

Sex differences in anti-allodynic, anti-hyperalgesic and anti-edema effects of Δ^9 -tetrahydrocannabinol in the rat

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ABSTRACT

Cannabinoid agonists such as Δ^9 -tetrahydrocannabinol (THC) are more potent and/or efficacious antinociceptive agents in female than male rats using acute pain models. We tested the hypothesis that THC is more effective in females than males using a model of longer-lasting, inflammatory pain. THC's anti-allodynic, anti-hyperalgesic, and anti-edema effects were examined 1, 3, and 7 days after injection of complete Freund's adjuvant (CFA) into the hind paw. Systemically administered THC (0.32–3.2 mg/kg, intraperitoneally [i.p.], same dose each day) was significantly more effective in females than males in attenuating CFA-induced thermal hyperalgesia, but was also more sedative in females. When administered locally into the inflamed hind paw, THC (250–500 μ g intraplantar, i.pl.) did not affect locomotor activity in either sex, yet produced greater anti-allodynic and anti-hyperalgesic effects in females than males. Despite THC's greater anti-allodynic and anti-hyperalgesic effects in females, both i.p. and i.pl. THC reduced hind paw thickness (edema) more in males. The anti-hyperalgesic effect of i.p. THC was blocked by the CB1 receptor-selective antagonist rimonabant in both sexes. Similarly, i.pl. rimonabant antagonized i.pl. THC's effects in both sexes; in contrast, the CB2 antagonist SR144528 significantly attenuated i.pl. THC's anti-allodynic effect only in females. Intraplantar SR144528 also antagonized i.pl. THC's anti-edema effect in males. This study suggests that cannabinoids may be better at reducing edema in males while being more effective against inflammatory pain in females. Furthermore, sex differences in THC's peripheral effects against inflammatory pain may be a result of activation of both types of cannabinoid receptors in females, in contrast to predominantly CB1 receptors in males.

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1. Introduction

Sex differences in behavioral effects of cannabinoids have been investigated by several groups, in experimental animals and to a lesser extent in humans. For example, female rodents have been found to be more sensitive than males to the antinociceptive [9,28,30], motoric [29,32], hypothermic [2,33], reinforcing [12], and cognition-impairing [5] effects of cannabinoid agonists. In human studies, female marijuana users report significantly lower “mental quality of life” than male users [20], and on laboratory measures of cognition, women are more impaired than men [26]. In contrast, a handful of studies demonstrate greater sensitivity to cannabinoids in males compared with females, for example in studies of cannabinoid-induced hyperphagia in rodents [11,21], and marijuana-induced “high” in humans [15,24]. Therefore, although a number of sex difference studies conducted thus far

suggest greater sensitivity to cannabinoid agonists in females than males, sex differences may depend on the effect examined.

In animal studies of cannabinoid-induced antinociception, sex differences in motoric effects of cannabinoid agonists are often observed in parallel with sex differences in their antinociceptive effects [9,29,33]. Thus, it is possible that greater cannabinoid-induced antinociception in females than males simply reflects greater cannabinoid-induced sedation in females, as a longer latency to respond to a noxious stimulus, which can be caused by sedation, is interpreted as antinociception. Additionally, given that sex differences in cannabinoid-induced antinociception have been examined using animal models of acute pain only, the significance of this finding to clinical treatment of pain – which is typically chronic and involving inflammation, neuropathy or both – may be limited. Thus, the purpose of the present study was to determine whether sex differences in cannabinoid-induced antinociception would also be observed using a model of longer-lasting, inflammatory pain. Both systemic (intraperitoneal, i.p.) and local (intraplantar, i.pl., injections into the inflamed hind paw) routes of THC administration were examined. If antinociception is partly

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a result of sedation, then local THC administration – which presumably does not produce sedation – should produce more equivalent antinociception in females and males than would systemic THC administration. Antagonist experiments also were conducted to examine possible sex differences in CB1 vs CB2 receptor mediation of THC's effects.

2. Method

2.1. Subjects

Adult male and female Sprague-Dawley rats, 60–90 days old, were used (bred in-house from Taconic stock, Germantown, NY). Rats were housed in same-sex pairs, under a 12:12 light:dark cycle (lights on at 6 am), in a room maintained at 21 ± 2 °C. Rats were treated in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* (1996). They were assigned randomly to treatment groups, with the exception that we avoided assigning same-sex siblings to any group that had 6 or fewer rats.

2.2. Apparatus

Thermal (heat) sensitivity was assessed using a Hargreaves apparatus (Ugo Basile Plantar Test, Model 7371, Collegeville, PA). Mechanical sensitivity was assessed using an electronic von Frey anesthesiometer (IITC Inc, Woodland Hills, CA). Horizontal locomotor activity was measured using a photobeam apparatus (Optovarimex, Columbus Instruments, Columbus, OH): 15 photobeams cross the width of a $20 \times 40 \times 23$ -cm clear Plexiglas rodent cage, with photobeams spaced 2.5 cm apart and 8 cm above the cage floor. Paw edema was quantified by measuring maximal dorsal-ventral hind paw thickness, in mm, with calipers.

2.3. Drugs

Rimonabant (SR141716A), SR144528, and THC (National Institute on Drug Abuse, Bethesda, MD; plus some SR144528 was purchased from Cayman Chemical Co, Ann Arbor, MI) were dissolved in a 1:1:18 ethanol:cremophor:saline solution, which served as the vehicle. When administered i.p., injection volumes were 1 mL/kg. When administered i.pl., THC (or vehicle) injection volume was 50 μ L. When THC and antagonist were both administered i.pl., a single syringe was filled with both compounds and the total injection volume was 50 μ L (40 μ L [400 μ g] THC or vehicle + 10 μ L [20 μ g] antagonist or vehicle). This volume did limit the dose of THC that could be administered i.pl.: solubility limitations allowed for a maximal THC dose of 500 μ g i.pl. CFA (5 mg/mL) was purchased from Sigma-Aldrich Chemical Co (St. Louis, MO).

2.4. Behavioral procedures

Behavioral testing was conducted during the light part of the light:dark cycle, between 9 am and 5 pm. In all experiments, baseline measurements on several tests were obtained on Day 0 (just before CFA injection). Rats were placed into hanging wire cages for approximately 20 minutes to habituate. The threshold at which a rat responded when the von Frey probe was applied to the plantar surface of the hind paw was recorded in g. Three assessments were made for each hind paw several minutes apart, and the order of testing was counterbalanced across rats (for approximately half of rats/group, the right hind paw was tested first; for the other half of rats/group, the left hind paw was tested first). The Hargreaves test was conducted next: latency to respond in seconds was recorded for each hind paw, with 3 assessments/hind paw, each assessment several minutes apart and order of hind paw testing

counterbalanced across rats. Rats were then placed into locomotor chambers, and the number of photobeam breaks in 5 minutes was recorded. Finally, maximal dorsal-ventral thickness of each hind paw was measured in mm. Immediately after all baseline measures were taken, rats were briefly anesthetized with isoflurane, and 0.1 mL CFA was injected into the right plantar hind paw.

In Experiment 1 (i.p. THC), 1 day after CFA injection, vehicle or THC (0.32, 1.0, or 3.2 mg/kg) was injected i.p. Thirty minutes later, the von Frey, Hargreaves and locomotor tests were conducted as described for Day 0, and thickness of each hind paw was recorded. The same procedure was repeated 3 days and 7 days after CFA injection; rats received the same pretreatment each day (ie, rats injected with vehicle on Day 1 received vehicle again on Days 3 and 7; rats injected with 1.0 mg/kg THC on Day 1 received this same dose again on Days 3 and 7, etc.). In Experiment 2 (i.pl. THC), the same procedure was used except THC (0, 250, or 500 μ g) was administered i.pl. instead of i.p. In Experiments 3 and 4 (i.p. and i.pl. antagonist studies, respectively), rats were tested only on Day 3 post-CFA.

2.5. Determination of estrous cyclicity

Vaginal cytology samples were taken by lavage in all females on the baseline day and on each test day, after all behavioral testing was complete. Proestrus was identified by the predominance of nucleated epithelial cells, estrus by the presence of dense sheets of cornified epithelial cells, diestrus-1 was identified by the presence of scattered, nucleated or cornified epithelial cells and leukocytes, and diestrus-2 was identified by a relative lack of any cells [13].

2.6. Data analysis

The mean of 3 trials/hind paw was calculated for each rat, for the von Frey and Hargreaves tests. In most cases, THC significantly altered dependent measures made in the CFA-treated (right) hind paw but not in the untreated hind paw, in both sexes. Therefore, some von Frey, Hargreaves, and paw thickness values are presented as the difference between the 2 hind paws (right hind paw score – left hind paw score), to simplify data presentation. Cases in which THC significantly altered dependent measures in the left hind paw are noted in the results.

There was a significant sex difference in locomotor activity in the absence of drug treatment, in all experiments. Thus, before analyzing for sex differences in drug effect (on Days 1, 3, and 7 post-CFA), each drug-treated rat's locomotor activity scores were converted to percent of the mean same-sex, vehicle-treated group's locomotor activity score: [$\#$ photobeam breaks on Day 1 (or 3 or 7) in drug-treated rat/mean $\#$ photobeam breaks on Day 1 (or 3 or 7) in same-sex vehicle-treated group] \times 100.

Dose- and day-effect data for THC i.p. or i.pl. (raw scores for Hargreaves, von Frey, and paw thickness tests, and % control locomotor scores) were analyzed using a 4-way ANOVA, with factors of sex (2 levels), THC dose (3–4 levels), day (3–4 levels, repeated), and paw (2 levels, repeated). For antagonist + THC analyses, a 3-way ANOVA was used for each antagonist, with factors of sex (2 levels), antagonist dose (2 levels), and THC dose (2 levels). Tukey's (or Dunnett's, for multiple comparisons to a control group) tests were used for post-hoc determination of significance. Significance level was $P \leq 0.05$ for all statistical tests.

3. Results

3.1. Sex differences in systemic (i.p.) THC effects

Fig. 1 shows mechanical thresholds (von Frey test) in the left vs right hind paws of female and male rats, on the baseline day

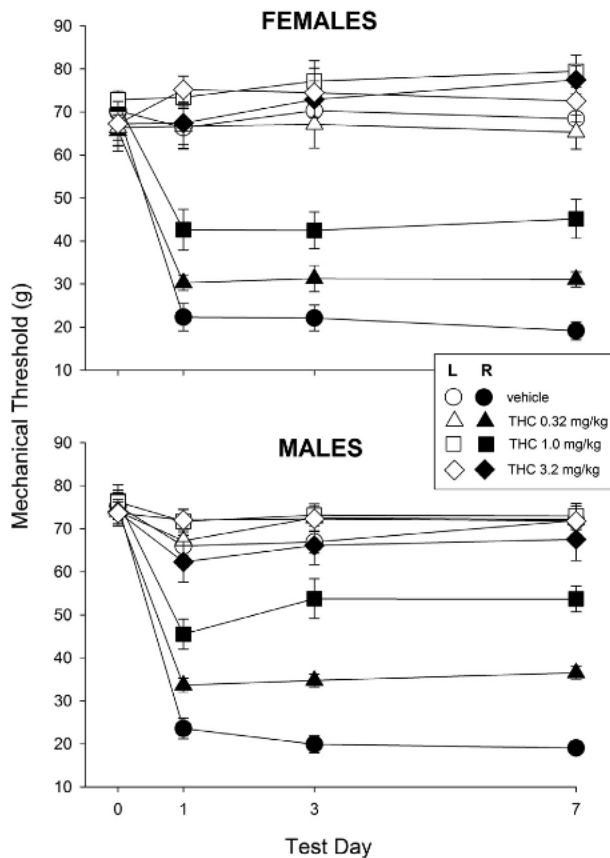


Fig. 1. The effect of systemically administered THC on mechanical thresholds (von Frey test) in female vs male rats. Baseline mechanical threshold was determined on Day 0, immediately before injecting CFA into the right hind paw. On Days 1, 3, and 7 post-CFA, vehicle or THC was administered i.p. 30 minutes before re-determining mechanical thresholds in the left (L) and right (R) hind paws. The same dose of THC was administered to the same rats each test day. Each point is the mean \pm 1 SEM of 10–11 rats.

(before CFA, Day 0), and 1, 3, and 7 days after CFA was injected into the right hind paw. CFA decreased mechanical thresholds in the right hind paw to approximately the same extent in female and male rats (closed circles; Day \times Paw: $F(3,222) = 168.21$, $P < 0.001$, with no Day \times Paw \times Sex interaction). When administered i.p. 30 minutes before testing on Days 1, 3, and 7 post-CFA, THC dose-dependently reversed CFA-induced allodynia in the right hind paw (Day \times Paw \times THC: $F(9,222) = 18.83$, $P < 0.001$), with no significant sex difference (Sex \times THC \times Day: $F(9,222) = 1.54$, $P = 0.13$). Although THC slightly increased mechanical threshold – by a maximum of 6–8 g in the “control” (left) hind paw of females – this increase was not statistically significant (left paw only: Day \times THC: $F(9,108) = 0.74$, n.s.).

Fig. 2 shows latency to respond to a noxious thermal stimulus (Hargreaves test) in the left vs right hind paws of female and male rats, on the baseline day (before CFA, Day 0), and 1, 3, and 7 days after CFA was injected into the right hind paw. CFA decreased latency to respond to approximately the same extent in females and males (closed circles; Day \times Paw: $F(3,222) = 84.01$, $P < 0.001$, with no Sex interaction). When administered i.p. 30 minutes before testing on Days 1, 3, and 7 post-CFA, THC dose-dependently reversed CFA-induced hyperalgesia, with greater effects in females than males (Sex \times THC: $F(3,74) = 4.06$, $P = 0.01$). THC slightly lengthened response latencies – by approximately 2.3 seconds maximally in the control (left) hind paw of females; this effect was marginally significant (left paw only: Day \times THC: $F(9,108) = 1.94$, $P = 0.054$).

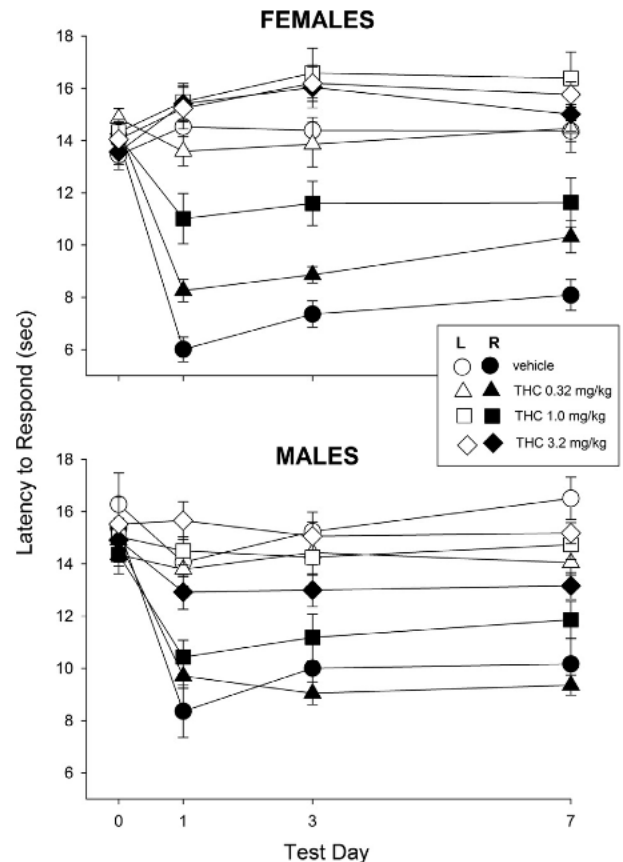


Fig. 2. The effect of systemically administered THC on latency to respond to a noxious thermal stimulus (Hargreaves test) in female vs male rats. Baseline latency to respond was determined on Day 0, immediately before injecting CFA into the right hind paw. On Days 1, 3, and 7 post-CFA, vehicle or THC was administered i.p. 30 minutes before re-determining response latencies to noxious heat in the left (L) and right (R) hind paws. The same dose of THC was administered to the same rats each test day. Each point is the mean \pm 1 SEM of 10–11 rats.

Fig. 3 shows the effects of CFA on locomotor activity in vehicle- vs THC-treated females and males. Examination of raw data (number of photobeam breaks, top panel) shows that females were more active than males in the absence of drug (vehicle-treated rats only, Sex: $F(1,18) = 19.40$, $P < 0.001$). Thus, data from THC-treated rats were converted to percent of same-sex, vehicle-treated control values to analyze for sex differences in THC effect. The bottom panel of **Fig. 3** shows that THC dose-dependently decreased locomotor activity in both sexes (THC: $F(2,56) = 223.69$, $P < 0.001$), with greater decreases in females than males (Sex: $F(1,56) = 5.36$, $P = 0.024$).

Fig. 4 shows the effects of CFA on paw thickness (a measure of edema) in vehicle- vs THC-treated female and male rats. CFA significantly increased thickness of the right hind paw in both sexes; although the increase was greater in males than females in terms of absolute paw thickness (Paw \times Day \times Sex: $F(3,222) = 9.99$, $P < 0.001$), the relative increase was very similar. That is, the right hind paw increased ~ 1.8 -fold in thickness relative to the left hind paw, in both sexes. THC significantly decreased paw thickness in males only (Sex \times THC: $F(3,74) = 2.86$, $P = 0.043$).

3.2. Sex differences in peripheral (i.p.) THC effects

Figs. 5–8 show the effects of THC given locally into the CFA-treated hind paw of females and males, 1, 3, and 7 days after CFA. **Fig. 5** shows mechanical thresholds (von Frey test) in the left vs right hind paw of female and male rats, on the baseline day (before CFA, Day 0),

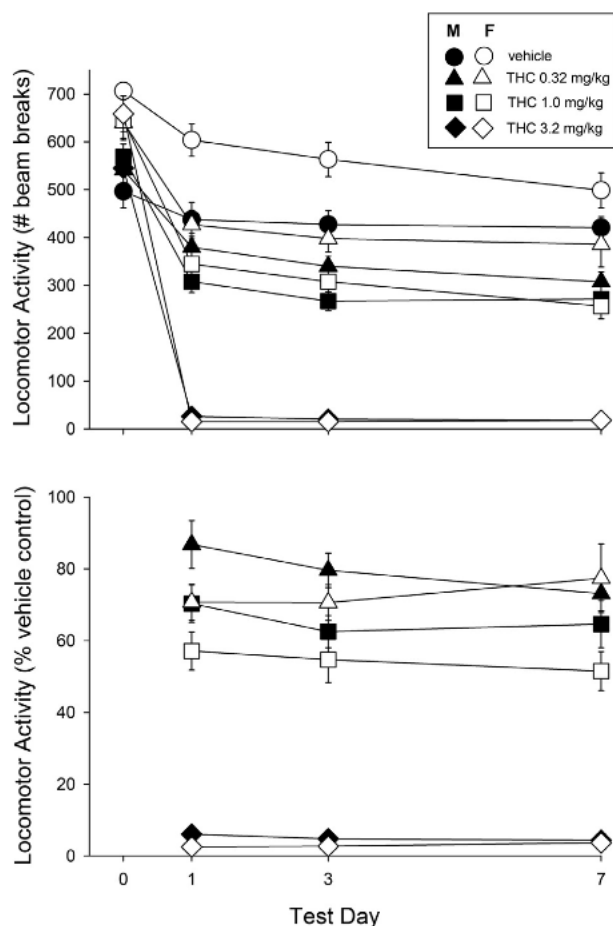


Fig. 3. The effect of systemically administered THC on locomotor activity in female (F) vs male (M) rats. Baseline locomotor activity (# photobeams broken in 5 minutes) was determined on Day 0, immediately before injecting CFA into the right hind paw. On Days 1, 3, and 7 post-CFA, vehicle or THC was administered i.p. 30 minutes before re-testing locomotor activity. The same dose of THC was administered to the same rats each test day. Top panel: Locomotor activity as raw # photobeam breaks. Bottom panel: Locomotor activity in THC-treated rats as percent of same-sex, vehicle-treated control values (as females locomote significantly more than males, in the absence of drug). Each point is the mean \pm 1 SEM of 10–11 rats.

and 1, 3, and 7 days after CFA was injected into the right hind paw. CFA decreased mechanical thresholds in the right hind paw in female and male rats (closed circles; Day \times Paw: $F(3,186) = 330.86$, $P < 0.001$). When administered i.p. 30 minutes before testing on Days 1, 3, and 7 post-CFA, THC dose-dependently reversed CFA-induced allodynia (Day \times Paw \times THC: $F(6,186) = 39.22$, $P < 0.001$), with the highest dose being more effective in females than males (Sex \times Paw \times THC: $F(2,62) = 6.84$, $P = 0.002$).

Fig. 6 shows latency to respond to a noxious thermal stimulus (Hargreaves test) on the baseline day (before CFA, Day 0), and 1, 3, and 7 days post-CFA, in i.p. vehicle- vs THC-treated female and male rats. CFA decreased latency to respond in the right hind paw to approximately the same extent in females and males (closed circles; Day \times Paw: $F(3,186) = 104.76$, $P < 0.001$). When administered i.p. into the right hind paw 30 minutes before testing on Days 1, 3, and 7, THC dose-dependently reversed CFA-induced hyperalgesia, with greater effects in females than males (Sex \times THC: $F(3,74) = 4.06$, $P = 0.01$). THC slightly lengthened response latency – by approximately 1.6 seconds maximally in the control (left) hind paw of males; this effect was not statistically significant (left paw only; Day \times THC: $F(6,99) = 0.55$, n.s.).

Fig. 7 shows locomotor activity in i.p. vehicle- vs THC-treated females and males. Examination of raw data (number of photo-

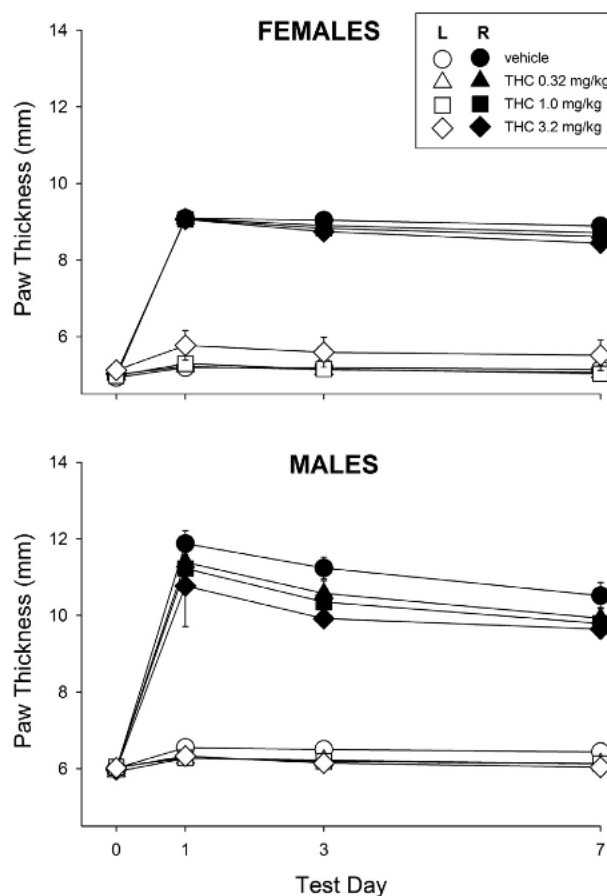


Fig. 4. The effect of systemically administered THC on paw edema in female vs male rats. Thickness of the left (L) and right (R) hind paw was measured on Day 0, immediately before injecting CFA into the right hind paw. On Days 1, 3, and 7 post-CFA, vehicle or THC was administered i.p. 30 minutes before re-assessing paw thickness. The same dose of THC was administered to the same rats each test day. Each point is the mean \pm 1 SEM of 10–11 rats.

beam breaks, top panel) shows that females were more active than males in the absence of drug (vehicle-treated rats only, Sex: $F(1,22) = 6.33$, $P = 0.02$). Thus, data from THC-treated rats were converted to percent of same-sex, vehicle-treated control values to analyze for sex differences in THC effect. The bottom panel of **Fig. 7** shows that i.p. THC did not significantly affect locomotor activity in either sex (THC: $F(1,40) = 0.02$, n.s.).

Fig. 8 shows the effects of i.p. THC on paw thickness, 1, 3, and 7 days post-CFA. CFA significantly increased thickness of the right hind paw in both sexes, but to a greater extent in males than females (Paw \times Day \times Sex: $F(3,186) = 337.96$, $P < 0.001$). In this experiment CFA caused a greater relative increase in thickness of the right hind paw in males compared with females: the right hind paw was approximately 2.2-fold thicker than the left in males but only 1.8-fold thicker in females 1 day after CFA injection. On Days 3 and 7 post-CFA, i.p. THC significantly decreased thickness of the right paw, to a significantly greater extent in males than females (Paw \times Day \times Sex \times THC: $F(6,186) = 43.676$, $P < 0.001$).

3.3. Antagonism of systemic THC effects

Fig. 9 shows antagonism of the anti-allodynic, anti-hyperalgesic and locomotor-decreasing effects of i.p. THC in female vs male rats. The CB1 and CB2 receptor-selective antagonists, rimonabant and SR144528, respectively, were administered i.p. 15 minutes before vehicle or 3.2 mg/kg THC (which was given 30 minutes before testing) on Day 3 post-CFA. Given alone, THC significantly decreased

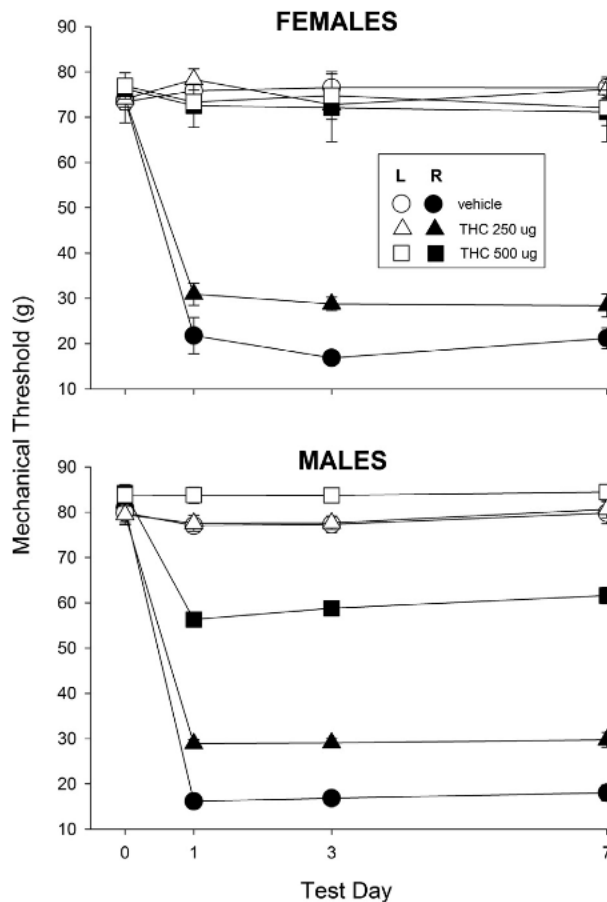


Fig. 5. The effect of locally administered THC (intraplantar, i.pl.) on mechanical thresholds (von Frey test) in female vs male rats. Baseline mechanical threshold was determined on Day 0, immediately before injecting CFA into the right hind paw. On Days 1, 3, and 7 post-CFA, vehicle or THC was administered i.pl. 30 minutes before re-determining mechanical thresholds in the left (L) and right (R) hind paws. The same dose of THC was administered to the same rats each test day. Each point is the mean \pm 1 SEM of 10–12 females or 11–13 males.

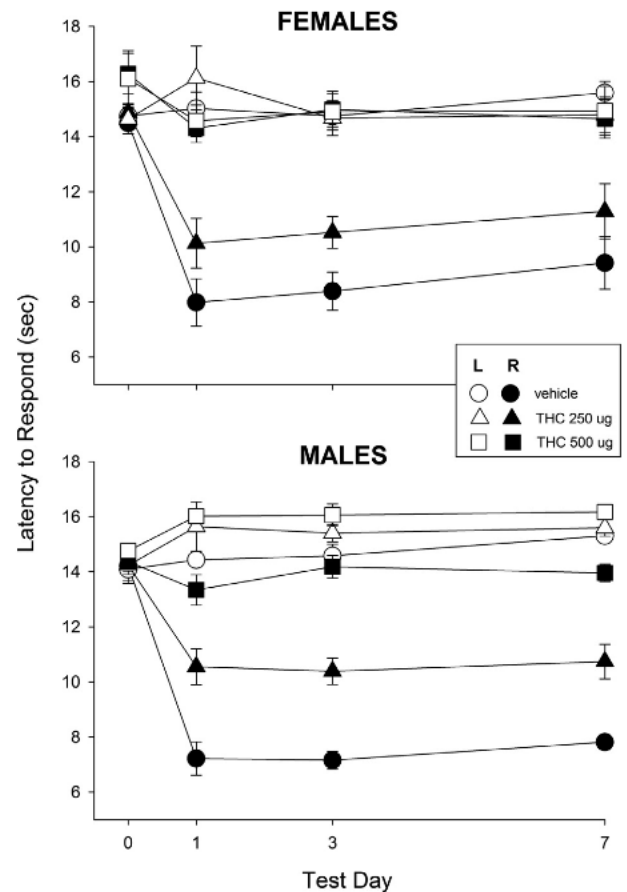


Fig. 6. The effect of locally administered THC (intraplantar, i.pl.) on latency to respond to a noxious thermal stimulus (Hargreaves test) in female vs male rats. Baseline latency to respond was determined on Day 0, immediately before injecting CFA into the right hind paw. On Days 1, 3, and 7 post-CFA, vehicle or THC was administered i.pl. 30 minutes before re-determining response latencies to noxious heat in the left (L) and right (R) hind paws. The same dose of THC was administered to the same rats each test day. Each point is the mean \pm 1 SEM of 10–12 females or 11–13 males.

mechanical allodynia only in females (Sex \times THC: $F(1,69) = 4.16$, $P = 0.045$), and this effect was not reversed by either antagonist. In contrast, THC significantly decreased thermal hyperalgesia in both sexes ($F(1,69) = 15.38$, $P < 0.001$), and only rimonabant significantly antagonized THC's anti-hyperalgesic effects in both sexes (Antagonist \times THC: $F(1,69) = 4.34$, $P = 0.041$). THC by itself also decreased locomotor activity only in females, and this effect also was antagonized by rimonabant (Antagonist \times THC: $F(1,35) = 7.54$, $P = 0.009$) but not SR144528. In this experiment, THC did not significantly reduce paw edema in either sex (data not shown).

3.4. Antagonism of peripheral THC effects

Fig. 10 shows antagonism of the anti-allodynic and anti-hyperalgesic of i.pl. THC in female vs male rats. Similar to the previous i.pl. experiment, anti-edema effects of i.pl. THC occurred only in males, so this measure is not shown for females. The CB1 and CB2 receptor-selective antagonists rimonabant and SR144528, respectively, were co-administered with vehicle or 400 μ g THC into the right hind paw 30 minutes before testing on Day 3 post-CFA. In both sexes, i.pl. THC significantly decreased mechanical allodynia ($F(1,84) = 10.69$, $P = 0.002$) and thermal hyperalgesia ($F(1,84) = 12.99$, $P = 0.001$), but only attenuated CFA-induced paw edema in males (males: $F(1,46) = 20.82$, $P < 0.001$; female data not shown). Rimonabant significantly antagonized THC's anti-allo-

dynic ($F(1,25) = 52.05$, $P < 0.001$) and anti-hyperalgesic ($F(1,25) = 22.36$, $P < 0.001$) effects in both sexes, as well as antagonizing THC's anti-edema effect in males ($P = 0.005$). In contrast, SR144528 significantly antagonized THC's anti-allodynic effect in females only ($P = 0.004$); antagonism of THC's anti-hyperalgesic effect was similar but not statistically significant ($P = 0.06$). SR144528 also significantly antagonized THC's anti-edema effect in males ($P = 0.002$). Neither THC nor the antagonists significantly altered locomotor activity in either sex (data not shown).

4. Discussion

The main findings of this study can be summarized as follows. Intraplantar CFA produced mechanical allodynia, thermal hyperalgesia and edema in the hind paws of adult female and male rats. When administered systemically (i.p.) or locally (i.pl.), THC produced dose-dependent anti-allodynic and anti-hyperalgesic effects in both sexes; however, in several cases THC was more effective in females than males. In contrast, THC produced greater anti-edema effects in males than females. Systemically administered THC also produced greater decreases in locomotor activity in females than males; thus, the greater anti-allodynic and anti-hyperalgesic effects of i.p. THC in females may be a result of THC's greater sedative effect in females. However, the same argument cannot be made for the greater anti-allodynic/hyperalgesic effects of locally

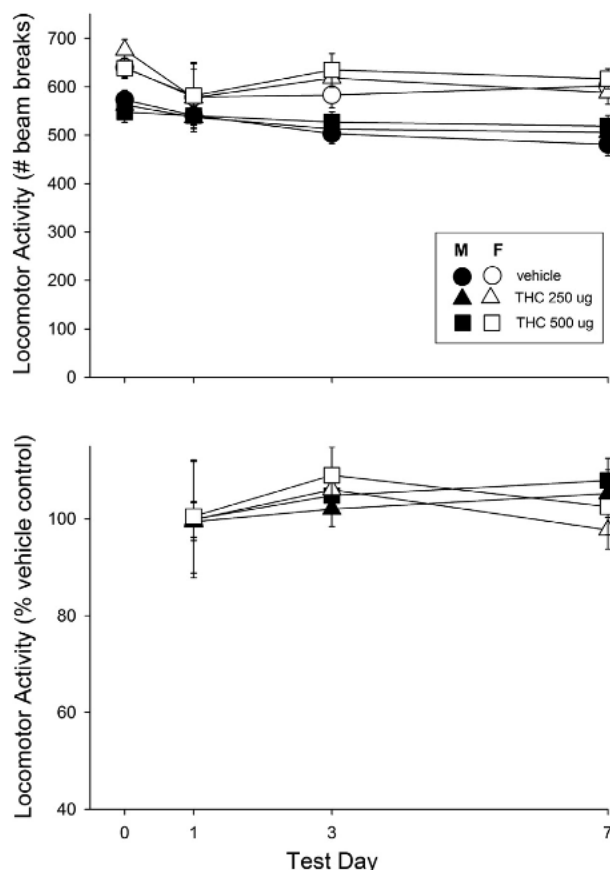


Fig. 7. The effect of locally administered THC (intraplantar, i.p.) on locomotor activity in female (F) vs male (M) rats. Baseline locomotor activity (# photobeams broken in 5 minutes) was determined on Day 0, immediately before injecting CFA into the right hind paw. On Days 1, 3, and 7 post-CFA, vehicle or THC was administered i.p. 30 minutes before re-testing locomotor activity. The same dose of THC was administered to the same rats each test day. Top panel: Locomotor activity as raw # photobeams broken. Bottom panel: Locomotor activity in THC-treated rats as percent of same-sex, vehicle-treated control values (as females locomote significantly more than males, in the absence of drug). Each point is the mean \pm 1 SEM of 10–12 females or 11–13 males.

administered THC, as i.p. THC did not decrease locomotor activity in either sex yet still produced greater anti-allodynic/hyperalgesic effects in females. Finally, most of THC's effects were CB1 receptor-mediated in both sexes, with two exceptions: in females, CB2 receptors also mediated the anti-allodynic/hyperalgesic effects of locally administered THC, and in males, CB2 receptors also mediated the anti-edema effects of locally administered THC.

In the present study, there were no consistently observed sex differences in inflammatory pain measures after hind paw CFA injection. CFA decreased mechanical thresholds by approximately 40–60 g, and shortened latency to respond to noxious heat by approximately 7–9 seconds, with no consistent sex differences across the four experiments. Edema, measured as percent increase in paw thickness from before to after CFA, was greater in males than in females in 1 of the 4 experiments (Experiment 2), but this was not accompanied by significantly greater allodynia or hyperalgesia in males compared with females. A few previous studies also report no sex differences in mechanical allodynia in chronic pain models (eg, CFA injection into masseter muscle [22]; post-incisional pain [18]). However, other studies report greater allodynia or thermal hyperalgesia in female compared with male rats after CFA injection (tail injection [6]; paw injection [31]), and after nerve injury [8,19]. Chronic CFA-induced pain is known to fluctuate across the estrous cycle: female rats in proestrus showed

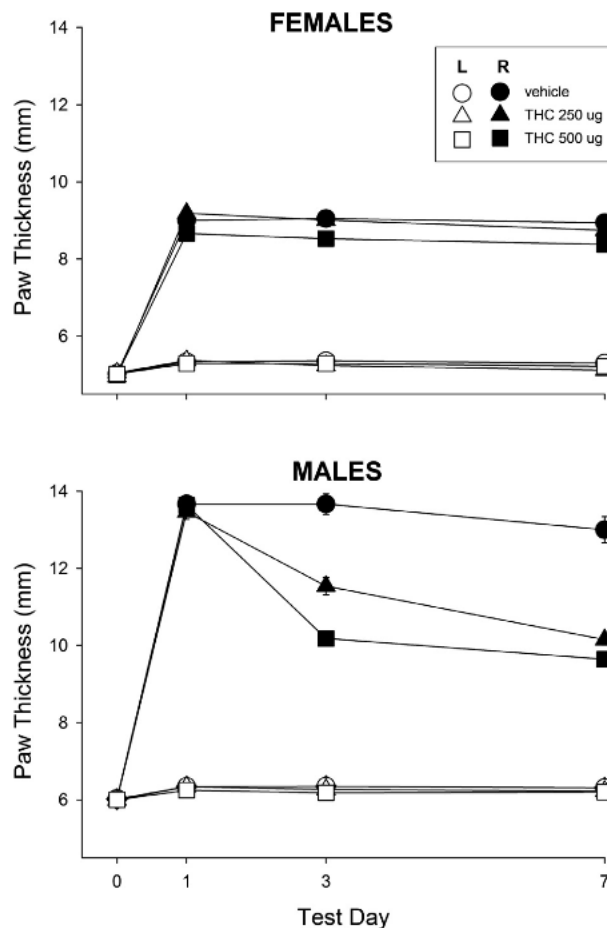


Fig. 8. The effect of locally administered THC (intraplantar, i.p.) on paw edema in female vs male rats. Thickness of the left (L) and right (R) hind paw was measured on Day 0, immediately before injecting CFA into the right hind paw. On Days 1, 3, and 7 post-CFA, vehicle or THC was administered i.p. 30 minutes before re-assessing paw thickness. The same dose of THC was administered to the same rats each test day. Each point is the mean \pm 1 SEM of 10–12 females or 11–13 males.

significantly greater CFA-induced hyperalgesia than females in other estrous stages (and males) [3]. In the present study, tracking of estrous stage revealed that very few females were in proestrus at the time of testing. For example, in the vehicle-treated groups in Experiments 1 and 2, only 2 of 22 females were in proestrus on Day 3 post-CFA. Had we tested more females in proestrus, greater allodynia and hyperalgesia may have been observed in females than in males. However, this study was not designed to characterize estrous stage effects on inflammatory pain; thus conclusions cannot be made regarding the possible influence of estrous stage on any measure.

The present finding of sex differences in anti-allodynia/hyperalgesia produced by THC in a longer-lasting, inflammatory pain model extends our previous reports of greater cannabinoid antinociception in females than males using acute pain tests [9,30]. Given the potential confounding of evoked pain (eg, latency-to-respond) measures by drug-induced sedation, and the fact that female rats are more sensitive than males to cannabinoid-induced motoric effects [9,30], it is possible that the greater antinociception observed in females is a result of, at least in part, greater cannabinoid-induced sedation. In fact, in the present study systemic THC administration produced significantly greater decreases in locomotor activity in females than males. When THC was administered locally into the inflamed hind paw, however, there were no sex differences in locomotor activity – in both sexes, i.p. THC did

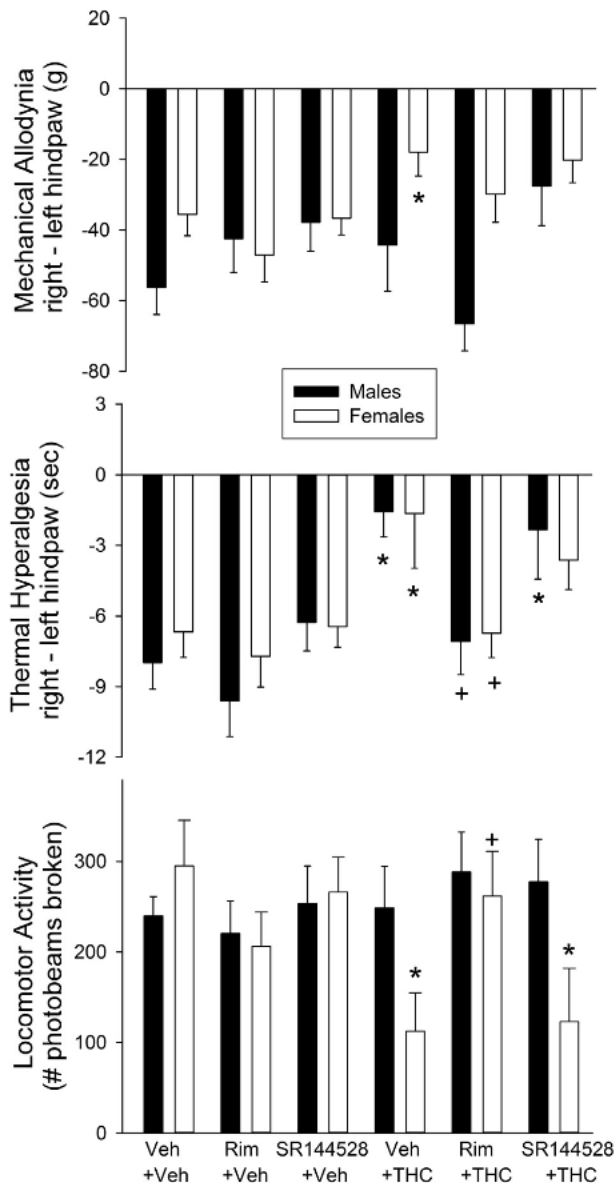


Fig. 9. Antagonism of the anti-allodynic, anti-hyperalgesic, and locomotor-decreasing effects of systemically administered THC in male and female CFA-treated rats, by the systemically administered CB1 antagonist rimonabant (Rim) and the CB2 antagonist SR144528. Top panel: Mechanical allodynia as measured by the von Frey test, shown as the difference in threshold between the right and left hind paws. Bars in the negative direction indicate that the right (CFA-injected) hind paw had a lower mechanical threshold than the left hind paw. Middle panel: Thermal hyperalgesia as measured by the Hargreaves test, shown as the difference between the right and left hind paws. Bars in the negative direction indicate that latency to respond was shorter for the right (CFA-injected) hind paw than for the left hind paw. Bottom panel: Locomotor activity. Each bar is the mean \pm 1 SEM of 9–10 rats. *Significant THC effect (different from same-sex, Veh+Veh control group), $P \leq 0.05$; +Significant antagonism of THC effect (different from same-sex, Veh+THC group), $P \leq 0.05$.

not affect locomotor activity compared with i.pl. vehicle. This result suggests that the greater anti-allodynia/hyperalgesia observed in females given i.pl. THC reflects greater pain reduction in females than in males. Still, one alternative explanation must be considered: given that female rats' hind paws are smaller than those of males, it is possible that the i.pl. THC injection distributed more fully throughout the paws of females compared with the paws of males. We plan to address this possibility in the future by adjusting i.pl. injection volume according to paw volume (ie, using a larger injection volume in males or a smaller injection

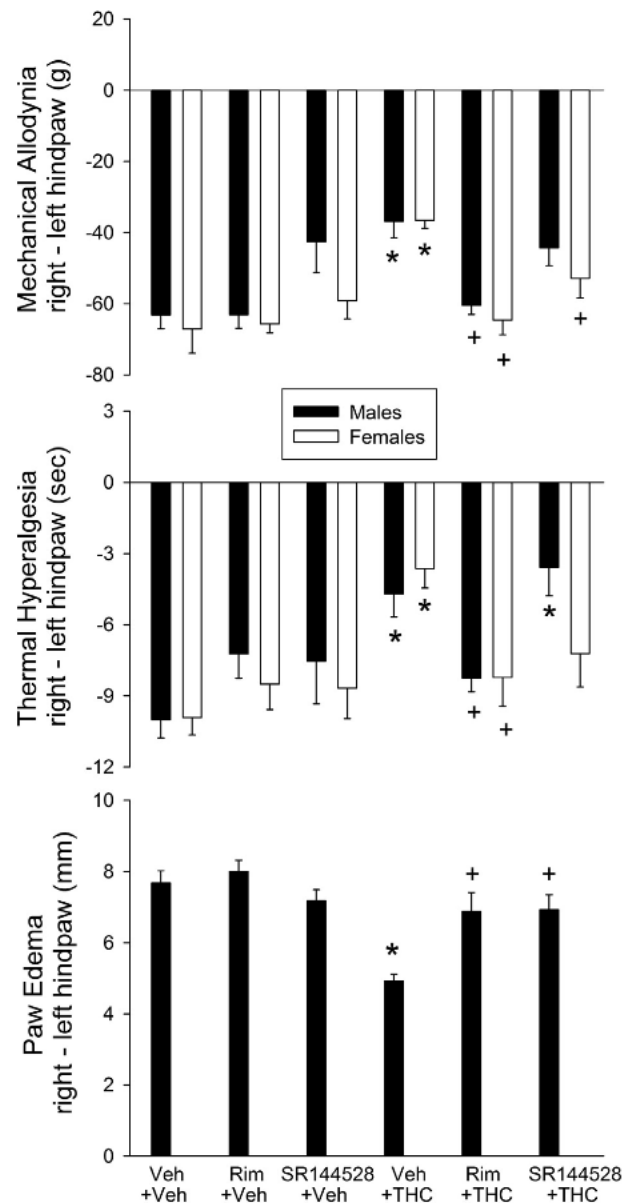


Fig. 10. Antagonism of the anti-allodynic, anti-hyperalgesic, and anti-edema effects of locally administered THC in male and female CFA-treated rats, by the locally administered CB1 antagonist rimonabant (Rim) and the CB2 antagonist SR144528. Top panel: Mechanical allodynia as measured by the von Frey test, shown as the difference in threshold between the right and left hind paws. Bars in the negative direction indicate that the right (CFA-injected) hind paw had a lower mechanical threshold than the left hind paw. Middle panel: Thermal hyperalgesia as measured by the Hargreaves test, shown as the difference between the right and left hind paws. Bars in the negative direction indicate that latency to respond was shorter for the right (CFA-injected) hind paw than for the left hind paw. Bottom panel: Paw edema, as measured by the difference in thickness between the right (CFA-injected) hind paw and the left hind paw. Paw edema is not shown for females because THC did not affect CFA-induced increases in paw thickness in this sex. Each bar is the mean \pm 1 SEM of 5–10 males or 6–12 females. *Significant THC effect (different from same-sex, Veh+Veh control group), $P \leq 0.05$; +Significant antagonism of THC effect (different from same-sex, Veh+THC group), $P \leq 0.05$.

volume in females, to test the same dose of i.pl. THC). It should be noted as well that solubility issues limited the examination of local doses of THC $>500 \mu\text{g}$. This dose did not completely attenuate allodynic/hyperalgesic effects of CFA in males. Thus, whether the sex difference in THC's effect reflects a difference in THC's potency or also a difference in efficacy cannot be determined from the present data.

One surprising finding – particularly given the greater THC-induced anti-allodynia/hyperalgesia in females – was the greater anti-edema effect of THC in males. This sex difference was particularly striking after injection of THC directly into the inflamed paw, suggesting a peripheral locus of the sex difference in THC's anti-edema effect. Anti-inflammatory effects of THC have been reported previously: for example, i.p. THC reduced tissue inflammation in a (male) rat model of colitis [17], and per os (p.o.) THC reduced carrageenan-induced paw edema in male rats [29] and lung tissue inflammation in female mice [4]. A literature search revealed no studies comparing any anti-edema effect of cannabinoids between the sexes. The suggestion from the present study that peripheral edema may be more effectively treated with THC in males than females bears further investigation.

The present study reveals a possible sex difference in receptor mediation of THC's effects against inflammatory pain. Whereas THC's anti-allodynic/hyperalgesic effects were fully blocked by the CB1 antagonist rimonabant in both males and females, i.p. THC's effects were also attenuated by the CB2 antagonist SR144528 in females. CB2 receptor antagonism was only observed when the antagonist was administered locally into the inflamed paw, suggesting that females may possess more peripheral CB2 receptors than do males. THC is known to bind non-selectively to both CB1 and CB2 receptors [25]. In male rats, THC's antinociceptive effects in acute pain models have typically been found to be CB1 receptor-mediated; however, peripheral CB2 receptor involvement has been reported under conditions of more long-lasting, inflammatory pain [7]. In our previous study examining sex differences in antagonism of cannabinoid-induced antinociception – the only such sex difference study, to our knowledge – we observed limited antagonism of THC (but not CP55,940-) induced antinociception by SR144528 only in females, using acute pain models [9].

There are several studies documenting sex differences in brain cannabinoid (typically CB1) receptors (for review, see [10]), but we are aware of only two studies examining sex differences in peripheral cannabinoid receptors. First, cannabinoid receptor density in leukocytes was found to be greater in women than men [23]. More recently, Niu and colleagues [22] reported that although CB1 receptor mRNA levels in trigeminal ganglia were not different between female and male rats under non-inflamed conditions, CFA injection into the masseter muscle significantly increased CB1 receptor mRNA in males only (CB2 receptors were not examined). Given that there are numerous studies in male rodents demonstrating a role for both CB1 and CB2 receptors in inflammatory pain conditions [1,14,16,27,34], further comparison of both types of peripheral cannabinoid receptors in females vs males is warranted, to determine, for example, whether inflammation induces greater increases in peripheral CB2 receptor density in females than in males. It should be noted that the one previous study reporting antagonism of THC-induced mechanical hyperalgesia by SR144528 in arthritic male rats required an antagonist dose of 10 mg/kg [7]. Thus, in the present study, failure to observe CB2 receptor antagonism in males may be explained by a sex difference in potency of SR144528 (perhaps reflecting lesser CB2 receptor density in males), in that a dose of SR144528 higher than that tested in the present study may have attenuated THC's effect in males as well as females.

We are aware of only one previous study in which the effects of a cannabinoid against inflammatory pain were compared in females vs males. Contrary to the present findings, the selective CB1 agonist ACPA was significantly more potent in males than females in reducing mechanical allodynia, 3 days post-CFA (masseter muscle injection: [22]). ACPA's action at CB1 but not CB2 receptors was confirmed in males, and the authors suggest that ACPA's comparatively weak anti-allodynic effect in females may have been CB2 receptor-mediated, although they did not test this possibility

[22]. Taken together with the present results, in which a non-selective cannabinoid agonist was more potent in females than males, and its peripheral effects were antagonized by CB2 as well as CB1 receptor blockade, these studies point to a potentially greater role for peripheral CB2 receptors in females than in males, under conditions of CFA-induced inflammatory pain. Given the previous studies in male rodents demonstrating a role for CB2 receptors in other inflammatory pain conditions (eg, [7,14,16]), however, further comparison is needed to determine the conditions under which sex differences in CB2 receptor mediation of inflammatory pain occur.

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