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Sex and cannabinoid CB1 genotype differentiate palatable food and cocaine self-administration behaviors in mice

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Abstract

Both cannabinoid CB1 receptor knockout and antagonism produce well-established attenuation of palatable food and drug self-administration behavior. Although cannabinoid drugs have received attention as pharmacotherapeutics for various disorders, including obesity and addiction, it is unclear whether these agents produce equivalent behavioral effects in females and males. In this study, acquisition of 32% corn oil or 10% Ensure self-administration, and maintenance of corn oil, Ensure, or 0.56 mg/kg/infusion cocaine self-administration under both fixed ratio (FR)-1 and progressive ratio (PR) schedule of reinforcement, was compared in male and female wild type (WT) and CB1 knockout (KO) mice. Furthermore, the effect of pretreatment with the CB1 antagonist SR141716 (0.3–3.0) on Ensure self-administration in male and female WT and CB1 KO mice was assessed. CB1 genotype and sex significantly interacted to produce an attenuation of acquisition and maintenance of Ensure self-administration and PR self-administration for both Ensure and cocaine in male CB1 KO mice. In contrast, male CB1 KO mice showed no deficit in acquisition and maintenance of FR-1 responding or in PR responding maintained by corn oil. Sex differences also arose within genotypes for responding maintained under all three reinforcers. Lastly, pretreatment with SR141716 attenuated Ensure self-administration in WT and CB1 KO mice but was approximately five-fold more potent in WT mice than in CB1 KOs. The present data add to a small but growing literature suggesting that the cannabinoid system may be differentially sensitive in its modulation of appetitive behavior in males versus females.

Keywords

CB1 receptor; knockout; mouse; palatable food; self-administration; sex differences; SR141716

Introduction

The endogenous cannabinoid (CB) system has been implicated in a variety of physiological functions, including appetite and reward (see Solinas *et al.*, 2003 for review). CB drugs are widely used recreationally and have received much interest recently as potential pharmacotherapeutics for the treatment of obesity (Vemuri *et al.*, 2008) and addiction (Parolaro *et al.*, 2005). Furthermore, although it has become clear within the last decade that

many drugs do not produce equivalent behavioral effects in females and males, it is not yet known whether CB drugs produce consistently different effects in men and women (see Craft, 2005 for review). For example, although it is well-known that CBs found in *Cannabis sativa* (a.k.a. marijuana) can increase appetite, particularly for sweet and palatable foods (Abel, 1975; Foltin *et al.*, 1988), whether sex differences exist in the appetite-stimulating effects of cannabis has not been systematically studied in humans. It has been reported, however, that men describe greater subjective effects of marijuana intoxication than women (Penetar *et al.*, 2005). To date, two animal studies have compared behavioral effects of CB agonists in male versus female laboratory animals; Miller *et al.* (2004) reported that the CB agonist CP 55,940 was more potent in male than female rats in increasing intake of sweetened condensed milk, and similarly Diaz *et al.* (2009) reported that the CB agonist WIN 55212 produced more pronounced hyperphagia in male versus female guinea pigs with free access to laboratory chow. Taken together, these studies suggest that the CB1 receptor system may be preferentially involved in the regulation of appetite and reward in males versus females.

There is now a great deal of preclinical and clinical evidence that antagonism of the cannabinoid CB1 receptor system decreases motivation for palatable foods and drugs of abuse. For example, palatable food consumption and self-administration by laboratory animals are attenuated by antagonism of CB1 receptors (Arnone *et al.*, 1997; Higgs *et al.*, 2003; McLaughlin *et al.*, 2003; Thornton-Jones *et al.*, 2005; Ward and Dykstra, 2005), and the CB1 receptor antagonist SR141716 (Rimonabant) produces weight loss in obese human subjects (Van Gaal *et al.*, 2005; Pi-Sunyer *et al.*, 2006), although its clinical utility has been compromised by the production of serious psychiatric side effects (Traynor *et al.*, 2007). CB1 receptor antagonism also attenuates the reinforcing effects of several drugs of abuse in laboratory animals (see Le Foll and Goldberg, 2005 for review), increases rates of nicotine cessation in humans (Anthenelli and Despres, 2004), and is currently being investigated for its ability to reduce voluntary alcohol consumption by nontreatment-seeking heavy drinkers. Whether CB1 receptor antagonism is more potent at attenuating appetite and/or drug reward in males versus females remains relatively unexplored [but see Foltin and Haney (2007); Diaz *et al.*, 2009].

Operant responding for palatable foods and addictive drugs is also altered in mice with a genetic invalidation of the CB1 receptor (CB1 KO) (Poncelet *et al.*, 2003; Sanchis-Segura *et al.*, 2004; Ward and Dykstra, 2005; see Le Foll and Goldberg, 2005 for review). More specifically, we have recently reported that male CB1 receptor knockout (KO) mice show significant deficits in responding for the sweet food reinforcer Ensure. However, these mice acquire self-administration of the pure fat reinforcer corn oil and will continue to respond for corn oil under both fixed ratio (FR) and progressive ratio (PR) schedules of reinforcement, and also fully reinstate corn-oil-seeking behavior after extinction (Ward and Dykstra, 2005; Ward *et al.*, 2007). In addition, although initial studies showed that cocaine self-administration under an FR schedule was not impaired in CB1 KO mice (Cossu *et al.*, 2001), Soria *et al.* (2005) showed significant deficits in CB1 KO mice self-administering cocaine under a PR schedule of reinforcement. Taking these results together with the previously mentioned sex difference studies, we hypothesized that male CB1 KO mice

would show more robust impairments in sweet palatable food and cocaine self-administration behavior as compared with female CB1 KO mice. We also hypothesized that, as is the case with male CB1 KO mice, female CB1 KO mice would not show a significant impairment of corn oil self-administration. Lastly, we hypothesized that the CB1 receptor antagonist SR141716 would produce more robust attenuation of self-administration behavior in males versus females. To test these hypotheses, acquisition of 32% corn oil or 10% Ensure self-administration and maintenance of corn oil, Ensure, or 0.56 mg/kg/infusion cocaine self-administration, under both an FR-1 and PR schedule of reinforcement, was compared in male and female wild type (WT) and CB1 KO mice. In addition, the effect of pretreatment with the CB1 antagonist SR141716 on Ensure self-administration in male and female WT and CB1 KO mice was assessed.

Methods

Subjects

Twenty male WT, 19 male CB1 KO, 17 female WT, and 21 female CB1 KO mice were used for these experiments. The CB1 knockout mice were generated on a full C57Bl/6 background by Zimmer *et al.* (1999) at the National Institutes of Health by a targeted mutation of the large single coding sequence of the CB1 receptor gene. Nucleic acids that code for amino acids 32 through 448 were replaced with a PGK-neo cassette through homologous recombination in embryonic stem cells (Zimmer, 1992). Heterozygous breeding pairs were obtained from a colony at Virginia Commonwealth University, and were bred and genotyped at Temple University to obtain male and female WT and CB1 KO knockout mice. After weaning and genotyping, all mice were singly housed under a 12-h light/dark cycle with lights off at 07.00 h, so that all experimental testing occurred during the dark cycle of the animals' diurnal cycle. During the acquisition phase of the study, animals were maintained at 90% of their free-feeding body weight (approximately 2.5 g daily Purina Rodent Chow Diet 5001, Ralston-Purina, St. Louis, Missouri, USA); after acquisition all mice were given free access to food and water throughout the duration of the operant studies. Animal protocols were approved by the institutional animal care and use committee, and the methods were in accordance with the Guide for the Care and Use of Laboratory Animals in Neuroscience and Behavioral Research (National Research Council 2003).

Apparatus

Food and cocaine self-administration experiments were conducted in mouse operant conditioning chambers (21.6 cm × 17.8 cm × 12.7 cm, Model ENV-307W, Med Associates, Georgia, Vermont, USA) located within ventilated sound attenuation chambers. The operant conditioning chambers were equipped with two nose-poke holes (1.2 cm diameter) with internal amber stimulus lights (ENV-313W), a house light (ENV-315M), ventilator fan, tone generator (ENV-323AW), and motor-driven dippers (ENV-302W) for liquid food presentation. The receptacle opening for access to the food was located between the two nose-poke holes, and an amber stimulus light was located above the receptacle opening (ENV-221M). For cocaine experiments, drug delivery was controlled by an electronic circuit that operates a computer-controlled syringe that is connected to a single-channel fluid swivel mounted on a counter-balanced arm above the operant chamber (MED-307A-CT-B2).

Experiment 1: acquisition of corn oil-reinforced and Ensure-reinforced

responding—Separate groups of food-restricted male and female WT and CB1 KO mice were trained to nose-poke for either 32% corn oil or 10% vanilla-flavored Ensure under an FR-1 schedule, during daily 30-min acquisition sessions. Ensure is a sweet-tasting food comprised of mixed macronutrients [2.5 g of carbohydrates and 0.4 g of fats per tablespoon is diluted in room temperature tap water. Corn is a pure fat (approximately 14 g per tablespoon) and is brought to a stable emulsion in 3% xanthan gum dissolved in tap water]. We have previously shown that both liquids maintain operant responding in C57BL/6 mice (Ward and Dykstra, 2005; Ward *et al.*, 2007; Ward *et al.*, 2008). In the corn oil study, the number of mice used were as follows: male WT ($n=10$), male KO ($n=12$), female WT ($n=9$), and female KO ($n=13$). In the Ensure study, the numbers of mice used were as follows: male WT ($n=10$), male KO ($n=7$), female WT ($n=8$), and female KO ($n=9$). Upon a response on the active, illuminated nose-poke hole, the adjacent cue light was illuminated, and liquid food was available for 20 s. The maximum number of reinforcers available per session was set at 10. Responses on the inactive nose-poke hole and entry into the dipper receptacle were recorded, but were without scheduled consequences. Acquisition of liquid food self-administration was defined as 10 reinforced responses in the active nose-poke hole, with the total responses including at least 75% active nose-poke hole responding. After individual mice reached these acquisition criteria, they proceeded to the FR-1 maintenance phase of the study. Mice that failed to reach the acquisition criteria after 15 days of acquisition testing did not continue on to the FR-1 maintenance phase and received a score of 15 days to meet the criteria for data analysis.

Experiment 2: maintenance of corn oil-reinforced and Ensure-reinforced responding and effect of SR141716

—Once individual mice met acquisition criteria, they continued to respond for either 32% corn oil or 10% Ensure under an FR-1 schedule during daily 1-h sessions, for 7 days. Each response on the active, illuminated nose-poke hole resulted in the presentation of liquid food for 8 s, paired with illumination of the stimulus light above the food receptacle and a 1-s tone. The house light and fan remained on throughout the 1 h test session. No maximum number of reinforcers was set. In a subset of mice from the Ensure study ($n=4-5$ /group), SR141716 (0.3–3.0) or vehicle was administered intraperitoneally (i.p.) in a randomized manner 15 min before starting FR-1 sessions, in addition to the seven nontreatment sessions, and each treatment session was separated by at least 3 days of nontreatment sessions. An average of 5 days separated the last SR141716 injection and the commencement of the PR experiment.

Experiment 3: progressive ratio responding for corn oil and Ensure

—After 7 days of FR-1 responding, all mice continued to respond for either 32% corn oil or 10% Ensure under a PR schedule of reinforcement, during daily 1 h sessions for 5 days, and the following progression of response requirements for reinforcement within each session was used: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, etc. (as described in Richardson and Roberts, 1996). Breaking point was defined as the number of infusions obtained by the end of the 1-h test session. Responses on the active, illuminated nose-poke hole resulted in the presentation of liquid food for 8 s, paired with illumination of the

stimulus light above the food receptacle and a 1-s tone. The house light and fan remained on throughout the session.

Experiment 4: maintenance and progressive ratio responding for cocaine—

After completion of experiment 3, chronically indwelling jugular cannulae were surgically implanted (as described by Caine *et al.*, 1999) into mice ($n=17$) from the Ensure study, and they were then trained to self-administer 0.56 mg/kg/infusion cocaine under an FR-1 schedule of reinforcement, during daily 2-h sessions for 7 days. Each response on the active, illuminated nose-poke hole resulted in the infusion of cocaine paired with illumination of the stimulus light above the food receptacle and a 1-s tone. After FR-1 responding, mice were given access to 0.56 mg/kg/infusion cocaine under the PR schedule of reinforcement described above. Breaking point was defined as the number of infusions obtained by the end of the 4-h test session or until 20 min elapsed with no active-hole nose-pokes.

Drugs

SR141716 (Research Triangle Institute, RTP, North Carolina, USA) was dissolved in a vehicle of 100% ethanol, cremophor (Sigma-Aldrich, St. Louis, Missouri, USA), and saline in a ratio of 1: 1: 18 and was injected i.p. at a volume of 0.1 ml/10 g 15 min before behavioral testing. Cocaine hydrochloride (National Institute on Drug Abuse, Rockville, Maryland, USA) was dissolved in saline at a concentration of 1.8 mg/ml for intravenous administration. Pump time was adjusted for each mouse so that the proper infusion was delivered for the administration of 0.56 mg/kg/infusion, with an average pump time of approximately 1 s.

Data analysis

To determine whether genotype and sex (or genotype and SR141716) produced a significant effect on liquid food or cocaine self-administration, and whether they produced a significant interaction, two-way analyses of variance (ANOVAs) were conducted on the following measures: (i) the number of days to acquire self-administration, (ii) the number of responses/session under an FR-1 schedule on day 7 of maintenance responding, (iii) effect of SR141716 on maintenance responding, (iv) breaking point achieved on day 5 of PR responding, and (v) body weight. Bonferroni post-hoc tests were run to identify specific effects of genotype in males and females, as well as differences in responding between males and females of like genotype. All statistical analyses were performed using GraphPad Prism 4 (GraphPad Software, La Jolla, California, USA).

Results

Effect of CB1 genotype and sex on 32% corn oil self-administration

During the initial acquisition phase, all mice, with the exception of one female WT mouse and two female CB1 KO mice, acquired 32% corn oil self-administration within 15 days (Fig. 1a). Two-way ANOVA revealed a significant effect of genotype [$F(1,40)=14.43$, $P<0.01$] and sex [$F(1,40)=18.41$, $P<0.01$] on the number of days to acquire corn oil self-administration, and a significant interaction [$F(1,40)=3.972$, $P<0.05$]. Bonferroni post-test revealed a significant effect of genotype in female mice ($P<0.01$) but not males, and

revealed a non-significant trend of sex in WT mice ($P=0.09$) and a significant effect of sex in CB1 KO mice ($P<0.05$).

Mice that met the acquisition criterion within 15 days were then maintained on 32% corn oil under an FR-1 schedule for 7 days. On the last day of the maintenance phase (Fig. 1b), two-way ANOVA revealed no significant effect of genotype on the number of reinforcers earned, but a significant effect of sex [$F(1,37)=12.83$, $P<0.01$], and no significant interaction. Bonferroni post-test revealed no significant effect of genotype in male or female mice, and a significant effect of sex in WT and CB1 KO mice ($P<0.05$). One male CB1 KO mouse and two female CB1 KO mice died of unknown causes during the maintenance phase, leaving final group sizes of 10 male WT (MWT), 11 male KO (MKO), eight female WT (FWT), and nine female KO (FKO).

The same mice were then trained to self-administer 32% corn oil under the PR schedule. On day 5 of the breaking point phase (Fig. 1c), two-way ANOVA revealed no significant effect of genotype or sex, and no significant interaction on the maximal breaking point was attained. Bonferroni post-test revealed no significant effect of genotype in male or female mice and no significant effect of sex in WT or CB1KO mice. Two male CB1 KO mice died of unknown causes during the breaking point phase.

Overall, genotype produced marginal effects on 32% corn oil responding, with female CB1 KO mice showing a deficit in acquisition as compared with female WT mice. There was a significant effect of sex in CB1KO mice on acquisition of corn oil self-administration, with males acquiring faster than females, and in WT and CB1 KO mice on maintenance, with males responding more under the FR-1 schedule. Final sample sizes were 10 MWT, nine MKO, eight FWT, and nine FKO.

Effect of CB1 genotype and sex on 10% Ensure self-administration

During the initial acquisition phase, all mice, with the exception of two male CB1 KO mice and 1 female CB1 KO mouse, acquired 10% Ensure self-administration within 15 days (Fig. 2a). Two-way ANOVA revealed no significant effect of genotype or sex on the number of days to acquire Ensure self-administration, but a significant interaction of genotype and sex [$F(1,30)=6.19$, $P<0.05$]. Bonferroni post-test revealed a significant effect of genotype in male mice ($P<0.05$) but not females and a nonsignificant trend for sex in either the WT ($P=0.058$) or CB1 KO mice.

Mice that met the acquisition criterion within 15 days were then maintained on 10% Ensure under an FR-1 schedule for 7 days. On the seventh day of the maintenance phase (Fig. 2b), two-way ANOVA revealed no significant effect of genotype or sex on the number of reinforcers earned, however, a significant interaction was revealed [$F(1,27)=4.78$, $P<0.05$]. Bonferroni post-test revealed a significant effect of genotype in male mice ($P<0.05$) but not females, and a significant effect of sex in WT mice ($P<0.05$) but not in CB1 KO mice. Two male CB1 KO mice died of unknown causes after the maintenance phase. Final sample sizes were eight MWT, seven MKO, eight FWT, and eight FKO.

The same mice from the maintenance phase were then trained to self-administer 10% Ensure under the PR schedule of Ensure delivery to assess breaking point. On day 5 of the 'breaking point' phase (Fig. 2c), two-way ANOVA revealed a significant effect of genotype on the average breaking point achieved [$F(1,25)=7.82$, $P<0.01$] but no significant effect of sex, and a significant interaction [$F(1,25)=4.09$, $P<0.05$] of genotype and sex on breaking point. Bonferroni post-test revealed a significant effect of genotype in male mice ($P<0.05$) but not females, and no significant effect of sex in WT or CB1KO mice. Final sample sizes were eight MWT, five MKO, eight FWT, and eight FKO.

To summarize, genotype and sex produced a significant interaction on 10% Ensure self-administration, with CB1 KO producing overall deficits in responding in male but not female mice. In addition, sex produced partial effects on 10% Ensure responding, with a trend toward faster acquisition in male WT mice versus females, and male WT responding more than female WT mice during the maintenance phase.

Effect of SR141716 on maintenance of Ensure self-administration in male and female WT and CB1 KO mice

A subset of mice from the Ensure self-administration group were intermittently pretreated with vehicle or SR141716 (0.3–3.0 mg/kg i.p.) on additional test days during their maintenance phase. Pretreatment with SR141716 produced dose-dependent decreases in FR-1 responding for 10% Ensure in all groups (Fig. 3). In WT mice, two-way ANOVA revealed a significant effect of SR141716 [$F(3,20)=23.01$, $P<0.05$] on the number of reinforcers earned, but no significant effect of sex or interaction. In KO mice, two-way ANOVA revealed a significant effect of SR141716 [$F(3,16)=13.03$, $P<0.05$] but no significant effect of sex, and no significant interaction [$F(3,12)=10.13$, $P<0.05$]. Calculated SR141716 effective dose of SR that produces a 50% decrease in FR1 responding for each group (ED_{50} s) to produce decreases in responding for MWT=0.48 mg/kg, MKO=2.40 mg/kg, FWT=0.78 mg/kg, and FKO=3.00 mg/kg, showing a five-fold shift in SR141716 potency between male WT and CB1 KO mice, and a four-fold shift between female WT and CB1 KO mice. SR administration did not have a lasting effect in any group, as response levels returned to baseline the day after treatment. For example, on the day before administration with 3.0 SR141716, the average number of active responses in MWT was 107.5 (± 16.5), and on the day after administration of this dose, responding averaged 111.3 (± 12.5).

Effect of CB1 genotype and sex on 0.56 mg/kg/infusion cocaine self-administration

After the completion of acquisition, maintenance, and PR responding for 10% Ensure, a subset of mice was cannulated and trained to self-administer 0.56 mg/kg/ infusion cocaine under an FR-1 schedule for 7 days. On the last day of the maintenance phase (Fig. 4a), two-way ANOVA revealed no significant effect of genotype or sex, and no significant interaction on the number of cocaine infusions earned. One male and one female CB1 KO mouse died after the maintenance phase of cocaine self-administration. Final sample sizes were five MWT, four MKO, four FWT, and four FKO.

The same mice were then trained to self-administer 0.56 mg/kg/infusion cocaine under the PR schedule to assess breaking point. On day 5 of the breaking point phase (Fig. 4b), two-way ANOVA revealed a significant effect of genotype on the mean breaking point achieved [$F(1,7)=14.00$, $P<0.01$], but not of sex, and a significant interaction [$F(1,7)=31.50$, $P<0.01$]. Bonferroni post-test revealed a significant effect of genotype in male mice, and a significant effect of sex in WT and CB1 KO mice ($P<0.05$). Two male and one female WT mice lost cannula patency and did not complete the PR study. Final sample sizes were three MWT, three MKO, three FWT, and three FKO.

Overall, genotype and sex produced limited effects on cocaine self-administration, with male CB1 KO mice and female WT mice showing lowered breaking point maintained by cocaine.

Effect of CB1 genotype and sex on body weight in C57Bl/6 mice

All mice were weighed daily, and two-way ANOVA of body weights mid-way through the maintenance phase revealed significant effects on body weight of genotype [$F(1,80)=9.38$, $P<0.01$] and sex [$F(1,80)=49.53$, $P<0.01$], but no significant interaction (Fig. 5). Bonferroni post-test revealed a significant effect of genotype in male mice ($P<0.01$), with WT mice weighing more than CB1 KO mice, but not in females. Bonferroni post-test also revealed a significant effect of sex in WT and CB1KO mice, with male mice weighing more than females.

Discussion

The present results are, to the best of the authors' knowledge, the first to show a CB1 genotype $\times$ sex interaction on palatable food or cocaine self-administration in operant responding paradigms. Overall, CB1 genotype and sex significantly interacted to produce an attenuation of acquisition and maintenance of Ensure self-administration and PR self-administration of both Ensure and cocaine in male CB1 KO mice. In addition, CB1 KO led to lower body weight in male mice; in comparison, female CB1 KO mice showed no significant deficits on these measures compared with their WT controls. These data are in agreement with a recent report by Diaz *et al.* (2009) showing a significant interaction between CB1 KO and sex in freely available laboratory chow consumption, wherein CB1 KO produced hypophagia in male but not female mice. Interestingly, this group has also identified differences in cell signaling in the arcuate nucleus of the hypothalamus (a site involved in appetite) between male and female guinea pig brain slices, and has shown differential modulation of postsynaptic potentials in this region by the application of CB agonist, suggesting that the sexually dimorphic nature of hypothalamic nuclei may contribute to these sex differences (Diaz *et al.*, 2009). In addition, Gerald *et al.* (2008) reported significant interactions between genotype and sex on the gene expression of several receptor types within the striatum (a region involved in reinforcement processes), including both μ -opioid and δ -opioid and D₁, D₂, and D₅ dopamine receptors. Lastly, as we have previously reported (Ward and Dykstra, 2005; Ward *et al.*, 2007), male CB1 KO mice showed no deficit in acquisition and maintenance of FR-1 responding or in PR responding maintained by corn oil; however, female CB1 KO mice did show a significant deficit in the

acquisition of corn oil self-administration in this study as compared with female WT controls.

Sex differences also arose within genotypes for responding maintained under all three reinforcers. Female WT mice self-administered less Ensure than male WT mice under an FR-1 schedule, and female WT and CB1 KO mice self-administered less corn oil than male WT and CB1 KO mice under an FR-1 schedule, although no sex differences were observed when palatable food self-administration was maintained under the PR schedule. Conversely, no sex differences arose in mice self-administering cocaine under an FR-1 schedule, although a trend toward increased cocaine self-administration in females of both genotypes was seen. This is consistent with a larger literature suggesting that low FR schedules are relatively insensitive to sex differences in the reinforcing effects of psychostimulants, in that although there is a trend toward reports of increased intake under FR schedules, several studies report no significant sex differences (see Roth *et al.*, 2004 for review). Sex differences in psychostimulant self-administration become more apparent under more challenging reinforcement schedules, however, such as PR schedules. For example, female rats reach significantly higher breaking points than males under a PR schedule for several stimulant drugs, including cocaine (Roberts *et al.*, 1989; Carroll *et al.*, 2002; Lynch, 2008). This is in contrast with the present result, wherein female WT mice maintained significantly lower cocaine breaking points under the PR schedule as compared with male WT controls. This may be a species difference, as our result is in agreement with another recent report in C57Bl/6 mice by Griffin *et al.* (2007), who reported no sex differences in cocaine self-administration under an FR-2 schedule, but a significant attenuation in PR responding for cocaine in female versus male mice. Lastly, as would be expected, female WT and CB1 KO mice also weighed significantly less than their male counterparts.

Pretreatment with the CB1 antagonist SR141716 produced dose-dependent attenuation of Ensure intake under an FR-1 schedule of reinforcement in both male and female WT and CB1 KO mice; however, SR141716 was four to five fold more potent in WT mice than in CB1 KO mice. Haller *et al.* (2002) report a similar finding, in that 1.0 mg/kg SR141716 produced no effect on anxiety in CB1 KO mice but produced a significant anxiolytic effect in CB1 KO mice at the 3.0 mg/kg dose. Moreover, higher doses of SR141716, but not lower doses, reverse deficient suckling behavior in CB1 KO mice (Fride *et al.*, 2003). These behavioral studies add to a larger literature suggesting that although SR141716 exerts its behavioral effects primarily at the CB1 receptor, effects at higher doses are also likely mediated by other receptor mechanisms, with a novel CB receptor being the most likely candidate (Járai *et al.*, 1999; Zimmer *et al.*, 1999; Di Marzo *et al.*, 2000; Breivogel *et al.*, 2001; see Haller *et al.*, 2002 for review).

The present results with SR141716 failed to show sex differences in CB modulation of palatable food self-administration within each genotype. This is in agreement with Foltin and Haney (2007), who reported no sex differences in the anorectic effect of SR141716 on self-administration of either palatable food or food pellets in male versus female baboons. Conversely, Diaz *et al.* (2009) showed that the CB1 receptor antagonist AM251 produced a significantly more pronounced hypophagia in freely fed male versus female guinea pigs.

This discrepancy with the present data may be a result of species differences, an operant versus free feeding model, or the CB1 antagonists used.

In summary, the present data add to a small but growing literature suggesting that the CB system may show differential sensitivity in its modulation of appetitive behavior in males versus females. In addition, these results support previous work from our laboratory showing a relative lack of involvement of the CB1 receptor system in the reinforcing efficacy of palatable fats, and extend these findings to female mice. This set of experiments also reemphasizes the fact that sex is an important and complex determining factor in reinforced behavior, in that sex modulates the various phases of self-administration differently (acquisition, maintenance, breaking point) and in a reinforcer-dependent manner (palatable fat, palatable sweet, cocaine). Taken together, these findings highlight the importance of characterizing the role of specific receptor systems in appetitive behavior across sexes, reinforcer types and reinforcement schedules, and species. Lastly, we report behavioral effects of the highest dose of the CB1 antagonist SR141716 tested in CB1 KO mice, supporting the wider hypothesis that some of the effects of SR141716 can be mediated by other receptors, possibly a yet-undesignated CB receptor subtype. Further work is necessary to characterize in more detail whether and how the CB system differs in males versus females in its involvement in appetite and reward, and to consider the role of sex in future applications of CB drugs in the clinical setting.

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Fig. 1.

Sex×CB1 genotype interactions on acquisition, maintenance, and breaking point in male and female wild type (WT) (dark bars) and CB1 knockout (KO) (light bars) mice trained to self-administer 32% corn oil. (a) Mean (±SEM) number of days to acquire 32% corn oil self-administration. (b) Mean (±SEM) number of 32% corn oil-reinforced nose-pokes per 1 h fixed ratio-1 session. (c) Mean (±SEM) number of 32% corn oil-reinforced nose-pokes per 2 h progressive ratio session. *Significantly different from WT control ($P<0.05$). #Significantly different from male mice of like genotype.

Fig. 2.

Sex $\times$ CB1 genotype interactions on acquisition, maintenance, and breaking point in male and female wild type (WT) (dark bars) and CB1 knockout (light bars) mice trained to self-administer 10% Ensure. (a) Mean ($\pm$ SEM) number of days to acquire 10% Ensure self-administration. (b) Mean ($\pm$ SEM) number of 10% Ensure-reinforced nose-pokes per 1 h fixed ratio-1 session. (c) Mean ($\pm$ SEM) number of 10% Ensure-reinforced nose-pokes per 2 h progressive ratio session. *Significantly different from WT control ($P < 0.05$). #Significantly different from male mice of like genotype.

Fig. 3. SR141716 attenuates 10% Ensure self-administration in male (squares) and female (triangles) wild type (solid) and CB1 knockout (open) mice. Data depict mean ($\pm$ SEM) number of 10% Ensure-reinforced nose-pokes per 1 h fixed ratio-1 session after administration of vehicle or SR141716 (0.3–3.0 mg/kg intraperitoneal).

Fig. 4.

Sex $\times$ CB1 genotype interactions on maintenance and breaking point in male and female wild type (WT) (dark bars) and CB1 knockout (light bars) mice trained to self-administer 0.56 mg/kg/infusion cocaine. (a) Mean ($\pm$ SEM) number of cocaine-reinforced nose-pokes per 2 h fixed ratio-1 session. (b) Mean ($\pm$ SEM) number of cocaine-reinforced nose-pokes per 4 h progressive ratio session. *Significantly different from WT control ($P < 0.05$). #Significantly different from male mice of like genotype.

Fig. 5. Sex×CB1 genotype interactions on body weight in male and female wild type (WT) (dark bars) and CB1 knockout (KO) (light bars) mice trained to self-administer 32% corn oil. Data depict mean (+SEM) body weights.