

**Sex Differences in CB<sub>1</sub> vs. CB<sub>2</sub> Receptor-Selective Antagonism of  
Antinociception Produced by  $\Delta^9$ -Tetrahydrocannabinol and CP55,940 in the  
Rat**

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**Abbreviations:** %MPE = percent maximum possible effect; CP55,940 = 5-(1,1-

Dimethylheptyl)-2-[5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]phenol; Rimonabant = 5-(4-

chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-N-1-piperidinyl-1H-pyrazole-3-carboxamide; SR144528  
= 5-(4-chloro-3-methylphenyl)-1-[(4-methylphenyl)methyl]-N-[(1S, 2S, 4R)-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl]-1H-pyrazole-3-carboxamide

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## ABSTRACT

The purpose of this study was to determine whether sex differences in cannabinoid-induced antinociception and motoric effects can be attributed to differential activation of CB<sub>1</sub> or CB<sub>2</sub> receptors. Rats were injected i.p. with vehicle, rimonabant (SR141716A, 0.1-10 mg/kg, a putative CB<sub>1</sub> receptor-selective antagonist) or SR144528 (1.0-10 mg/kg, a putative CB<sub>2</sub> receptor-selective antagonist). Thirty min later,  $\Delta^9$ -tetrahydrocannabinol (THC, 1.25-40 mg/kg) or CP55,940 (0.05-1.6 mg/kg) was injected. Paw pressure and tail withdrawal antinociception, locomotor activity and catalepsy were measured. Rimonabant dose-dependently antagonized THC and CP55,940 in each test, but was up to 10 times more potent in females than males on the nociceptive tests; estimates of rimonabant affinity (apparent pK<sub>B</sub>) for the CB<sub>1</sub> receptor were approximately 0.5-1 mol/kg higher in females than males. SR144528 partially antagonized THC-induced tail withdrawal antinociception and locomotor activity in females, but this antagonism was not dose-dependent or consistent; no SR144528 antagonism was observed in either sex tested with CP55,940. Neither the time course of rimonabant antagonism nor plasma levels of rimonabant differed between the sexes. Rimonabant and SR144528 did not antagonize morphine-induced antinociception, and naloxone did not antagonize THC-induced antinociception in either sex. These results suggest that THC produces acute antinociceptive and motoric effects via activation of CB<sub>1</sub> – and perhaps under some conditions, CB<sub>2</sub> receptors – in females, whereas THC acts primarily at CB<sub>1</sub> receptors in males. Higher apparent pK<sub>B</sub> for rimonabant in females suggests that cannabinoid drugs bind with greater affinity to CB<sub>1</sub> receptors in females than males, likely contributing to greater antinociceptive effects observed in female compared to male rats.



## INTRODUCTION

Sex differences in a variety of cannabinoid effects have been demonstrated in animals. For example, cannabinoids such as  $\Delta^9$ -tetrahydrocannabinol (THC) are more potent in female than male rodents in producing antinociception (Tseng and Craft, 2001; Romero et al., 2002), hypothermia (Borgen et al., 1973; Wiley et al., 2007), and motoric effects (Cohn et al., 1972; Tseng and Craft, 2001). Female rats also acquire cannabinoid self-administration faster than males, and maintain higher rates of responding (Fattore et al., 2007). In contrast, male rodents are more sensitive than females to the hyperphagic effect of cannabinoid agonists (Diaz et al., 2009). Sex differences in cannabinoid effects also have been demonstrated in humans. For example, dronabinol (synthetic THC) retarded gastric emptying to a greater extent in women than men (Esfandyari et al., 2006), and women reported more dizziness than men after cannabinoid intake (Mathew et al., 2003). In contrast, men reported greater ratings of “high” as well as some other subjective effects after cannabinoid intake (Haney, 2007). Sex differences in cannabinoid analgesia have not yet been examined in humans, but a growing number of controlled clinical studies demonstrates that chronic pain is alleviated by cannabinoids (Russo, 2008).

Cannabinoids are known to produce antinociception via supraspinal, spinal and peripheral mechanisms, and by acting at CB<sub>1</sub> and CB<sub>2</sub> receptors (Anand et al., 2009). It is not known which of these mechanisms contribute to greater cannabinoid antinociception in females compared to males. Antinociception produced by systemically administered THC can be attenuated by the CB<sub>1</sub> receptor-selective antagonist rimonabant (SR141716A, also shown to be an inverse agonist), administered either i.c.v. or i.t., in both male and female rats (Tseng and Craft, 2004), demonstrating supraspinal and spinal CB<sub>1</sub> receptor involvement in both sexes. In

male rodents, antinociception produced by CB<sub>2</sub> receptor activation can occur spinally and peripherally (Gutierrez et al., 2007; Romero-Sandoval and Eisenach, 2007). Although antinociception via supraspinal CB<sub>2</sub> receptor activation has yet to be demonstrated, CB<sub>2</sub> receptors have been detected in pain-relevant brain areas such as the periaqueductal gray, albeit at a lower density than CB<sub>1</sub> receptors (Gong et al., 2006).

The purpose of the present study was to test the hypothesis that sex differences in cannabinoid antinociception are mediated by both CB<sub>1</sub> and CB<sub>2</sub> receptors. Gonadally intact male and female rats were pretreated with the putative CB<sub>1</sub> or CB<sub>2</sub> receptor-selective antagonist, rimonabant or SR144528, respectively (Shire et al., 1999), in combination with vehicle or a cannabinoid agonist, and then tested over a 4-hr period on two nociceptive and two motor activity assays. Time course analyses were conducted for the primary psychoactive compound in cannabis, THC, and for the synthetic cannabinoid agonist CP55,940. Both of these cannabinoids are generally characterized as non-selective, mixed CB<sub>1</sub>/CB<sub>2</sub> agonists; however, CP55,940 has significantly greater efficacy than THC at CB<sub>1</sub> receptors (Govaerts et al., 2004). The acute antinociceptive effects of both agonists appear to be mediated by CB<sub>1</sub> receptors in male rats (Lichtman and Martin, 1997) and mice (Varvel et al., 2005). To investigate a pharmacodynamic mechanism underlying sex differences in agonist/antagonist interaction in the present study, complete agonist dose-effect curves were obtained, alone and in combination with the most effective dose(s) of each antagonist. *In vivo* apparent pK<sub>B</sub> values (Negus et al., 1993; Rowlett and Woolverton, 1996) were calculated from these curves to test the hypothesis that sex differences in behavioral effects of cannabinoid drugs are due to sex differences in the affinity of cannabinoid drugs for cannabinoid receptors. The time course of antagonist effect as well as plasma levels of antagonist also were examined to determine whether sex differences in

antagonism of cannabinoid agonist-induced behaviors could be attributed to sex differences in antagonist duration of action or absorption. Finally, potential sex differences in the actions of THC at opioid receptors (naloxone antagonism), and in the actions of morphine at cannabinoid receptors, were examined to determine the specificity of sex differences in cannabinoid pharmacology.

## METHODS

**Subjects.** Adult (60-85 day old) male and female Sprague-Dawley rats were used (bred in-house from Taconic stock, Germantown, NY). Rats were housed under a 12:12 hr light:dark cycle (lights on at 0600 hr), in a room maintained at  $21\pm 2^{\circ}\text{C}$ . Rats were treated in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* (1996). Rats were assigned randomly to treatment groups, with the exception that we avoided assigning same-sex siblings to any group that had six or fewer rats. Each rat was tested with a single drug combination.

**Apparatus.** Tail withdrawal antinociception was assessed using a 2.5-L water bath (Precision Scientifics Inc., Winchester, VA) set to  $50\pm 0.5^{\circ}\text{C}$ ; latency to withdraw the tail was timed with a hand-held stopwatch, and cutoff was 12 sec. 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/sec to a maximum of 750 g (15-sec cutoff). Horizontal locomotor activity was measured using a photobeam apparatus (Opto-varimex, Columbus Instruments, Columbus, OH): 15 photobeams cross the width of a 20 X 40 X 23-cm clear Plexiglas rodent cage, with photobeams spaced 2.5 cm apart and 8 cm above the cage floor. Catalepsy was measured using a bar test: a ring stand with a 1.5-cm diameter horizontal bar set at 13 cm (female) or 15.5 cm (male) above the countertop.

**Drugs.** Rimonabant, SR144528, THC and CP55,940 (National Institute on Drug Abuse, Bethesda, MD) were dissolved in a 1:1:18 ethanol:cremophor:saline solution, which served as the vehicle. Naloxone hydrochloride and morphine sulfate (Sigma-Aldrich Co., St. Louis, MO) were dissolved in physiological saline. All cannabinoid drugs were administered i.p., and opioids were administered s.c., all in volumes of 1 mL/kg, except for the highest doses of the

cannabinoid agonists (20-40 mg/kg THC and 0.4-1.6 mg/kg CP55,940), which were administered in larger volumes (2-8 mL/kg) due to solubility limitations. The 8 mL/kg volume of the 1:1:18 vehicle was tested in two female and two male rats to determine whether the greater amount of ethanol (approximately 0.35 g/kg) would produce significant antinociceptive or motoric effects on any of the four tests, and it did not (data not shown). Finally, two different batches of SR144528 were used during the course of these experiments, and the older batch (kept over 10 years in a freezer) used in initial experiments partially antagonized THC on some measures in females, whereas the newer batch did not. We hypothesized that the older batch had degraded such that it was no longer CB<sub>2</sub> receptor-selective; however, analysis of the two batches showed that they were identical and purely SR144528 (Dr. H. Seltzman, Research Triangle Institute, personal communication).

***Behavioral procedure.*** Baseline nociception was measured by testing each rat on the tail withdrawal and paw pressure tests, in that order, twice. After baseline testing, vehicle or a single dose of rimonabant (0.1-10 mg/kg), SR144528 (1.0-10 mg/kg) or naloxone (1.0 mg/kg) was injected. In most cases, 30 min later (rimonabant, SR144528 pretreatment) or 5 min later (naloxone pretreatment), vehicle or agonist (THC, CP55,940, morphine) was injected. In most experiments, rats were then tested on tail withdrawal and paw pressure tests at 15, 30, 60, 120 and 240 min post-injection. For the tail withdrawal assay, the distal 5 cm of the tail was submerged in the warm water bath and latency to withdraw the tail was recorded; if the rat did not respond by the 12-sec cutoff, the test was terminated and 12 sec was recorded. For the paw pressure test, latency to withdraw or attempt to withdraw the hindpaw was recorded; if the rat did not respond by the 15-sec cutoff, the test was terminated and 15 sec was recorded. Horizontal locomotor activity was measured as the number of photobeams broken in a 5-min period,

beginning immediately after nociceptive testing at 30, 60, 120 and 240 min post-injection. Catalepsy was assessed immediately after the locomotor test, at the 60-min time point only. Latency to withdraw both forepaws from the bar or jump onto the bar was recorded; if the rat did not respond by 12 sec, the test was terminated and 12 sec was recorded. If a rat moved its forepaws across the bar (approximately 1% of all rats tested), it was not considered cataleptic and its score was dropped from the dataset before analysis. Rats were returned to their home cages between testing periods.

Exceptions to the above method were: (1) morphine dose-effect curves were obtained using a cumulative dosing procedure with 15-min injection/test intervals; (2) for the antagonist time course determination, vehicle, rimonabant or SR144528 was injected 5, 30, 60 or 90 min before THC.

The experimenter who collected approximately 50% of the THC data (J.D.L.) was blinded to treatment assignment: A.A.W. prepared drugs and filled syringes, which were labeled only with a rat number, and J.D.L. injected and tested rats. Most other testing was conducted without blinding; THC data collected by J.D.L. and A.A.W. were not significantly different, so these data were pooled.

***Determination of estrous cycle.*** Immediately after behavioral testing, a vaginal smear was obtained from each of the females. Slides were later stained with Giemsa (Sigma-Aldrich) and scored under the microscope, as follows: Proestrus was identified by the predominance (approximately 75% or more of cells in the sample) of nucleated epithelial cells; proestrus to estrus (sometimes referred to as “late proestrus”) was identified by approximately equal proportions of nucleated and cornified epithelial cells; estrus was identified by the presence of

dense sheets of cornified epithelial cells; and diestrus was identified by scattered nucleated and cornified epithelial cells and leukocytes (diestrus-1) or a relative lack of any cells (diestrus-2).

***Determination of plasma rimonabant levels.*** To determine whether sex differences in rimonabant antagonism could be due to sex differences in drug absorption, a separate group of female and male rats was injected with 1.0 mg/kg rimonabant; rats were euthanized 60 min post-injection and plasma levels of rimonabant were determined by high performance liquid chromatography. The method of Hurtado and colleagues (2010) was followed, with minor changes: a mobile phase of 75% acetonitrile and 25% water (v/v) was used, with a flow rate of 1.0 mL/min; pterostilbene was used as the internal standard.

***Data Analysis.*** Baseline nociceptive latencies for each rat on the tail withdrawal and paw pressure tests were calculated as the mean of the two pre-injection trials. To adjust for individual differences in baseline latency to respond, response latencies following drug administration were converted to percent maximum possible effect (%MPE) in each rat:  $(\text{drug latency} - \text{mean baseline latency}) / (\text{cutoff latency} - \text{mean baseline latency}) \times 100$ . For catalepsy %MPE calculations, the mean catalepsy score of same-sex, vehicle-treated rats was used as the baseline latency. Because there was a trend towards sex differences in locomotor activity in vehicle-treated rats (see Results), locomotor activity data in drug-treated rats (# photobeams broken) were converted to percent of the same-sex, vehicle control group, at each time point:  $(\# \text{ photobeam breaks in drug-treated rat} / \text{mean } \# \text{ photobeam breaks in same-sex vehicle control group}) \times 100$ .

Time course data for THC and CP55,940 alone (%MPE tail withdrawal and paw pressure scores, and % control locomotor scores) were analyzed using a 3-way ANOVA (sex (2 levels),

dose (4-5 levels), time (4-5 levels, repeated), with estrous stage entered as a covariate). Catalepsy data (latency in sec) for THC and CP55,940 alone were analyzed using a 2-way ANOVA (sex, dose), with estrous stage entered as a covariate. For antagonist + THC time course analyses, a 2-way ANOVA was used in each sex, for each antagonist: factors were antagonist dose (3-4 levels) and time (4-5 levels, repeated). Catalepsy data (in sec) were analyzed using ANOVA in each sex (antagonist dose, 4-5 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.

To analyze antagonism in terms of change in agonist potency, agonist-antagonist interactions were examined at the time of peak agonist effect, which was determined to be 30-60 min post-injection (see Results). Thus, for THC and CP55,940, the mean %MPE on each nociceptive test and the mean % control locomotor activity scores at the 30- and 60-min time points were calculated for each individual, and dose-effect curves for the agonist alone and in combination with each antagonist were constructed from these data. For catalepsy data, %MPE catalepsy scores were calculated (only measured at 60-min post-injection) for each individual. The agonist dose that produced 50% effect ( $ED_{50}$ ) alone and in the presence of each dose of rimonabant was then estimated by log-linear regression, for antinociception data (peak %MPE values), locomotor activity data (peak % control values), and catalepsy data (%MPE values) (Pharm/PCS, Version 4.2). Potency ratios were calculated to determine whether there were sex differences in the degree to which rimonabant shifted agonist dose-effect curves (Pharm/PCS, Version 4.2). Finally, apparent  $pK_B$  values were determined to provide an estimate of rimonabant affinity in females vs. males:  $pK_B = -\log [B/(dose\ ratio - 1)]$ , where B=antagonist dose



in mol/kg and dose ratio= $ED_{50}$  antagonist+agonist/ $ED_{50}$  vehicle+agonist (Negus et al., 1993; Rowlett and Woolverton, 1996).

## RESULTS

***Sex differences in baseline measurements.*** Sex differences in baseline latencies to respond on the nociceptive tests were examined in rats in the cannabinoid antagonist + THC time course experiment, as this was the largest dataset. Nociceptive latencies were significantly shorter in females than males:  $3.73 \pm 0.08$  vs.  $4.09 \pm 0.09$  sec in females vs. males, respectively, on the tail withdrawal test ( $t(306)=-2.87, p=0.004$ ), and  $3.90 \pm 0.09$  vs.  $4.90 \pm 0.11$  sec on the paw pressure test ( $t(306)=-7.15, p<0.001$ ). On the locomotor activity test, vehicle-treated females were slightly but not significantly more active than vehicle-treated males ( $F(1,26)=2.25, p=0.15$ ; Table 1). On the catalepsy test, there were no sex differences in vehicle-treated rats: mean latency to remove both paws from the bar (or jump up on the bar) was approximately 1.0-1.5 sec in both sexes (Fig. 2, right panel).

***Sex differences in behavioral effects of THC.*** Figure 1 shows time-effect curves for THC alone (vehicle + THC) in female vs. male rats, on the two nociceptive tests. THC produced dose- and time-dependent antinociception in both sexes on both tests, but was more potent in females than males. For example, whereas 10 mg/kg THC produced near-maximal paw pressure antinociception in females, 20 mg/kg was required to produce a similar effect in males (left panels). Statistical comparison of the THC doses that were tested in both sexes (1.25-10 mg/kg) yielded a significant sex difference on the paw pressure test ( $F(1,108)=6.27, p=0.014$ ) and on the tail withdrawal test ( $F(1,108)=29.58, p<0.001$ ). THC's antinociceptive effects generally peaked at 30-60 min post-injection in both sexes on both tests. Estrous stage accounted for a significant portion of the variance in response to THC on the tail withdrawal but not paw pressure test, with females in estrus showing the greatest THC-induced antinociception ( $F(1,85)=5.06, p=0.027$ ; data not shown).

Figure 2 shows THC-induced suppression of locomotor activity (left panels) and catalepsy (right panel). Statistical comparison of the THC doses that were tested in both sexes (1.25-10 mg/kg) indicated greater locomotor suppression in females than males at some doses and time points (e.g., at 5 mg/kg, 120-240 min post-injection; sex x time x THC dose:  $F(9,246)=2.62, p=0.007$ ), and greater catalepsy in females at 5 and 10 mg/kg (sex x THC dose:  $F(4,106)=4.45, p=0.002$ ). Estrous stage did not significantly influence females' response to THC on tests of motor activity (data not shown).

***CB<sub>1</sub> vs. CB<sub>2</sub> antagonism of THC-induced antinociception and sedation: time-effect curves.*** Figure 3 shows antagonism of 5 mg/kg THC on the paw pressure test, by rimonabant (top panels) and SR144528 (bottom panels), in female vs. male rats. Rimonabant dose-dependently antagonized THC-induced antinociception in both sexes (females:  $F(3,37)=7.99, p<0.001$ ; males:  $F(3,34)=5.21, p=0.005$ ); however, nearly complete antagonism was observed at 1.0 mg/kg in females vs. 10 mg/kg in males. SR144528 (1-10 mg/kg) did not antagonize THC-induced paw pressure antinociception in males ( $F(3,37)=0.44, n.s.$ ); in females, antagonism by SR144528 was not statistically significant ( $F(3,38)=2.59, p=0.07$ ).

Figure 4 shows antagonism of 5 mg/kg THC by rimonabant and SR144528 on the tail withdrawal test, in females vs. males. Rimonabant dose-dependently antagonized THC-induced tail withdrawal antinociception in females at relatively low doses ( $F(3,37)=17.19, p<0.001$ ), with nearly complete antagonism at 0.32 mg/kg. In males, antagonism was also dose-dependent, and essentially complete at 3.2 mg/kg ( $F(3,34)=3.12, p=0.039$ ). SR144528 partially antagonized THC-induced tail withdrawal antinociception in females ( $F(3,38)=3.63, p=0.021$ ), but not in males ( $F(3,37)=0.59, n.s.$ ).

Figure 5 shows antagonism of 5 mg/kg THC-induced suppression of locomotor activity. Rimonabant antagonized THC-induced decreases in locomotor activity in both sexes; however, the potency and time course of antagonism differed between the sexes. In females, 0.1-1.0 mg/kg rimonabant dose-dependently antagonized THC's effect ( $F(3,37)=7.02, p=0.001$ ), and there was no dose by time interaction. In males, rimonabant antagonized THC's locomotor-suppressant effect only at 30-60 min post-THC injection (rimonabant dose x time:  $F(9,102)=2.64, p=0.009$ ). SR144528 attenuated THC's locomotor-suppressant effect in females ( $F(3,38)=3.99, p=0.015$ ) but only partially and only at the intermediate dose, 3.2 mg/kg. In contrast, SR144528 tended to exacerbate THC-induced locomotor suppression in males ( $F(3,37)=3.08, p=0.039$ ).

Figure 6 shows antagonism of catalepsy produced by 5 mg/kg THC, which was measured 60 min post-THC injection. In females, 0.1-10 mg/kg rimonabant dose-dependently antagonized THC-induced catalepsy ( $F(4,40)=6.73, p<0.001$ ). In males, rimonabant antagonism was not significant ( $F(3,34)=0.67, n.s.$ ). SR144528 (1.0-10 mg/kg) did not antagonize THC-induced catalepsy in rats of either sex (Fig. 6).

***CB<sub>1</sub> antagonism of THC-induced antinociception and sedation: THC dose-effect curves.*** To better quantify apparent sex differences in CB<sub>1</sub> receptor-selective antagonism of THC's behavioral effects, doses of THC higher and lower than 5 mg/kg were examined in combination with the antagonist doses that were the most effective in each sex (determined from the first antagonist experiment, Fig. 3). Agonist-antagonist interactions were graphed and statistically compared only at the time of peak agonist + antagonist effect (i.e., 30-60 min post-THC injection, see Methods). Figure 7 shows THC dose-effect curves alone and combination with two doses of rimonabant on the two nociceptive tests. The ED<sub>50</sub> values derived from each

dose-effect curve, and relative potencies of antagonist + THC vs. vehicle + THC are shown in Table 2. In females, 0.32 and 1.0 mg/kg rimonabant shifted the THC dose-effect curve to the right on both nociceptive tests (Fig. 7), with 1.0 mg/kg increasing the ED<sub>50</sub> for THC approximately 6- to 7-fold (Table 2). In males, 1.0 and 10 mg/kg rimonabant shifted the THC dose-effect curve to the right on both nociceptive tests (Fig. 7); however, 1.0 mg/kg rimonabant only increased the THC ED<sub>50</sub> approximately 3-fold (Table 2). This sex difference in potency ratio was statistically significant on the paw pressure and tail withdrawal tests (Table 2). In males, 10 mg/kg rimonabant shifted the THC dose-effect curves farther to right, to nearly the same extent that 1.0 mg/kg did in females (Fig. 7; Table 2).

Figure 8 shows THC dose-effect curves on the two tests of motor function, alone and in combination with the two doses of rimonabant in each sex. On the locomotor activity test, the THC dose-effect curve was shifted rightward by rimonabant (0.32 and 1.0 mg/kg in females, and 1.0 and 10 mg/kg in males). The magnitude of the shift produced by 1.0 mg/kg rimonabant did not differ between females and males (see potency ratios, Table 2). On the catalepsy test, rimonabant produced dose-dependent rightward shifts in the THC dose-effect curve that looked very similar to those observed on the nociceptive tests: whereas 1.0 mg/kg rimonabant produced a 4.5-fold increase the THC ED<sub>50</sub> in females, it produced essentially no change in THC potency in males (Fig. 8). Although ED<sub>50</sub> values for THC could not be calculated for the catalepsy measure in males due to the shallowness of the dose-effect curve, the higher dose of rimonabant tested in males, 10 mg/kg, appeared to shift the THC dose-effect to the right (Fig. 8).

Apparent pK<sub>B</sub> values, calculated in all cases in which potency ratios could be calculated, were higher in females than in males. The apparent pK<sub>B</sub> for rimonabant calculated from paw pressure data was 6.40-6.58 mol/kg (1.0 and 0.32 mg/kg rimonabant, respectively) in females vs.

5.31-5.98 mol/kg (10 and 1.0 mg/kg rimonabant, respectively) in males. A similar sex difference was obtained from tail withdrawal data: apparent  $pK_B$  values were 6.44-6.70 mol/kg in females vs. 5.92 mol/kg in males. In contrast, sex differences in apparent  $pK_B$  were smaller using locomotor activity and catalepsy data:  $pK_B$  estimates ranged from 6.08-6.32 vs. 5.80 mol/kg in females vs. males, respectively.

***CB<sub>1</sub> vs. CB<sub>2</sub> antagonism of CP55,940-induced antinociception and sedation.*** We next compared the ability of rimonabant and SR144528 to antagonize the antinociceptive and motoric effects of another mixed CB<sub>1</sub>/CB<sub>2</sub> (but higher potency and efficacy) cannabinoid agonist, CP55,940. Similar to THC, CP55,940's antinociceptive effects peaked at approximately 30-60 min post-injection in both sexes on both tests (data not shown). Figure 9 shows antagonism of CP55,940-induced antinociception by 1.0 and 10 mg/kg rimonabant in female vs. male rats. The ED<sub>50</sub> values derived from each dose-effect curve, and potency ratios of antagonist + CP55,940 vs. vehicle + CP55,940 are shown in Table 3. On the paw pressure test (Fig. 9, left panels), rimonabant shifted the CP55,940 dose-effect curve to the right in both sexes; however, the rightward shifts were greater in females than males (see Table 3). Rimonabant also tended to flatten the CP55,940 curves in females but not males. Similar effects were observed on the tail withdrawal test (Fig. 9, right panels): rimonabant shifted the CP55,940 dose-effect curve approximately 4- and 12-fold to the right in males (Table 3), whereas rimonabant flattened the CP55,940 curve in females such that ED<sub>50</sub> values could not be calculated (slopes of CP55,940 curves were significantly different between vehicle- and rimonabant-treated females,  $p < 0.05$ ). SR144528 did not shift the CP55,940 curves to the right in either sex, on either nociceptive test (data not shown).

Figure 10 shows antagonism of CP55,940 by rimonabant on the two tests of motor function. The 1.0 and 10 mg/kg doses of rimonabant produced dose-dependent rightward shifts in the CP55,940 dose-effect curves in both sexes, and the shifts were comparable in magnitude (see Table 3). On the catalepsy test, rimonabant appeared to shift the CP55,940 curve farther to the right in females than males (Fig. 10), but the potency ratios could not be compared quantitatively due to the low efficacy of CP55,940 in males (and given the limitations on dose ranges that could be tested due to drug insolubility). Estrous stage did not significantly influence females' responses to CP55,940, on any of the four behavioral tests (data not shown).

Apparent  $pK_B$  values, calculated in all cases in which potency ratios could be calculated, tended to be higher in females than in males. For example, the apparent  $pK_B$  for rimonabant (1.0 mg/kg dose) on the paw pressure test was 6.02 vs. 5.46 mol/kg in females vs. males, respectively. In contrast, sex differences in apparent  $pK_B$  were very small on the locomotor and catalepsy tests:  $pK_B$  estimates (1.0 mg/kg rimonabant dose) were 6.06-6.10 vs. 5.92-5.98 mol/kg in females vs. males.

***Time course of rimonabant and SR144528 antagonism.*** To test whether sex differences in antagonism of THC could be due to a different time course of antagonist effect in females vs. males, separate rats were pretreated with vehicle, rimonabant (1.0 mg/kg), or SR144528 (3.2 mg/kg) either 5, 30, 60 or 90 min before THC (5 mg/kg) was administered. Figures 11 and 12 show the time course of antagonism of the antinociceptive and motoric effects of THC, respectively, at the time of peak agonist effect (30-60 min after THC injection, see Methods). There were no significant sex differences in the time course of antagonism of THC-induced antinociception (Fig. 11; sex x antagonist x pretreatment time:  $F(6,155)=0.95$  (paw pressure),  $F(6,155)=1.43$  (tail withdrawal), *n.s.*). In both sexes, rimonabant was maximally effective when

given 5- to 30-min before THC – that is, when antinociceptive effects were assessed 35-90 min after antagonist administration – and was clearly waning by the 90-min pretreatment time (i.e., when behavioral effects were assessed 120-150 min after antagonist administration). The time course of rimonabant antagonism of THC's motoric effects was very similar, although again, antagonism of catalepsy was not statistically significant in males. SR144528 did not significantly antagonize any behavioral effect of THC at any pretreatment time, in either sex.

**Plasma levels of rimonabant.** When measured at 60 min post-injection, there were no sex differences in plasma levels of rimonabant: mean  $\pm$  1 S.E.M. levels were  $4.40 \pm 0.50$   $\mu\text{g/mL}$  in females vs.  $4.91 \pm 0.47$   $\mu\text{g/mL}$  in males ( $t(18)=0.79$ , *n.s.*).

**Effects of  $CB_1$  and  $CB_2$  antagonists alone.** Neither rimonabant nor SR144528 given alone (in combination with vehicle) produced antinociception or hyperalgesia on the paw pressure or tail withdrawal tests, and neither antagonist produced catalepsy in either sex (see points over "V" on Figs. 7 & 8). However, Table 1 shows that both antagonists decreased locomotor activity to some extent, particularly in males. Rimonabant decreased locomotor activity more in males than females ( $F(1,43)=4.96$ ,  $p=0.031$ ); this sex difference was significant at the highest antagonist dose tested, 10 mg/kg ( $p=0.024$ ). SR144528 also decreased locomotor activity more in males than females ( $F(1,53)=10.67$ ,  $p=0.002$ ); this sex difference was significant at 1.0 mg/kg ( $p=0.024$ ) and 3.2 mg/kg ( $p=0.039$ ). The fact that both antagonists decreased locomotor activity to some extent when given alone would presumably interfere with their ability to antagonize the locomotor-suppressant effects of the cannabinoid agonists.

**$CB_1$  antagonism of morphine-induced antinociception.** Greater potency of rimonabant in females than males was not expected. Therefore, rimonabant was examined in combination



with the mu opioid agonist morphine, to test whether sex differences in cannabinoid antagonism were specific to the cannabinoid system. Table 4 shows that morphine by itself was more potent in males than females on the paw pressure and tail withdrawal tests. Rimonabant (1.0 mg/kg) failed to significantly antagonize morphine-induced antinociception on either the tail withdrawal or paw pressure tests, in either sex (Table 4). SR144528 (3.2 mg/kg) also failed to antagonize morphine-induced antinociception in rats of either sex (data not shown).

***Naloxone antagonism of THC-induced antinociception.*** To further test whether sex differences in antagonism of THC were confined to the cannabinoid system, the ability of an opioid antagonist to block the effects of 5 mg/kg THC was examined in female vs. male rats. Naloxone (1.0 mg/kg) did not significantly alter THC-induced paw pressure ( $F(1,24)=1.28$ , *n.s.*) or tail withdrawal ( $F(1,24)=0.47$ , *n.s.*) antinociception in either sex (data not shown). Naloxone also did not alter THC-induced suppression of locomotor activity in either sex ( $F(1,24)=0.37$ , *n.s.*; data not shown). Naloxone attenuated the catalepsy produced by 5 mg/kg THC, but this effect was not statistically significant ( $F(1,24)=3.36$ ,  $p=0.08$ ; data not shown).

## DISCUSSION

The main finding of this study is that the CB<sub>1</sub> receptor-selective antagonist rimonabant was up to 10 times more potent in female than male rats in blocking the antinociceptive effects of THC and CP55,940. Estimates of rimonabant affinity (apparent pK<sub>B</sub>) for the CB<sub>1</sub> receptor calculated from the behavioral data were approximately 0.5-1 mol/kg higher in females than males. Neither the time course of rimonabant antagonism of THC nor plasma levels of rimonabant differed between the sexes, suggesting that the sex difference in antagonism is not due to peripheral pharmacokinetic factors. The sex difference in rimonabant antagonism did not extend to the opioid agonist morphine, and the opioid antagonist naloxone did not significantly attenuate THC's effects in either sex, indicating that sex differences in antagonism are specific to the cannabinoid system. Taken together, these results suggest that cannabinoid drugs bind with greater affinity to CB<sub>1</sub> receptors in females than males, which may contribute to greater cannabinoid agonist effects observed in female compared to male rats.

*Sex differences in cannabinoid agonist effects.* THC and CP55,940 alone were more potent in producing behavioral effects in females compared to males. Specifically, ED<sub>50</sub> values for THC (Table 2) were significantly lower in females than males on paw pressure, tail withdrawal and locomotor tests, and the THC dose-effect curve for catalepsy was clearly steeper in females than males (Fig. 2), suggesting greater efficacy in females. For CP55,940, sex differences were smaller, but potency was still significantly greater in females than males on the paw pressure and catalepsy tests (Table 3). Similar sex differences in cannabinoid agonist potency have been reported previously by our lab (Tseng and Craft, 2001; 2004) and by others (Cohn et al., 1972; Romero et al., 2002; also see Wiley et al., 2007; Wiley and Evans, 2009). The antinociceptive tests in this and previous studies all used a delayed or absent withdrawal

response as the antinociceptive endpoint. Thus it is possible that greater antinociception in females results from greater motor impairment in females compared to males: in females, each agonist was nearly equipotent in producing catalepsy and antinociception, whereas in males, each agonist was significantly more potent in producing antinociception than catalepsy (Tables 2 and 3). If “antinociception” simply results from motor impairment in females, nociceptive and motor measures would be expected to change in tandem. One finding in the present study argues against this: rimonabant antagonism of THC-induced antinociception was greater than its antagonism of motor impairment (Table 2). Furthermore, we reported previously that ovarian hormones modulate antinociceptive but not motoric effects of THC, using the same behavioral tests (Craft and Leidl, 2008; Wakley and Craft, 2011). Although antinociception and catalepsy do not always change in tandem, motor impairment may still contribute to longer latencies on the nociceptive tests. To determine whether sex differences exist in cannabinoid antinociception per se, antinociception produced by local cannabinoid administration in a peripheral pain assay (e.g., Ko and Woods, 1999), or by a peripherally restricted cannabinoid agonist (Yu et al., 2010) could be compared in females and males, as cannabinoid-induced motor impairment does not typically occur using these approaches. We are pursuing such strategies to better distinguish sex differences in cannabinoid-induced antinociception vs. motoric effects.

***Sex differences in antagonism of cannabinoid agonist effects.*** As expected, rimonabant dose-dependently antagonized nearly all behavioral effects of THC and CP55,940 in both sexes. The only exception was THC-induced catalepsy in males; however, the failure to observe significant antagonism of this behavior is likely due to the fact that THC alone produced only minimal catalepsy in males. The effective dose range of rimonabant against other agonist-induced behavior changes in males, 1.0-10 mg/kg, agrees with previous reports of systemic

rimonabant potency against a range of behavioral effects produced by THC and other cannabinoid agonists in male rats (e.g., Lichtman and Martin, 1997; Järbe et al., 2010). The greater potency of rimonabant (higher apparent  $pK_B$ ) observed in female rats was unexpected. We are aware of only one previous study comparing the potency of rimonabant between females and males. In that study, i.t. and i.c.v. rimonabant (1-1000  $\mu$ g) antagonized paw pressure antinociception and catalepsy produced by 10 mg/kg i.p. THC in both sexes to approximately the same extent, and with similar potency in most cases (Tseng and Craft, 2004). The fact that sex differences in rimonabant potency were observed when rimonabant was administered systemically but not when it was administered centrally suggests that peripheral cannabinoid pharmacology may differ between males and females. Peripheral mechanisms may be particularly vital to antinociception produced by cannabinoids (Agarwal et al., 2007; Kunos et al., 2009). We are currently examining sex differences in peripheral cannabinoid antinociception, to determine to what extent these may contribute to sex differences in antinociception after systemic cannabinoid administration.

Another unexpected finding in the present study was the attenuation of THC's effects by the putative  $CB_2$  receptor-selective antagonist, SR144528. SR144528 antagonism was not dose-dependent and was observed only inconsistently: in the first experiment (Figs. 3-6), the intermediate dose of SR144528, 3.2 mg/kg, partially but significantly antagonized the tail withdrawal antinociception and depressed locomotion produced by THC – in females only. In contrast, in the antagonist time course experiment, 3.2 mg/kg SR144528 did not antagonize any effects of THC in either sex. At present we cannot explain the inconsistent antagonism. Although THC is known to bind to  $CB_1$  and  $CB_2$  receptors with similar affinity (Govaerts et al., 2004), its acute antinociceptive and motoric effects – as previously characterized in males –

appear to be exclusively CB<sub>1</sub> receptor-mediated (for review, see Pertwee, 2008). The present results suggest that THC acts at CB<sub>1</sub> receptors -- and sometimes CB<sub>2</sub> receptors -- in females, however, given the inconsistency of the SR144528 antagonism, this result must be interpreted with caution.

*Pharmacokinetic vs. pharmacodynamic mechanisms.* One possible explanation for the greater potency of rimonabant observed in females compared to males is a pharmacokinetic difference. For example, perhaps rimonabant is absorbed or transported to target receptor sites more readily, or it is metabolized or excreted more slowly, in females than males. To address some of these possibilities, we first examined the time course of rimonabant antagonism of THC, and found no sex difference. In all cases, rimonabant was maximally effective in both sexes when injected 5 or 30 min before THC (behavioral effects being measured 35-65 or 60-90 min, respectively, after rimonabant injection: Figs. 11 and 12). Antagonism waned steadily thereafter, such that in most cases it was no longer significant at the 90-min pretreatment time (i.e., when behavior was measured 120-150 min after rimonabant injection: Figs. 11 and 12). This time course agrees with a recent study in which rimonabant antagonism of THC's discriminative stimulus effects was maximal at 20-60 min post-injection and almost completely gone by 4 hr post-injection in male rats (Järbe et al., 2010), as well as a study in male mice showing that [<sup>3</sup>H]rimonabant binding site displacement was greatest at 30-60 min post-injection and waned by 3-4 hr post-injection (Rinaldi-Carmona et al., 1996).

Plasma rimonabant levels also were compared between females and males. There was no sex difference, suggesting again that sex differences in rimonabant antagonism of THC and CP55,940 were not due to greater antagonist absorption in females. However, it is possible that transport of rimonabant into the CNS was greater in females than in males, as we have

previously suggested for THC (Tseng et al., 2004). We are not aware of any published sex comparisons of rimonabant pharmacokinetics in any species, so this possibility remains to be addressed.

Alternatively, sex differences in the endogenous cannabinoid system may explain the present results. Endocannabinoid production is greater in female than male rats, in brain regions such as the pituitary, hypothalamus, striatum and midbrain (Gonzalez et al., 2000; Bradshaw et al., 2006). Additionally, brain CB<sub>1</sub> receptor density and affinity may differ between female and male rats, although the direction of these differences appears to be brain site-specific. For example, cannabinoid receptor density is greater in females than males in the amygdala (Riebe et al., 2010) but lower in females than males in the hypothalamus (Riebe et al., 2010) and mesencephalon (Rodriguez de Fonseca et al., 1994). Higher binding affinity also has been observed in females, in striatum, limbic forebrain and mesencephalon (Rodriguez de Fonseca et al., 1994), and in males in hypothalamus (Riebe et al., 2010). One study in humans also reported greater peripheral cannabinoid receptor expression in women than men (Onaivi et al., 1999), although these were presumably CB<sub>2</sub> receptors given that they were on leukocytes.

In conclusion, sex differences in *in vivo* apparent pK<sub>B</sub> values calculated from the rimonabant + cannabinoid agonist dose-effect curves support the hypothesis that sex differences in cannabinoid antinociceptive potency are pharmacodynamic in nature. Specifically, the results suggest that one mechanism underlying greater cannabinoid antinociception in females compared to males is greater CB<sub>1</sub> receptor affinity in females compared to males. Given the potential of supraspinal, spinal and peripheral cannabinoid receptor involvement in systemic cannabinoid antinociception, in the future it will be important to compare CB<sub>1</sub> receptor affinity and density in females vs. males at all levels of the neuraxis. Additionally, sex differences in the actions of

cannabinoids at receptors other than CB<sub>1</sub> and CB<sub>2</sub> (e.g., GPR55: Ryberg et al., 2007) may contribute to sex differences in cannabinoid antinociception.

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## **AUTHORSHIP CONTRIBUTIONS**

*Participated in research design:* Craft and Laggart

*Conducted experiments:* Wakley, Tsutsui and Laggart

*Performed data analysis:* Craft

*Wrote or contributed to the writing of the manuscript:* Craft, Wakley, Tsutsui and Laggart

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## FOOTNOTES

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## LEGENDS FOR FIGURES

**Fig. 1.** Time course of the antinociceptive effects of THC on the paw pressure (left panels) and tail withdrawal (right panels) tests in female rats (top panels) vs. male rats (bottom panels). Vehicle was administered 30 min before a second injection of vehicle (“vehicle”) or a single dose of THC. Each point is the mean  $\pm$  1 S.E.M., N=10-14 rats.

**Fig. 2.** Time course of the motoric effects of THC on the locomotor activity (left panels) and catalepsy (right panel) tests in female vs. male rats. Locomotor data are presented as % of same-sex, vehicle-treated controls. The mean number of photobeam breaks in vehicle-treated rats was 652, 412, 334 and 286 (females), and 592, 311, 258 and 175 (males) at the 30-, 60-, 120- and 240-min time points, respectively. Each point is the mean  $\pm$  1 S.E.M., N=8-12 rats. \*time spent on bar significantly greater than in same-sex, vehicle-treated controls; +time spent on bar significantly greater in females than in males,  $p < 0.05$ .

**Fig. 3.** Dose-dependent antagonism of 5 mg/kg THC on the paw pressure test in female (left panels) vs. male rats (right panels), by the CB1 receptor-selective antagonist rimonabant (0.1-10 mg/kg; top panels) or the CB2 receptor-selective antagonist SR144528 (1.0-10 mg/kg; bottom panels). Antagonists were administered 30 min before THC (45 min before the first nociceptive response was measured). Each point is the mean  $\pm$  1 S.E.M., N=6-13 rats.

**Fig. 4.** Dose-dependent antagonism of 5 mg/kg THC on the tail withdrawal test in female (left panels) vs. male rats (right panels), by the CB1 receptor-selective antagonist rimonabant (0.1-3.2 mg/kg; top panels) or the CB2 receptor-selective antagonist SR144528 (1.0-10 mg/kg; bottom panels). Antagonists were administered 30 min before THC (45 min before the first nociceptive response was measured). Each point is the mean  $\pm$  1 S.E.M., N=6-13 rats.



**Fig. 5.** Dose-dependent antagonism of 5 mg/kg THC on the locomotor activity test in female (left panels) vs. male rats (right panels), by the CB1 receptor-selective antagonist rimonabant (0.32-10 mg/kg; top panels) or the CB2 receptor-selective antagonist SR144528 (1.0-10 mg/kg; bottom panels). Locomotor data are presented as % of same-sex, vehicle-treated controls. The mean number of photobeam breaks in vehicle-treated rats was 652, 412, 334 and 286 (females), and 592, 311, 258 and 175 (males) at the 30-, 60-, 120- and 240-min time points, respectively. For clarity, not all antagonist doses tested are shown (e.g., rimonabant 0.1 mg/kg in females). Antagonists were administered 30 min before THC (60 min before locomotor activity was first measured). Each point is the mean  $\pm$  1 S.E.M., N=6-13 rats.

**Fig. 6.** Dose-dependent antagonism of the cataleptic effect of 5 mg/kg THC on the bar test in female vs. male rats, by the CB1 receptor-selective antagonist rimonabant (0.1-10 mg/kg) or the CB2 receptor-selective antagonist SR144528 (1.0-10 mg/kg). Antagonists were administered 30 min before THC; catalepsy was measured 60 min after THC was administered. Each bar is the mean  $\pm$  1 S.E.M., N=5-14 rats. \*significantly longer time spent on bar than the same-sex vehicle+vehicle (“0 + 0”) group,  $p \leq 0.05$ . +significant antagonism: less time spent on bar than the same-sex group treated with 5 mg/kg THC alone (“5 + 0”),  $p \leq 0.05$ .

**Fig. 7.** Rightward shifts of the THC dose-effect curve produced by the CB1 receptor-selective antagonist rimonabant, on the paw pressure (left panels) and tail withdrawal (right panels) tests, in female (top panels) vs. male rats (bottom panels). Vehicle or a single dose of antagonist was administered 30 min before THC; THC dose-effect curves were constructed from data obtained at the time of peak THC effect (30-60 min, see Methods). Points above “V” are the effects of vehicle or antagonist administered alone (in combination with vehicle). Each point is the mean  $\pm$  1 S.E.M., N=6-14 rats.

**Fig. 8.** Rightward shifts of THC dose-effect curve produced by the CB1 receptor-selective antagonist rimonabant, on the locomotor activity (left panels) and catalepsy tests (right panels), in female (top panels) vs. male rats (bottom panels). Vehicle or a single dose of antagonist was administered 30 min before THC; THC dose-effect curves were constructed from data obtained at 30-60 min (locomotion) or 60 min (catalepsy) after THC injection. Locomotor data are presented as % of same-sex, vehicle-treated controls; the mean number of photobeam breaks in vehicle-treated rats was 532 (females) and 452 (males). Points above “V” are the effects of vehicle or antagonist administered alone (in combination with vehicle). Each point is the mean  $\pm$  1 S.E.M., N=8-12 rats.

**Fig. 9.** Rightward shifts of the CP55,940 dose-effect curve produced by the CB1 receptor-selective antagonist rimonabant, on the paw pressure (left panels) and tail withdrawal (right panels) tests, in female (top panels) vs. male rats (bottom panels). Vehicle or a single dose of antagonist was administered 30 min before CP55,940; CP55,940 dose-effect curves were constructed from data obtained at the time of peak CP55,940 effect (30-60 min, see Methods). Points above “V” are the effects of vehicle or antagonist administered alone (in combination with vehicle). Each point is the mean  $\pm$  1 S.E.M., N=6-8 rats.

**Fig. 10.** Rightward shifts of the CP55,940 dose-effect curve produced by the CB1 receptor-selective antagonist rimonabant, on the locomotor activity (left panels) and catalepsy tests (right panels), in female (top panels) vs. male rats (bottom panels). Vehicle or a single dose of antagonist was administered 30 min before CP55,940; CP55,940 dose-effect curves were constructed from data obtained 30-60 min (locomotion) or 60 min (catalepsy) after THC injection. Locomotor data are presented as % of same-sex, vehicle-treated controls; the mean number of photobeam breaks in vehicle-treated rats was 517 (females) and 466 (males). Points

above “V” are the effects of vehicle or antagonist administered alone (in combination with vehicle). Each point is the mean  $\pm$  1 S.E.M., N=6-8 rats.

**Fig. 11.** Time course of antagonist effect in females vs. males: antinociception. Vehicle, rimonabant (1.0 mg/kg) or SR144528 (3.2 mg/kg) was administered 5, 30, 60 or 90 min before 5 mg/kg THC. Paw pressure and tail withdrawal antinociception were assessed 15-240 min after THC injection; the means of the 30- and 60-min time points (time of peak THC effect) are plotted. Each point is the mean  $\pm$  1 S.E.M., N=7-8 rats.

**Fig. 12.** Time course of antagonist effect in females vs. males: motoric effects. Vehicle, rimonabant (1.0 mg/kg) or SR144528 (3.2 mg/kg) was administered 5, 30, 60 or 90 min before 5 mg/kg THC. Locomotor data are presented as % of same-sex, vehicle-treated controls; the mean number of photobeam breaks in vehicle-treated rats was 532 (females) and 398 (males). Locomotor activity was assessed 30-240 min after THC injection; the means of the 30- and 60-min time points (time of peak THC effect) are plotted. Catalepsy was assessed 60 min post-THC injection; thus, the antagonist pretreatment times for this measure were 65, 90, 120 and 150 min. Each point is the mean  $\pm$  1 S.E.M., N=7-8 rats.

**TABLE 1. Effects of vehicle or rimonabant and SR144528 given alone (in combination with vehicle) on locomotor activity.**

Data are the mean  $\pm$  1 S.E.M. number of photobeam breaks, N=14/sex, vehicle-treated groups; N=5-6/sex/dose, antagonist-treated groups.

| Antagonist/sex    | Time after second (vehicle) injection <sup>a</sup> |              |              |               |
|-------------------|--|--------------|--------------|---------------|
|                   | 30 min   | 60 min       | 120 min      | 240 min       |
| <b>Vehicle</b>    |  |              |              |               |
| females           | 652 $\pm$ 55                                       | 412 $\pm$ 68 | 334 $\pm$ 59 | 286 $\pm$ 56  |
| males             | 592 $\pm$ 24                                       | 311 $\pm$ 33 | 258 $\pm$ 41 | 175 $\pm$ 34  |
| <b>rimonabant</b> |  |              |              |               |
| 1.0 mg/kg         |  |              |              |               |
| females           | 606 $\pm$ 31                                       | 338 $\pm$ 49 | 337 $\pm$ 46 | 322 $\pm$ 83  |
| males             | 633 $\pm$ 45                                       | 320 $\pm$ 27 | 243 $\pm$ 60 | 268 $\pm$ 44  |
| 10 mg/kg          |  |              |              |               |
| females           | 624 $\pm$ 95                                       | 359 $\pm$ 36 | 367 $\pm$ 42 | 256 $\pm$ 57  |
| males*            | 420 $\pm$ 76                                       | 232 $\pm$ 39 | 194 $\pm$ 38 | 141 $\pm$ 70  |
| <b>SR144528</b>   |  |              |              |               |
| 1.0 mg/kg         |  |              |              |               |
| females           | 618 $\pm$ 62                                       | 412 $\pm$ 98 | 307 $\pm$ 20 | 332 $\pm$ 77  |
| males*            | 509 $\pm$ 76                                       | 220 $\pm$ 48 | 172 $\pm$ 47 | 145 $\pm$ 54  |
| 3.2 mg/kg         |  |              |              |               |
| females           | 648 $\pm$ 52                                       | 476 $\pm$ 70 | 375 $\pm$ 70 | 319 $\pm$ 106 |

|          |          |          |           |          |
|----------|----------|----------|-----------|----------|
| males*   | 561 ± 55 | 257 ± 66 | 194 ± 57  | 195 ± 71 |
| 10 mg/kg |          |          |           |          |
| females  | 662 ± 80 | 455 ± 52 | 408 ± 72  | 428 ± 85 |
| males    | 563 ± 83 | 392 ± 77 | 304 ± 103 | 263 ± 89 |

<sup>a</sup>Antagonist was administered 30 min prior to vehicle injection, then locomotor activity was recorded for 5 min at 30, 60, 120 and 240 min after vehicle injection; thus, the time points shown are 60, 90, 180 and 300 min after antagonist administration.

\*Significant sex difference, pooled across time: less locomotor activity in males than females treated with the same dose,  $p \leq 0.05$ .

**TABLE 2. ED<sub>50</sub> values (95% C.I.) and potency ratios (95% C.I.) for THC in combination with rimonabant on the paw pressure, tail withdrawal, locomotor activity and catalepsy tests.**

|                    | FEMALES                  |                            | MALES                    |                            |
|--------------------|--------------------------|----------------------------|--------------------------|----------------------------|
|                    | ED <sub>50</sub> (mg/kg) | Potency Ratio <sup>a</sup> | ED <sub>50</sub> (mg/kg) | Potency Ratio <sup>a</sup> |
| <b>vehicle+THC</b> |                          |                            |                          |                            |
| Paw Pressure       | 3.12 (2.26, 4.17)        |                            | 5.30 (3.57, 7.13)*       |                            |
| Tail Withdr.       | 4.27 (3.38, 5.62)        |                            | 14.79 (10.26, 30.06)*    |                            |
| Locomotor          | 2.71 (1.77, 3.74)        |                            | 4.33 (3.36, 5.69)*       |                            |
| Catalepsy          | 4.48 (3.56, 5.89)        |                            | -- <sup>b</sup>          |                            |
| <b>Rimonabant</b>  |                          |                            |                          |                            |
| <b>0.32 + THC</b>  |                          |                            |                          |                            |
| Paw Pressure       | 12.25 (7.06, 60.81)      | 3.59 (2.12, 6.08)          |                          |                            |
| Tail Withdr.       | 25.18 (16.52, 61.24)     | 4.44 (3.01, 6.56)          |                          |                            |
| Locomotor          | 6.18 (2.37, 10.33)       | 2.44 (1.43, 4.14)          |                          |                            |
| Catalepsy          | 10.94 (8.05, 16.90)      | 2.35 (1.64, 3.36)          |                          |                            |
| <b>1.0 + THC</b>   |                          |                            |                          |                            |
| Paw Pressure       | 20.04 (14.69, 30.97)     | 6.44 (4.20, 9.89)          | 16.18 (11.19, 27.67)     | 3.07 (1.82, 5.19)*         |
| Tail Withdr.       | 41.50 (23.71, 237.14)    | 6.87 (4.55, 10.37)         | 45.50 (30.48, 97.05)     | 2.79 (1.81, 4.29)*         |
| Locomotor          | 9.98 (7.06, 14.06)       | 3.59 (2.28, 5.65)          | 11.99 (7.98, 26.79)      | 2.37 (1.54, 3.64)          |
| Catalepsy          | 26.49 (17.02, 105.20)    | 4.56 (3.17, 6.57)          | 24.04 (15.10, 75.51)     | --                         |
| <b>10 + THC</b>    |                          |                            |                          |                            |
| Paw Pressure       |                          |                            | 29.04 (18.32, 90.36)     | 5.36 (3.02, 9.49)          |
| Tail Withdr.       |                          |                            | -- <sup>b</sup>          | --                         |

|           |  |  |                     |                 |
|-----------|--|--|---------------------|-----------------|
| Locomotor |  |  | 18.49 (5.24, 15.92) | -- <sup>c</sup> |
| Catalepsy |  |  | -- <sup>b</sup>     | --              |

<sup>a</sup>relative potency of antagonist + agonist vs. vehicle + agonist

<sup>b</sup>ED<sub>50</sub> could not be estimated (mean maximum effect < 35 %MPE at highest dose tested)

<sup>c</sup>slopes significantly different,  $p < 0.05$ , so potency ratio could not be estimated

\*significant sex difference (mean for males lies outside of the C.I. around the mean for females)

**TABLE 3. ED<sub>50</sub> values (95% C.I.) and potency ratios (95% C.I.) for CP55,940 in combination with rimonabant on the paw pressure, tail withdrawal, locomotor activity and catalepsy tests.**

|                      | FEMALES                  |                            | MALES                    |                            |
|----------------------|--------------------------|----------------------------|--------------------------|----------------------------|
|                      | ED <sub>50</sub> (mg/kg) | Potency Ratio <sup>a</sup> | ED <sub>50</sub> (mg/kg) | Potency Ratio <sup>a</sup> |
| <b>veh+CP55,940</b>  |                          |                            |                          |                            |
| Paw Pressure         | 0.09 (0.07, 0.11)        |                            | 0.12 (0.06, 0.18)*       |                            |
| Tail Withdr.         | 0.13 (0.09, 0.24)        |                            | 0.11 (0.07, 0.19)        |                            |
| Locomotor            | 0.10 (0.04, 0.20)        |                            | 0.09 (0.06, 0.12)        |                            |
| Catalepsy            | 0.19 (0.15, 0.49)        |                            | 0.55 (0.24, >200)*       |                            |
| <b>Rimonabant</b>    |                          |                            |                          |                            |
| <b>1.0 +CP55,940</b> |                          |                            |                          |                            |
| Paw Pressure         | 0.32 (0.18, 4.05)        | 3.26 (1.95, 5.45)          | 0.18 (0.10, 0.26)        | 1.62 (0.96, 2.76)*         |
| Tail Withdr.         | -- <sup>b</sup>          | -- <sup>c</sup>            | 0.52 (0.30, 3.46)        | 4.34 (2.14, 8.84)          |
| Locomotor            | 0.40 (0.17, 1.00)        | 3.74 (1.34, 10.47)         | 0.22 (0.09, 0.38)        | 2.78 (1.57, 4.92)          |
| Catalepsy            | 0.94 (0.64, 1.92)        | 3.49 (2.00, 6.09)          | 2.81 (0.94, >200)*       | 3.08 (1.23, 7.69)          |
| <b>10 + CP55,940</b> |                          |                            |                          |                            |
| Paw Pressure         | 0.87 (0.51, 91.41)       | 8.48 (4.96, 14.49)         | 0.72 (0.50, 1.22)        | 6.26 (3.57, 10.97)         |
| Tail Withdr.         | -- <sup>b</sup>          | -- <sup>c</sup>            | 1.90 (0.94, 103.28)      | 12.51 (5.65, 27.72)        |
| Locomotor            | 0.80 (0.35, 59.70)       | 7.36 (2.58, 20.99)         | 0.53 (0.31, 0.85)        | -- <sup>c</sup>            |
| Catalepsy            | -- <sup>b</sup>          | -- <sup>c</sup>            | 3.02 (1.63, 53.2)        | 6.92 (3.11, 15.39)         |

<sup>a</sup>relative potency of antagonist + agonist vs. vehicle + agonist

<sup>b</sup>ED<sub>50</sub> could not be estimated (mean maximum effect < 35 %MPE at highest dose tested)

<sup>c</sup>slopes significantly different,  $p < 0.05$ , so potency ratio could not be estimated



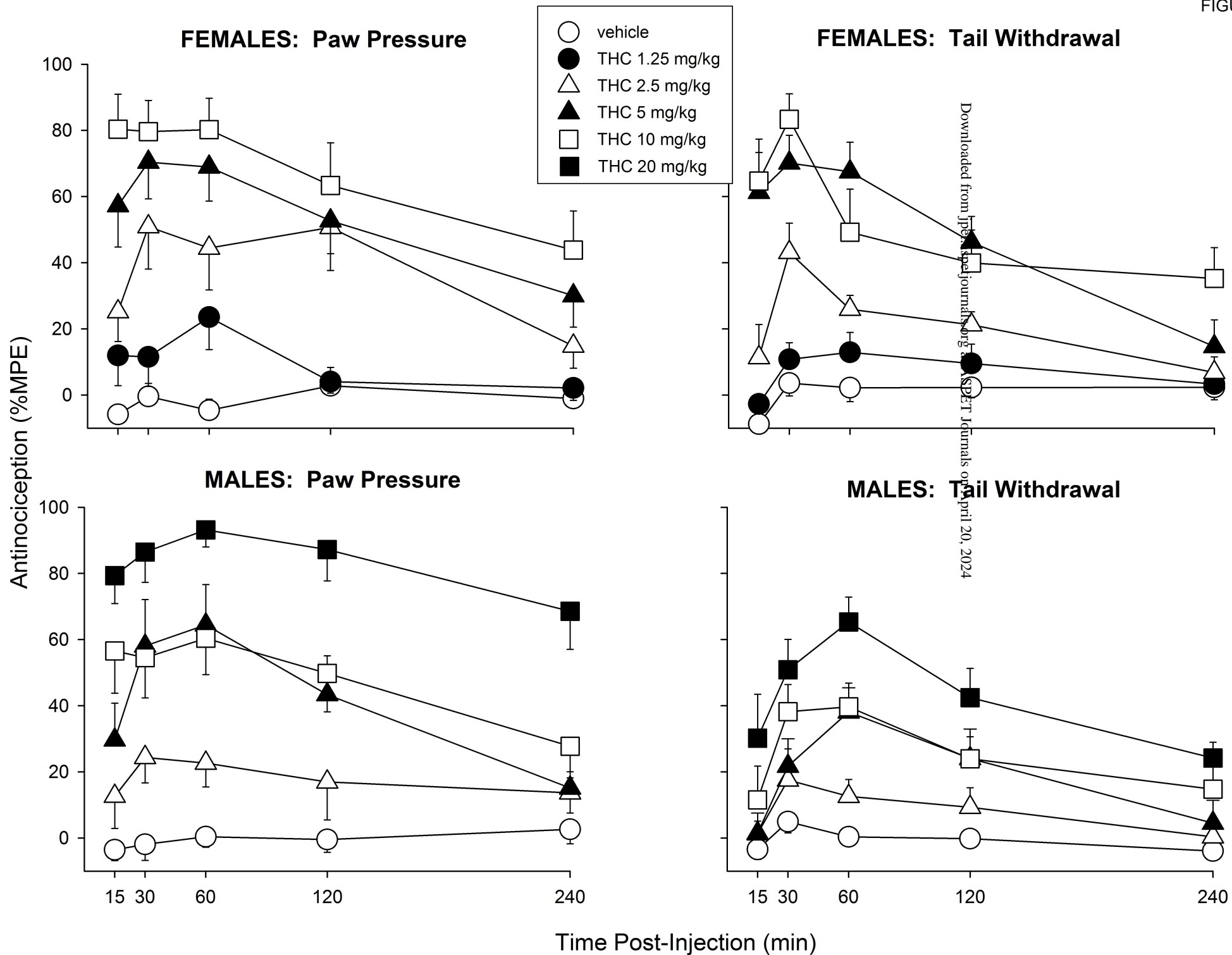
\*significant sex difference (mean for males lies outside of the C.I. around the mean for females)

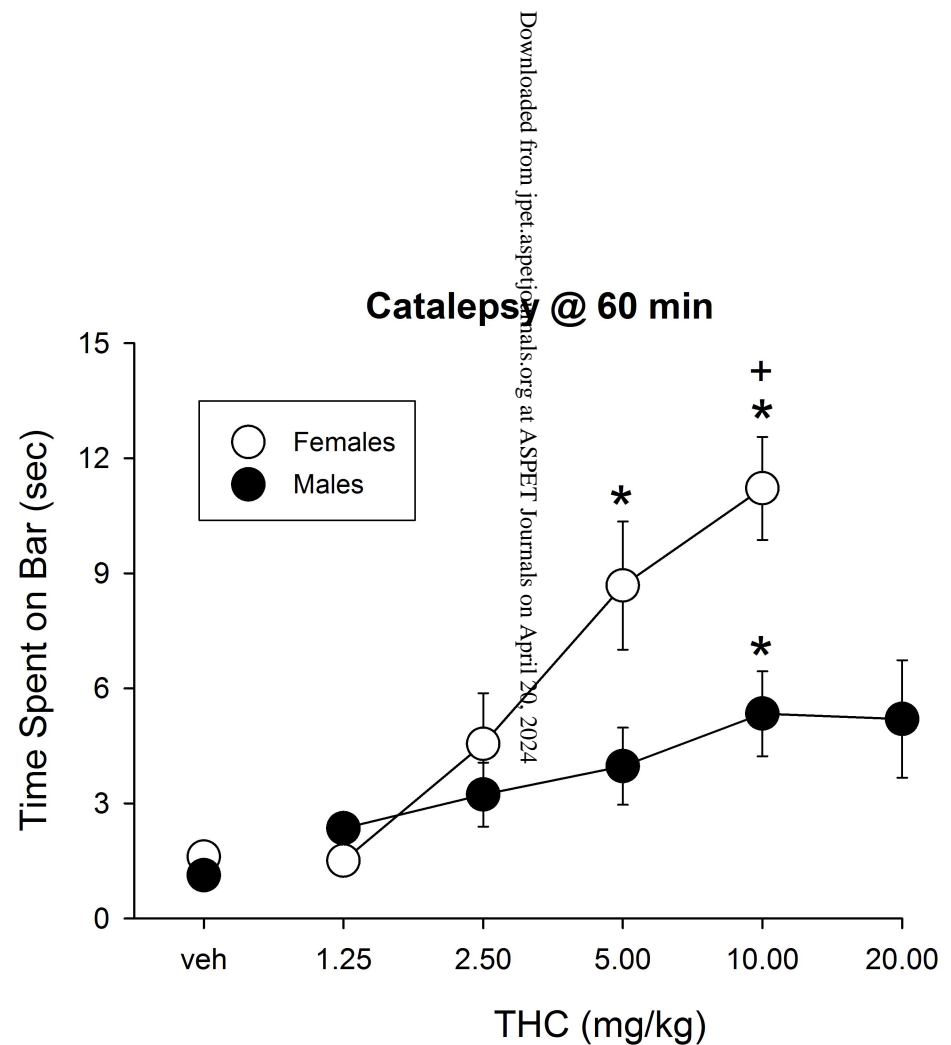
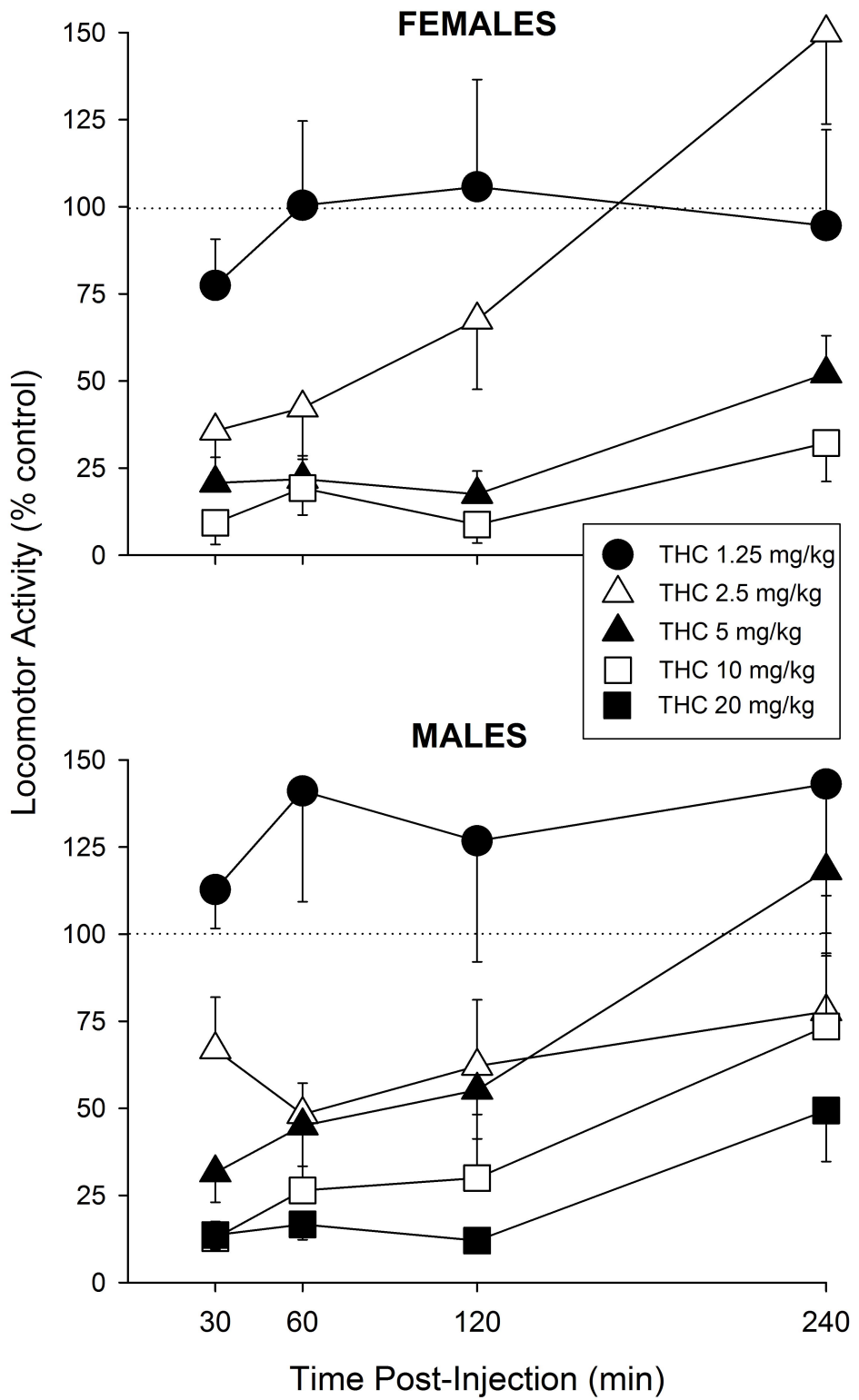
**TABLE 4. ED<sub>50</sub> values (95% C.I.) and potency ratios (95% C.I.) for morphine in combination with rimonabant on the paw pressure and tail withdrawal tests.**

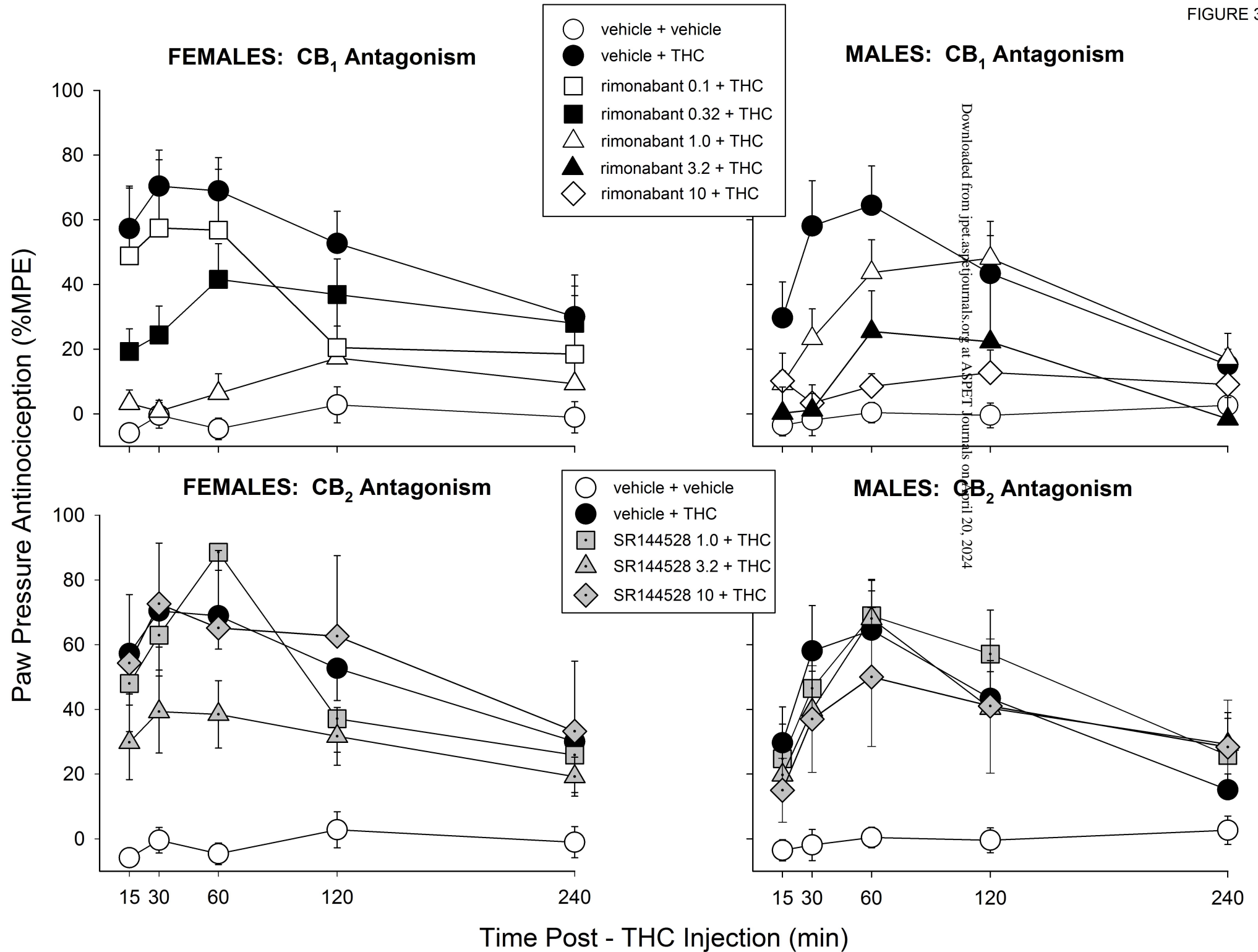
|                      | FEMALES                  |                            | MALES                    |                            |
|----------------------|--------------------------|----------------------------|--------------------------|----------------------------|
|                      | ED <sub>50</sub> (mg/kg) | Potency Ratio <sup>a</sup> | ED <sub>50</sub> (mg/kg) | Potency Ratio <sup>a</sup> |
| <b>veh+morphine</b>  |                          |                            |                          |                            |
| Paw Pressure         | 7.80 (6.34, 9.66)        |                            | 6.22 (5.24, 7.48)*       |                            |
| Tail Withdrawal      | 6.58 (5.40, 8.15)        |                            | 4.25 (3.60, 5.00)*       |                            |
| <b>Rimonabant</b>    |                          |                            |                          |                            |
| <b>1.0 +morphine</b> |                          |                            |                          |                            |
| Paw Pressure         | 6.08 (5.13, 7.26)        | 0.77 (0.60, 1.00)          | 4.65 (3.85, 5.68)*       | 0.75 (0.59, 0.96)          |
| Tail Withdrawal      | 5.93 (5.15, 6.85)        | 0.91 (0.72, 1.14)          | 4.99 (4.21, 5.85)*       | 1.14 (0.95, 1.45)          |

<sup>a</sup>relative potency of antagonist + agonist vs. vehicle + agonist

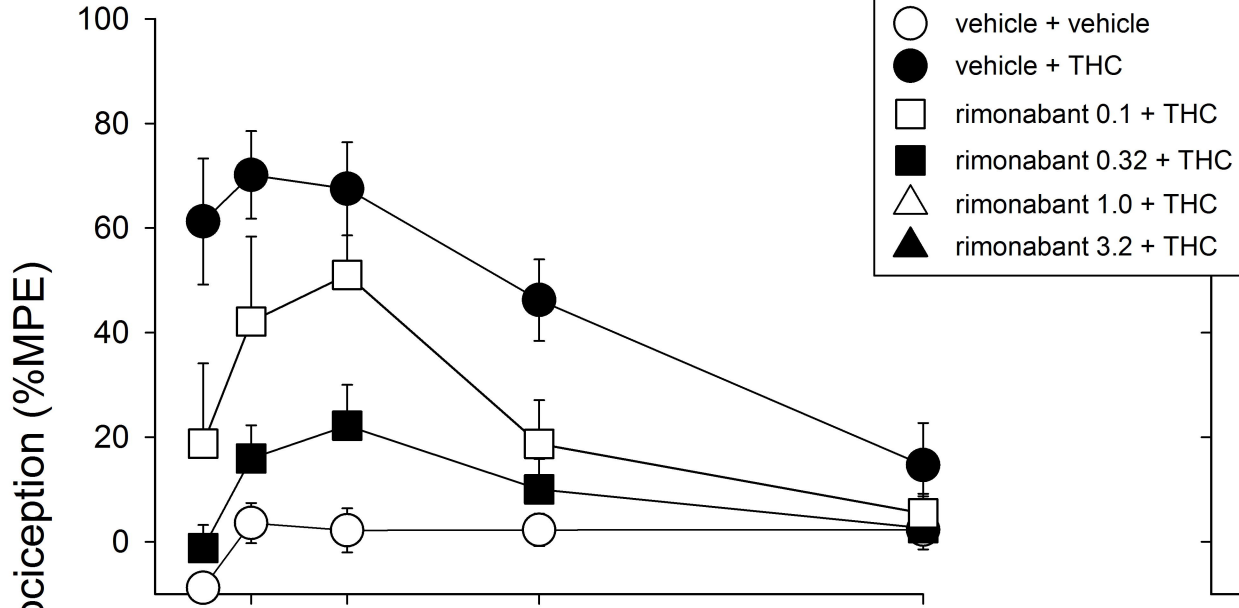
\*significant sex difference (mean for males lies outside of the C.I. around the mean for females)



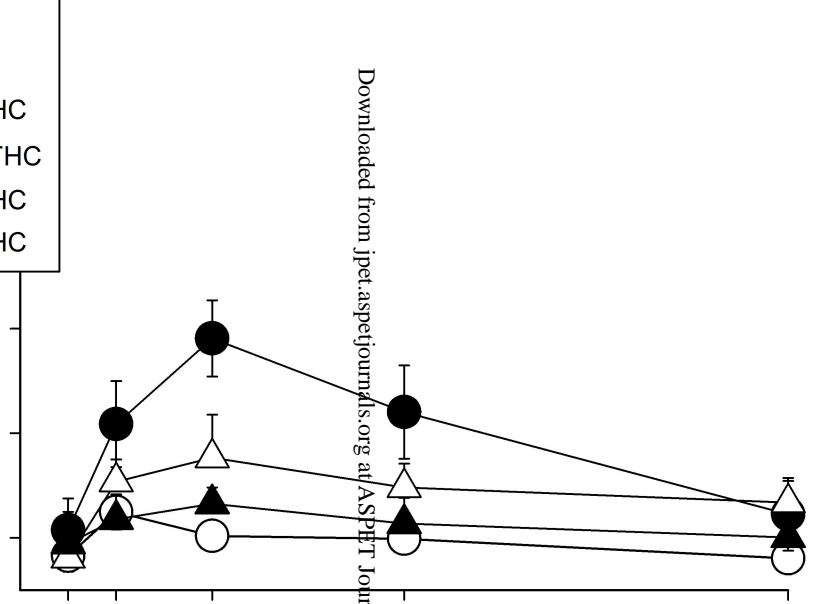




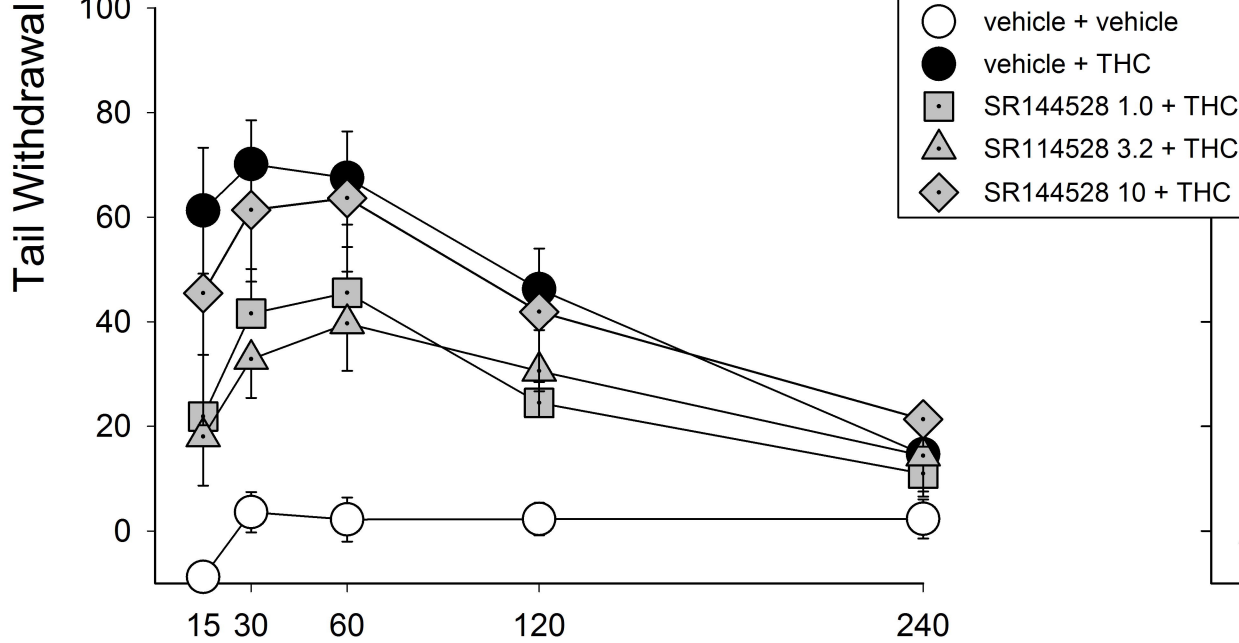
**FEMALES: CB<sub>1</sub> Antagonism**



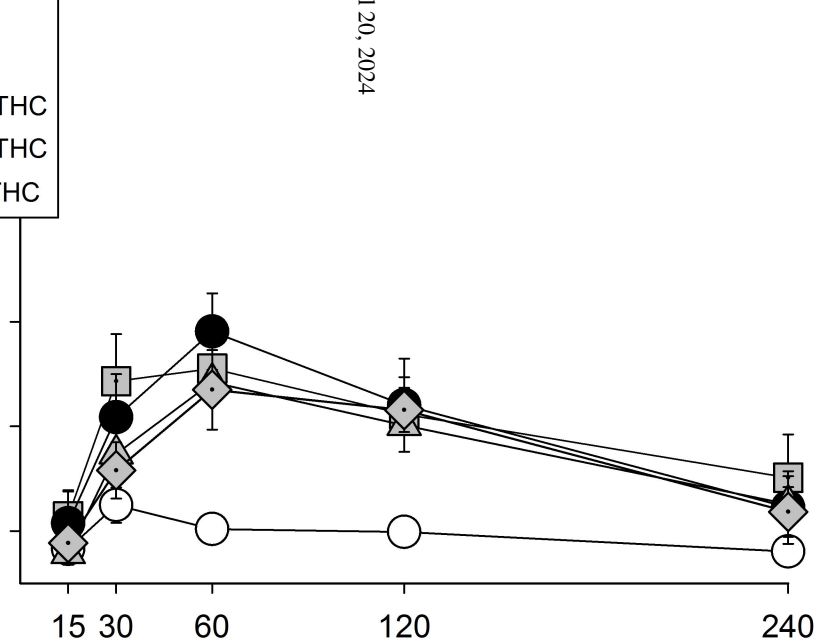
**MALES: CB<sub>1</sub> Antagonism**



**FEMALES: CB<sub>2</sub> Antagonism**



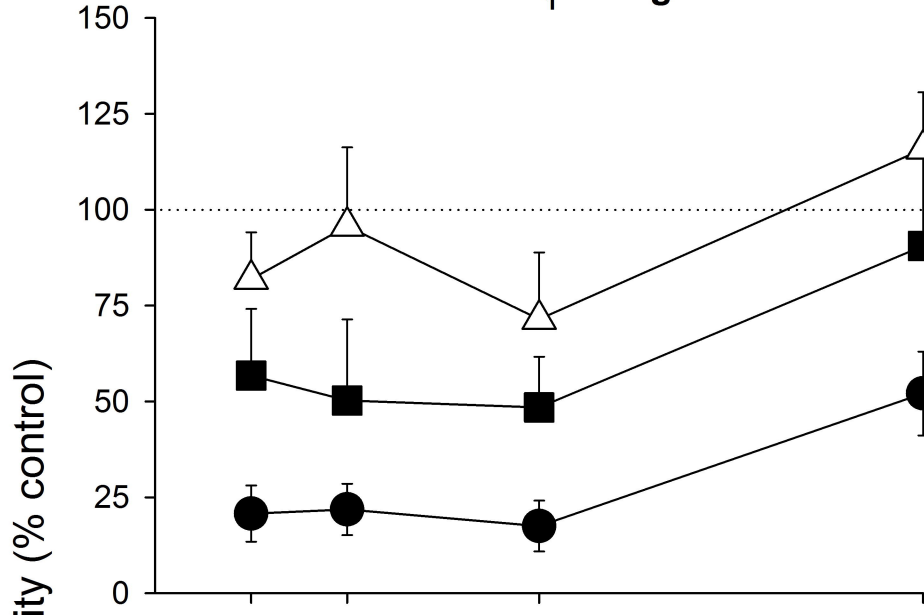
**MALES: CB<sub>2</sub> Antagonism**



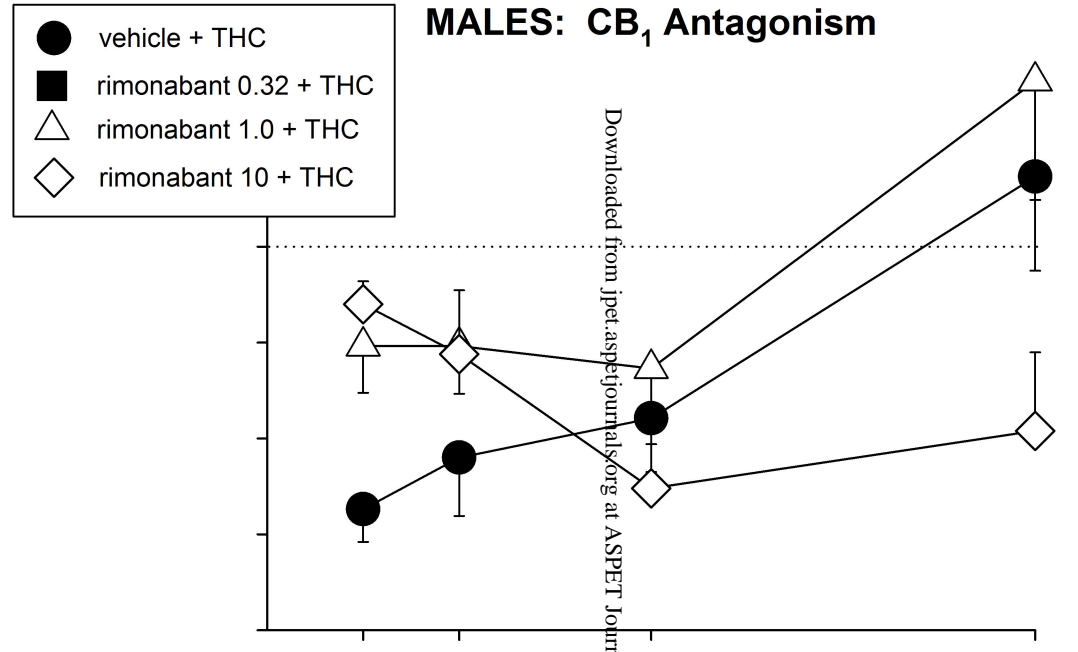
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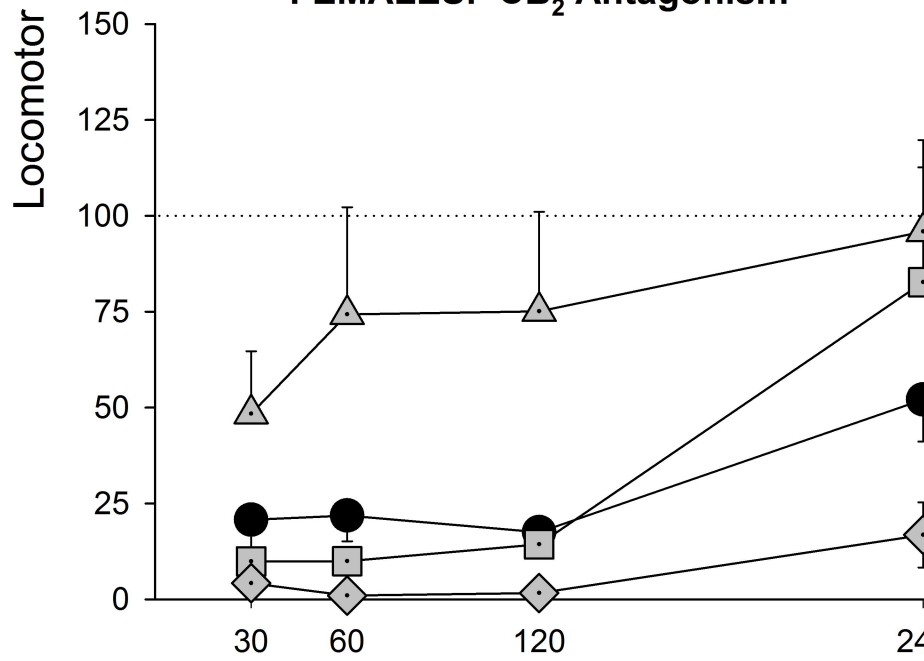
**FEMALES: CB<sub>1</sub> Antagonism**



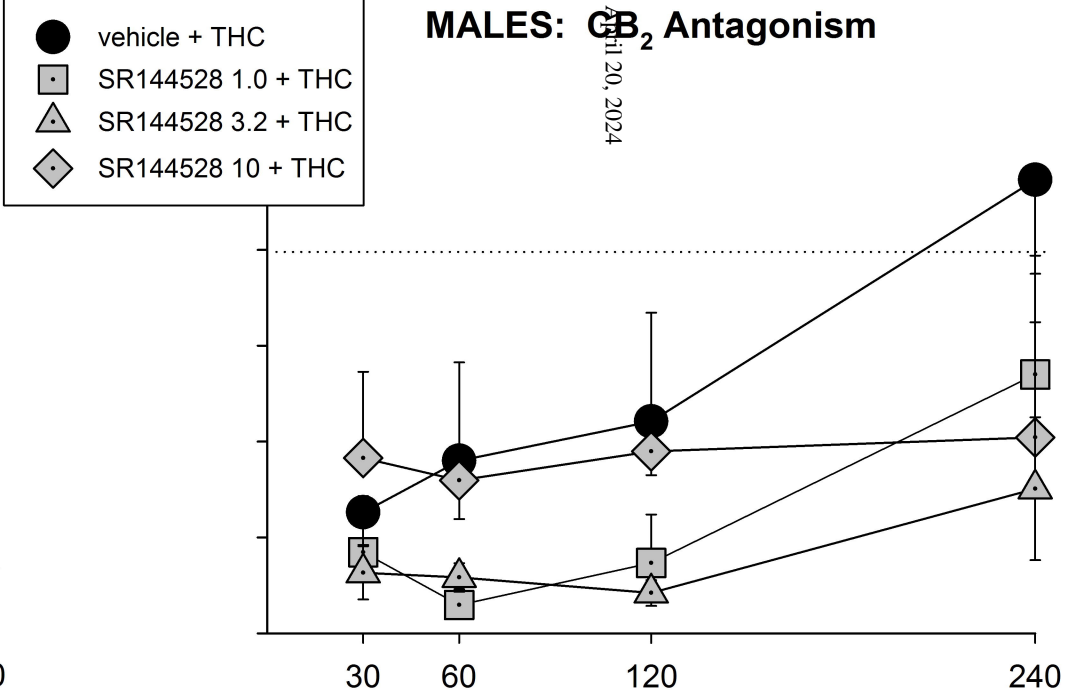
**MALES: CB<sub>1</sub> Antagonism**



**FEMALES: CB<sub>2</sub> Antagonism**



**MALES: CB<sub>2</sub> Antagonism**



Time Post - THC Injection (min)

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