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Comparison of the discriminative stimulus and response rate effects of Δ^9 -tetrahydrocannabinol and synthetic cannabinoids in female and male rats*

Jenny L. Wiley¹, Timothy W. Lefever¹, Julie A. Marusich¹, and Rebecca M. Craft²

¹RTI International, 3040 Cornwallis Road, Research Triangle Park, NC 27709-2194

²Department of Psychology, Washington State University, Pullman, WA 99164-4820

Abstract

Background—Women report greater sensitivity to the subjective effects of Δ^9 -tetrahydrocannabinol (THC). Similarly, female rodents tend to be more sensitive to some pharmacological effects of THC and synthetic cannabinoids. This study examined sex differences in discriminative stimulus and response rate effects of THC and synthetic cannabinoids in rats.

Methods—A cumulative dosing THC discrimination procedure was utilized to evaluate sex differences in the discriminative stimulus effects of THC and three synthetic cannabinoids: CP47,497, WIN55,212-2, and JWH-018. Sex differences in the effects of these four compounds and a degradant of A-834735 on response rates also were assessed in a food-reinforced discrete dosing procedure.

Results—Females required a lower training dose than males for acquisition of the discrimination. Further, THC was more potent at producing rimonabant-reversible discriminative stimulus and response rate effects in females. While synthetic cannabinoids were more potent in producing THC-like effects than was THC in female rats, greater discrepancies were observed in male rats. Similar sensitivity to the response rate-decreasing effects induced by most, but not all (A-834735 degradant), synthetic cannabinoids was seen in both sexes.

Conclusions—This study represents one of the first direct comparisons of sex differences in THC discrimination. Females were more sensitive to THC's effects, which may be related, in part, to sex differences in THC metabolism. Synthetic cannabinoids were more potent than THC in both

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Corresponding author: Jenny L. Wiley, Ph.D., RTI International, 3040 Cornwallis Road, Research Triangle Park, NC 27709-2194, 919-541-7276 (phone), 919-541-6499 (fax), jwiley@rti.org.

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Contributors

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Conflict of Interest

The authors declare no conflicts of interest.

sexes, but were considerably more so in male than in female rats. Future research should emphasize further characterization of sex differences in cannabinoid pharmacology.

Keywords

drug discrimination; response rate; sex differences; synthetic cannabinoids; tetrahydrocannabinol

1.0 Introduction

Early in 2016, the National Institutes of Health (NIH) issued a new requirement for inclusion of animals of both sexes in most NIH-funded preclinical biological research. Shortly thereafter, Becker and Koob (2016) published an elegant overview of sex differences in animal models of different stages of addiction for several classes of abused substances, including cannabinoids. Although research in this area is sparse, a few studies suggest that female rodents are more sensitive than males to some of the pharmacological effects of Δ^9 -tetrahydrocannabinol (THC), the primary psychoactive constituent of cannabis. For example, compared to male rats, females show a greater anxiogenic effect during spontaneous withdrawal from THC (Harte-Hargrove and Dow-Edwards, 2012), although sex differences were not consistently observed in precipitated withdrawal (Marusich et al., 2015; Marusich et al., 2014). THC also is more potent in inducing acute antinociception and reducing inflammatory pain in female than in male rats (Craft et al., 2013a; Tseng and Craft, 2001). Preclinical results are consistent with findings from the human laboratory showing that women report greater sensitivity to the subjective effects of smoked cannabis (Cooper and Haney, 2014) and to lower doses of orally administered THC (Fogel et al., 2016); however, they are less sensitive than men to the subjective effects of higher doses of THC, an effect that the authors suggest may be related to greater tolerance at these doses (Fogel et al., 2016). Hence, although fewer women than men use cannabis for recreational purposes (SAMHSA, 2014), women may be more susceptible to developing addiction once use is initiated, a phenomenon that has been referred to as “telescoping” (Becker and Hu, 2008).

While there are no large epidemiological studies on sex differences in the prevalence of synthetic cannabinoid use, a review of several small studies conducted between 2011 and 2012 found that the majority of synthetic cannabinoid users were male (68–83%) (Castaneto et al., 2014). Despite lower incidence of use, women initiated use at a significantly younger age, as reported in a convenience sample of college students at the University of Cincinnati (Vidourek et al., 2013). In addition, while most emergency room (ER) visits due to synthetic cannabinoid use involve young men, occasional ER admission of young women has been reported (Tait et al., 2016). These preliminary findings suggest that synthetic cannabinoids represent a significant health threat for individuals of both sexes, a hypothesis supported by results from preclinical research. Fattore and colleagues reported that female rats acquired self-administration of the synthetic cannabinoid WIN55,212-2 faster than males (Fattore et al., 2007), responded for more infusions (Fattore et al., 2010), and showed greater cue-induced reinstatement (Fattore et al., 2010). These results would predict that, while women may use synthetic cannabinoids at lower rates, they may be more sensitive to the psychological effects of these compounds.

The purpose of the present study was to examine sex differences in the discriminative stimulus and response rate effects of THC and synthetic cannabinoids. To date and despite demonstration of WIN55,212-2 self-administration (Fattore et al., 2007), the reinforcing effects of THC itself have not been amenable to development of a rodent model of self-administration (Lefever et al., 2014). However, the subjective effects of cannabis have been successfully modeled in rodents through the use of THC discrimination procedures (Balster and Prescott, 1992). In the present study, a cumulative dosing THC discrimination procedure in rats was utilized to evaluate sex differences in the discriminative stimulus effects of THC and three synthetic cannabinoids: CP47,497 (bicyclic cannabinoid), WIN55,212-2 (aminoalkylindole), and JWH-018 (indole-derived cannabinoid commonly referred to as “Spice”) (Figure S1¹). In addition, separate groups of female and male rats were trained to respond for food reinforcement in a discrete dosing procedure. Potency in this procedure is not dependent upon THC training dose and rats lacked the extensive history of cannabinoid administration required by the THC discrimination procedure. Hence, potential sex differences in the response rate effects of synthetic cannabinoids could be examined independently of THC. Sex differences in the response rate effects of THC, the three synthetic cannabinoids, and the ring open degradant of a tetramethylcyclopropyl ketone indole synthetic cannabinoid, A-834735 (Figure S1²), were assessed. These synthetic cannabinoids not only exhibit greater affinity for the CB₁ receptor (see Table 1), but also show nearly 2-fold or higher efficacy for stimulation of this receptor than does the partial agonist THC (Grim et al., 2016). Because of their potency and ability to occupy a greater number of CB₁ receptors (i.e., high efficacy), these compounds may be useful tools for examination of sex differences in pharmacodynamic mechanisms underlying the effects of cannabinoids.

2.0 Materials and Methods

2.1 Subjects

Adult female (175–200 g) and male (275–325 g) Sprague-Dawley rats (Harlan, Frederick, MD) were housed individually in standard rat cages in a temperature-controlled (20–22°C) vivarium with a 12 h light-dark cycle (lights on at 6 am). Behavioral sessions were conducted during the light portion of the cycle. Rats were maintained at 85 – 90% of free-feeding body weights by restricting the daily ration of rodent chow. Water was available *ad libitum* in home cages. All animals were cared for in accordance with federal and state regulatory guidelines and the study was approved by the RTI Institutional Animal Care and Use Committee.

2.2 Apparatus

Rat operant chambers (MED Associates, St. Albans, VT or Coulbourn Instruments, Whitehall, PA), enclosed in light- and sound-attenuating isolation cubicles, were equipped with house lights, two retractable levers, and a food cup. A pellet dispenser delivered 45 mg pellets (Bioserv Inc., Frenchtown, NJ, USA) into the food cup. MED-PC software (MED

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Associates) or Graphic State (Coulbourn) was used to control schedule contingencies and record data for the drug discrimination and response rate experiments, respectively.

2.3 THC Discrimination Procedure

Rats were trained and tested using a cumulative dosing THC discrimination procedure. A flowchart of the phases in this procedure is shown in Figure S2³. During the initial stage of acquisition, each rat was placed daily into an operant chamber and trained to press either of two levers according to a fixed ratio (FR) 1 schedule of food reinforcement, as described previously (Wiley et al., 1993). When rats were reliably pressing both levers, daily training sessions were continued with a double alternation schedule of pre-session THC and vehicle injection administered i.p. 30 min prior to the start of the session. On days when THC was administered, lever presses on one lever produced a food pellet, whereas lever presses on the other lever produced a food pellet on days when vehicle was administered. To control for possible olfactory cues (Extance and Goudie, 1981), the levers were cleaned thoroughly between animals and at the end of the session. In addition, the position of the THC-associated lever (right vs. left) was counterbalanced across rats assigned to each chamber. An attempt was made to train rats of both sexes to discriminate 3 mg/kg THC from vehicle. Responding was suppressed following injection with this dose during early acquisition in both sexes. Males recovered quickly, and successfully acquired the discrimination. In contrast, most females stopped responding during THC sessions. Subsequently, a new group of females (n=8) was successfully trained to discriminate 1 mg/kg THC from vehicle. Half of the original male group (n=4) was maintained on the original 3 mg/kg THC training dose and a fading procedure (Järbe et al., 1998) was used to re-train the other half (n=4) at a training dose of 1 mg/kg.

After responding with the training dose was established, the FR value was gradually increased on each lever to the final FR10 schedule of reinforcement, in which 10 consecutive responses were required for delivery of a food pellet. Daily 15-min training sessions were conducted Monday through Friday until the rats met three criteria during 8 of 10 consecutive sessions: (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 exceeded 0.2 responses/s. When the three criteria were met, multiple component training began. A multiple component schedule was used to prepare rats for the extended multiple component schedule that comprised each cumulative dosing test session (see below). Unlike with discrete dosing drug discrimination procedures, rats undergoing cumulative dosing procedures must be trained to switch levers during the overall session as the internal stimulus changes to “not like the training drug” to “drug-like.”

During multiple component training, rats were exposed to two components of FR10 discrimination training. The components were configured to last either 5 min or until the rat had received 35 pellets (350 correct responses), whichever occurred first. Incorrect responses had no programmed consequence. If rats received 35 pellets before the 5 min period ended, they remained in the operant chamber until the 5 min had elapsed. Before the first session

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component, rats received an injection of vehicle or the THC training dose. They were placed into the chamber 25 min later and the first component began 5 min after entry into the chamber. After the first component, rats were immediately injected again and returned to their home cages, where they had access to water. The second component began 30 min later, with component parameters as described above. Component sequences were of three types, presented in counterbalanced order: vehicle only (2 vehicle injections), THC only [THC training dose, sham injection (i.e., handling and needle poke only)], and stimulus switches (vehicle, THC training dose). As during their initial training, only the injection-appropriate lever was active (on a FR10 schedule of food reinforcement) during each component and responses on the incorrect lever re-set the ratio on the correct lever. Two-component training continued until rats met the acquisition criteria (see preceding paragraph) on 8 of 10 components. Subsequently, rats were tested in a series of two-component probe tests with both levers active. This series included the following sequences: vehicle/vehicle, vehicle/THC, and THC/sham. The regular two-component training schedule was continued on intervening days.

After completion of the control tests, cumulative dose-effect curve determinations were initiated. Rats were tested up to twice per week using the cumulative dosing procedure, which was comprised of up to 7 components. To avoid satiation, each component was configured to end after 10 pellets had been received or 5 min elapsed, whichever came first. Timing of cumulative dosing and testing was the same as on the training days: injection followed by a 30 min pre-treatment, 5 min component, injection, 30 min pre-treatment, etc. This pattern was continued until rats completed the planned doses or failed to respond during a component. For example, a THC dose-effect curve determination started with a vehicle injection, followed by increasing doses of THC (0.1, 0.2, 0.7, 2, 7, and 20 mg/kg) for final cumulative doses of 0.1, 0.3, 1.0, 3.0, 10 and 30 mg/kg. Both levers were active during the cumulative dosing components. To minimize the number of daily injections, rats that failed to complete at least one ratio (i.e., 10 responses) during a component were not injected with subsequent doses or tested further on that day.

After completion of the first THC dose-effect curve, the laboratory in which this research was conducted moved to a new facility. Following the move, rats were re-tested once or twice with THC using the same cumulative dosing method described above. Subsequently, the CB₁ and CB₂ receptor antagonists, rimonabant and SR144528, respectively, were evaluated in both sexes (rimonabant) or in females only (SR144528). The procedure for evaluation of antagonists was similar to the cumulative dosing procedure for THC, except a dose of antagonist (instead of vehicle) was injected 30 min prior to the start of the first component. Female rats and the group of male rats trained to discriminate 3 mg/kg THC were also tested using a cumulative dosing procedure with CP47,497, WIN55,212-2, and JWH-018. Because the performance of male rats trained to discriminate 1 mg/kg THC became unreliable during later control tests, they were not tested with these additional compounds. SR144528 was not tested in male rats due to limited supply of the compound. In addition, previous research had shown that SR144528 did not alter THC's discriminative stimulus effects in male rodents (Järbe et al., 2006).

2.4 Discrete Dosing Fixed Ratio Procedure

Because sex differences in training dose in the drug discrimination paradigm could cause sex differences in the potency of substituted drugs, the effects of synthetic cannabinoids on responding of female and male rats for food reinforcement were examined. Separate groups of adult female (n=7) and male (n=8) rats were trained to respond on a single lever under a FR1 schedule of food reinforcement. The FR was gradually increased to 10 during daily (Monday–Friday) 15-min sessions. Training continued until responding was stable (i.e., did not differ by more than 10% over 6 sessions) (Schoenfeld et al., 1956). Subsequently, rats were tested with THC and the synthetic cannabinoids, JWH-018, CP47,497, WIN55,212-2, and the ring open degradant of A-834735. Test sessions occurred no more than twice a week, with continued training on the intervening weekdays. Injections for the response rate study were administered 30 min prior to the start of the session.

2.5 Drugs

THC (National Institute on Drug Abuse; NIDA, Rockville, MD), rimonabant (NIDA), SR144528 (NIDA), JWH-018 (NIDA), CP47,497 (NIDA), WIN55,212-2 (Cayman Chemical, Ann Arbor, MI), and the ring open degradant of A-834735 (Cayman Chemical) [Fig. S1] were suspended in a vehicle of 7.8 % Polysorbate 80 N.F. (VWR, Radnor, PA, USA) and sterile saline USP (Butler Schein, Dublin, OH, USA). All compounds were administered intraperitoneally (i.p.) at a volume of 1 ml/kg.

2.6 Data Analysis

For each discrimination test session, mean ($\pm$ SEM) percent responding on the drug lever and rate of responding (responses/s) were calculated for each session component. When appropriate, ED₅₀ values (and 95% confidence limits) were calculated separately for each dose-effect curve using least-squares linear regression, including data from the linear part of the curve for percent drug-lever responding, plotted against log₁₀ transformation of the dose. Because rats that responded less than 10 times during a test session component 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. Response-rate data were analyzed using repeated-measures ANOVA across dose. For the discrete dosing fixed ratio responding procedure, mean ($\pm$ SEM) response rate (responses/s) during vehicle sessions was calculated for each rat. These mean rates were used to calculate percentage of mean control responding for each drug dose. Response-rate data were analyzed using mixed design ANOVA (sex as between-subjects factor and dose as repeated measure). Significant ANOVAs for both studies were followed by Tukey post hoc tests (α =0.05). Number Cruncher Statistical Systems (NCSS) software (2004 version, Kaysville, UT) was used to perform all statistical analyses.

3.0 Results

Figure 1 shows the results of control tests and dose-effect curve determinations with THC in female rats trained to discriminate 1 mg/kg THC (Fig. 1, left panels) and in male rats trained to discriminate either 1 or 3 mg/kg THC (Fig. 1, middle and right panels, respectively). During control tests (Fig. 1, left side of each top panel), rats of both sexes responded

predominantly on the vehicle-associated lever when injected with vehicle prior to each 5-min probe session (Veh-Veh), whereas they responded primarily on the THC-associated lever when injected with THC before the first 5-min probe session or when sham-injected before the second 5-min probe session (THC-Sham). When the stimulus condition was switched between sessions (Veh-THC), rats reliably switched most of their responses from the vehicle- to the THC-associated lever. In the first dose-effect curve determination, THC fully and dose-dependently substituted in all groups of rats (Fig. 1, top panels), with a rank order potency of females > males (1 mg/kg) > males (3 mg/kg) [Table 1]. During the second dose-effect curve determination, maximal responding on the THC-associated lever decreased in female and male rats trained to discriminate the 1 mg/kg dose (Fig. 1, top left and middle panels, respectively), but not in male rats trained to discriminate the 3 mg/kg dose (Fig. 1, top right panel). In each group, potencies were lower during the second dose-effect curve [Table 1]. Notably, the minimal effective dose for producing full substitution was higher for all groups during the second dose-effect curve, suggesting drift of stimulus control. Nevertheless, responding showed dose-dependence, 95% confidence limits were overlapping, and rank order potency among the three groups was the same. Higher doses of THC significantly decreased overall response rates in all groups during both THC dose-effect curve determinations [Fig. 1, bottom panels].

In female rats, the CB₁ receptor antagonist rimonabant (3 and 10 mg/kg) shifted the THC dose-effect curve for substitution to the right (Fig. 2, left top panel), without producing any substantial responding on the THC-associated lever by itself at either dose (Fig. 2, left side of left top panel). THC potency was decreased by 6.7- and 8.4-fold in the female rats by the 3 and 10 mg/kg doses of rimonabant, respectively (Table 1). The dose-effect curve for response rate was also shifted to the right by 3 mg/kg rimonabant: a 10-fold higher THC dose (30 vs. 3 mg/kg) was required for significant reduction of responding in the female rats receiving rimonabant than in those injected only with THC [Fig. 2, left bottom panel]. In contrast, the CB₂ receptor antagonist SR144528 (3 and 10 mg/kg) produced slight leftward shifts in the THC dose-effect curve (Fig. 2, top right panel), with a decrease in the minimum effective THC dose for complete substitution (> 80% THC lever responding) from 3 mg/kg for THC alone to 1 mg/kg for THC + 10 mg/kg SR144528. SR144528 also did not shift the THC dose-effect curve for response rate [Fig. 2, bottom right panel].

In males trained to discriminate 1 mg/kg THC from vehicle, neither 3 nor 10 mg/kg rimonabant affected the dose-effect curves for THC substitution (Fig. S3). Similar THC potency was observed regardless of whether or not rimonabant was co-administered (Table 1); however, full substitution (> 80% THC-lever responding) was not observed at any THC alone dose, suggesting that discriminative control was compromised in this group. Hence, these rats were not tested with additional compounds. In male rats trained to discriminate 3 mg/kg THC, 3 and 10 mg/kg rimonabant decreased THC potency 1.2- and 2.3-fold, respectively (Table 1; Fig. 2, middle panels).

As shown in Figure 3, CP47,497, WIN55,212-2, and JWH-018 fully substituted for and were slightly to substantially more potent than THC, in both female rats (Fig. 3, top left panel) and male rats (Fig. 3, top right panel). Table 1 shows potencies for each compound. In male rats, the indole-derived cannabinoids (WIN55,212-2 and JWH-018) produced

approximately 50% responding on the THC-associated lever at the lowest dose tested (0.03 mg/kg), as did the vehicle dose tested during the JWH-018 cumulative dosing session. This degree of substitution at lower doses, and particularly for the vehicle dose, suggests that nonspecific factors may have influenced responding on the THC-associated lever. For this reason, potencies for JWH-018 and WIN55,212-2 tested previously in discrete dosing 3 mg/kg THC discrimination procedures in male rats (Wiley et al., 1995; Wiley et al., 2014a) have been included in Table 1 for comparison purposes. These previous results suggest similarity in the potencies of the indole-derived synthetic cannabinoids across sex, despite the between-sex difference in training dose. An enhanced degree of THC-lever choice did not occur with lower doses of the bicyclic cannabinoid CP47,497 and its potencies across sex are also similar. However, comparisons within each sex to the respective THC dose-effect curve reveal that potencies of the synthetic cannabinoids show greater similarity to that of THC for female rats (1.8- to 3.8-fold greater potencies for synthetics) than they do for male rats. In males, the synthetic cannabinoids exhibit dramatic (10- to 65-fold) increases in potencies compared to THC (Table 1). Reduction of response rates occurred at the same dose of JWH-018 in both sexes, but at a one-log unit lower dose in females than in males for CP47,497 and WIN55,212-2 [Fig. 3, bottom left panel], although response rate data for 10 mg/kg WIN55,212-2 in males was highly variable [Fig. 3, bottom right panel].

Figure 4 shows the results of the discrete dosing experiment with THC and 4 synthetic cannabinoids. All compounds produced dose-dependent suppression of responding. THC was more potent in females than in males (Fig. 4, top panel). Analysis of the A-834735 degradant results revealed a trend for greater decreases in females than in males (Fig. 4, top panel). In contrast, sex differences in the response suppressing effects of JWH-018, CP47,497, and WIN55,212-2 were not observed (Fig. 4, bottom panel).

4.0 Discussion

One of the most notable observations in this study was that the THC training dose that we (Wiley et al., 2014b) and others (Gatch and Forster, 2014; Jarbe and McMillan, 1980; Solinas and Goldberg, 2005) have traditionally used for male rats (3 mg/kg) was too high for female rats. Despite differences in training dose, however, THC produced dose-dependent substitution in all groups, with rank order potency dependent upon training dose and sex: 1 mg/kg THC in females > 1 mg/kg THC in males > 3 mg/kg THC in males. While the role of training dose is well-characterized in cannabinoid discrimination (Järbe et al., 1998), the present study represents the first to examine sex differences in THC discrimination in rats. Winsauer *et al.* (2012) tested female rats only and reported that they required lower THC training doses (0.56–1.8 mg/kg) than those generally reported for male rats, which is consistent with the data reported here.

After completion of the first THC dose-effect curve in all rats, a relocation of the RTI animal facility provided an opportunity for a natural experiment. The move and set-up of the lab required two weeks, immediately following which a second THC dose-effect curve was completed in the same rats. In all groups, THC was less potent in producing discriminative stimulus effects after the move (i.e., ED₅₀s were 1.4 to 2.4-fold higher; Table 1). In addition, male rats trained to discriminate 1 mg/kg THC never regained full substitution at any THC

dose up to a dose (30 mg/kg) that substantially suppressed responding. Hence, it appears that maintenance of a reliable 1 mg/kg THC discrimination using a cumulative dosing procedure in male rats may not be not feasible. [By comparison, cumulative THC dose-effect curves exhibited considerable overlap in adult male rats trained to discriminate 3 mg/kg THC following exposure to repeated vehicle injection for 14 days during suspended training (Wiley et al., 1993).] Relocation of the animal facility was undoubtedly a stressful experience for the rats, since numerous environmental factors (e.g., sounds, lights, smells) affecting rodent behavior (Castelhano-Carlos and Baumans, 2009) changed during the move. Given the demonstrated role of endocannabinoids in modulation of the stress response (Hill et al., 2010), underlying alterations of brain endocannabinoid levels and/or CB₁ receptors represent plausible (but unconfirmed) contributors to observed differences in THC potency after relocation.

As reported previously for male rats (Järbe et al., 2006; Wiley et al., 1995), THC's discriminative stimulus effects in females are CB₁ receptor mediated, as shown by the rimonabant-induced rightward shift of the THC dose-effect curve. By contrast, the rimonabant-induced rightward shift was minor in male rats trained to discriminate 3 mg/kg THC from vehicle and absent in male rats assigned to the 1 mg/kg THC training dose condition, suggesting that rimonabant's potency to antagonize THC's discriminative stimulus effects is lower in males than females, although this interpretation must be tempered somewhat by the lower degree of stimulus control displayed by the male rats in the 1 mg/kg THC group and differences in training doses between females and males trained to discriminate 3 mg/kg THC. Rimonabant's decreased potency in males also has been noted with respect to reversal of cannabinoid-induced antinociceptive effects (Craft et al., 2012). As reported previously for male rodents (Järbe et al., 2006; Wiley et al., 2002), the CB₂ antagonist SR144528 failed to antagonize THC's discriminative stimulus effects in female rats, and small leftward shifts in the THC dose-response curve were noted, raising the possibility that pharmacological elimination of THC's CB₂ agonist properties may enhance the drug's CB₁ receptor mediated effects. This result is consistent with the previously suggested hypothesis that involvement of CB₂ receptors may be greater in mediation of THC's pharmacological effects in female (vs. male) rats (Craft et al., 2013a; Craft et al., 2012).

Like THC, many synthetic cannabinoids are dual CB₁/CB₂ agonists, but most abused synthetic cannabinoids have stronger CB₁ receptor affinity than THC (Manera et al., 2008), including those tested here (Table 1). Not surprisingly, all three compounds fully substituted for THC in rats of both sexes, as has been reported previously in THC-discriminating male rats tested with a variety of synthetic cannabinoids (Gatch and Forster, 2014; Wiley et al., 2016). Further, they were more potent than THC and were considerably more so for males than for females. Although absolute differences in potency were observed between the sexes for WIN55,212-2 and JWH-018, stimulus control was disrupted at lower doses in males for these two compounds, suggesting a possible contributory role for non-specific factors. Alternatively, between-sex differences in potencies in this study could have been due to sex differences in THC training dose, which has been shown to affect the degree to which similar compounds will substitute (and their potencies when substitution is observed) (Jarbe et al., 2014), albeit the predicted direction of differences would have been that compounds

would have been more potent in the rats trained to discriminate a lower dose of THC (i.e., females). Between-sex potencies for JWH-018 and WIN55,212-2 were more similar when potencies for these compounds in females were compared to those obtained in previous studies in males trained to discriminate 3 mg/kg THC in discrete dosing (vs. cumulative dosing) procedures (Wiley et al., 1995; Wiley et al., 2014a). Nevertheless, these findings demonstrate for the first time that synthetic cannabinoids possess THC-like discriminative stimulus effects in female rats, and they appear to be more potent than THC in both sexes.

Because differences in training dose complicated sex comparisons of drug potency in the drug discrimination paradigm, we also compared the effects of synthetic cannabinoids on simple FR responding for food reinforcement. In this procedure, females were more sensitive than males to THC-induced suppression of responding, a difference that may be related to sex differences in the metabolism of THC in rats (Tseng et al., 2004; Wiley and Burston, 2014) or to sex differences in pharmacodynamic factors (Burston et al., 2010; Craft et al., 2013b; Fattore et al., 2007). By contrast, sex differences in the effects of CP47,497, WIN55,212-2, and JWH-018 on responding were not observed. The latter results contrast with the increased sensitivity of female rats to the reinforcing effects of WIN55,212-2 (Fattore et al., 2007). Given that differences in pharmacokinetics cannot account for this differential pattern of sensitivity across behavioral assays (i.e., greater sensitivity of female rats to WIN55,212-2's reinforcing effects vs. no sex differences in WIN55,212-2's effects on response rates in the FR schedule), the possibility of underlying sex differences in pharmacodynamic factors (e.g., receptor density or distribution, hormonal influences) must be considered. Interestingly, female rats exhibited a trend towards increased sensitivity to the rate decreasing effects of a ring open degradant of A-834735, a synthetic cannabinoid with extremely high efficacy for stimulation of CB₁ receptors (Grim et al., 2016; Thomas et al., 2016). "Super" agonists such as the A-834735 degradant might be expected to be more potent in rats with lower CB₁ receptor reserves in relevant brain areas. Although brain areas mediating response rates have not been elucidated, CB₁ receptor densities have been shown to be lower in the prefrontal cortex and amygdala of female (vs male) rats (Castelli et al., 2014).

In summary, this study represents one of the first direct comparisons of THC discrimination in female and male rats. Its primary findings are that, compared to male rats, female rats require a lower training dose for acquisition of stimulus control in a cumulative dosing THC discrimination procedure and exhibit correspondingly greater potency for THC's discriminative stimulus and response rate-decreasing effects. Further, THC's discriminative stimulus effects in female rats are reversible by rimonabant, but not by SR144528, as has been reported previously for male rats. Previous research suggests that this greater sensitivity in females may be related, in part, to sex differences in the metabolism of THC. While synthetic cannabinoids (WIN55,212-2, JWH-018, and CP47,497) were more potent in producing THC-lever responding than was THC in female rats, considerably greater discrepancies in the potencies of these compounds compared to that of THC was observed in male rats. To the extent that these findings may be generalized to humans, they suggest that women and men may experience THC-like effects at similar doses of synthetic cannabinoids; however, for men, these doses may be substantially lower than their usual THC dose. For women, doses may be more similar. By contrast with THC, female and male

rats show similar sensitivity to the response rate-decreasing effects induced by some (WIN55,212-2, JWH-018, CP47,497), but not all (A-834735 degradant), synthetic cannabinoids. The lack of sex differences in WIN55,212-2's response rate suppressive effects contrasts with the enhanced sensitivity of female rats to its reinforcing effects. This assay selectivity in sex differences suggests that pharmacokinetic factors cannot completely account for the latter finding. Finally, results with SR144528 tentatively suggest that CB₂ receptors may contribute to THC's discriminative stimulus effects in female rats, an idea that requires additional research for confirmation. Future research should emphasize further characterization of sex differences in cannabinoid pharmacology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Effects of THC on percentage of THC-lever responding (top panels) and response rates (bottom panels) in female and male rats ($n=8$ and 4 , respectively) trained to discriminate 1 mg/kg THC (left and middle panels, respectively) and in male rats ($n=4$) trained to discriminate 3 mg/kg THC (right panels). Unfilled circles show effects of a cumulative dose-effect curve with THC before a laboratory relocation and filled circles show effects of the same cumulative doses of THC after the laboratory move. At the left side of each panel, points above veh and veh represent the results of a two-component control test with vehicle; points above THC and sham show results of a two-component control test with the training dose of THC followed by a sham injection; and the points above veh and THC show results of a two-component control test with vehicle followed by the training dose of THC. For each dose-effect curve determination, values represent means ($\pm$ SEM). * indicates significant response rate difference compared to 0 mg/kg dose for the same dose-effect curve. Female rats: $F(5,35)=21.49$, $p<0.05$ (THC-1); $F(5,30)=43.72$, $p<0.05$ (THC-2). Male rats trained with 1 mg/kg THC: $F(6,18)=12.18$, $p<0.05$ (THC-1); $F(6,18)=26.23$, $p<0.05$ (THC-2). Male rats trained with 3 mg/kg THC: $F(6,18)=17.50$, $p<0.05$ (THC-1); $F(6,18)=12.82$, $p<0.05$ (THC-2).

Figure 2.

Effects of THC alone and in combination with the CB₁ receptor antagonist rimonabant (left and middle panels) or the CB₂ receptor antagonist SR144528 (right panels) on percentage of THC-lever responding (top panels) and response rates (bottom panels), each as a function of cumulative THC dose. Data for female rats trained to discriminate 1 mg/kg THC from vehicle are shown in the left and right panels; data for male rats trained to discriminate 3 mg/kg THC from vehicle are shown in the middle panels. The left side of each panel shows responding after vehicle (first dose for THC alone curve) and 3 or 10 mg/kg doses of the respective antagonist (first dose of antagonist combination curves). For each dose-effect curve determination, values represent means ($\pm$ SEM) for 7-8 female or 4 male rats for rimonabant (left and middle panels, respectively) and 4-5 female rats for SR144528 (right panels). * indicates significant response rate difference compared to vehicle for THC alone or to the antagonist dose alone (left side of panel). Rimonabant in females: $F(5,30)=43.72$, $p<0.05$ (THC alone); $F(7,49)=11.14$, $p<0.05$ (THC+3 mg/kg rimonabant); $F(7,42)=5.82$, $p<0.05$ (THC+10 mg/kg rimonabant). Rimonabant in males: $F(6,18)=12.82$, $p<0.05$ (THC alone); $F(7,21)=3.27$, $p<0.05$ (THC+3 mg/kg rimonabant); $F(7,21)=1.59$, $p>0.05$ (THC+10 mg/kg rimonabant). SR144528: $F(5,29)=35.79$, $p<0.05$ (THC alone); $F(6,24)=16.31$, $p<0.05$ (THC+3 mg/kg SR144528); $F(6,18)=3.12$, $p<0.05$ (THC+10 mg/kg SR144528).

Figure 3.

Effects of cumulative doses of synthetic cannabinoids (JWH-018, CP47,497, and WIN55,212-2) on percentage of THC-lever responding (top panels) and response rates (bottom panels) in female ($n=6-7$) and male ($n=4$) rats trained to discriminate 1 or 3 mg/kg THC, respectively (left and right panels, respectively). A cumulative THC dose-effect curve is also reproduced from Figure 1. At the left side of each panel, points above V show results after injection with vehicle (first dose of each cumulative dose-effect curve). For each dose-effect curve determination, values represent means ($\pm$ SEM). * indicates significant response rate difference compared to the vehicle dose for the same dose-effect curve. Female rats: $F(5,30)=11.97$, $p<0.05$ (JWH-018); $F(5,30)=32.22$, $p<0.05$ (CP47,497); $F(6,30)=44.78$, $p<0.05$ (WIN55,212-2). Male rats: $F(5,15)=5.65$, $p<0.05$ (JWH-018); $F(5,15)=60.11$, $p<0.05$ (CP47,497); $F(6,18)=2.64$, $p>0.05$ (WIN55,212-2).

Figure 4.

Effects of discrete doses of THC and the synthetic cannabinoids (JWH-018, CP47,497, WIN55,212-2, and the ring open degradant of A-834735) on responding under a FR10 schedule for food reinforcement. Response rate (responses/s) during all vehicle sessions was averaged and served as a basis for calculation of percentage of mean vehicle control for each rat at each dose. Values represent means ($\pm$ SEM) for data from 5-8 rats, with filled and unfilled symbols showing data for female and male rats, respectively. \$ indicates significant main effect or trend of sex for THC [$F(1,5)=14.08$, $p<0.05$] or A-834735 degradant [$F(1,6)=4.92$, $p=0.068$], respectively. # indicates significant main effect of dose for THC [$F(3,15)=26.53$, $p<0.05$], A-834735 degradant [$F(4,24)=19.46$, $p<0.05$], JWH-018 [$F(4,24)=8.47$, $p<0.05$], CP47,497 [$F(3,18)=20.4$, $p<0.05$], and WIN55,212-2 [$F(4,24)=26.61$, $p<0.05$].

Table 1Cannabinoid potency in female and male rats trained to discriminate THC^a

Test Condition	Females (1 mg/kg)	Males (1 mg/kg)	Males (3 mg/kg)	Males ^b (3 mg/kg)
THC-1	0.39 (0.15 – 1.03)	0.60 (0.18 – 1.99)	0.98 (0.27 – 3.58)	1.13 ^c (0.89 – 1.43)
THC-2	0.53 (0.27 – 1.04)	1.45 (0.61 – 3.49)	1.97 (1.09 – 3.55)	n/d
CB ₁ K _i = 41 nM ^d CB ₂ K _i = 36 nM ^d				
CB ₁ Receptor Antagonism THC + Rim (3 mg/kg)	3.56 (2.32 – 5.48)	1.26 (0.33 – 4.81)	2.31 (1.05 – 5.08)	n/d
THC + Rim (10 mg/kg)	4.49 (2.18 – 9.23)	~ 1.0	4.60 (2.92 – 7.22)	n/d
CB ₂ Receptor Antagonism THC + SR144528 (3 mg/kg)	0.34 (0.20 – 0.58)	n/d	n/d	n/d
THC + SR144528 (10 mg/kg)	0.37 (0.24 – 0.58)	Potency THC-2/ Synthetic [*]	n/d	n/d
Synthetic Cannabinoids		Female	Male	
CP47,497	0.14 (0.08 – 0.23)	3.79	10.37	n/d
CB ₁ K _i = 2.2 nM ^e CB ₂ K _i = n/d			0.19 (0.09 – 0.40)	
WIN55, 212-2	0.30 (0.21 – 0.41)	1.77	19.7	0.2 ^f (0.09 – 0.4)
CB ₁ K _i = 1.89 nM ^d CB ₂ K _i = 0.3 nM ^d			~ 0.1	
JWH-018	0.19 (0.11 – 0.33)	2.80	65.67	0.23 ^c (0.15 – 0.35)
CB ₁ K _i = 9 nM ^d CB ₂ K _i = 3 nM ^d			~ 0.03	

^a ED₅₀ values (95% confidence limits) are expressed as mg/kg. THC training dose for each group of rats is noted at the head of each column. CB₁ and CB₂ receptor affinities are provided (in parentheses) beneath the name of each cannabinoid agonist. n/d = not determined.

^b Results from previous studies using discrete dosing procedures with male rats.

^c (Wiley et al., 2014a)

^d (Showalter et al., 1996)

* Ratio of potencies of THC-2: synthetic cannabinoid within sex.

^f (Wiley et al., 1995)

^e (Huffman et al., 2008)

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