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Evaluation of sex differences in cannabinoid dependence

Julie A. Marusich[a], Timothy W. Lefever[a], Kateland R. Antonazzo[a], Rebecca M. Craft[b], Jenny L. Wiley[a,*]

[a] RTI International, Research Triangle Park, NC 27709-2194, USA
[b] Department of Psychology, Washington State University, Pullman, WA 99164-4820, USA

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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.

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

* Corresponding author at: RTI International, 3040 Cornwallis Road, Research Triangle Park, NC 27709-2194, USA. Tel.: +1 919 541 7276; fax: +1 919 541 6499.
E-mail address: jwiley@rti.org (J.L. Wiley).

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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 CB_1 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 40 female Sprague–Dawley rats (Harlan, Dublin, VA) were housed in polycarbonate cages in a temperature-controlled (20–22 °C) environment with a 12 h 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 h apart for days 1–6. On day 7, the second injection of the day was given approximately 4 h 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.

Table 1
Substance administered for each injection for all drug groups.

Treatment group	Days 1–6		Day 7	
	1st injection	2nd injection	1st injection	2nd 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)

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 minutes 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 hours later, the second injection (challenge condition) was given. Five minutes 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.