Published in final edited form as:

Neuroscience. 2007 November 30; 150(1): 14–21.

Corticotropin releasing factor (CRF)-1 receptor antagonist, CP-154,526, blocks the expression of ethanol-induced behavioral sensitization in DBA/2J mice

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Abstract

Rationale—Manipulation of glucocorticoid receptor signaling has been shown to alter the acquisition and expression of ethanol-induced locomotor sensitization in mice. It is unknown if other components of the hypothalamic-pituitary-adrenal (HPA)-axis modulate locomotor sensitization resulting from repeated ethanol administration. In the present investigation, we determined if pretreatment with an intraperitoneal (i.p.) injection of CP-154,526, a selective corticotropin releasing factor (CRF) type-1 receptor antagonist, would block the acquisition and/or expression of ethanol-induced locomotor sensitization in male DBA/2J mice.

Methods—To assess the role of the CRF₁ receptor in the acquisition of behavioral sensitization, mice were pretreated with an i.p. injection of CP-154,526 30 minutes before each of 10 sensitizing i.p. injections of ethanol. To determine the role of the CRF₁ receptor in modulating the expression of ethanol-induced sensitization, mice that had previously been sensitized to the locomotor stimulant effects of ethanol were pretreated with CP-154,526 30 minutes before an i.p. injection of ethanol on the test day. In a third study, ethanol-naïve mice were pretreated with CP-154,526 30 minutes before an initial i.p. injection of ethanol to determine the combined effects of the CRF₁ receptor antagonist and ethanol on locomotor activity. Blood ethanol concentrations were assessed at the termination of sensitization studies.

Results—Pretreatment with CP-154,526 blocked the expression of ethanol-induced locomotor sensitization in DBA/2J mice but did not prevent the acquisition of sensitization. The ability of CP-154,526 to block the expression of ethanol-induced locomotor sensitization was not attributable to alterations in blood ethanol levels or possible sedative effects produced by the combined administration of CP-154,526 and ethanol.

Conclusions—These data provide novel evidence that CRF₁ receptor signaling modulates the expression of ethanol-induced locomotor sensitization, and adds to a growing literature suggesting a role for neurochemicals and hormones associated with the HPA-axis in behavioral sensitization resulting from repeated exposure to drugs of abuse.

Keywords

ethanol; corticotropin releasing factor; CRF₁ receptor; locomotor activity; sensitization

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INTRODUCTION

Behavioral sensitization is defined as the long-lasting and progressive enhancement of the locomotor and motivational responses to a drug following repeated administration (Kalivas and Stewart, 1991). Over the past 20 years, numerous studies have demonstrated that repeated ethanol exposure elicits locomotor sensitization in mice (Crabbe et al., 1992; Cunningham and Noble, 1992; Lister, 1987; Phillips et al., 1995). Although recent studies have expanded our understanding of this complex phenomenon, the neurochemical basis of ethanol-induced sensitization is still not fully understood. However, numerous neurotransmitter systems have been implicated in ethanol-induced locomotor sensitization, including dopamine (Broadbent et al., 2005; Palmer et al., 2003), gamma-aminobutyric acid (GABA) (Broadbent and Harless, 1999), opioids (Nestby et al., 1997; Pastor and Aragon, 2006), and glutamate (Broadbent et al., 2003; Kotlinska et al., 2006; Szumlinski et al., 2005).

Additional candidates that may modulate drug-induced behavioral sensitization are the neurochemicals and hormones associated with hypothalamic-pituitary-adrenal (HPA)-axis signaling. Exposure to a variety of psychological stressors promotes robust activation of the HPA-axis (Smith and Vale, 2006). Interestingly, stress can substitute for drug administration in cross-sensitization studies. Thus, stress induced by forced restraint (Roberts et al., 1995), social defeat (Yap et al., 2005), maternal separation (Kikusui et al., 2005), social isolation (Frances et al., 2000), and other stressors (Lepsch et al., 2005) have been shown to augment the locomotor stimulant effects induced by various drugs of abuse. Importantly, manipulation of systems associated with the HPA-axis, such as glucocorticoid receptors, alters the development of behavioral sensitization to ethanol as well as other drugs of abuse (Johnson et al., 1995; Ortiz et al., 1995; Rivet et al., 1989; Roberts et al., 1995; Stohr et al., 1999; Wedzony and Czzyrak, 1994).

Corticotropin-releasing factor (CRF), a 41 amino acid polypeptide with high concentrations in the paraventricular nucleus of the hypothalamus (PVN) (Swanson et al., 1983) and a key component of the HPA-axis (Smith and Vale, 2006), has also been implicated in behavioral sensitization induced by psychostimulants (Erb and Brown, 2006; Przegalinski et al., 2005). Given that the CRF system modulates some of the neurobiological responses to ethanol, CRF is an obvious candidate in the modulation of ethanol-induced behavioral sensitization. Surprisingly, this question has not been addressed. However, there are data indicating that acute and chronic ethanol exposure activate central CRF (Koob et al., 1993; Rasmussen et al., 2000; Rivier et al., 1984) and increased levels of CRF are observed in the brain during ethanol withdrawal (Merlo Pich et al., 1995). The anxiogenic effect of ethanol withdrawal (Breese et al., 2004; Knapp et al., 2004; Overstreet et al., 2004; Rassnick et al., 1993) and ethanol self-administration in dependent animals (Funk et al., 2007; Valdez et al., 2002) also involve CRF receptor signaling. Because of the central role of CRF receptor signaling in modulating neurobiological responses to ethanol, here we determined if the CRF₁ receptor (CRF₁R) is involved in the acquisition and/or expression of ethanol-induced locomotor sensitization. To address these questions, male DBA/2J mice were pretreated with the peripherally bioavailable and selective CRF₁ receptor antagonist CP-154,526 (Lundkvist et al., 1996; Schulz et al., 1996) 30 minutes before each of 10 sensitizing injections of ethanol (acquisition experiment) or 30 minutes before an injection of ethanol on the test day in mice that had previously been sensitized to the locomotor stimulant effects of ethanol (expression experiment). We chose CP-154,526 because of its ability to block the sensitized anxiety-like response associated with repeated ethanol withdrawals (Knapp et al., 2004; Overstreet et al., 2004). Since acute and chronic ethanol exposure activate central CRF (Koob et al., 1993; Rasmussen et al., 2000; Rivier et al., 1984), and because glucocorticoid receptor antagonists block the acquisition of ethanol-induced locomotor sensitization (Roberts et al., 1995), we predicted that pre-treatment

with CP-154,526 would block the acquisition and/or expression of ethanol-induced behavioral sensitization.

MATERIALS AND METHODS

Animals

Subjects were 130 male DBA/2J mice (Jackson Laboratory, Bar Harbor, ME) obtained at 8 weeks of age and weighing between 20 and 26 grams at the beginning of the experiment. Mice were individually housed for two weeks with *ad libitum* access to standard rodent chow (Teklad, Madison, WI) and water and maintained at 22°C on a 12h:12h light:dark cycle. All experiments were initiated 1 hour after the beginning of the light cycle, and experiments were conducted in compliance with the National Institutes of Health guidelines and protocols were approved by the University of North Carolina Animal Care and Use Committee.

Drug Preparation

CP-154,526 (butyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-ethylamine) was donated by Pfizer (Groton, CT), and was suspended in a vehicle of 0.5% carboxymethylcellulose (CMC). Each dose of CP-154,526 was mixed in a concentration that yielded an injection volume of 5 ml/kg. CP-154,526 displays high affinity for the CRF₁ receptor ($K_i < 10$ nM) and blocks CRF-stimulated adenylate cyclase activity in rodent pituitary and cortical membranes (Lundkvist et al., 1996; Schulz et al., 1996).

The Effect of CP-154,526 on the Acquisition of Ethanol-Induced Locomotor Sensitization

Mice were randomly assigned to one of the 5 treatment conditions ($n = 10$ per group) in which two intraperitoneal (i.p.) injections were administered with 30 minutes between each injection (CMC + Saline; CMC + EtOH; 5 mg/kg CP-154,526 + EtOH; 10 mg/kg CP-154,526 + EtOH; 10 mg/kg CP-154,526 + Saline). The general procedure was as follows: On days 1–10, mice were individually removed from their cage and pre-treated with an i.p. injection of CMC or CP-154,526 (5 or 10 mg/kg). Thirty minutes later, mice received an i.p. injection of either a 2.5 g/kg dose of ethanol (20% w/v solution, mixed in 0.9% NaCl saline) or an equivolume (12.5 ml/kg) injection of isotonic saline (0.9% NaCl). Mice were then returned to their homecages and left undisturbed aside from routine animal husbandry. On day 11, all animals were transported to the testing room in their home cages and allowed to habituate for at least 35-minutes prior to testing. Mice were then given an i.p. injection of a 1.5 g/kg (7.5 ml/kg) dose of ethanol prior to being placed into the center of an open-field arena that automatically recorded activity via photo beam breaks (Harvard Apparatus, Inc., Holliston, MA) in 5-minute units over 20-minutes. The open field arena measured 40.64 cm by 40.64 cm by 30.48 cm and was made of clear Plexiglas. Several cm of corncob bedding were placed into the open field chamber to aid in cleaning and to prevent the buildup of odor. Soiled bedding was removed after each locomotor activity session. The room in which locomotor activity testing occurred was dimly lit by overhead lighting (average light intensity from samples taken in the 8 locomotor chambers was 11.4 Fc). Following the completion of locomotor activity testing, mice immediately received a tail nick for the collection of 6 μ l of blood in Fisher Scientific heparinized micro-hematocrit capillary tubes (Pittsburgh, PA).

The Effect of CP-154,526 on the Expression of Ethanol-Induced Locomotor Sensitization

The paradigm used in this study has been described previously (Fee et al., 2006) and was adapted from a design commonly used to study ethanol-induced behavioral sensitization (Lessov et al., 2001). On test day 1–3, all mice received an i.p. injection of isotonic saline (7.5 ml/kg), based on the equivalent volume for a 1.5 g/kg dose of ethanol, prior to being placed into the center of the open-field arena. On day 4, all mice received an i.p. injection of a 1.5 g/

kg dose of ethanol prior to placement into the locomotor chamber to establish a baseline of ethanol-induced locomotor activity. Mice were then assigned to 1 of 4 treatment groups ($n = 10$ per group) equated for locomotor activity following the initial ethanol injection (Day 4). All mice then underwent 10 consecutive days in which a 2.5 g/kg (12.5 ml/kg) dose of ethanol (i.p.) was administered in their home cage (one injection per day). The site of i.p. injection (left or right) was alternated daily to minimize discomfort. On day 15, 30 minutes before i.p. injection of a 1.5 g/kg dose of ethanol mice were pretreated with CMC or a 5, 10, or 20 mg/kg dose of CP-154,526. Following completion of the locomotor session, mice immediately received a tail nick for the collection of blood samples.

The Effect of CP-154,526 on Basal Ethanol-Induced Locomotor Activity

On test day 1–3, all mice received an i.p. injection of isotonic saline (7.5 ml/kg) prior to being placed into the center of the open-field arena. Mice were then assigned to 1 of 4 treatment groups ($n = 10$ per group) based on average locomotor activity over the 3 days with saline injections. On day 4, the mice were then pretreated with an i.p. injection of CMC or CP-154,526 (5, 10, or 20 mg/kg). Thirty minutes later, all mice received an i.p. injection of a 1.5 g/kg dose of ethanol immediately before locomotor activity testing. This study allowed for a determination of the effect of CP-154,526 on baseline ethanol-induced locomotor activity before the development of behavioral sensitization (i.e., after the first injection).

Assessment of Blood Ethanol Concentrations

Six μ l of whole blood from tail nicks were collected into capillary tubes and dispensed into 12×75 mm borosilicate glass tubes containing 375 μ l of water and 0.5 g of NaCl. These liquid samples were capped and refrigerated until processing by gas chromatography. Liquid ethanol standards (also 6 μ l, 0–400 mg%) and samples were similarly prepared and heated in a water bath at 55 °C for 10 minutes. Subsequently, a 1.5 ml sample of headspace gas was removed from the glass tubes with a plastic 3.0 ml syringe and injected directly into a SRI 8610C gas chromatograph (Torrance, CA) equipped with an external syringe adapter and 1.0 ml external loading loop. Samples were run at 140°C through a Haysep D column and detected with flame ionization at approximately 2 minutes post-injection. Hydrogen gas, carrier gas (also hydrogen), and internal air generator flow rates were 13.3, 25, and 250 ml/minute, respectively. Area under the curve for blood samples were analyzed with SRI PeakSimple software for Windows running on a Dell Inspiron 3500 ® laptop computer.

Data Analyses

For each experiment, locomotor activity data were analyzed using analyses of variance (ANOVA). All data are presented as mean $\pm$ S.E.M. LSD tests were conducted for all post-hoc analyses (Winer et al., 1991). Significance was set at $P < 0.05$ (two-tailed).

RESULTS

The Effect of CP-154,526 on the Acquisition of Ethanol-Induced Locomotor Sensitization

For the acquisition experiment, locomotor activity data from the test day are presented in Fig. 1. Daily treatment with CP-154,526 prior to a sensitizing dose of ethanol (2.5 g/kg) failed to block the acquisition of locomotor sensitization in DBA/2J mice. Two ANOVAs were performed to analyze these data. A 2×2 (CMC or 10 mg/kg dose of CP-154,526 $\times$ saline or ethanol injection) ANOVA performed on locomotor activity data revealed a significant main effect of ethanol treatment [$F(1, 36) = 16.29$, $p < .001$]. Neither the antagonist pretreatment nor interaction effects achieved statistical significance. The main effect of ethanol treatment shows that repeated injections of the 2.5 g/kg dose of ethanol successfully induced a sensitized response to ethanol relative to saline treated mice. A one-way ANOVA comparing the 3 groups

pretreated with CMC, 5 mg/kg of CP-154,526, or 10 mg/kg of CP-154,526 before each 2.5 g/kg ethanol injection failed to achieve statistical significance, verifying that pretreatment with CP-154,526 did not block the expression of ethanol-induced sensitization. Data representing blood ethanol concentrations (BECs) from the acquisition study are depicted in the top portion of Table 1. Neither a 2×2 nor a one-way ANOVA performed on BEC data were significant, confirming that increased locomotor activity in sensitized groups was not attributable to changes in ethanol metabolism. Additionally, these data suggest that chronic treatment with CP-154,526 did not influence blood ethanol levels on the test day.

The Effect of CP-154,526 on the Expression of Ethanol-Induced Locomotor Sensitization

For the expression experiment, locomotor activity data are presented in Fig. 2. For each group that received a different dose of CP-154,526, data are presented representing average baseline locomotor activity following saline injections, locomotor activity following the initial injection of ethanol, and locomotor activity on the test day when mice were pretreated with the CRF₁R antagonist 30 minutes prior to ethanol injection. Relative to baseline activity, the initial injection of a 1.5 g/kg dose of ethanol did not alter locomotor activity. Furthermore, repeated injections of ethanol increased the locomotor stimulant effects of ethanol in mice pretreated with CMC or the low (5 mg/kg) dose of CP-154,526. Pretreatment with the 10 and 20 mg/kg doses of CP-154,526 prior to i.p. injection of a 1.5 g/kg dose of ethanol on the test day effectively blocked the expression of a sensitized locomotor response that was observed in mice pretreated with CMC or the 5 mg/kg dose of CP-154,526. These observations were confirmed by a 3×4 (day $\times$ dose of CP-154,526) repeated-measures ANOVA performed on locomotor activity data. The analysis revealed a significant main effect of day [$F(2, 72) = 37.367, p < .001$] and a significant interaction effect between day and dose of CP-154,526 [$F(6, 72) = 6.327, p < .001$]. The dose main effect was not significant. Post hoc LSD tests run on test day (12th ethanol injection) data revealed significantly lower levels of locomotor activity in groups pretreated with the 10 and 20 mg/kg doses of CP-154,526 relative to CMC treated mice. There was no significant difference between mice treated with CMC or the 5 mg/kg dose of CP-154,526. Furthermore, LSD tests showed that locomotor activity on the test day was significantly greater than locomotor activity following baseline saline injections and after the first ethanol injection in groups pretreated with CMC or the 5 mg/kg dose of CP-154,526. However, locomotor activity on the test day did not differ significantly from locomotor activity following baseline saline injections or after the first ethanol injection in groups pretreated with the 10 mg/kg and 20 mg/kg doses of CP-154,526.

Data representing BECs collected at the end of the locomotor activity test of the expression study are depicted in the bottom half of Table 1. A one-way ANOVA performed on these data did not achieve statistical significance, suggesting that reductions in ethanol-induced locomotor activity on the test day by groups pretreated with CP-154,526 were not related to alterations of blood ethanol levels.

The Effect of CP-154,526 on Basal Ethanol-Induced Locomotor Activity

Locomotor activity data by ethanol naïve mice pretreated with one of four doses of CP-154,526 30 minutes before an initial i.p. injection of 1.5 g/kg dose of ethanol are presented in Fig. 3. A one-way ANOVA performed on locomotor activity data failed to achieve statistical significance, indicating that CP-154,526, at the doses tested, did not alter locomotor activity when presented in combination with an initial injection of ethanol.

DISCUSSION

Here, we found that pretreatment with 10 and 20 mg/kg (i.p.) doses of the selective CRF₁R antagonist CP-154,526 effectively blocked the expression of ethanol-induced locomotor

sensitization in male DBA/2J mice that had previously been sensitized to the locomotor stimulant effects of ethanol. Altered expression of ethanol-induced locomotor sensitization by CP-154,526 was not associated with changes in BECs, suggesting that the effects of the CRF₁R antagonist were not secondary to alterations of ethanol metabolism. Additionally, ethanol naïve DBA/2J mice, when pretreated with CP-154,526 before an initial i.p. injection of ethanol, showed no alterations of ethanol-induced locomotor activity. These data guard against the possibility that CP-154,526 and ethanol, when administered in combination, synergistically induced sedation or inhibited general locomotor activity. On the other hand, DBA/2J mice that were pretreated with an i.p. injection of CP-154,526 30 minutes before each of 10 sensitizing i.p. injections of ethanol demonstrated a sensitized locomotor response comparable to that observed in the absence of CP-154,526, indicating that pretreatment with CP-154,526 did not block the acquisition of ethanol-induced behavioral sensitization. Collectively, these data suggest that the plastic changes associated with the acquisition of ethanol-induced locomotor sensitization can be achieved independent of CRF₁R activity. However, once established, the behavioral output reflecting the sensitized response is modulated by CRF₁R signaling.

While the present study suggests that CRF₁R signaling does not modulate the acquisition of ethanol-induced behavioral sensitization, such a conclusion must be tempered in light of possible limitations of the present procedures. Since the maximum dose of CP-154,526 used in the acquisition study was 10 mg/kg, it is possible that higher doses may have blocked the acquisition of ethanol-induced locomotor sensitization. However, repeated treatment with doses greater than 10 mg/kg was avoided because of potential aversive side-effects associated with chronic administration of high doses of CP-154,526 (Arborelius et al., 2000). It is also possible that when given peripherally in an i.p. injection, CP-154,526 did not sufficiently reach the critical brain regions that are involved with the acquisition of ethanol-induced behavioral sensitization. Limiting this concern are the observations that peripheral administration of CP-154,526 has been shown to cross the blood-brain barrier and reach peak brain concentrations 20 minutes after administration with significant levels of the drug observed in the cortex, striatum, cerebellum, and hippocampus (Keller et al., 2002). Additionally, i.p. injection of CP-154,526 in the dose ranged examined here appears to produce antidepressant-like and anxiolytic-like effects in rodents (Breese et al., 2004; Chen et al., 1997; Lundkvist et al., 1996; Mansbach et al., 1997), indicating functional central actions when given peripherally.

An additional issue worth noting is that there were procedural differences between the acquisition and expression experiments. Mice were repeatedly pre-exposed (following 3 days of saline injections and 1 day with an ethanol injection) to the open-field apparatus before the sensitization test in the expression study, but no such pre-exposures were given in the acquisition study. It is thus possible that habituation to the testing environment is necessary for CP-154,526 to block the expression (and perhaps acquisition) of ethanol-induced behavioral sensitization.

These data extend a small but growing literature demonstrating that manipulation of neurochemicals and hormones associated with the HPA-axis alters drug-induced locomotor sensitization. Pretreatment with glucocorticoid receptor antagonists have been shown to block the behavioral sensitization caused by repeated administration of ethanol (Roberts et al., 1995) and MK-801 (Wedzony and Czyrak, 1994). Adrenalectomy blocked amphetamine- (Rivet et al., 1989) and nicotine- (Johnson et al., 1995) induced locomotor sensitization, effects that were reversed by glucocorticoid receptor agonist treatment. On the other hand, pretreatment with corticosterone or synthetic corticosteroids sensitized locomotor activation caused by administration of morphine (Stohr et al., 1999) and cocaine (Ortiz et al., 1995). Most relevant for to the present project are the findings that blockade of CRF receptor signaling prevented (Erb and Brown, 2006; Przegalinski et al., 2005), and i.c.v. administration of CRF

augmented (Erb and Brown, 2006), cocaine-induced behavioral sensitization in rats. Additionally, repeated central administration of CRF induced a sensitized locomotor response to D-amphetamine (Cador et al., 1993) and pretreatment with CRF antiserum significantly attenuated the development of D-amphetamine-induced behavioral sensitization (Cole et al., 1990a). Similarly, stress-induced sensitization of D-amphetamine-induced stereotypy behavior was attenuated by treatment with central infusion of a CRF receptor antagonist (Cole et al., 1990b). The present results extend these findings by provide evidence that CRF₁R signaling is involved in the expression of ethanol-induced behavioral sensitization.

It is of interest to consider the possible mechanism(s) by which the CRF₁ receptor modulates the expression, but not acquisition, of ethanol-induced behavioral sensitization. One theory suggests that the acquisition of stimulant-induced behavioral sensitization involves plastic changes in dopaminergic cell bodies of the ventral tegmental area (VTA), while the expression of sensitized behavior involves changes in dopamine transmission in axon terminal fields of the nucleus accumbens (NAc) (Cador et al., 1995; Kalivas and Stewart, 1991). Interestingly, central administration of the CRF₁R antagonist CRA-0450 significantly reduced cocaine-induced dopamine overflow in the NAc, indicating that the CRF₁R modulates dopamine activity in the NAc (Lodge and Grace, 2005). Assuming that dopamine activity in the NAc is involved in ethanol-induced behavioral sensitization, the above observation raises the interesting possibility that CP-154,526 blocked the expression of ethanol-induced locomotor sensitization by acting on CRF₁R located in the NAc. On the other hand, *in vitro* application of CRF in midbrain slices from mice induced a potentiation of N-methyl-D-aspartate (NMDA) receptor-mediated synaptic transmission in dopamine neurons of the ventral tegmental area (VTA), an effect that was blocked by the CRF₂ receptor antagonist antisauvagine-30 but not the CRF₁R antagonist CP-154,526 (Ungless et al., 2003). Since the VTA dopamine system is thought to be a critical region in the acquisition of drug-induced behavioral sensitization (Cador et al., 1995; Kalivas and Stewart, 1991), it is not entirely surprising that CP-154,526 did not block the acquisition of ethanol-induced locomotor sensitization in light of the observations by Ungless et al. (2003).

Alternatively, given the critical role of HPA-axis signaling in drug-induced behavioral sensitization, CP-154,526 may block the expression of ethanol-induced locomotor sensitization via its ability to prevent a normal HPA-axis response (Arborelius et al., 2000; Xu et al., 2005). However, it remains unclear why attenuation of normal HPA-axis signaling via glucocorticoid receptor blockade would block the acquisition of ethanol-induced behavioral sensitization (Roberts et al., 1995) while blocking CRF activity upstream of corticosterone release is only effective at altering the expression of ethanol-induced behavioral sensitization. Direct examination of HPA-axis activity (e.g., plasma adrenocorticotrophic hormone or corticosterone levels) in future sensitization studies will help to determine if the present results are attributable to CRF manipulations involved in HPA-axis signaling.

In summary, the present data provide direct evidence that CRF₁R signaling modulates the expression of ethanol-induced locomotor sensitization in DBA/2J mice. The ability of CP-154,526 to block the expression of ethanol-induced locomotor sensitization was not attributable to alterations in blood ethanol levels or possible sedative effects produced by the combined administration of CP-154,526 and ethanol. Additional work with CRF₂ receptor antagonists as well as site-directed manipulation of CRF receptor signaling are necessary to gain a more complete understanding of the role of CRF in ethanol-induced behavioral sensitization.

Acknowledgements

This work was supported by NIH grants AA015877, AA015875, AA013573, AA015148, and the Department of Defense grant PR054214. We thank Pfizer Inc. for kindly donating the CP-154,526 used in the present work. We also thank Darin Knapp for his expert assistance with blood ethanol analyses.

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Fig. 1.

Effects of CP-154,526 on the acquisition of ethanol-induced locomotor sensitization in male DBA/2J mice. Data represent test day ethanol-induced locomotor activity. On each of 10 acquisition days, mice received either pretreatment with an i.p. injection of CP-154,526 (the vehicle CMC, 5, or 10 mg/kg) 30 minutes prior to an i.p. injection of a 2.5 g/kg dose of ethanol, or pretreatment with an i.p. injection of CP-154,526 (CMC or 10 mg/kg) 30 minutes prior to i.p. injection of saline. On the test day, mice received an i.p. injection of a 1.5 g/kg dose of ethanol prior to placement in the locomotor chamber. All values reported are mean $\pm$ SEM. Symbol (*) denotes that non-sensitized groups (CMC-Saline and 10 mg/kg-Saline) were significantly less active than sensitized groups (CMC-EtOH, 5 mg/kg-EtOH, and 10 mg/kg-EtOH) ($P < 0.05$).

Expression Test

Fig. 2.

Effects of CP-154,526 on the expression of ethanol-induced locomotor sensitization in male DBA/2J mice. Data represent baseline locomotor activity (Baseline), locomotor activity following the first i.p. injection of a 1.5 g/kg dose of ethanol (1st Ethanol), and locomotor activity on the test day in which mice were pretreated with CP-154,526 (0, 5, 10, or 20 mg/kg) followed 30 minutes later by i.p. injection of a 1.5 g/kg dose of ethanol (12th Ethanol + CP-154,526). Between the 1st and 12th ethanol injection, all mice were given 10 daily i.p. injections of a 2.5 g/kg dose of ethanol to induce behavioral sensitization. Symbol (*) denotes that pretreatment with the 10 and 20mg/kg doses of CP-154,526 prior to ethanol injection significantly reduced ethanol-induced locomotor activity on the test day relative to mice pretreated with CMC or the 5 mg/kg dose of CP-154,526. Symbol (+) denotes that ethanol-induced locomotor activity was significantly greater following the 12th ethanol injection relative to the baseline activity and the 1st ethanol injection in mice pretreated with CMC or the 5 mg/kg dose of CP-154,526.

Fig. 3.

Data represent locomotor activity in male DBA/2J mice that were pretreated with an i.p. injection of CP-154,526 (0, 5, 10, or 20 mg/kg) followed 30 minutes later by an initial i.p. injection of a 1.5 g/kg dose of ethanol. All values reported are mean $\pm$ SEM.

Table 1

Blood Ethanol Concentrations (BEC; mg%) from the Sensitization Test Day of the Acquisition and Expression Experiments.

Acquisition Experiment	n	BEC
CMC-Saline	10	130.53 ± 4.16
CMC-EtOH	10	123.70 ± 4.74
5 mg/kg-EtOH	10	127.76 ± 4.44
10 mg/kg-Saline	10	126.18 ± 4.66
10 mg/kg-EtOH	10	123.42 ± 2.24
Expression Experiment		
CMC	10	130.93 ± 4.92
5 mg/kg	10	133.72 ± 5.52
10 mg/kg	10	133.09 ± 8.94
20 mg/kg	10	135.09 ± 6.40

Sample size indicated by *n*. Mice were pre-treated with carboxymethylcellulose (CMC) or a dose (mg/kg) of CP-154,526 before intraperitoneal injection of saline or EtOH (Acquisition Experiment) or EtOH (Expression Experiment). All BEC values reported are mean ± SEM.