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Sex differences in antinociceptive tolerance to delta-9-tetrahydrocannabinol in the rat

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Abstract

Background—Sex differences in cannabinoid effects have been reported in rodents, with adult females typically being more sensitive than adult males. The present study compared the development of antinociceptive tolerance to delta-9-tetrahydrocannabinol (THC) in adult, gonadally intact female vs. male rats.

Methods—Cumulative dose-effect curves were obtained for THC (1.0–18 mg/kg *i.p.*) on warm water tail withdrawal and paw pressure tests. Vehicle or the sex-specific ED₈₀ dose for THC was administered twice daily for 9 days; THC dose-effect curves were then re-determined.

Results—On the pre-chronic test day, THC was significantly more potent in females than males in producing antinociception on the tail withdrawal and paw pressure tests. After 9 days of twice-daily THC treatment (5.4 mg/kg/injection in females, 7.6 mg/kg/injection in males), THC potency on both tests decreased more in females than males. On the tail withdrawal test, chronic THC produced 4.2- vs. 2.8-fold increases in ED₅₀ values in females vs. males, respectively. On the paw pressure test, chronic THC produced 4.4- vs. 2.9-fold increases in ED₅₀ values in females vs. males, respectively. Chronic THC treatment did not significantly disrupt estrous cycling in females.

Conclusions—These results demonstrate that – even when sex differences in acute THC potency are controlled for – females develop more antinociceptive tolerance to THC than males. Given the importance of drug tolerance in the development of drug dependence, these results suggest that females may be more vulnerable than males to developing dependence after chronic cannabinoid exposure.

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Contributors

Authors Craft, Wiley and Wakley participated in the research design. Author Wakley conducted experiments and performed data analysis. Authors Wakley, Craft and Wiley wrote or contributed to the writing of the manuscript and approved the final manuscript. All authors discussed the results and commented on the manuscript.

Conflicts of Interest

The authors have not conflicts of interest to declare.

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Keywords

Sex Differences; Cannabinoid; Tolerance; Antinociception; Locomotion; Pain; Estrous Cycle

1. INTRODUCTION

Sex bias in biomedical research is now well-documented. For example, a majority of non-human animal studies published in 2009 in the fields of neuroscience and pharmacology included only male subjects (Beery and Zucker, 2011). Even in fields such as pain, in which there is a predominance of women in clinical populations, most preclinical research has been conducted in males (Mogil and Chanda, 2005). Given the known sex differences in pain (Fillingim et al., 2009; Unruh, 1996) and drug abuse/dependence (Carroll et al., 2004), examination of sex differences in the effects of analgesics with abuse liability is warranted. The purpose of the present study was to determine whether females and males develop differential tolerance to the antinociceptive effects of a cannabinoid. Characterizing drug tolerance is important not only as it relates to a drug's abuse/dependence potential (Marusich et al., 2013), but also, in the case of analgesics, to their ability to continue to relieve pain with repeated use.

Sex differences in the behavioral effects of cannabinoids have been reported in rodents. Females are more sensitive than males to the reinforcing, anxiogenic, spatial memory-impairing, hypothermic, antinociceptive and sedative effects of cannabinoids (Cha et al., 2007; Craft et al., 2012; Fattore et al., 2007; Harte-Hargrove and Dow-Edwards, 2012; Romero et al., 2002; Tseng and Craft, 2001). For example, *i.p.* -9-tetrahydrocannabinol (THC) was 2–3 times more potent in female compared to male rats against acute thermal and mechanical pain (Craft et al., 2012; Tseng and Craft, 2001), and significantly more effective in females than males against thermal hyperalgesia in a chronic inflammatory pain model (Craft et al., 2013).

Despite the known sexual dimorphism in acute cannabinoid effects, cannabinoid tolerance has been investigated primarily in males (Bass and Martin, 2000; McKinney et al., 2008; Whitlow et al., 2003; Wiley et al., 1993). The few exceptions were all from the same lab (Wiley, 2003; Wiley et al., 2011, 2007). In the first study (Wiley, 2003), female mice were more sensitive than males to acute THC-induced hyperlocomotion; after 2.5 days of twice-daily THC injections, only females showed dose-dependent changes in THC's effect. A later study in rats focused primarily on developmental differences (adolescent vs. adult), but the inclusion of both sexes allowed for the assessment of sex differences (Wiley et al., 2007). This study reported no significant sex differences between age-matched males and females in the tetrad (i.e., hypolocomotion, antinociception, hypothermia and catalepsy); however, adult females were more sensitive to THC-induced antinociception and catalepsy and also developed greater tolerance to THC's antinociceptive effects than adolescent females (Wiley et al., 2007). In contrast, adult males were more sensitive than adolescent males to locomotor suppressant and hypothermic effects following chronic THC administration, suggesting that age-dependent sex differences are also assay-dependent. In this study, quantification of tolerance development was difficult. First, tolerance was so great in the adult rats on the post-chronic test day that many of the THC dose-effect curves were

flattened (highest dose produced < 50% Maximum Possible Effect (MPE)), precluding accurate quantification of potency changes over time. Second, the same dose of THC was used to induce tolerance in both sexes, which may not be optimal given sex differences in acute THC potency. Previous work on sex differences in opioid tolerance has shown that individual differences in acute morphine potency predict individual (including sex) differences in the magnitude of tolerance development to morphine (Barrett et al., 2001). Thus, to compare tolerance development between groups that differ in acute drug sensitivity, it may be appropriate to adjust the tolerance-inducing dose.

The aim of the present study was to determine if adult male and female rats develop differential tolerance to THC-induced antinociception. Using a cumulative dosing procedure, THC's antinociceptive and sedative effects were measured on the pre-chronic test day. Then rats were given THC twice-daily for 9 days to induce tolerance. On the post-chronic test day, THC potency was reevaluated. Because there are sex differences in THC's acute antinociceptive potency (Craft et al., 2012; Tseng and Craft, 2001), sex-specific ED₈₀ doses (determined in a pilot study) were administered twice-daily to induce tolerance. Additionally, because little previous work has examined estrous cycling during chronic THC treatment, vaginal cytology was assessed to determine THC's effects on estrous cycling in the female rat.

2. METHODS

2.1 Subjects

Male and female Sprague-Dawley rats (60–100 days old) were used (Taconic, Germantown, NY). *Ad libitum* access to food and water was provided except during testing. The vivarium was maintained at 21±2°C on a 12:12 h light:dark cycle with lights on at 0700. Rats used in this study were treated in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

2.2 Apparatus

Tail withdrawal antinociception was assessed using a 2.5-L water bath (Precision Scientifics Inc., Winchester, VA) maintained at 50±0.5°C. Paw pressure antinociception was assessed using an analgesy-meter (Ugo-Basile, Varese, Italy). The pressure on the paw began at 30 g and increased at a constant rate of 48 g/s to a maximum of 750 g. Catalepsy was measured using a bar test (1.5-cm diameter horizontal bar set at 12 cm for females and 15 cm for males above the table surface). Horizontal locomotor activity was measured using a photobeam apparatus (Opto-Varimex, Columbus Instruments, Columbus, OH) in which 15 photobeams cross the width of a 20 X 40 X 23-cm clear Plexiglas rodent cage. Photobeams were spaced 2.5 cm apart and 8 cm above the cage floor.

2.3 Drug

THC (200 mg in 5 mL of ethanol; National Institute on Drug Abuse, Bethesda, MD) was dissolved in 1:1:18 (ethanol:cremophor:saline) solution, which also served as the vehicle. Vehicle or THC was administered *i.p.* in a volume of 1 mL/kg, except doses > 10 mg/kg, which were administered in larger volumes of a 10 mg/mL THC solution, due to solubility

limitations. Because the amount of ethanol and cremophor increased with the volume of vehicle injected for doses larger than 10 mg/kg, larger volumes of vehicle were tested in separate rats to determine whether the greater amount of ethanol and cremophor in the vehicle solution produced antinociceptive or motoric effects.

2.4 Determination of estrous stage

Immediately after behavioral testing, and daily during the chronic administration period, a vaginal cell sample was obtained from each female via lavage. Classification of vaginal cytology for each stage has been described previously (see Bradshaw et al., 2006; Freeman, 1988; Wakley and Craft, 2011)

2.5 Behavioral procedure

THC dose-effect curves were obtained in 4 rats/sex and the ED₈₀ was calculated for each rat on each of the two nociceptive tests. Sex-specific ED₈₀ values were calculated as the mean of eight individual ED₈₀ values (4 rats x 2 tests); these means were 5.4 mg/kg (females) and 7.6 mg/kg (males). For the tolerance experiment, 10 rats/sex were assigned to each condition (chronic vehicle vs. chronic THC). On the pre- and post-chronic test days, testing began at 0800 h with the lights on during testing. Three baselines were obtained on each nociceptive test. For the tail withdrawal assay, the rat was lightly wrapped in a towel and held vertically while the distal 5 cm of the tail was submerged in the warm water bath and latency to withdraw the tail was measured to the nearest 0.01 s with a cutoff of 15 s. For the paw pressure test, the rat was also lightly restrained with a towel and placed horizontally on a flat surface while the latency to withdraw or attempt to withdraw the hindpaw was measured to the nearest 0.1 s and a cutoff of 15 s (750 g) was used. Cumulative dosing, using ¼ log unit dose intervals, commenced after the third baseline test, with the following THC doses injected at 15-min intervals: 1.0, 0.8, 1.4, 2.4, 4.4 and in some cases 8.0 mg/kg, reaching a total cumulative dose of 10–18 mg/kg THC. Fifteen min after each injection, rats were tested on both nociceptive tests. Once the rat reached cutoff on both tests, catalepsy and locomotor activity were assessed. For the catalepsy test, the rat's forepaws were placed on the bar and latency to remove both paws from the bar or jump onto the bar was recorded to the nearest 0.01 s (cutoff 15 s). Following catalepsy testing, rats were placed into locomotor chambers, and activity was measured as the number of photobeams broken in a 15-min period. Rats were placed on a chronic injection regimen the next morning.

To induce tolerance, vehicle or the predetermined ED₈₀ for THC was administered *i.p.* twice-daily (at 0800 h and 1700 h) for 9 days. THC-treated females received 5.4 mg/kg/injection and THC-treated males received 7.6 mg/kg/injection. Vaginal cell samples were obtained from females every morning; males were handled daily to ensure similar amounts of handling. On the morning of the post-chronic test day (starting 15 h after the last chronic injection), the THC dose-effect curve was re-determined. Specifically, 1.8–32.0 mg/kg THC (chronic vehicle group) or 18–180 mg/kg (chronic THC group) was administered via cumulative dosing, and behavioral testing was conducted as described above. To compare the effects of THC to a no-drug condition on motor behaviors, a separate group of rats (9/sex) was treated and tested only with vehicle. On Day 1, these rats were given vehicle (1 mL/kg, *i.p.*) and then tested 15 min later on catalepsy and locomotor activity only. During

chronic administration, these rats received vehicle injections twice-daily for 9 days; on the post-chronic test day, rats were once again given vehicle and catalepsy and locomotor activity were re-assessed.

2.6 Data analysis

Baseline nociceptive latencies for each rat on the tail withdrawal and paw pressure tests were calculated as the mean of the three pre-injection trials. Because baseline responding changed from pre- to post-chronic testing, individual response latencies following drug were converted to % MPE on each day: $(\text{drug latency} - \text{baseline latency}) / (\text{cutoff latency} - \text{baseline latency}) \times 100$, using the baseline latency on the same day on which drug was tested. The dose that produced 50% effect (ED_{50}) in each rat was estimated by log-linear interpolation, using data points from the linear portion of the dose-effect curve (typically between 15 – 85% MPE). ED_{50} values (in log units) were compared among groups using three-way ANOVA, with factors of sex, chronic treatment (vehicle vs. THC), and time (pre-chronic vs. post-chronic, repeated). Catalepsy (latency in s) and locomotor activity (number of photobeam breaks) were analyzed using three-way ANOVA, with factors of sex, treatment-test group (vehicle-vehicle, vehicle-THC, THC-THC), and time (pre-chronic vs. post-chronic, repeated). Group differences were considered significant if $P \leq 0.05$.

To examine THC's effect on estrous cycling in females, daily vaginal cytology was used to estimate the time spent in proestrus to estrus stages over the course of the experiment. The percent of days in proestrus to estrus was calculated for each rat using the following formula: $[\text{number of vaginal cell samples identified as proestrus or estrus (or in between the two phases)} / \text{total number of vaginal cell samples}] \times 100$. An independent samples t -test was used to compare estrous cycling between chronic treatment groups (vehicle vs. THC).

3. RESULTS

3.1 Baselines

No sex differences in baseline tail withdrawal latencies were observed on the pre-chronic test day (sex: $F(1,36)=0.08$, *n.s.*) and latencies were similar between rats assigned to chronic vehicle and chronic THC treatment groups (chronic treatment: $F(1,36)=1.31$, *n.s.*). Baseline latencies decreased from pre-chronic to post-chronic tests, more in females than males (sex $\times$ time: $F(1,36)=7.12$, $p<0.05$). Females' latencies decreased from 6.31 ± 0.14 to 5.48 ± 0.13 s, and males' latencies decreased from 6.26 ± 0.14 to 6.11 ± 0.12 s. On the paw pressure test, on the pre-chronic test day, females had significantly shorter baseline latencies compared to males (5.15 ± 0.14 vs. 6.15 ± 0.15 s, respectively; sex: $F(1,36)=24.50$, $p<0.001$), but there were no differences in baselines between rats assigned to vehicle- vs. THC-treated groups (chronic treatment: $F(1,36)=0.02$, *n.s.*). Similar to tail withdrawal, paw pressure baseline latencies decreased from pre-chronic to post-chronic, but to approximately the same extent in both sexes (to 4.59 ± 0.13 s for females and 5.65 ± 0.14 s for males; $F(1,36)=20.38$, $p<0.001$).

3.2 Antinociceptive tolerance to THC

Figure 1 shows the THC dose-effect curves on pre-chronic and post-chronic test days for tail withdrawal (panels A and B) and paw pressure tests (panels C and D). The ED₅₀ values derived from pre- vs. post-chronic dose-effect curves are shown in Figure 2. At the pre-chronic test, THC was more potent in females compared to males (N=20 rats/sex), on each nociceptive test (tail withdrawal, sex: $F(1,36)=22.24$, $p<0.001$; paw pressure, sex: $F(1,36)=5.21$, $p<0.05$). Figures 1A (vehicle-treated) and 1B (THC-treated) show that on the tail withdrawal test, the THC dose-effect curve shifted to the right from pre-chronic to post-chronic; as expected, this shift was significantly greater in THC- than in vehicle-treated rats. That is, ED₅₀ values for THC on the tail withdrawal test increased 1.4- to 1.6-fold in vehicle-treated rats compared to 3- to 4-fold in THC-treated rats (see Fig. 2; chronic treatment x time: $F(1,36)=269.47$, $p<0.001$). Females tended to show greater reductions in THC potency after chronic treatment compared to males (sex x chronic treatment x time: $F(1,36)=3.66$, $p=0.06$).

On the paw pressure test, the THC dose-effect curve shifted to the right from pre-chronic to post-chronic, with greater shifts observed in THC- compared to vehicle-treated rats (compare Figs. 1C and 1D). The ED₅₀ values derived from these dose-effect curves increased 1.3- to 1.6-fold vs. 3- to 4-fold in chronic vehicle- vs. chronic THC-treated rats, respectively (see Fig. 2; chronic treatment x time: $F(1,36)=237.82$, $p<0.001$). The THC dose-effect curve shifted farther to the right in THC-treated females than in THC-treated males: ED₅₀ values increased 4.4-fold in females vs. 2.9-fold in males (sex x chronic treatment x time: $F(1,36)=4.49$, $p<0.05$).

Solubility limits for THC required that high doses of THC (> 10 mg/kg) be administered in larger volumes than lower doses; thus, injections of higher doses of THC contained more ethanol and cremophor than injections of lower doses of THC. To examine potential effects of larger amounts of ethanol/cremophor, increasing volumes of vehicle (1.8 – 8.0 mL/kg) were administered using cumulative dosing in a separate group of 4 rats/sex. Large volumes of vehicle (containing approximately 0.035 – 0.35 g/kg of ethanol) did not produce antinociception on either tail withdrawal (vehicle volume: $F(4,24)=1.17$, *n.s.*) or paw pressure tests (vehicle volume: $F(4,24)=1.24$, *n.s.*) (Table 1). Additionally, the largest vehicle volume tested (8 mL/kg) resulted in low catalepsy scores (1.07 ± 0.51 in females, 1.65 ± 0.92 in males) and no obvious suppression of locomotor activity (1473 ± 297 beam breaks in females, 883 ± 175 in males).

3.3 Tolerance to THC's effects on locomotor activity and catalepsy

Because motor behaviors were examined after rats reached cutoff on the nociceptive tests, not all rats were tested on catalepsy and locomotor activity tests after the same dose of THC. Table 2 shows that motor behaviors for a majority of the rats were tested after 18 mg/kg on the pre-chronic test day and after 32 mg/kg (chronic vehicle-treated rats) or 180 mg/kg (chronic THC-treated rats) on the post-chronic test day. To compare catalepsy and locomotor activity after THC to a no-drug condition, a separate group of rats was chronically treated and tested with vehicle only (see Methods).

Figure 3A shows catalepsy scores for all three groups at the pre-chronic and post-chronic tests. At the pre-chronic test, catalepsy scores for vehicle-vehicle control females and males (first two bars) were similarly low, and they did not change significantly from pre-chronic to post-chronic tests. Compared to these vehicle-vehicle controls, THC produced catalepsy in rats assigned to the chronic vehicle- and chronic THC-treated groups (Fig. 3A; group: $F(1,52)=12.26, p<0.001$). Catalepsy scores decreased from pre-chronic to post-chronic tests (time: $F(1,52)=9.09, p<0.01$). At the post-chronic test, compared to chronic vehicle-treated rats, chronic THC-treated rats had significantly lower catalepsy scores (group: $F(2, 57)=6.71, p<0.05$). Neither sex nor group significantly influenced catalepsy scores on either test day (sex x time: $F(1,52)=0.16, n.s.$; group x time: $F(2,52)=2.58, p=0.086$; sex x group x time: $F(2,52)=0.62, n.s.$).

Locomotor activity for all three groups is shown in Figure 3B. In the vehicle-vehicle control group (first two bars), no sex differences were observed, and locomotor activity did not change significantly from pre-chronic to post-chronic tests. At the pre-chronic test, THC decreased locomotor activity in rats assigned to chronic vehicle-treated and chronic THC-treated groups compared to vehicle-vehicle controls (Fig. 3B; group: $F(2,52)=93.73, p<0.001$), with no sex difference. After 9 days of THC treatment, THC decreased locomotor activity less in the chronic THC-treated group than it had pre-chronically (group x time: $F(2,52)=3.17, p=0.05$). Additionally, at the post-chronic test, chronic THC-treated rats showed significantly less locomotor suppression than chronic vehicle-treated rats after both groups had been injected with THC ($p<0.05$). Sex did not significantly influence locomotor activity on either test day (sex x time: $F(1,52)=0.81, n.s.$; sex x group x time: $F(2,52)=0.12, n.s.$).

3.4 Estrous cycling during chronic administration

During the 11-day experiment, females in both chronic vehicle and chronic THC groups were in vaginal proestrus or estrus (or in between) approximately the same amount of time: the percent of days in proestrus to estrus was 31.8 ± 6.3 vs. 30.0 ± 6.6 in chronic vehicle- vs. chronic THC-treated groups, respectively (chronic treatment: $t(18)=0.21, n.s.$).

4. DISCUSSION

Similar to previous reports (Craft et al., 2012; Tseng and Craft, 2001; Wakley and Craft, 2011), in this study females were more sensitive than males to the acute antinociceptive effects of THC. Recently, we reported lower ED₅₀ values in females than males for both tail withdrawal and paw pressure assays using a single THC dose/rat procedure (Craft et al., 2012). The present study replicates and extends this finding to a cumulative dosing regimen. Enhanced sensitivity to THC in females has been attributed to estradiol: estradiol-replaced ovariectomized female rats (Craft and Leidl, 2008) and proestrous-estrous female rats (Wakley and Craft, 2011) show enhanced THC-induced antinociception compared to ovariectomized control females and gonadally intact males. This evidence suggests that acute THC-induced antinociception is enhanced in females during and immediately after high-estradiol states, and sex differences in THC sensitivity can be attributed to greater estradiol in females than males.

Females also developed greater tolerance than males to the antinociceptive effects of THC, as evidenced by a significantly greater reduction in THC potency in females than males on the paw pressure test. A similar trend was found on the tail withdrawal test. To date, sex differences in development of tolerance to THC-induced antinociception have been examined in only one other study (Wiley et al., 2007). Contrary to the present results, sex differences were not found, likely due to differences in methodology, including use of different rat strains, same vs. different doses in each sex for tolerance induction, and different pre-test injection intervals. For example, in the previous study tail-flick was assessed 45 min after THC injection and dose intervals were ½-log unit apart. In contrast, the present study used a 15-min pretreatment with ¼-log unit dose intervals. Antinociception produced by moderate doses of *i.p.* THC (i.e., approximately 3–6 mg/kg) peaks at 15–30 min post-injection and lasts about 120 min in both sexes (Craft et al., 2012; Tseng and Craft, 2001). Therefore, the pre-treatment time and dosing intervals were optimized in the present study to detect sex differences in THC potency and development of THC tolerance.

Previous work suggests that sex differences in tolerance development may be due to sex differences in acute drug potency. When the same tolerance-inducing dose of morphine was administered to rats of both sexes, the greater antinociceptive tolerance to morphine that developed in males could be attributed to males' greater sensitivity to acute morphine (Barrett et al., 2001). That is, the sex that is initially more sensitive to drug would be expected to show greater tolerance development given the same chronic drug treatment regimen, but this sex difference results from administering a functionally higher chronic dose in one sex compared to the other, rather than a true sex difference. In the present study, we adjusted for sex differences in acute THC potency by administering less THC to females than to males during the chronic regimen, and a sex difference in tolerance development was still observed. These results suggest that there are in fact sex differences in the development of cannabinoid tolerance.

In the present study, tolerance to the motoric effects of THC also developed, evidenced by a reduction in THC-induced catalepsy and locomotor suppression from pre-chronic to post-chronic tests in THC-treated rats, but not in vehicle-treated rats. This reduction occurred despite the higher final test dose of THC administered to the chronic THC groups as compared to the chronic vehicle groups (180 vs. 18 or 32 mg/kg, respectively). While the present design did not allow for determination of sex differences in motoric effects of acute THC, greater acute THC-induced locomotor suppression and catalepsy in females than males has been observed when motor activity was examined over a 2–4 h period (Craft et al., 2012; Tseng and Craft, 2001). Following chronic treatment with vehicle or THC, rats in each of the chronic treatment groups received the same (THC groups) or similar (vehicle groups) THC doses, yet sex differences in motoric effects of THC were not observed. To optimize the experimental design for discerning sex differences in antinociceptive potency of THC, THC's effects on motor behaviors were assessed only once at a single dose, after rats reached cutoff on the nociceptive tests. Therefore, the absence of sex differences in tolerance to THC's motoric effects may be due to the reduced power inherent in a single-dose experimental design.

Estrous cycling appeared to be somewhat suppressed in both chronic vehicle- and THC-treated females in the present study. The length of a normal estrous cycle in most female rats is 4–5 days, and females are in proestrus to estrus approximately 40–45% of the time (Feder, 1981; Freeman, 1988; Haim et al., 2003). In the present study, the average percent time in proestrus to estrus was 30% -- approximately 10–15% lower than would be expected in normally cycling females. Because chronic vehicle and chronic THC groups had similar percentages, suppressed cycling likely resulted from handling stress rather than THC. Females used in the present study were not handled or habituated to daily vaginal lavage prior to the start of the study. Although chronic oral cannabinoid administration has not been shown to produce long-term disruption of estrous cycling in rats, cannabinoid exposure prior to the luteinizing hormone surge can delay the onset of estrus (O'Connell et al., 1987). In contrast, chronic *s.c.* THC administration has been shown to disrupt estrous cycling in female rats (Marusich et al., 2014). Research in humans (Mendelson et al., 1986) and primates (Smith et al., 1983) also suggests that cannabinoid exposure can alter luteinizing hormone levels and menstrual cycling and that tolerance to this effect develops. Given that the chronic THC dose used in the present study was lower than those used in previous studies in which cycle disruption was observed (5.4 mg/kg THC twice-daily in the present study vs. 25–30 mg/kg THC once- or twice-daily in previous studies (Marusich et al., 2014; O'Connell et al., 1987)), the chronic THC dose likely contributes to differences among study outcomes. Future work will need to examine dose- and time-dependent effects of chronic THC on reproductive cycling to more comprehensively characterize this effect.

Some evidence suggests that there are sex differences in (and estradiol influence on) THC pharmacokinetics (Narimatsu et al., 1991; Tseng et al., 2004) – which could contribute to sex differences in THC tolerance. In fact, Wiley and Burston (in press) recently reported that after chronic THC administration, blood levels of the major active metabolite 11-OH-THC increased in female rats but dropped in males. In male rodents, tolerance to the behavioral effects of THC following repeated THC administration is known to result from desensitization and down-regulation of cannabinoid subtype-1 (CB₁) receptors in specific regions of the brain (Breivogel et al., 1999; Rodriguez De Fonseca et al., 1994; Romero et al., 1998a, 1998b, 1997; Sim-Selley and Martin, 2002). β -arrestin2, the non-visual arrestin involved in internalization of G protein-coupled receptors (Ibrahim and Kurose, 2012), partially contributes to these receptor changes during tolerance development. For example, deletion of β -arrestin2 reverses, but does not eliminate desensitization and down-regulation of the CB₁ receptor and tolerance to THC-induced antinociception (Breivogel et al., 2008; Nguyen et al., 2012). Interestingly, estradiol increases β -arrestin2 recruitment in cell membranes (Sandén et al., 2011), which may explain greater THC tolerance in females. Although a previous study found that CB₁ receptor desensitization in the periaqueductal gray was greater in male than female rats (Burston et al., 2010), the hormonal state of the females was not known. Because hormonal state influences females' sensitivity to THC-induced antinociception (Craft and Leidl, 2008; Craft et al., 2012; Tseng and Craft, 2001; Wakley and Craft, 2011), this could be an important factor when examining desensitization or other changes in the CB₁ receptor during the development of tolerance. Hence, inclusion of hormone manipulation in future studies may be beneficial for determining the mechanisms responsible for sex differences in THC tolerance development.

In conclusion, the present study demonstrates sex differences in the antinociceptive potency of acute THC and in the development of tolerance to the antinociceptive effects of THC. Specifically, females were initially more sensitive to THC than males, and – despite the chronic administration of approximately 30% less THC to females than males, to adjust for sex differences in acute THC potency – females developed greater tolerance than males. The estrous stage results suggest that a low dose of chronic THC does not disrupt reproductive cycling in female rats. Further investigation is needed to determine the exact role that gonadal hormones play in modulating the development of cannabinoid tolerance.

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Highlights

- Females show greater THC tolerance than males on two nociceptive assays.
- Greater tolerance developed in females given a 30% lower tolerance induction dose.
- A moderate dose of chronic THC did not disrupt estrous cycling.

Figure 1.

Antinociceptive effects of THC on pre-chronic and post-chronic test days for the tail withdrawal test (panels A and B) and the paw pressure test (panels C and D), in female (F) and male (M) rats treated twice-daily for 9 days with vehicle (panels A and C) or THC (panels B and D). Each point is the mean $\pm$ 1 SEM of 10 rats.

Figure 2.

ED₅₀ values for THC on pre-chronic (open bars) and post-chronic (diagonal line bars) tests on the tail withdrawal test (panel A) and the paw pressure test (panel B), in female (white bars) and male (grey bars) rats treated twice-daily for 9 days with vehicle (VEH) or THC. Each bar is the mean $\pm$ 1 SEM of 10 rats. *Significantly different from pre-chronic ED₅₀ value of the same-sex, same-chronic treatment group, P 0.05. +Significantly different from males in same chronic treatment group, P 0.05.

Figure 3.

Effects of THC on catalepsy (panel A) and locomotor activity (panel B) in female vs. male rats before (pre-chronic) and after (post-chronic) treatment with vehicle (Veh) or THC twice-daily for 9 days. Vehicle-vehicle control rats (first two bars in each panel) were treated and tested only with vehicle. Each bar is the mean $\pm$ 1 SEM of 9–10 rats.

*Significantly different from same-sex, vehicle-vehicle control group, one-way ANOVA, $P < 0.05$. #Significantly different from chronic vehicle-treated rats, one-way ANOVA, $P < 0.05$.

Average antinociception (mean $\pm$ 1 SEM %MPE) on tail withdrawal and paw pressure tests following cumulative dosing with increasing volumes of 1:1:18 (ethanol:cremophor:saline) solution. Negative values indicate that latency to respond sometimes dropped slightly below baseline.

Table 1

	Vehicle Volume (mL/kg) ^a					
	1.8	1.4	2.4	4.4	8.0	
Females						
Tail Withdrawal	-1.39 $\pm$ 5.37	-4.21 $\pm$ 3.19	-1.47 $\pm$ 3.47	-2.74 $\pm$ 6.07	0.34 $\pm$ 6.24	
Paw Pressure	-5.30 $\pm$ 4.92	0.27 $\pm$ 4.39	-5.23 $\pm$ 6.42	-6.55 $\pm$ 4.51	-2.70 $\pm$ 4.45	
Males						
Tail Withdrawal	-4.05 $\pm$ 6.13	-4.38 $\pm$ 6.93	-8.67 $\pm$ 5.22	-0.47 $\pm$ 2.89	2.40 $\pm$ 4.48	
Paw Pressure	-3.21 $\pm$ 3.14	7.54 $\pm$ 10.03	-1.46 $\pm$ 9.72	7.95 $\pm$ 10.51	2.01 $\pm$ 6.02	

^a As cumulative dosing was used, the total volume after each injection was 1.8, 3.2, 5.6, 10.0 and 18.0 mL/kg of vehicle solution (containing approximately 0.035–0.35 g/kg ethanol).

Table 2

Number of rats tested on locomotor and catalepsy assays at each THC dose.

Pre-chronic	THC (mg/kg)					
	1.8	3.2	5.6	10.0	18.0	TOTAL
Females, Veh			1	1	8	10
Males, Veh				1	9	10
Females, THC				4	6	10
Males, THC				1	9	10
Post-chronic	18.0	32.0	56.0	100.0	180.0	TOTAL
Females, Veh	1	9				10
Males, Veh		10				10
Females, THC					10	10
Males, THC					10	10