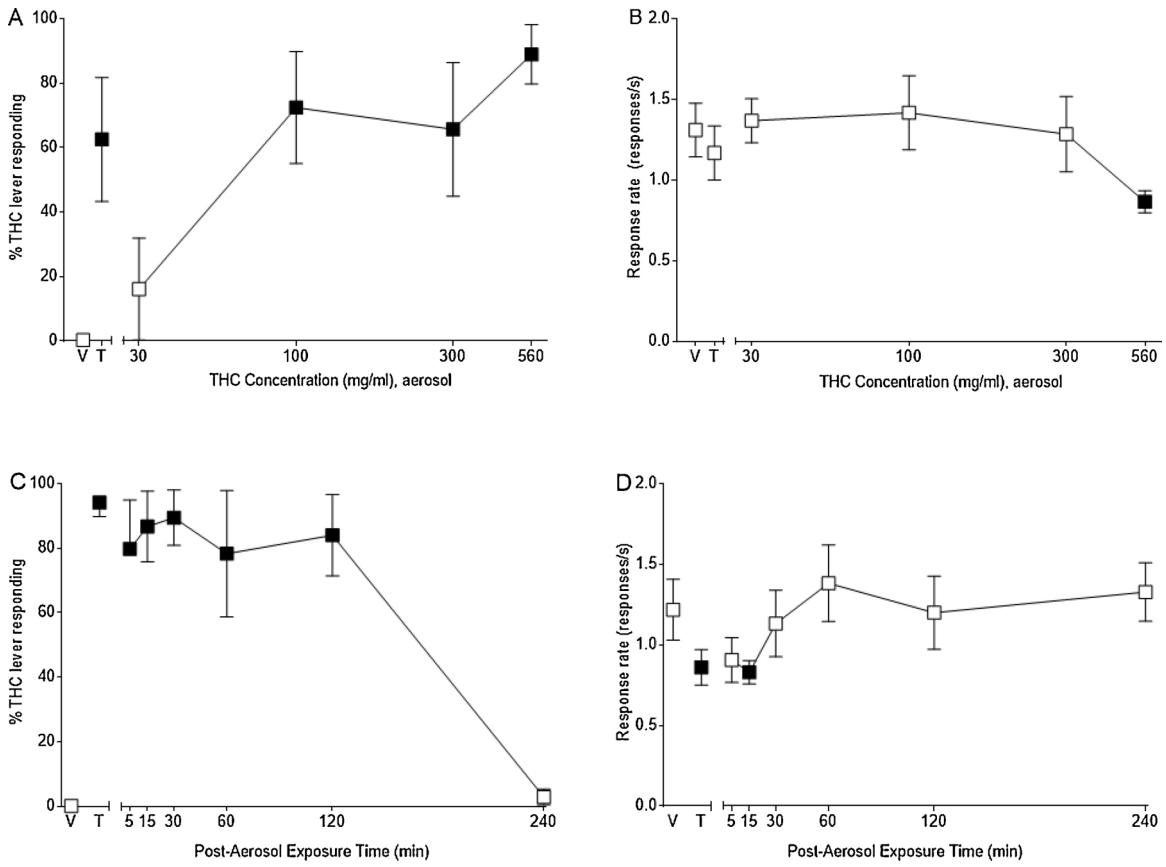

Fig. 3. Top panels show the effects of aerosolized THC as a function of concentration on percentage of responses that occurred on the THC-associated lever (panel A) and response rates (panel B) in adult male Long-Evans rats trained to discriminate 3 mg/kg THC (i.p.) from vehicle in a two-lever drug discrimination procedure. Bottom panels show the effects of 560 mg/mL aerosolized THC on percent THC-lever responding (panel C) and response rates (panel D). Control tests with vehicle (V; administered via aerosol exposure 15 min pre-session) and 3 mg/kg THC (T; administered i.p. 30 min pre-session) were conducted prior to the concentration-effect curve and the time course, with results shown at the left side of the panels. Each point represents the mean (± SEM) of data for 5 or 6 male rats in the top and bottom panels, respectively. Black filled symbols indicate a significant post hoc difference from vehicle for the indicated concentration or time ($p < 0.05$).

During test sessions following acquisition, THC produced full and dose-dependent substitution for the 3 mg/kg training dose with i.p., p.o., and s.c. administration in rats of both sexes. Further, across the two routes of administration that involved first-pass metabolism, potencies (ED_{50}s) were similar for both routes, with overlapping confidence limits. While the study did not have sufficient sample size for each sex to provide a definitive assessment of sex differences in THC's discriminative stimulus effects, the threshold dose for substitution was ½ log dose lower in female than in male rats (1 vs. 3 mg/kg, respectively) for the i.p. and p.o. routes. In addition, orally delivered THC produced enhanced responding on the THC-associated lever for longer durations in females compared to males, suggesting prolonged THC-like psychoactivity. Several previous studies have reported enhanced sensitivity of female rats to the pharmacological effects of i.p. THC, including its discriminative stimulus (Wiley et al., 2021, 2017) and antinociceptive (Craft et al., 2013; Moore et al., 2021) effects. Further, greater subjective response to oral THC and smoked cannabis in women than men at lower doses has been reported (Fogel et al., 2007; Matheson et al., 2020; Sholler et al., 2020). Nevertheless, in the present study, ED_{50} values were overlapping for the dose-effect curve and there was considerable variability across subjects at the lower dose and across time for these routes of administration, suggesting caution in interpretation of sex as the basis for these between-subject variations.

Compared to results with i.p. or oral dosing, THC was less potent when administered s.c. Further, substantial overlap of the s.c. THC dose-effect curves across sex was observed. During the time-course experiment, percentage of responding on the THC-associated lever after s.c. injection reached 80 % or more (i.e., full substitution) by 30 min post-injection and remained substantially elevated (~80–95%) in both sexes at all subsequent time points up to 4 h post-injection. In contrast, the percentage of THC-associated lever responding steadily declined over time after i.p. and oral injections. First-pass metabolism of THC to 11−OH-THC may account for the slow decline in its discriminative stimulus effects following i.p. and oral administration as this psychoactive metabolite is further metabolized to inactive compounds. Because first-pass metabolism does not occur with s.c. administration, inactivation of THC via metabolic processes is slower and duration of action is extended. Consequently, tolerance induction to THC frequently relies on a regimen of repeated s.c. injections in rats (Bass and Martin, 2000; Beardsley and Martin, 2000; McKinney et al., 2008). Although tolerance also develops when THC is administered chronically via other routes of administration (Moore et al., 2010; Nguyen et al., 2018; Wakley et al., 2014; Winsauer et al., 2015), the ability of s.c. THC to elicit THC-like discriminative stimulus effects for a longer time span suggests that this route of administration may result in maintenance of threshold brain levels of psychoactive cannabinoids (e.g., THC and its psychoactive metabolite, 11−OH-THC) over a more extended period than the other two routes of systemic administration tested herein. Hence, s.c. administration may have an advantage for use in rodent models where sustained cannabinoid exposure is required.

While the study was underpowered for conclusive determination of sex differences in the discriminative stimulus effects of THC, sex differences in response rates were robust, with female rats consistently responding at lower rates than males. This difference occurred with

vehicle and across most doses of THC, regardless of route of administration (i.p., p.o., and s.c.), suggesting that its cause was not rooted in the pharmacological effects of THC per se. While we have observed lower response rates in females previously in THC discrimination with mice, this effect has not been consistently observed in rats (Wiley et al., 2021, 2019; Wiley et al., 2017). Notably, because this study used a fixed ratio schedule of food reinforcement, the number of pellets earned during test sessions was primarily a function of response rate, suggesting that decreased response rates observed with females might be related to lower body weights and associated decreases in amount of food required for satiation. To examine this possibility further, we calculated the amount of food earned during test sessions as a function of body weight (mg food/g body weight) [data not shown]. Results showed that females continued to earn lower amounts of food even when body weight was taken into account, suggesting that sex differences in weight may not fully explain the difference in baseline response rates observed in this study.

In the final experiment of the overall study, the effects of THC following aerosol exposure were investigated, but only in male rats, as gradual attrition of rats over the course of this long study resulted in an insufficient number of female animals for adequate evaluation. In males, aerosol exposure to THC resulted in concentration-dependent increases in responding on the THC-associated lever, with a maximum of 89 % at the 560 mg/mL concentration. Further, responding on the THC lever at this concentration reached near maximal level at 5 min after exposure and sharply declined between 2–4 h after exposure. While we previously reported that aerosolized synthetic cannabinoids engendered THC-like responding in a drug discrimination paradigm in mice (Wiley et al., 2019), to our knowledge, this study is the first to demonstrate that aerosolized THC substitutes for i.p. THC in rats trained to discriminate THC from vehicle. These results are consistent with previous studies showing that aerosolized THC induces a profile of other pharmacological effects in rodents characteristic of systemically administered psychoactive cannabinoids (Martin et al., 1991), including antinociception (Javadi-Paydar et al., 2018; Moore et al., 2021; Nguyen et al., 2016b; Taffe et al., 2020), hypothermia (Javadi-Paydar et al., 2018; Moore et al., 2021; Nguyen et al., 2016b; Taffe et al., 2020), hypoactivity (Javadi-Paydar et al., 2018; Nguyen et al., 2016b), increased feeding (Manwell et al., 2014b), and conditioned place preference (Manwell et al., 2014a). Aerosolized THC-rich cannabis extract also supported self-administration in rats (Freels et al., 2020), an animal model of the reinforcing effects of substances (O'Connor et al., 2011). Together, these studies support use of this translationally relevant model to investigate THC and other vaped substances of abuse.

In summary, this study provides an overview of potency and time course in THC-like discriminative stimulus effects across route of administration in adult female and male Long-Evans rats. As with all studies, it has limitations, including the inability to assess aerosolized THC in females due to subject attrition, failure to test the full duration of THC-like effects for each route of administration, small sample size for evaluation of sex differences in THC's discriminative stimulus effects, and the use of a single dose and single route of administration for training in the THC discrimination procedure. Further, the present findings cannot be generalized to other rodent species or strains without additional research, as previous studies have demonstrated that species and strain and their interaction with sex may affect THC's pharmacological profile (Moore et al., 2021; Wiley et al., 2021). Despite these limitations, however, the results of this systematic examination of the effects of route of administration on an abuse-related effect of THC parallel similar ongoing pharmacokinetic studies with phytocannabinoids in humans (Russell et al., 2018; Sholler et al., 2020; Spindle et al., 2020, 2019). The latter studies are in response to the growing use of cannabis for medicinal and recreational purposes and diversification in its route of administration (e.g., smoking, vaping, edibles, topicals) (Russell et al., 2018; Schauer et al., 2016) and seek to provide guidance on factors that may affect regulatory decisions concerning cannabis products. Results of preclinical studies may increase the impact of these clinically based studies through determination of mechanisms underlying THC's pharmacological profile. In concert, clinical studies and translational preclinical work have the potential to enhance the scientific basis for policy decisions affecting public health in this arena.

Contributors

All authors have read and approved the final version of the manuscript.

Participated in research design: Wiley, Taylor.

Performed data analysis: Wiley.

Wrote or contributed to the writing of the manuscript: Wiley, Marusich, Taylor.

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Declaration of Competing Interest

No conflict declared.

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