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Evaluation of Sex Differences in Cannabinoid Dependence

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Abstract

Background—Chronic recreational marijuana users often report withdrawal symptoms when trying to quit, with some reports suggesting withdrawal may be more pronounced in women. In animal models, female rodents show enhanced sensitivity to acute Δ^9 -tetrahydrocannabinol (THC) administration, but chronic administration has been studied little.

Methods—Sex differences in THC dependence in rats were examined. Adult male and female Sprague-Dawley rats were administered 30 mg/kg THC or vehicle twice daily for 6.5 days. On day 7, rats were challenged with vehicle or rimonabant, counterbalanced across dosing groups, and were assessed for withdrawal-related behaviors.

Results—During chronic THC dosing, disruption of estrous cycling and weight loss (both sexes) were observed. Whereas overt signs of withdrawal were minimal in THC-treated rats challenged with vehicle, rimonabant precipitated a pronounced withdrawal syndrome in THC-dependent rats that was characterized by changes in a number of domains, including somatic (paw tremors, head twitches, and retropulsion), early-stage cognition (lack of locomotor habituation, disrupted prepulse inhibition), and affective (increased startle reactivity). With the exception of increased retropulsion in female rats, sex differences were not noted. In vehicle-treated rats, rimonabant induced puritis.

Conclusions—This study represents the first examination of THC dependence in adult rats of both sexes, extends previous findings to females, and revealed some sex differences. The results suggest that the changes that occur during precipitated withdrawal from THC extend beyond somatic signs to more nuanced disruptions of cognitive and affective functioning. The breadth of withdrawal signs observed in rodents mirrors those that have been observed in humans.

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Contributions

Authors Craft, Lefever, Marusich, and Wiley participated in research design. Authors Antonazzo, Lefever, and Marusich conducted experiments. Authors Antonazzo, Craft, Lefever, and Wiley performed data analysis. Authors Craft, Marusich, and Wiley wrote or contributed to the writing of the manuscript. All authors contributed to the manuscript, and have approved the final version of the manuscript.

Conflict of Interest

The authors have no conflicts of interest.

Keywords

Δ^9 -tetrahydrocannabinol; dependence; rimonabant; sex differences

1. INTRODUCTION

Among the numerous phytocannabinoids contained in the *Cannabis sativa* plant, Δ^9 -tetrahydrocannabinol (THC) is the primary psychoactive ingredient, and in plants bred for recreational use, it is often the most prevalent cannabinoid. Even before it was recently legalized for recreational use in two states in the U.S., cannabis was the most widely used illicit substance of abuse in the U.S. (Substance Abuse and Mental Health Services Administration, 2013). In addition, cannabis has been used therapeutically to treat a variety of ailments including chronic pain (Aggarwal et al., 2009), chemotherapy-induced nausea and vomiting (Walsh et al., 2003), and various neurological disorders (Williamson and Evans, 2000; Hill et al., 2012). Because recreational and therapeutic use of cannabinoids commonly involves chronic use, dependence may develop. A large percentage of marijuana users (44–91%) report experiencing some withdrawal symptoms when trying to quit (Hasin et al., 2008; Levin et al., 2010). Although women are less likely than men to report regular marijuana use, women are more likely to report withdrawal symptoms (Levin et al., 2010), which may contribute to their greater propensity for relapse when they stop using a drug (Becker and Hu, 2008).

While sex differences in the pharmacology of other abused substances such as psychostimulants and opioids have been increasingly examined over the past two decades (Carroll et al., 2004; Becker and Hu, 2008), less has been done in the cannabinoid field (see Craft et al., 2013a for review). Acutely, cannabinoids are more potent in producing antinociception in female than male rats (Tseng and Craft, 2001; Craft et al., 2013b), and more potent in suppressing locomotion and in producing catalepsy in female rats compared to males (Tseng and Craft, 2001; Craft et al., 2013b). A few studies have examined sex differences in the effects of repeated administration of cannabinoids. Several findings of note are that female rodents are more sensitive than males to the reinforcing and discriminative stimulus effects of cannabinoids (Fattore et al., 2007; Wiley et al., 2011), and that withdrawal from THC produced more anxiety related behaviors in adolescent female rats compared to males (Harte-Hargrove and Dow-Edwards, 2012). The latter results are consistent with the hypothesis that more intense withdrawal may be a significant factor in the increased rate of relapse to drug-taking in women.

The purpose of this study was to examine sex differences in withdrawal from THC using a wide range of behavioral measures in rats. Given that the overt signs of spontaneous withdrawal in THC-dependent rodents are subtle (Compton et al., 1990), withdrawal was precipitated through administration of the CB₁ receptor antagonist/inverse agonist, rimonabant. A number of previous studies have demonstrated that rimonabant-precipitated withdrawal is characterized by robust somatic signs in rodents, including paw flutters/tremors and head twitches (Tsou et al., 1995; Cook et al., 1998). In addition to systematic observation of these somatic signs, the battery of assays in the present study included commonly used measures of cannabinoid action (body temperature, locomotor activity, and antinociception), as well as measures that have been used to assess affective and rudimentary cognitive processes (sensorimotor reactivity and gating, and habituation).

2. MATERIALS AND METHODS

2.1 Subjects

Forty male and forty female Sprague-Dawley rats (Harlan, Dublin VA) were housed in polycarbonate cages in a temperature-controlled (20–22°C) environment with a 12 hr light-dark cycle (lights on at 6 am). Each rat was pair housed with a rat of the same sex and in the same drug group. All animals were approximately 60 days of age at the beginning of the experiment and were gonadally intact. Rats had *ad libitum* access to food (Purina® Certified 5002 Rodent Chow, Barnes Supply, Durham NC) and water while in their home cages. All experiments 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 the Institutional Animal Care and Use Committee at RTI.

2.2 Apparatus

Measurement of locomotor activity occurred in standard Plexiglas locomotor activity chambers (47 cm × 25.5 cm × 22 cm). Beam breaks were recorded by San Diego Instruments Photobeam Activity System software (model SDI: V-71215, San Diego, CA, USA) on a computer located in the experimental room. The apparatus contained two 4-beam infrared arrays that measured horizontal movement. A glass beaker filled with water heated to 50°C was used for the warm water tail withdrawal procedure. A digital thermometer (Physitemp Instruments, Inc., Clifton, NJ, USA) was used to measure rectal temperature. Startle sessions were conducted in an enclosed, clear Plexiglas rectangular chamber (15 cm × 10 cm, with ceiling adjustable from 5 to 11 cm high) which rested on a force sensing plate (SM100; Kinder Scientific, Poway, CA, USA). Plexiglas chambers and sensing plates were located inside sound attenuating cabinets. Acoustic stimuli were produced by a noise generator, mounted 8 cm above the top of the chamber. Cabinets were illuminated by a 6-W house light mounted on the ceiling above the chamber. A Dell computer with Kinder Scientific software and interface was used to present stimuli and to record data.

2.3 Procedures

Rats were randomly assigned to one of four drug groups described below (n=10 rats of each sex per group). All rats were dosed twice daily for 7 days, with injections occurring approximately 8 hrs apart for days 1–6. On day 7, the second injection of the day was given approximately 4 hrs after the first injection. Table 1 shows the sequence of injections for each of the four treatment groups. The vehicle/vehicle (Veh/Veh) group was administered vehicle for both daily injections for all 7 days. The vehicle/rimonabant group (Veh/Rim) received vehicle twice daily for 6 days and once on the morning of day 7; 10 mg/kg rimonabant was the second injection on day 7. The THC/vehicle (THC/Veh) group received 30 mg/kg THC twice daily for 6 days and once on the morning of day 7; vehicle was the second injection on day 7. The THC/rimonabant (THC/Rim) group received 30 mg/kg THC twice daily for 6 days and once on the morning of day 7; 10 mg/kg rimonabant was the second injection on day 7. All injections were given subcutaneously (s.c.) except the second injection on day 7, which was given intraperitoneally (i.p.). The chronic dosing regimen, including route of administration, was based upon procedures established in numerous previous studies of cannabinoid tolerance and dependence in rodents (Beardsley and Martin, 2000; Falenski et al., 2010; Schlosburg et al., 2011; Wiley et al., 2007). Vaginal smears were collected once daily from female rats beginning 7 days prior to the start of drug administration, and ending 8 days after the last day of drug administration (22 days total). Male rats were handled daily for a comparable amount of time.

On days 1 and 6 of the repeated dosing regimen, baseline measures of temperature and tail withdrawal latency were collected, followed by administration of the first injection of the

day. Thirty min later, rats were placed in the locomotor activity chambers for a 5-min session. Immediately thereafter, temperature and tail withdrawal latency were measured again.

On Day 7, dependence was assessed. Baseline measures of temperature and tail withdrawal latency were collected, followed by injection with the last dose of the repeated dosing regimen. Four hrs later, the second injection (challenge condition) was given. Five min after the injection rats were placed in the locomotor activity chambers for a 15-min session, with data collected in 5-min bins. Immediately thereafter, temperature and tail withdrawal latency were measured again. Rats were then placed in the observation arena for 30 min and their overt behavior was observed. All observations were made by one trained technician who was blind to the challenge condition for each rat. The number of times the following behaviors occurred was recorded: forepaw tremors, head twitches, “wet dog” shakes (entire body), grooming, sniffing, scratching with hind paw, ptosis (eyelid closure), writhing, piloerection (hair erection), retropulsion (walking backwards), and audible vocalizations. Following observations, rats were exposed to one startle session, described in detail below, and then returned to their home cages.

Auditory startle sessions lasted approximately 20 min. Sessions began with a 5-min adaptation period, during which rats were exposed to 69-dB background noise. This background noise continued throughout the session. Each startle session consisted of 61 trials (average intertrial interval = 15 s). Sessions were comprised of four trial types, presented in mixed order. On one type of trial, the rats were exposed to a 120-dB acoustic stimulus (pulse trials). Startle amplitudes during these trials indicate the degree of sensorimotor reactivity. A second type of trial consisted of an 85-dB prepulse (20-ms duration) followed by 120-dB pulse (prepulse + pulse trials). The other two types of trials consisted of exposure to an 85-dB prepulse alone (prepulse trials) or to 69-dB background noise (no-stimulation trials). The latter were control trials used to measure the degree of “noise” in the procedure. Startle amplitudes during the prepulse alone and no-stimulation trials were typically very low and are not presented. Startle sessions began with a single pulse trial, followed by three blocks of 20 trials per block (five trials of each of the four types). Startle pulse duration was held constant at 40 ms. A 100-ms delay was imposed between prepulse and pulse stimuli.

2.4 Determination of estrous cyclicity

The stage of estrous cycle was determined cytologically following vaginal lavage. Vaginal lavage was conducted at the beginning of each day prior to any drug administration or testing. The particular stage was based on the topography of the cells in a sample. Proestrus was identified when cells were predominantly (approximately 75% or more) nucleated epithelial cells. A predominance of cornified epithelial cells classified the estrus stage. Diestrus-1 (metestrus) was recognized by scattered, nucleated or cornified epithelial cells and leukocytes, and diestrus-2 was recognized by a relative lack of any type of cells (Freeman, 1988).

2.5 Drugs

Δ^9 -Tetrahydrocannabinol (THC) [National Institute on Drug Abuse (NIDA), Bethesda, MD, USA] and rimonabant (NIDA) were suspended in a vehicle of 7.8 % Polysorbate 80 N.F. (VWR, Radnor, PA, USA) and 92.2% sterile saline USP (Butler Schein, Dublin, OH, USA). Doses of all drugs are expressed as mg/kg. All drugs were administered at a volume of 1 ml/kg.

2.6 Data Analysis

Locomotor activity was measured as the total number of photocell beam interruptions during the entire 5-min sessions on days 1 and 6 and in separate 5-min bins during the 15-min session on day 7. Startle score was defined as the average of the maximum Newtons of pressure exerted during all pulse-alone trials within a session. Prepulse inhibition (PPI) was calculated for prepulse + pulse trials as a percentage of pulse-alone scores [(mean startle amplitude for pulse-alone trials - mean startle amplitude for prepulse + pulse trials)/mean startle amplitude for pulsealone trials] $\times$ 100. During behavioral observation sessions on day 7, the mean number ($\pm$ SEM) of incidents was calculated for each of the 12 behaviors recorded and data were subjected to analysis as described below.

For the tolerance component of the study (days 1 and 6), data for rats from all challenge groups that received a given repeated treatment (i.e., vehicle or 30 mg/kg THC) were grouped for data analysis. Each dependent measure was separately analyzed with a mixed three-way (sex, treatment, day) analysis of variance (ANOVA), with day (1 and 6) as the repeated factor and sex (male and female) and treatment (vehicle and THC) as between-subjects factors. In the event of a 3-way interaction, separate two-factor (sex, treatment) ANOVAs were conducted for each test day. When these ANOVAs were significant, Tukey post hoc tests ($\alpha = 0.05$) were used to analyze the data further.

For the dependence component of the study (day 7), data for each dependent measure were grouped by sex for each of the four treatment/challenge conditions: Veh/Veh, Veh/Rim, THC/Veh, and THC/Rim. Locomotor activity data from day 7 were analyzed with a mixed model factorial (sex, group, bin) ANOVA, with bin as the repeated factor. Each of the other dependent measures (antinociception, temperature, startle, % PPI, and each observational measure) was analyzed with separate two-factor (sex, group) ANOVAs. Significant ANOVAs were followed by Tukey post hoc tests ($\alpha = 0.05$) to specify differences among means for the main effects and/or interaction.

Throughout the study, body weights of all rats were recorded each morning before any treatment or testing. Analysis of body weight data was undertaken for selected days: day 1, day 7, and day 15, with day 1 being the first day of repeated treatment with vehicle or THC, day 7 being the day on which dependence was assessed, and day 15 being the last day on which vaginal smears were taken (the final day of the experiment for all rats). Body weight data were analyzed separately in each sex using mixed model (group, day) ANOVAs, with group as the between-subjects factor and day as the within-subjects factor. Tukey post hoc tests ($\alpha = 0.05$) were used to specify differences among means, as appropriate.

Time spent in proestrus to estrus stages was calculated as an indicator of normal estrous cycling, for rats in each treatment group (Veh/Veh, Veh/Rim, THC/Veh, THC/Rim), in each phase of the study. The “pre-chronic” phase was the 8-day period before the start of daily vehicle/THC injections (this included the first day of daily injections since lavage was conducted prior to any injections); the “chronic” phase was the 6-day period during which vehicle or THC was administered daily (days 2–7 of daily dosing); the “post-chronic” phase was the 8-day period following the chronic phase, during which no injections were given. Thus, percent days in proestrus to estrus = [number of vaginal cell samples identified as proestrus or estrus (or in between the two phases) / total number of vaginal cell samples] $\times$ 100, calculated for each rat during each phase of the study. These data were analyzed by ANOVA, with between-subjects factors of THC (vehicle or THC) and rimonabant (vehicle or rimonabant) and the within-subjects factor of phase (pre-chronic, chronic, post-chronic). Another statistic that was used to examine the effect of THC on estrous cycling was simply the number of rats that showed evidence of normal estrous cycling. Normal cycling was

defined as at least one vaginal cytology sample identified as proestrus or estrus (or in between the two phases) every 4–5 days.

3. RESULTS

Figure 1 shows the effects of vehicle and 30 mg/kg THC (s.c.) in male and female rats on locomotor activity during a 5-min session after acute administration (morning of day 1) and after the last injection of twice daily injections for 5.5 days (morning of day 6). Acutely, 30 mg/kg THC significantly decreased locomotor activity in males, compared to males that received vehicle and compared to females that received either vehicle or THC [sex $\times$ treatment $\times$ day interaction: $F(1,68)=5.13$, $p<0.05$]. With repeated injection, tolerance developed to this effect in the males, as no difference in locomotor activity between vehicle- and THC-treated male rats was noted on day 6. THC did not significantly affect locomotor activity in females on either day. THC (30 mg/kg, s.c.) also did not significantly alter rectal temperature or warm water tail withdrawal latency on either day, in either sex (data not shown). Neither antinociception nor hyperalgesia was observed, and therefore data from these measures are not presented.

Figure 2 shows the effects of day 7 challenge with vehicle or rimonabant (10 mg/kg) on locomotor activity in male and female rats (top and bottom panels, respectively) following repeated treatment with either vehicle or 30 mg/kg THC. Data from the 15-min session are grouped in three consecutive 5-min bins. During the first bin, the locomotor activity of rats in the four treatment groups did not differ in either sex; however, in each subsequent bin, THC-treated rats of both sexes challenged with rimonabant exhibited significantly greater locomotor activity than did rats in any of the other treatment groups [group $\times$ bin interaction: $F(6,144)=11.92$, $p<0.05$]. Whereas rats in these other groups exhibited habituation across bins (compared to bin 1), the activity of THC-treated rats challenged with rimonabant was sustained throughout the entire session. Challenge with rimonabant produced negligible changes in rectal temperature and did not alter tail withdrawal latencies (data not shown).

Following challenge with vehicle or rimonabant on day 7, the incidences of most behaviors on the observation checklist were not significantly different compared to the Veh/Veh condition, including grooming, wet dog shakes, sniffing, ptosis, writhing, piloerection, audible vocalizations, urination and defecation (Table 2). Four exceptions were noted (Figure 3): scratching (panel A), forepaw tremors (panel B), head twitches (panel C), and retropulsion (panel D). Rimonabant increased bouts of scratching in rats of both sexes treated repeatedly with vehicle [main effect of group: $F(3,72)=8.61$, $p<0.05$], but not in those treated repeatedly with THC. Precipitated withdrawal in THC-treated male and female rats challenged with rimonabant was characterized by increases in the number of forepaw tremors [main effect of group: $F(3,72)=24.82$, $p<0.05$] and head twitches [main effect of group: $F(3,72)=12.22$, $p<0.05$], behaviors that occurred infrequently in the other groups. In female rats, but not in males, increased incidence of retropulsion was also observed during precipitated withdrawal [sex $\times$ treatment interaction: $F(3,72)=3.36$, $p<0.05$].

Figure 4 shows the effects of day 7 challenge with vehicle or rimonabant on acoustic startle response (top panel) and percent PPI (bottom panel) in male and female rats following repeated treatment with vehicle or 30 mg/kg THC. Compared to rats that received vehicle during the repeated dosing period, startle reactivity was enhanced in THC-treated male and female rats [main effect of group: $F(3,72)=8.20$, $p<0.05$] (Figure 4, top panel), regardless of whether they were challenged with vehicle or with rimonabant on day 7. In contrast, PPI showed significant disruption only in the THC/Rim group, and not in the THC/Veh group or in the groups treated repeatedly with vehicle [main effect of group: $F(3,72)=4.76$, $p<0.05$].

(Figure 4, bottom panel), suggesting that this effect was associated with precipitated withdrawal.

Figure 5 shows body weights of male (top panel) and female (bottom panel) rats on days 1, 7 (challenge day), and 15 (last day of study). Given the expected differences in baseline body weights, data for males and females were analyzed separately. On day 1, body weights of male rats did not differ across treatment condition (Figure 5, top panel). Over the course of the study, male rats repeatedly treated with vehicle (Veh/Veh and Veh/Rim groups) showed a steady and progressive increase in body weight. In contrast, male rats repeatedly treated with THC (THC/Veh and THC/Rim groups) lost weight during the period of THC treatment, resulting in significantly lower weights on day 7 (compared to their day 1 weights and compared to the weights of vehicle-treated males) [chronic treatment $\times$ day interaction: $F(6,72)=24.92$, $p<0.05$]. While these rats gained weight after THC treatment was stopped, post hoc tests revealed that their body weights remained significantly below the weights of the vehicle-treated male rats on day 15.

Similar to the male rats, female rats did not differ in body weight across groups on day 1 (Figure 5, bottom panel). Vehicle-treated female rats also gained weight over the course of the study. Although their rate of gain was slower than for males, vehicle-treated female rats were significantly heavier on day 15 than on day 1, whereas female rats repeatedly treated with THC (THC/Veh and THC/Rim groups) lost weight during the period of THC treatment, resulting in significantly lower weights on day 7 (compared to their day 1 weights and compared to the weights of vehicle-treated females) [chronic treatment $\times$ day interaction: $F(6,72)=5.58$, $p<0.05$]. While these rats gained weight after THC treatment was stopped, post hoc tests revealed that body weights for the THC/Veh group remained significantly below the weights of the vehicle-treated female rats on day 15. On the other hand, post hoc tests showed that body weights for the THC/Veh group remained significantly lower than vehicle-treated rats on day 15, and were slightly but not significantly greater than their day 1 weights.

Figure 6 shows the percent of days that female rats were in proestrus or estrus in each phase of the study, based on vaginal cytology samples that were obtained each morning for 22 days. Given that most rats' cycle duration is 4–5 days, rats would be expected to be in proestrus or estrus approximately 40–50% of the time (slightly less, since proestrus lasts less than one day) (Freeman, 1988). Thus, during the pre-chronic phase, an 8-day baseline period that preceded daily injections, rats in all groups except the Veh/Veh group spent a near-normal percent of days in proestrus/estrus. The number of females showing evidence of estrous cycling was also lower in the Veh/Veh group than in the other groups (Table 3). Estrous cycling in the Veh/Veh group recovered over the 22 days of the study, as evidenced by progressive increases in percent days in proestrus/estrus as well as in the number of females showing evidence of cycling from pre-chronic to post-chronic phases (Figure 6 and Table 3). In the other three groups, estrous cycling decreased from pre-chronic to chronic phases and then increased from chronic to post-chronic phases, measured by percent days in proestrus/estrus [phase $\times$ THC: $F(2,72)=3.85$, $p<0.05$], and by the number of females showing evidence of estrous cycling (Table 3). Post-hoc analyses on the percent days in proestrus/estrus data showed that THC treatment significantly decreased estrous cycling during both the chronic phase [$F(1,36)=4.52$, $p<0.05$] and the post-chronic phase [$F(1,36)=9.60$, $p<0.05$]. The number of females showing evidence of normal estrous cycling also dropped dramatically from the pre-chronic to chronic phases, and at least partially rebounded from chronic to post-chronic phases in THC-treated groups compared to vehicle-treated groups (Table 3).

4. DISCUSSION

Previous studies have shown that acute administration of THC produces a profile of effects in rodents of both sexes, including locomotor decreases, antinociception, hypothermia, and catalepsy (Martin et al., 1991; Wiley et al., 2007). This profile was not observed here in either sex, except that male rats showed significant locomotor decreases following acute THC. Female rats did not show effects in any of these assays (catalepsy was not assessed). Although several possible procedural differences may account for the absence of typical THC-like acute effects, the most probable explanation is the mismatch between the s.c. route of administration and the short (30 min) pre-testing injection interval. Previous studies examining THC in the tetrad model have primarily used i.p. (Wiley et al., 2007) or intravenous (Martin et al., 1991) administration, often with longer pre-testing injection intervals in the former instance. Hence, it is quite likely that more typical THC-like effects would have been evident with a longer delay between injection and testing. Importantly, however, administering 30 mg/kg THC s.c. did not incapacitate rats of either sex. Tolerance developed to the locomotor decreases observed after acute THC in male rats in the present study, which is consistent with previous studies showing rapid tolerance to pharmacological effects of THC (Bass and Martin, 2000).

Cannabinoid dependence has been difficult to detect due to the paucity of robust withdrawal signs upon termination of THC administration (Compton et al., 1990), although spontaneous withdrawal was noted with more sensitive measures in rhesus monkeys (Beardsley et al., 1986) and adolescent rats (Harte-Hargrove and Dow-Edwards, 2012). The discovery of rimonabant facilitated demonstration of dependence by producing precipitated withdrawal (Tsou et al., 1995; Cook et al., 1998); however, rimonabant produces effects when given alone, including pruritis (present study; Schlosburg et al., 2011), increased locomotion (Bass et al., 2002), and appetite suppression (Thornton-Jones et al., 2006). Somatic signs of precipitated withdrawal in the THC-treated rats were also observed, including paw tremors and head twitches. No sex differences in these signs were noted here or in mice (Falenski et al., 2010); however, here, another somatic sign (retropulsion) occurred more frequently in female rats. Retropulsion usually occurs with higher inducing doses of THC and has been reported only rarely during withdrawal (Aceto et al., 1996; Gonzalez et al., 2004). Retropulsion is associated with imbalance between dopamine and serotonin neurotransmission (Curzon et al., 1979; Dawbarn et al., 1981; Andrews et al., 1982), as may occur in THC-dependent rats due to decreases in mesolimbic dopamine neurotransmission (Diana et al., 1998; Tanda et al., 1999). Dopamine function may also be affected via dependence-induced changes in the endocannabinoid system (Spiga et al., 2011), which interacts with dopamine neurotransmission indirectly (Patel et al., 2003; Melis et al., 2004). A plethora of research has demonstrated that females are more sensitive to pharmacological manipulations of the dopamine system (e.g., Barbui et al., 2005; Walker et al., 2006; Becker and Hu, 2008; Kritzer and Creutz, 2008).

In contrast to previous studies showing rimonabant-induced locomotor increases in male rodents (Bass et al., 2002), rimonabant did not affect locomotion in vehicle-treated rats of either sex in the present study. These disparate results may be caused by the rimonabant dose, species differences (rat vs. mouse), and/or effects produced by extensive handling prior to rimonabant administration in the present study. During the first 5-min bin, locomotor activity was similar for all groups. Although habituation across bins occurred in the Veh/THC, Veh/Rim, and THC/Veh conditions, it was not observed in rats in the THC/Rim condition. No sex differences in this effect were observed.

Most cannabinoid dependence studies in rodents have focused on somatic signs of withdrawal (Tsou et al., 1995; Cook et al., 1998; Lichtman et al., 2001), with less emphasis

on learned behavior. Intrasession habituation reflects changes in behavior resulting from the rodent learning about its environment and is affected by genetic factors and several neurotransmitter systems, including serotonin, acetylcholine, and glutamate (Leussis and Bolivar, 2006). Interference of cannabinoid withdrawal in habituation-related learning may implicate endocannabinoid involvement in this process. Previous research suggests that precipitated withdrawal may also disrupt spatial memory in rodents, an effect accompanied by changes in endocannabinoid signaling mechanisms in the cerebellum (Wise et al., 2011).

Acoustic startle results in the present study suggest that early attentional processes may be impaired during THC withdrawal. Acoustic startle produces a reflex following a sudden loud noise and measures sensorimotor reactivity (Davis et al., 1980), whereas PPI is a decrease in the magnitude of this response after presentation of a weak acoustic stimulus milliseconds before the sudden loud noise (Hoffman and Ison, 1980). All groups treated chronically with THC exhibited increased startle amplitude, suggesting that spontaneous and precipitated withdrawal affected sensorimotor reactivity, which may be indicative of withdrawal-induced anxiogenesis. In support of this hypothesis, cannabinoid-dependent rodents undergoing withdrawal showed decreased open arm time in the elevated plus-maze (Huang et al., 2010; Harte-Hargrove and Dow-Edwards, 2012). Conversely, spontaneous withdrawal from the cannabinoid WIN55,212-2 did not alter startle amplitude in rats (Bortolato et al., 2005). The divergence between these results and those of the present study may be related to different treatment durations, different timing between injection and startle assessment, or differences in cannabinoid efficacy (i.e., the full agonist, WIN55,212-2, versus the partial agonist, THC).

Withdrawal-induced disruption of PPI was observed in the present study in both sexes in the THC/Rim group. PPI reflects integration of sensory and motor processes in the brain and is a measure of early pre-attentional processes (Swerdlow et al., 2000). Lack of an effect on PPI in the THC/Veh group is consistent with termination of repeated administration of WIN55,212-2 also failing to alter PPI in male rats (Bortolato et al., 2005). Interestingly, male rats repeatedly exposed to WIN55,212-2 during adolescence, but not during adulthood, exhibited long-lasting deficits in PPI (Schneider and Koch, 2003). Therefore, disruption of sensorimotor gating by cannabinoid dependence may occur under specific circumstances such as early life exposure or upon antagonist-precipitated withdrawal, with both sexes being vulnerable to this effect.

Of the three physiological measures included here (rectal temperature, nociception, and body weight), only body weight was affected by chronic THC treatment. Whereas steady weight gain over time was observed for vehicle-treated rats of both sexes, male and female rats lost weight during chronic THC treatment. Previous results suggest that THC produces a biphasic effect on food consumption in rodents (Wiley et al., 2005) with moderate doses increasing consumption, and higher doses (such as those used in this dependence procedure) decreasing consumption. Therefore, the weight loss observed during chronic THC administration in the present study is most likely related to decreases in food consumption, although this variable was not directly measured.

Another physiological correlate of chronic THC administration in the present study was suppression of estrous cycling. This finding corroborates previous studies demonstrating that cannabinoid exposure disrupted estrous cycling (O'Connell et al., 1987) and decreased sexual receptivity (Kostellow et al., 1980; Ferrari et al., 2000) in female rodents, and suppressed menstrual cycling in monkeys (Smith et al., 1983). Cannabinoids inhibit release of the luteinizing hormone from the hypothalamus, so that ovulation does not occur (Ayalon et al., 1977; Field and Tyrey, 1986). Similarly, marijuana smoking decreases luteinizing hormone levels in women (Mendelson et al., 1986). The fact that THC-treated rats showed

disrupted estrous cycling during the post-chronic phase suggests that either recovery takes longer than 8 days once THC treatment stops, and/or withdrawal from THC also disrupts estrous cycling. Other studies have shown that reproductive indices in females can take weeks to return to normal after repeated cannabinoid exposure (Smith et al., 1983; O'Connell et al., 1987). To our knowledge there are no published studies examining estrous cycling following precipitated cannabinoid withdrawal.

In summary, rimonabant precipitated a pronounced withdrawal syndrome in THC-dependent male and female rats. This syndrome was characterized by changes in a number of functional domains, including somatic (increased paw tremors, head twitches, and retropulsion), early-stage cognition (lack of locomotor habituation, disrupted PPI), and affective (increased acoustic startle reactivity). Sex differences in these effects were not observed, except female rats showed increased incidence of retropulsion. Additionally, estrous cycling in female rats was disrupted during THC dosing. This study represents the first systematic examination of THC dependence in gonadally intact adult rats of both sexes and extends previous findings in male rodents to females. The scope of changes that occur during antagonist-precipitated withdrawal from THC extends beyond somatic signs to disruptions of cognitive and affective functioning. The breadth of withdrawal signs observed in rodents mirrors those that have been observed in human cannabis users undergoing withdrawal, including physical signs (Gorelick et al., 2012), affective discomfort (Haney et al., 1999; Hasin et al., 2008), and cognitive deficits (Schreiner and Dunn, 2012), and may reflect the broad spectrum of physiological functions in which the endocannabinoid system is involved.

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REFERENCES

- Aceto MD, Scates SM, Lowe JA, Martin BR. Dependence on delta 9-tetrahydrocannabinol: studies on precipitated and abrupt withdrawal. *J. Pharmacol. Exp. Ther.* 1996; 278:1290–1295. [PubMed: 8819514]
- Aggarwal SK, Carter GT, Sullivan MD, ZumBrunner C, Morrill R, Mayer JD. Medicinal use of cannabis in the United States: historical perspectives, current trends, and future directions. *J. Opioid Manag.* 2009; 5:153–168. [PubMed: 19662925]
- Andrews CD, Fernando JC, Curzon G. Differential involvement of dopamine-containing tracts in 5-hydroxytryptamine-dependent behaviours caused by amphetamine in large doses. *Neuropharmacology.* 1982; 21:63–68. [PubMed: 6801538]
- Ayalon D, Nir I, Cordova T, Bauminger S, Puder M, Naor Z, Kashi R, Zor U, Harel A, Lindner HR. Acute effect of delta1-tetrahydrocannabinol on the hypothalamo-pituitary-ovarian axis in the rat. *Neuroendocrinology.* 1977; 23:31–42. [PubMed: 331132]
- Barbui C, Nose M, Bindman J, Schene A, Becker T, Mazzi MA, Kikkert M, Camara J, Born A, Tansella M. Sex differences in the subjective tolerability of antipsychotic drugs. *J. Clin. Psychopharmacol.* 2005; 25:521–526. [PubMed: 16282831]
- Bass CE, Griffin G, Grier M, Mahadevan A, Razdan RK, Martin BR. SR-141716A-induced stimulation of locomotor activity. A structure-activity relationship study. *Pharmacol. Biochem. Behav.* 2002; 74:31–40. [PubMed: 12376150]

- Bass CE, Martin BR. Time course for the induction and maintenance of tolerance to Delta(9)-tetrahydrocannabinol in mice. *Drug Alcohol Depend.* 2000; 60:113–119. [PubMed: 10940538]
- Beardsley PM, Balster RL, Harris LS. Dependence on tetrahydrocannabinol in rhesus monkeys. *J. Pharmacol. Exp. Ther.* 1986; 239:311–319. [PubMed: 3021952]
- Beardsley PM, Martin BR. Effects of the cannabinoid CB(1) receptor antagonist, SR141716A, after Delta(9)-tetrahydrocannabinol withdrawal. *Eur. J. Pharmacol.* 2000; 387:47–53. [PubMed: 10633159]
- Becker JB, Hu M. Sex differences in drug abuse. *Front. Neuroendocrinol.* 2008; 29:36–47.
- Bortolato M, Aru GN, Frau R, Orru M, Luckey GC, Boi G, Gessa GL. The CB receptor agonist WIN 55,212-2 fails to elicit disruption of prepulse inhibition of the startle in Sprague-Dawley rats. *Psychopharmacology (Berl.)*. 2005; 177:264–271. [PubMed: 15290008]
- Carroll ME, Lynch WJ, Roth ME, Morgan AD, Cosgrove KP. Sex and estrogen influence drug abuse. *Trends Pharmacol. Sci.* 2004; 25:273–279. [PubMed: 15120494]
- Compton DR, Dewey WL, Martin BR. Cannabis dependence and tolerance production. *Adv. Alcohol Subst. Abuse.* 1990; 9:129–147. [PubMed: 2165734]
- Cook SA, Lowe JA, Martin BR. CB1 receptor antagonist precipitates withdrawal in mice exposed to Delta9-tetrahydrocannabinol. *J. Pharmacol. Exp. Ther.* 1998; 285:1150–1156. [PubMed: 9618417]
- Craft RM, Marusich JA, Wiley JL. Sex differences in cannabinoid pharmacology: a reflection of differences in the endocannabinoid system? *Life Sci.* 2013a; 92:476–481. [PubMed: 22728714]
- Craft RM, Wakley AA, Tsutsui KT, Laggart JD. Sex differences in cannabinoid 1 vs. cannabinoid 2 receptor-selective antagonism of antinociception produced by delta9-tetrahydrocannabinol and CP55,940 in the rat. *J. Pharmacol. Exp. Ther.* 2013b; 340:787–800. [PubMed: 22182934]
- Curzon G, Fernando JC, Lees AJ. Backward walking and circling: behavioural responses induced by drug treatments which cause simultaneous release of catecholamines and 5-hydroxytryptamine. *Br. J. Pharmacol.* 1979; 66:573–579. [PubMed: 465894]
- Davis M, Strachan DI, Kass E. Excitatory and inhibitory effects of serotonin on sensorimotor reactivity measured with acoustic startle. *Science.* 1980; 209:521–523. [PubMed: 7394520]
- Dawbarn D, Long SK, Pycock CJ. Increased central 5-hydroxytryptamine receptor mechanisms in rats after chronic neuroleptic treatment. *Br. J. Pharmacol.* 1981; 73:149–156. [PubMed: 6116511]
- Diana M, Melis M, Muntoni AL, Gessa GL. Mesolimbic dopaminergic decline after cannabinoid withdrawal. *Proc. Natl. Acad. Sci. USA.* 1998; 95:10269–10273. [PubMed: 9707636]
- Falenski KW, Thorpe AJ, Schlosburg JE, Cravatt BF, Abdullah RA, Smith TH, Selley DE, Lichtman AH, Sim-Selley LJ. FAAH^{-/-} mice display differential tolerance, dependence, and cannabinoid receptor adaptation after delta 9-tetrahydrocannabinol and anandamide administration. *Neuropsychopharmacology.* 2010; 35:1775–1787. [PubMed: 20357755]
- Fattore L, Spano MS, Altea S, Angius F, Fadda P, Fratta W. Cannabinoid self-administration in rats: sex differences and the influence of ovarian function. *Br. J. Pharmacol.* 2007; 152:795–804. [PubMed: 17891164]
- Ferrari F, Ottani A, Giuliani D. Inhibitory effects of the cannabinoid agonist HU210 on rat sexual behavior. *Physiol. Behav.* 2000; 69:547–554. [PubMed: 10913795]
- Field E, Tyrey L. Tolerance to the luteinizing hormone and prolactin suppressive effects of Δ^9 -tetrahydrocannabinol develops during chronic prepubertal treatment of female rats. *J. Pharmacol. Exp. Ther.* 1986; 238:1034–1038. [PubMed: 3018216]
- Freeman, ME. The ovarian cycle of the rat. In: Knobil, E.; Neill, J., editors. *The Physiology of Reproduction*. New York, New York: Raven Press; 1988. p. 1893–1928.
- Gonzalez S, Fernandez-Ruiz J, Di Marzo V, Hernandez M, Arevalo C, Nicanor C, Cascio MG, Ambrosio E, Ramos JA. Behavioral and molecular changes elicited by acute administration of SR141716 to Delta9-tetrahydrocannabinol-tolerant rats: an experimental model of cannabinoid abstinence. *Drug Alcohol Depend.* 2004; 74:159–170. [PubMed: 15099659]
- Gorelick DA, Levin KH, Copersino ML, Heishman SJ, Liu F, Boggs DL, Kelly DL. Diagnostic criteria for cannabis withdrawal syndrome. *Drug Alcohol Depend.* 2012; 123:141–147. [PubMed: 22153944]
- Haney M, Ward AS, Comer SD, Foltin RW, Fischman MW. Abstinence symptoms following smoked marijuana in humans. *Psychopharmacology (Berl.)*. 1999; 141:395–404. [PubMed: 10090647]

- Harte-Hargrove LC, Dow-Edwards DL. Withdrawal from THC during adolescence: sex differences in locomotor activity and anxiety. *Behav. Brain Res.* 2012; 231:48–59. [PubMed: 22421367]
- Hasin DS, Keyes KM, Alderson D, Wang S, Aharonovich E, Grant BF. Cannabis withdrawal in the United States: results from NESARC. *J. Clin. Psychiatry.* 2008; 69:1354–1363. [PubMed: 19012815]
- Hill AJ, Williams CM, Whalley BJ, Stephens GJ. Phytocannabinoids as novel therapeutic agents in CNS disorders. *Pharmacol. Ther.* 2012; 133:79–97. [PubMed: 21924288]
- Hoffman HS, Ison JR. Reflex modification in the domain of startle: I. Some empirical findings and their implications for how the nervous system processes sensory input. *Psychol. Rev.* 1980; 87:175–189. [PubMed: 7375610]
- Huang P, Liu-Chen LY, Kirby LG. Anxiety-like effects of SR141716-precipitated delta9-tetrahydrocannabinol withdrawal in mice in the elevated plus-maze. *Neurosci. Lett.* 2010; 475:165–168. [PubMed: 20363293]
- Kostellow AB, Ziegler D, Kunar J, Fujimoto GI, Morrill GA. Effect of cannabinoids on estrous cycle, ovulation and reproductive capacity of female A/J mice. *Pharmacology.* 1980; 21:68–75. [PubMed: 6250172]
- Kritzer MF, Creutz LM. Region and sex differences in constituent dopamine neurons and immunoreactivity for intracellular estrogen and androgen receptors in mesocortical projections in rats. *J. Neurosci.* 2008; 28:9525–9535. [PubMed: 18799684]
- Leussis MP, Bolivar VJ. Habituation in rodents: a review of behavior, neurobiology, and genetics. *Neurosci. Biobehav. Rev.* 2006; 30:1045–1064. [PubMed: 16774787]
- Levin KH, Copersino ML, Heishman SJ, Liu F, Kelly DL, Boggs DL, Gorelick DA. Cannabis withdrawal symptoms in non-treatment-seeking adult cannabis smokers. *Drug Alcohol Depend.* 2010; 111:120–127. [PubMed: 20510550]
- Lichtman AH, Fisher J, Martin BR. Precipitated cannabinoid withdrawal is reversed by Delta(9)-tetrahydrocannabinol or clonidine. *Pharmacol. Biochem. Behav.* 2001; 69:181–188. [PubMed: 11420084]
- Martin BR, Compton DR, Thomas BF, Prescott WR, Little PJ, Razdan RK, Johnson MR, Melvin LS, Mechoulam R, Ward SJ. Behavioral, biochemical, and molecular modeling evaluations of cannabinoid analogs. *Pharmacol. Biochem. Behav.* 1991; 40:471–478. [PubMed: 1666911]
- Melis M, Perra S, Muntoni AL, Pillolla G, Lutz B, Marsicano G, Di Marzo V, Gessa GL, Pistis M. Prefrontal cortex stimulation induces 2-arachidonoyl-glycerol-mediated suppression of excitation in dopamine neurons. *J. Neurosci.* 2004; 24:10707–10715. [PubMed: 15564588]
- Mendelson JH, Mello NK, Ellingboe J, Skupny AS, Lex BW, Griffin M. Marijuana smoking suppresses luteinizing hormone in women. *J. Pharmacol. Exp. Ther.* 1986; 237:862–866. [PubMed: 3012072]
- O'Connell ME, Morrill GA, Fujimoto GI, Kostellow AB. Factors affecting the response of the female rat reproductive system to cannabinoids. *Toxicol. Appl. Pharmacol.* 1987; 88:411–417. [PubMed: 3033848]
- Patel S, Rademacher DJ, Hillard CJ. Differential regulation of the endocannabinoids anandamide and 2-arachidonoylglycerol within the limbic forebrain by dopamine receptor activity. *J. Pharmacol. Exp. Ther.* 2003; 306:880–888. [PubMed: 12808005]
- Schlosburg JE, O'Neal ST, Conrad DH, Lichtman AH. CB1 receptors mediate rimonabant-induced pruritic responses in mice: investigation of locus of action. *Psychopharmacology (Berl.)*. 2011; 216:323–331. [PubMed: 21340468]
- Schneider M, Koch M. Chronic pubertal, but not adult chronic cannabinoid treatment impairs sensorimotor gating, recognition memory, and the performance in a progressive ratio task in adult rats. *Neuropsychopharmacology.* 2003; 28:1760–1769. [PubMed: 12888772]
- Schreiner AM, Dunn ME. Residual effects of cannabis use on neurocognitive performance after prolonged abstinence: a meta-analysis. *Exp. Clin. Psychopharmacol.* 2012; 20:420–429. [PubMed: 22731735]
- Smith CG, Almirez RG, Berenber J, Asch RH. Tolerance develops to the disruptive effects of Δ^9 -tetrahydrocannabinol on primate menstrual cycle. *Science.* 1983; 219:1453–1455. [PubMed: 6298938]

- Spiga S, Lintas A, Diana M. Altered mesolimbic dopamine system in THC dependence. *Curr. Neuropharmacol.* 2011; 9:200–204. [PubMed: 21886590]
- Substance Abuse and Mental Health Services Administration. Results from the 2012 National Survey on Drug Use and Health: Summary of National Findings, in NSDUH Series H-46, HHS Publication No. (SMA) 13-4795. Rockville, MD: Substance Abuse and Mental Health Services Administration; 2013.
- Swerdlow NR, Braff DL, Geyer MA. Animal models of deficient sensorimotor gating: what we know, what we think we know, and what we hope to know soon. *Behav. Pharmacol.* 2000; 11:185–204. [PubMed: 11103873]
- Tanda G, Loddo P, Di Chiara G. Dependence of mesolimbic dopamine transmission on delta9-tetrahydrocannabinol. *Eur. J. Pharmacol.* 1999; 376:23–26. [PubMed: 10440085]
- Thornton-Jones ZD, Kennett GA, Benwell KR, Revell DF, Misra A, Sellwood DM, Vickers SP, Clifton PG. The cannabinoid CB1 receptor inverse agonist, rimonabant, modifies body weight and adiponectin function in diet-induced obese rats as a consequence of reduced food intake. *Pharmacol. Biochem. Behav.* 2006; 84:353–359. [PubMed: 16814374]
- Tseng AH, Craft RM. Sex differences in antinociceptive and motoric effects of cannabinoids. *Eur. J. Pharmacol.* 2001; 430:41–47. [PubMed: 11698061]
- Tsou K, Patrick S, Walker JM. Physical withdrawal in rats tolerant to Δ^9 -tetrahydrocannabinol precipitated by a cannabinoid receptor antagonist. *Eur. J. Pharmacol.* 1995; 280:R13–R15. [PubMed: 8566091]
- Walker QD, Ray R, Kuhn CM. Sex differences in neurochemical effects of dopaminergic drugs in rat striatum. *Neuropsychopharmacology.* 2006; 31:1193–1202. [PubMed: 16237396]
- Walsh D, Nelson KA, Mahmoud FA. Established and potential therapeutic applications of cannabinoids in oncology. *Support. Care Cancer.* 2003; 11:137–143. [PubMed: 12618922]
- Wiley JL, Burston JJ, Leggett DC, Alekseeva OO, Razdan RK, Mahadevan A, Martin BR. CB1 cannabinoid receptor-mediated modulation of food intake in mice. *Br. J. Pharmacol.* 2005; 145:293–300. [PubMed: 15778743]
- Wiley JL, Walentiny MD, Vann RE, Baskfield CY. Dissimilar cannabinoid substitution patterns in mice trained to discriminate Delta(9)-tetrahydrocannabinol or methanandamide from vehicle. *Behav. Pharmacol.* 2011; 22:480–488. [PubMed: 21712709]
- Wiley JL, O'Connell MM, Tokarz ME, Wright MJ Jr. Pharmacological effects of acute and repeated administration of {delta}9-tetrahydrocannabinol in adolescent and adult rats. *J. Pharmacol. Exp. Ther.* 2007; 320:1097–1105. [PubMed: 17172531]
- Williamson EM, Evans FJ. Cannabinoids in clinical practice. *Drugs.* 2000; 60:1303–1314. [PubMed: 11152013]
- Wise LE, Varvel SA, Selley DE, Wiebelhaus JM, Long KA, Middleton LS, Sim-Selley LJ, Lichtman AH. delta(9)-Tetrahydrocannabinol-dependent mice undergoing withdrawal display impaired spatial memory. *Psychopharmacology (Berl.).* 2011; 217:485–494. [PubMed: 21559804]

Figure 1.

Effects of vehicle and THC (30 mg/kg, s.c.) on locomotor activity in male (filled bars) and female (open bars) rats on days 1 and 6 (left and right sides of panel, respectively). Shown are mean ($\pm$ SEM) numbers of beam breaks during 5-min locomotor activity sessions for 9–10 rats of each sex. Day 1 shows the acute effects of vehicle and THC, whereas day 6 shows the effects of vehicle and THC after the repeated dosing paradigm. Three-way ANOVA showed a significant day $\times$ treatment $\times$ sex interaction, which was further analyzed with separate treatment $\times$ sex ANOVA for each day followed by Tukey post hoc tests when appropriate. # Tukey post hoc test indicates significant differences ($p < 0.05$) compared to vehicle for same sex and compared to vehicle and THC for females (i.e., compared to all of the other filled bars).

Figure 2.

Effects of day 7 challenge with vehicle or rimonabant (10 mg/kg, i.p.) on locomotor activity in male (top panel) and female (bottom panel) rats treated repeatedly with vehicle or with THC. Legend on graph shows treatment conditions and is in the format of repeated treatment (Veh or THC) / challenge (Veh or Rim). Shown are mean ($\pm$ SEM) numbers of beam breaks during three consecutive 5-min bins of a 15-min session for 9–10 rats of each sex. # indicates a significant difference compared to Veh/Veh condition during the same bin for both sexes, as based upon Tukey post hoc test ($p < 0.05$) following significant treatment $\times$ bin interaction in the three-way (treatment, bin, sex) ANOVA. In addition, a significant

effect of bin (compared to bin 1) was observed for all of the treatment conditions except for the THC/Rim condition.

Figure 3.

Effects of day 7 challenge with vehicle or rimonabant (10 mg/kg, i.p.) on overt behaviors in male (filled bars) and female (open bars) rats treated repeatedly with vehicle or THC. Shown are mean ($\pm$ SEM) numbers of scratching bouts (panel A), forepaw tremors (panel B), head twitches (panel C), and incidents of retropulsion (panel D) for 10 rats of each sex. * indicates significant main effect of treatment condition, as compared to Veh/Veh condition. # indicates significant difference from Veh/Veh condition for the same sex. \$ indicates significant difference compared to opposite sex in the same treatment group. Significant differences based upon significant ANOVA with follow up Tukey post hoc tests ($p < 0.05$).

Figure 4.

Effects of day 7 challenge with vehicle or rimonabant (10 mg/kg, i.p.) on acoustic startle response (top panel) and percent prepulse inhibition (bottom panel) in male (filled bars) and female (open bars) rats treated repeatedly with vehicle or THC. Mean values ($\pm$ SEM) are shown for 10 rats per sex. * indicates significant main effect of treatment condition, as compared to Veh/Veh condition ($p < 0.05$).

Figure 5.

Body weights of male (top panel) and female (bottom panel) rats at the beginning of the study (day 1), on challenge day (day 7) and on the last day of the study (day 15). Legend on graph shows treatment conditions and is in the format of repeated treatment (Veh or THC) / challenge (Veh or Rim). Shown are mean values ($\pm$ SEM) of data for 10 rats of each sex. Data for each sex were analyzed separately. * indicates significant difference compared to day 1 in the same group of rats (interaction $p < 0.05$). # indicates significant difference compared to Veh/Veh group on the same day (interaction $p < 0.05$). Significant differences based upon significant ANOVA with follow up Tukey post hoc tests ($p < 0.05$).

Figure 6.

Percent time female rats spent in proestrus or estrus, estimated by the percent of days on which vaginal cytology samples indicated proestrus or estrus (or in between the two stages), during each of the three phases of the study (N=10 rats/group). The pre-chronic phase was the 8-day period before the start of daily vehicle/THC injections (this included the first day of daily injections since lavage was conducted prior to any injections); the chronic phase was the 6-day period during which vehicle (open bars) or THC (gray bars) was administered daily (days 2–7 of chronic dosing); the post-chronic phase was the 8-day period following the chronic phase, during which no injections were given. * indicates significant suppression of cycling ($p < 0.05$) in THC-treated rats (THC/Veh and THC/Rim) compared to vehicle-treated rats (Veh/Veh and Veh/Rim). Significant differences based upon significant ANOVA with follow up Tukey post hoc tests ($p < 0.05$).

Table 1

Substance administered for each injection for all drug groups.

Treatment Group	Days 1–6		Day 7	
	1 st injection	2 nd injection	1 st injection	2 nd injection
Veh/Veh	Vehicle	Vehicle	Vehicle	Vehicle
Veh/Rim	Vehicle	Vehicle	Vehicle	Rimonabant (10 mg/kg)
THC/Veh	THC (30 mg/kg)	THC (30 mg/kg)	THC (30 mg/kg)	Vehicle
THC/Rim	THC (30 mg/kg)	THC (30 mg/kg)	THC (30 mg/kg)	Rimonabant (10 mg/kg)

Table 2

Incidents of specific behaviors in the functional observational battery in male and female rats during the challenge test on day 7.*

Treatment Group / Sex	Veh/Veh		Veh/Rim		THC/Veh		THC/Rim	
	M	F	M	F	M	F	M	F
Behaviors								
Grooming	11 (4.3)	11 (2.4)	15 (3.0)	18 (3.3)	6 (1.2)	10 (1.7)	17 (2.5)	16 (2.2)
Wet dog shakes	2 (1.2)	1 (0.6)	3 (0.7)	4 (1.5)	0 (0.1)	0 (0.1)	8 (4.3)	4 (1.1)
Sniffing	8 (1.3)	6 (1.1)	6 (1.1)	7 (1.3)	4 (0.7)	5 (1.4)	10 (2.0)	10 (2.0)
Prosis	5 (1.4)	3 (0.6)	8 (2.3)	5 (1.2)	5 (0.9)	7 (1.2)	6 (1.5)	7 (1.4)
Writhing	2 (1.3)	1 (1.0)	3 (1.8)	2 (1.6)	0 (0)	0 (0.1)	7 (4.4)	4 (2.7)
Piloerection	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0.1)	0 (0.2)
Vocalizations	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Urination	0 (0.1)	0 (0.2)	0 (0.2)	1 (0.2)	0 (0)	0 (0.2)	0 (0.2)	0 (0.2)
Defecation	0 (0)	1 (0.6)	1 (0.3)	1 (0.5)	0 (0.1)	0 (0)	0 (0.3)	0 (0)

* Data for each behavior expressed as number of incidences ($\pm$ SEM) that occurred during the functional observational battery assessment on Day 7 (see Procedures for further details).

Table 3

Number of females showing evidence of normal estrous cycling (out of 10 rats in each treatment group), during each phase of the study.

Treatment Group	Pre-chronic Phase	Chronic Phase	Post-chronic Phase
Veh/Veh	4	5	9
Veh/Rim	7	4	9
THC/Veh	7	1	7
THC/Rim	9	1	4