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Chronic Unpredictable Stress Enhances Cocaine Conditioned Place Preference in CB_1 Receptor Knockout Mice

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Abstract

Cannabinoid signaling via the CB_1 receptor modulates the effects of drugs of abuse and the response to exposure to stressors. In addition, exposure to stressors can alter the effects of drugs of abuse. The present study examined the effects of exposure to chronic unpredictable stress (CUS) in CB_1 receptor knockout mice (CB_1 KO) and their wild-type (WT) littermates, using cocaine conditioned place preference (CPP) to compare their response to cocaine. Mice were untreated or exposed to two weeks of CUS. Following this period, the acquisition of a cocaine CPP was examined with one of three doses (3.2, 10.0 or 17.0 mg/kg) of cocaine. Untreated CB_1 KO and WT mice both acquired the cocaine CPP; however, exposure to CUS enhanced the acquisition of the cocaine CPP in CB_1 KO mice, but did not significantly alter the effects of cocaine in WT mice. Taken together, these findings support previous evidence suggesting a role for the CB_1 receptor in the response to stress as well as in the effects of cocaine.

Keywords

chronic stress; CB1 receptor; cannabinoid receptor; endocannabinoid; knockout mouse; cocaine

INTRODUCTION

The cannabinoid system modulates numerous central nervous system (CNS) functions, including the classic cannabinoid tetrad of thermoregulation, antinociception, locomotor activity and catalepsy (Little et al., 1988), as well as feeding behavior (Wiley et al., 2005), food reinforcement (Ward and Dykstra, 2005), and cognition (for review see Riedel and Davies, 2005). Pharmacological (Rinaldi-Carmona et al., 1994) as well as genetic manipulations (Ledent et al., 1999; Zimmer et al., 1999) provide evidence that modulation of many of these behaviors occurs through signaling via the type 1 cannabinoid receptor (CB_1).

The endocannabinoid system, specifically signaling at the CB_1 receptor, also plays a role in modulating the effects of drugs of abuse. For instance, pharmacological studies (Norwood et al., 2003) and studies with CB_1 receptor knockout (KO) mice (Martin et al., 2000; Cossu et al., 2001) suggest a role of the endocannabinoid system in the effects of morphine and other drugs. However, studies regarding cocaine are less clear. For example, CB_1 KO mice acquire a conditioned place preference (CPP) to cocaine (Martin et al., 2000) and also self-administer cocaine (Cossu et al., 2001) under certain conditions, suggesting that the CB_1

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receptor may not be involved in the conditioned rewarding or primary reinforcing effects of cocaine. Moreover, pharmacological blockade of CB1 receptors does not alter cocaine self-administration under fixed-ratio schedules of reinforcement (Tanda et al., 2000; De Vries et al., 2001; Filip et al., 2006). In contrast, other work suggests that CB1 receptor antagonism attenuates motivation to self-administer cocaine as measured by a progressive ratio schedule of reinforcement (Soria et al 2005; Xi et al., 2007; S.J. Ward personal communication, 2008), cue-induced reinstatement of cocaine seeking (De Vries et al 2001; S.J. Ward personal communication, 2008) and the acquisition of cocaine CPP (Chaperon et al., 1998).

There is also evidence that cannabinoid agonists modulate emotional behaviors. For example, administration of cannabinoid agonists produce both anxiolytic (Valjent et al., 2002; Berrendero and Maldonado, 2002; Marco et al., 2004) or anxiogenic (Onaivi et al., 1990; Arevalo et al., 2001; Marin et al., 2003; Genn et al., 2004; Marco et al., 2004) effects depending on dosage and experimental conditions. Anxiolytic effects are also observed when levels of endogenous cannabinoids are enhanced either by inhibition of the reuptake of endogenous cannabinoids (Bortolato et al., 2006; Patel and Hillard, 2006) or by inhibition of fatty acid amide hydrolase (FAAH; Patel and Hillard, 2006; Hill et al., 2007), an enzyme involved in the degradation of endogenous cannabinoids. Moreover, CB_1 receptor antagonists produce anxiogenic effects in a variety of rodent models of anxiety (Navarro et al., 1997; Arevalo et al., 2001; Patel and Hillard, 2006), and studies utilizing CB_1 KO mice extend these findings (Haller et al., 2002; Martin, 2002; Haller et al., 2004a,b; Uriguen et al., 2004).

The CB_1 receptor has also been implicated in the responses seen after exposure to stressors. The CB_1 antagonist SR141716A increases, whereas the agonist CP 55,940 decreases, isolation-induced ultrasonic vocalizations in rats pups (McGregor, 1996). In addition enhancement of cannabinoid signaling produces antidepressant-like effects in the mouse tail-suspension test (Gobbi et al., 2005) and rat forced-swim test (Gobbi et al., 2005; Hill and Gorzalka, 2005; Bambico et al., 2007). In addition to the modulation of behavioural responses to stress, the endocannabinoid system has a regulatory role in the physiological effects of stress. For instance, Patel et al. (2004) demonstrated that stress-induced elevations in corticosterone levels are enhanced by CB_1 receptor antagonism and attenuated by manipulations that enhance cannabinoid signaling. Further, CB1 KO mice display greater stress-induced elevations in adrenocorticotropic hormone than WT mice (Haller et al., 2004a).

Another model of emotional behavior is the chronic unpredictable stress (CUS) paradigm which involves exposing mice to a variety of mild stressors in an unpredictable manner (for a review see Willner, 2005). The most commonly utilized endpoint to study the effects of exposure to CUS is the consumption of, or preference for, sweet or palatable foods, though there are also effects on grooming, aggression, and behaviors in the forced swim test (FST) and learned helplessness models. Though there are contrasting reports, exposure to stress paradigms such as these have been shown to decrease the consumption/preference of sweet foods, disrupt grooming, increase aggression, and potentiate immobility in the FST model and enhance learned helplessness following exposure to the CUS paradigm (reviewed in Willner, 2005). As a result of these observations, the CUS paradigm is often used as a model of depression and this is further substantiated by studies showing that these effects are reversible by antidepressant treatment (Willner et al., 1987).

The development of a conditioned place preference is also altered by exposure to the CUS paradigm. For example, CUS attenuates the development of food-induced CPP (Papp et al., 1991; Muscat et al., 1992; D'Aquila et al., 1997; Benelli et al., 1999) as well as the acquisition of a morphine CPP (Papp et al., 1992; Valverde et al., 1997) a *dl*-amphetamine

CPP (Papp et al., 1991) and quinpirole and amphetamine CPP (Papp et al., 1993). Conversely, cocaine-induced place preference ha been reported to be enhanced in rats exposed to CUS (Haile et al., 2001).

Recent work has demonstrated that the endocannabinoid system modulates the effects of exposure to CUS. For instance, Bortolato et al. (2007) have shown that the FAAH inhibitor URB597 produces effects that are similar to antidepressant treatment in rats exposed to a regimen of CUS. In addition, mice lacking the CB1 receptor are more sensitive to the effects of chronic unpredictable stress as indicated by a decrease in the consumption of a sucrose solution across a period of exposure to various stressors (Martin et al., 2002).

On the basis of findings regarding the role of stress in drug addiction (Sinha, 2001; Goeders, 2002) and the growing interest in the role of the cannabinoid system in emotional and drug abuse-related behaviors, the present study examined the effects of CUS in mice lacking the CB_1 receptor, utilizing cocaine-induced CPP as the endpoint. In addition, while it has been established that mice lacking the CB_1 receptor can acquire a cocaine CPP at high doses (i.e. 20 mg/kg), less is known about the development of cocaine CPP in CB_1 receptor KO mice when lower doses of cocaine are examined.

METHODS

Subjects

Male CB1-KO and wild-type (WT) mice were used for these experiments. The CB_1 KO mice were generated on a full C57Bl/6 background by Zimmer and colleagues at the NIH, by a targeted mutation of the large single coding sequence of the CB_1 receptor gene (Zimmer et al., 1999). 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). For initial experiments, heterozygous breeding pairs were obtained from a colony at Virginia Commonwealth University, and were bred and genotyped at the Julius L. Chambers Biomedical/Biotechnology Research Institute at North Carolina Central University animal facilities in order to obtain WT and CB_1 KO mice. Further experiments were conducted with mice bred and genotyped in the animal facilities of the Psychology Department at the University of North Carolina at Chapel Hill. Mice were group housed after weaning, and then individually housed once experiments were begun. Mice had free access to food and water throughout the duration of the study except where specified by the experimental protocol. Lights were programmed on a 12 h light/dark cycle with lights off at 07.00 h (for an exception see experimental procedures for mice exposed to chronic unpredictable stress). Conditioned place preference experiments occurred during the dark portion of the light cycle with minimal illumination only as necessary. Animal protocols were approved by the institutional animal care and use committee, and the methods were in accord with the *Guide for the Care and Use of Laboratory Animals*.

Experimental Procedures

Chronic Unpredictable Stress—Three weeks prior to the beginning of the chronic unpredictable stress (CUS) paradigm, mice were individually housed while all other aspects of housing remained as described above. Following habituation to individual housing, CB_1 KO and WT mice underwent two weeks of exposure to CUS or two weeks of standard care (untreated mice). CUS consisted of exposure to the following stressors: 1-hr in a mouse restraint tube, inversion of the light/dark cycle, 2-hr of access to an empty water bottle, 15-hr of food restriction, 30-min forced swim in 32±2°C water, and 10-min paired housing in damp bedding. During this two week period, mice were exposed to 1–3 stressors per day

(see table 1). The stress exposure regimen was mixed with the qualifier that exposures to the same stressor never occurred consecutively within a day.

Conditioned Place Preference—Assessment of conditioned place preference (CPP) occurred in a three-compartment apparatus (46.5cm L × 12.7cm W × 12.7cm H; Med Associates, St. Albans, VT, USA) consisting of a neutral center (gray walls, flat floor) and distinct side compartments (white walls, grid floor, cob bedding; black walls, bar floor, cotton bedding). Photocells lining the walls measured locomotor activity and time spent in each compartment.

The conditioned place preference procedure consisted of three phases: pre-conditioning, conditioning and testing. During the pre-conditioning phase, mice were initially placed into the neutral gray compartment and allowed access to the entire apparatus. The time spent in each compartment was measured for 30-min. During conditioning, stressed and untreated CB_1 KO and WT mice were confined to one of the distinct side compartments for 30 min immediately after injections of saline or cocaine (3.2, 10.0 or 17 mg/kg). Previous work in our laboratory suggested that this range of doses would include a low dose that was insufficient to produce a conditioned place preference and a higher dose that would produce a significant conditioned place preference. Saline injections were given on the first conditioning day and alternated with cocaine injections for six days, resulting in three pairings of saline and three of cocaine. During these sessions, locomotor activity was also recorded. A single test session occurred on the day after the final conditioning day. During the test session mice were placed into the neutral center compartment and allowed free access to the entire apparatus, and the time spent in each compartment was recorded for 30 min.

Drugs

Cocaine hydrochloride was provided by the National Institute on Drug Abuse (Bethesda, MD, USA). Cocaine was dissolved in 0.9% saline and injected intraperitoneally at a volume of 0.1 ml/10 g.

Data analysis

Locomotor activity counts were recorded throughout the 30-min conditioning sessions. Locomotor activity following cocaine administration was compared to locomotor activity observed following saline administration and expressed as a percentage (activity counts following cocaine/activity counts following saline*100) and then collapsed across conditioning sessions to provide one activity measure per dose of cocaine. Conditioned place preference was measured by determining the time spent in the cocaine-paired compartment after conditioning compared to the time spent in that compartment prior to conditioning and expressed as the difference between these measures. Data were analyzed by three-way ANOVA to determine the effects of genotype, dose of cocaine, stress condition and their interactions. Fisher's protected least significant difference (PLSD) analysis and simple effects analysis were conducted when appropriate. All analyses were conducted with an alpha level of significance of $P<0.05$.

RESULTS

Figure 1 shows the effects of knockout of the CB_1 receptor on cocaine-induced locomotor activity during conditioning sessions in untreated mice (top) and mice exposed to CUS (bottom). Three-way ANOVA revealed a significant main effect of the dose of cocaine that was administered during conditioning [$F(2,83) = 18.44$, $P < 0.05$], indicating that locomotor activity was increased in a dose-dependent manner. Locomotor activity increased as a

function of the dose of cocaine such that 3.2<10<17 mg/kg ($P < 0.05$ in all comparisons). However, for the dependent variable of locomotor activity there were no main effects of stress or genotype and there were no significant two-way or three-way interactions between any of the independent variables.

Figure 2 shows the effects of knockout of the CB_1 receptor on cocaine CPP in untreated mice (top) and mice exposed to CUS (bottom). In all groups tested, the lowest dose of cocaine (3.2 mg/kg) produced mean CPP scores with 95% confidence limits that included zero, suggesting that this dose did not produce a conditioned place preference. ANOVA revealed a main effect of dose on the change in time spent in the cocaine-paired compartment from pre-conditioning to test [$F(2,83) = 24.13$, $P < 0.05$]. Cocaine dose-dependently increased the amount of time spent in the cocaine-paired compartment after conditioning such that 3.2<10<17 mg/kg ($P < 0.05$ in all comparisons). Main effects of genotype and stress were not statistically significant; however, there was a significant interaction between genotype and stress [$F(1,83) = 4.79$, $P < 0.05$]. Simple effects analysis revealed that exposure to CUS significantly increased cocaine CPP in CB_1 KO mice [$F(1,46) = 4.03$, $P < 0.05$]. On the other hand, CUS tended to decrease cocaine CPP in WT mice relative to untreated mice, however, this difference was not statistically significant [$F(1,46) = 1.06$, $P > 0.05$].

Pairwise comparisons were performed at the 10 mg/kg dose based on predictions that exposure to stress would have no effect on cocaine CPP at 3.2 mg/kg or 17 mg/kg cocaine. We predicted that 3.2 would not be sufficient to produce a significant cocaine CPP regardless of treatment and 17 mg/kg would produce a significant CPP in all groups (Martin et al., 2000), potentially obscuring some of the effects of CUS. Exposure to CUS decreased cocaine CPP in WT mice conditioned with 10 mg/kg cocaine, but this effect was not significant [$t(16) = 1.50$, $P = 0.15$]. On the other hand, exposure to CUS significantly increased cocaine CPP in KO mice at this dose [$t(15) = 3.17$, $P = 0.01$]. These results are consistent with the simple effects analysis above, and the results of the three-way analysis which revealed that the interaction of genotype and dose approached, but did not reach statistical significance [$F(2,83) = 2.94$, $P = 0.06$]. There were no other significant two-way or three-way interactions between independent variables with regard to cocaine CPP.

DISCUSSION

One purpose of the present study was to determine if there are differences between both CB_1 WT and KO mice in their response to cocaine in the CPP model. Consistent with previous work (Martin et al., 2000), CB_1 KO and WT mice acquired a cocaine CPP. In addition, statistical analysis suggested that there were no differences between the two genotypes with regard to the effects of cocaine on locomotor activity. Nevertheless, examination of the data (see figure 2, 10mg/kg) and the fact that the effect of the interaction of genotype and dose approached statistical significance suggests that CB1 KO and WT mice may be differentially sensitive to the effects of cocaine as measured by CPP.

While previous research has provided evidence that the type 1 cannabinoid receptor plays a role in the reinforcing and conditioned rewarding properties of opioids and various other drugs of abuse, results regarding psychostimulants such as cocaine have been mixed. For instance, in some experimental preparations, neither pharmacological antagonism (Tanda et al., 2000; De Vries et al., 2001; Filip et al., 2006) nor deletion of the CB_1 receptor (Cossu et al., 2001) had an effect on cocaine self-administration maintained by fixed ratio schedules of reinforcement. In addition, CB_1 KO mice acquired a cocaine CPP when conditioned with 20 mg/kg cocaine (Martin et al., 2000). In contrast, other research has shown that altering CB_1 signaling by pharmacological means inhibited the acquisition of cocaine-induced CPP

(Chaperon et al., 1998) and disrupted cocaine self-administration (Xi et al., 2007; S.J. Ward personal communication, 2008). In addition, Soria et al., (2005) found that cocaine self-administration was disrupted by deletion of the CB_1 receptor and by CB1 receptor antagonism. Thus, the present study and growing literature in this area suggest that while the endocannabinoid system, specifically the CB_1 receptor, may not be essential to the behavioral effects of cocaine, it likely has a modulatory role.

The second goal of the present study was to utilize cocaine CPP as an endpoint to compare the effects of exposure to chronic unpredictable stress in CB_1 KO and WT mice. Although these mice did not differ with regards to the acquisition of a cocaine CPP, the data suggest that the genotypes responded differently to stress. Specifically, CPP was significantly increased in CB_1 KO mice exposed to CUS; however there was a non-significant decrease in WT mice exposed to CUS. While statistical analysis indicated that the dose of cocaine was not a factor in this interaction, results obtained at the 10 mg/kg dose of cocaine may illustrate the effect of CUS on CPP in the CB_1 KO mice.

Previous work has demonstrated that exposure to stress alters responses to cocaine administration in animal models relevant to drug abuse (for review see Goeders, 2002). Both acquisition (Goeders and Guerin, 1994; Haney et al., 1995; Miczek and Mutschler, 1996) and reinstatement (Erb et al., 1996; Ahmed and Koob, 1997; Mantsch and Goeders, 1999) of cocaine self-administration are modulated by exposure to acute or repeated stress. In addition, the nature of exposure to stress can be important. For instance, Haile et al. (2001) reported that stressors administered in an unpredictable manner enhanced cocaine-induced locomotor activity and place conditioning whereas predictable stress did not.

Interestingly, in the present study, CUS enhanced the cocaine conditioned place preference in CB_1 KO mice but had no effect on place conditioning in WT mice. The absence of enhanced place conditioning in WT mice in the present study is somewhat at odds with the results of the study of Haile et al. (2001), and while the cause of this inconsistency is unclear, it is not entirely surprising. The enhancement of cocaine conditioned place preference seen in CB1 KO mice in the present study and in rats in the Haile et al. study differs from the results of some other studies of drug-induced conditioned place preference that have demonstrated that exposure to chronic unpredictable stressors disrupts the acquisition of CPP with morphine (Papp et al., 1992; Valverde et al., 1997), *dl*-amphetamine (Papp et al., 1991), amphetamine and quinpirole (Papp et al. 1993). Further, researchers assessing the effects of CUS on other endpoints have reported results that are not always consistent with the usual profile of effects (for a review see Willner, 2005). In addition to the fact that CUS produces a variety of effects across a range of endpoints, there are also differences between the Haile et al. study and the present study (e.g. species and experimental design) that make direct comparison difficult.

The mechanisms underlying the effects of exposure to stressors on the effects of drugs such as cocaine are not entirely known; however, activation of the hypothalamic-pituitary-adrenal (HPA) axis and the resultant elevations in glucocorticoids has been associated with stress-induced changes in behavioral responses to cocaine (Marinelli and Piazza, 2002). For example, Goeders and Guerin (1996a) found that enhancement of cocaine self-administration in rats was accompanied by increases in corticosterone levels whereas cocaine self-administration does not occur in adrenalectomized rats (Goeders and Guerin, 1996b). In addition, experimenter-administered corticosterone facilitates cocaine self-administration (Mantsch et al., 1998).

The enhancement of cocaine conditioned place preference seen in the CB1 KO mice in the present study may have been a result of alterations in HPA axis function in these mice. CB_1

KO mice display increased basal and stress-induced activation of the HPA axis (Barna et al., 2004; Haller et al., 2004a; Cota et al., 2007). Further, the effects of pharmacological manipulations of the cannabinoid system on behavior in stress-exposure models extend to the physiological markers indicative of HPA axis activation. For instance, in mice exposed to 30 min of restraint, corticosterone levels are increased in a manner that is enhanced by the CB_1 antagonist SR141716A but attenuated by the CB_1 agonist CP 55,940 as well as the endogenous cannabinoid uptake inhibitor AM404, and the FAAH inhibitor URB597 (Patel et al., 2004).

In addition to cannabinoid system modulation of the effects of stress discussed above, exposure to stress also affects the functioning of the endocannabinoid system. Exposure to chronic stress decreases in 2-arachidonylglycerol levels and CB1 receptor expression in the hippocampus (Hill et al., 2005). Moreover, exposure to chronic stress increases CB1 mRNA in the prefrontal cortex and decreases it in the midbrain (Bortolato et al., 2007). These data sets provide further support for the importance of the endocannabinoid system in the stress response.

Although altered HPA axis function in CB1 KO mice may underlie the results of the present study, interactions between the status of the cannabinoid system, stress exposure, and the effects of cocaine complicate the task of identifying the exact mechanisms that underlie our results. For instance, there is evidence of co-localization of receptors and convergence of signal transduction mechanisms between the cannabinoid and dopamine systems (Meschler and Howlett, 2001). Moreover, the endogenous cannabinoid anandamide increases extracellular dopamine levels in the nucleus accumbens (Solinas et al., 2006), and the CB1 receptor antagonist SR141716A inhibits phasic dopamine release in response to cocaine (Cheer et al., 2007). Evidence that exposure to chronic stressors produces region-specific alterations in dopaminergic function (Bekris et al., 2005) further complicates the interpretation of our results. Clearly, further research is needed to explain the nature of the interactions between manipulations of the endocannabinoid system, stress, and cocaine.

In summary, the present results demonstrate that exposure to CUS enhanced the acquisition of a cocaine CPP in CB_1 KO mice. No such enhancement was seen in wild-type mice, suggesting that deletion of the CB1 receptor produced a greater susceptibility to the effects of the CUS paradigm employed in the present study. These findings support previous evidence suggesting a role for the CB_1 receptor in emotional behaviors as well as in the effects of drugs of abuse.

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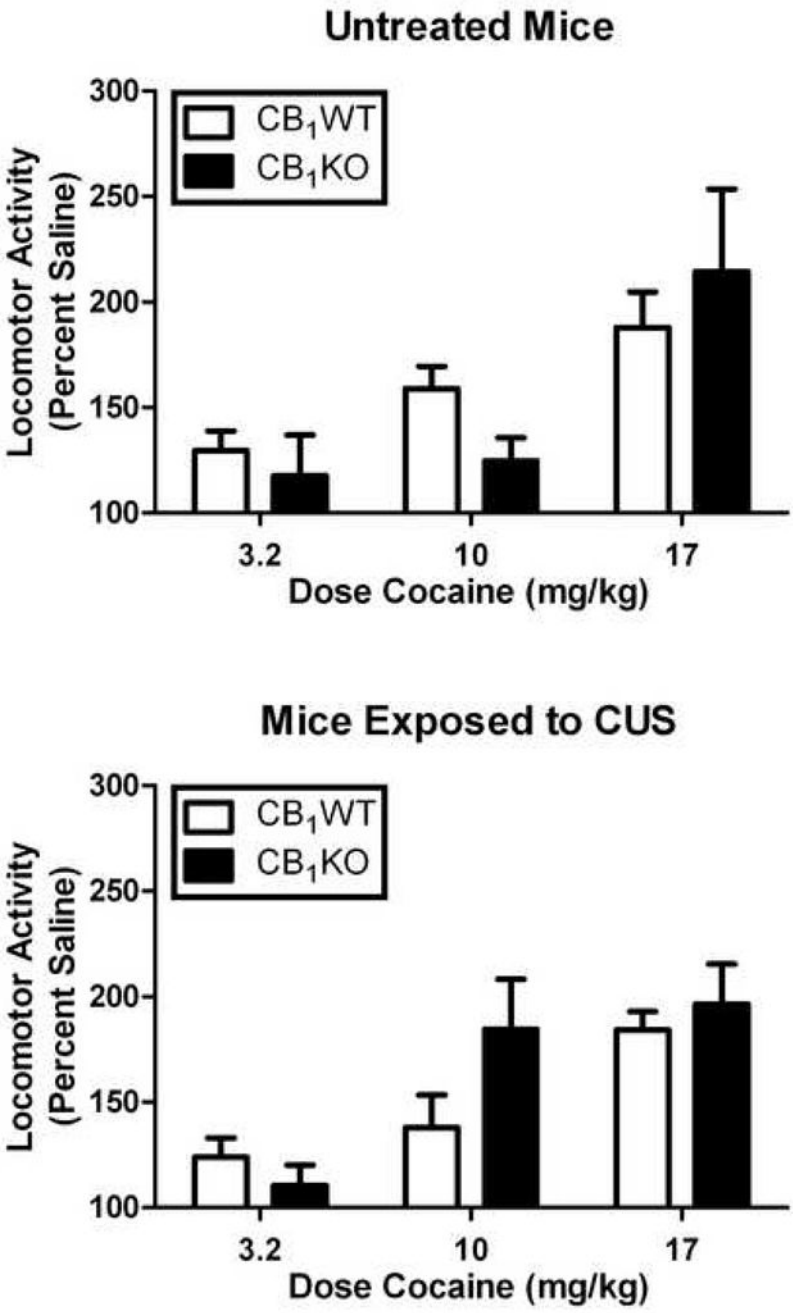

Fig. 1.

Cocaine-induced locomotor activity during conditioning in CB1 WT mice (open bars) and CB1 KO mice (closed bars). Mean (+ SEM) cocaine-induced locomotor activity during conditioning sessions expressed as percent of saline-induced locomotor activity for mice that were untreated (top) or exposed to CUS (bottom). The number of animals in each group was as follows: untreated WT mice: 3.2 mg/kg = 7, 10 mg/kg = 9, and 17 mg/kg = 8; untreated KO mice: 3.2 mg/kg = 8, 10 mg/kg = 11, and 17 mg/kg = 7; WT mice exposed to CUS: 3.2 mg/kg = 7, 10 mg/kg = 9, and 17 mg/kg = 8; KO mice exposed to CUS: 3.2 mg/kg = 8, 10 mg/kg = 7, and 17 mg/kg = 7.

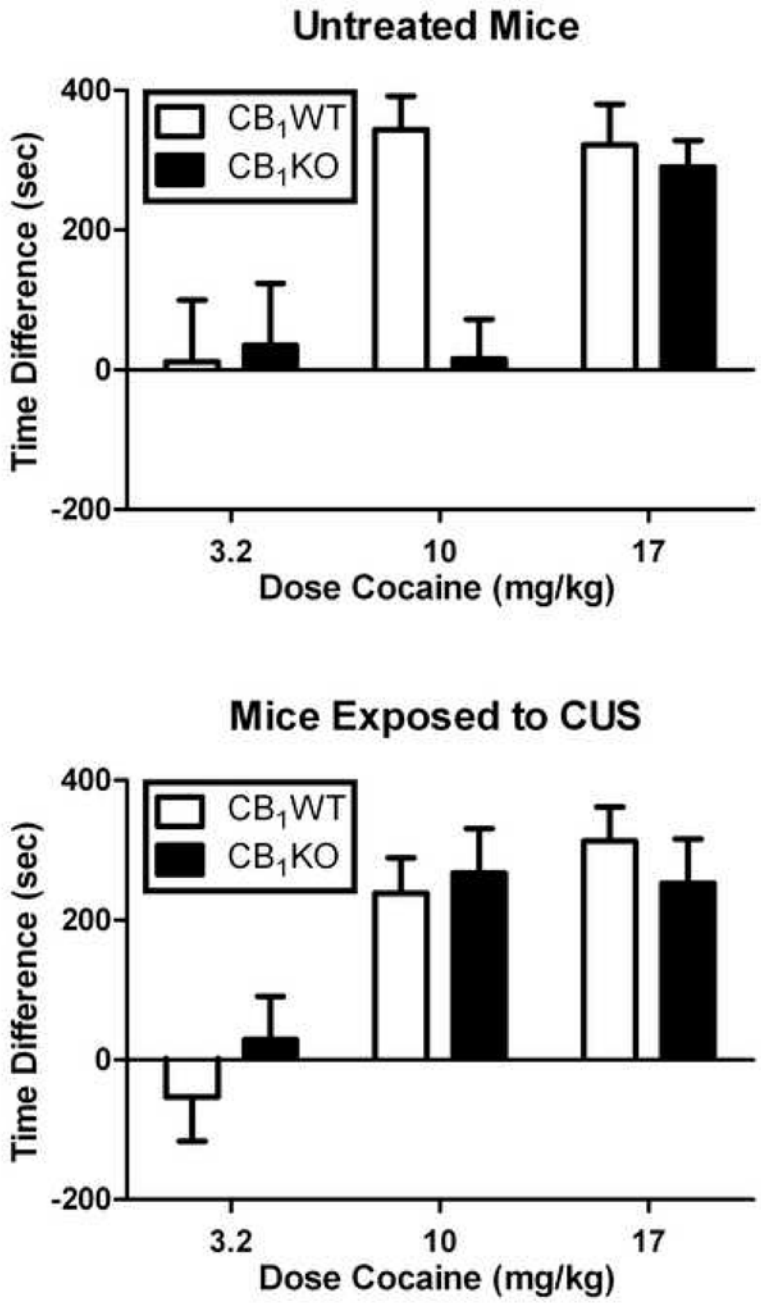

Fig. 2.

Cocaine CPP in CB1 WT mice (open bars) and CB1 KO mice (closed bars). Mean (± SEM) cocaine conditioned place preference expressed as the difference in time (s) spent in the cocaine-paired compartment before and after conditioning for mice that were untreated (top) or exposed to CUS (bottom). The number of animals in each group was as follows: untreated WT mice: 3.2 mg/kg = 7, 10 mg/kg = 9, and 17 mg/kg = 8; untreated KO mice: 3.2 mg/kg = 8, 10 mg/kg = 11, and 17 mg/kg = 7; WT mice exposed to CUS: 3.2 mg/kg = 7, 10 mg/kg = 9, and 17 mg/kg = 8; KO mice exposed to CUS: 3.2 mg/kg = 8, 10 mg/kg = 7, and 17 mg/kg = 7.

Table 1

Chronic unpredictable stress schedule

Day	Morning Stressor	Mid-day Stressor	Evening Stressor
1		Paired housing/damp bedding	
2		Inversion of light/dark cycle	
3	Confinement	Forced swim	Paired housing/damp bedding
4	Access to empty water bottle	Confinement	
5	Forced swim		Food restriction
6	Inversion of light/dark cycle	Paired housing/damp bedding	
7			Access to empty water bottle
8	Inversion of light/dark cycle		
9		Paired housing/damp bedding	
10	Forced swim	Confinement	Food restriction
11	Confinement	Paired housing/damp bedding	
12	Access to empty water bottle		Inversion of light/dark cycle
13	Paired housing/damp bedding	Forced swim	Confinement
14		Access to empty water bottle	