Published in final edited form as:

Alcohol. 2014 August ; 48(5): 427–431. doi:10.1016/j.alcohol.2014.01.013.

Effects of the Novel Cannabinoid CB1 Receptor Antagonist PF 514273 on the Acquisition and Expression of Ethanol Conditioned Place Preference

Melanie M. Pina and Christopher L. Cunningham

Department of Behavioral Neuroscience, Oregon Health & Science University, Portland, OR, USA
97239-3098

Abstract

The centrally expressed cannabinoid receptor (CB1) has been considered a potential therapeutic target in treating alcoholism. Though CB1 receptors have been shown to modulate primary and conditioned ethanol reward, much of this research employed animal models that require ethanol ingestion or oral routes of administration. This is problematic considering CB1 antagonist drugs have high anorectic liability and have been used clinically in the treatment of obesity. Therefore, the present study examined CB1 antagonism in DBA/2J mice using an unbiased ethanol-induced conditioned place preference (CPP) procedure, a paradigm that does not require ethanol ingestion. To evaluate the role of CB1 receptors in primary ethanol reward, the highly potent and selective novel CB1 antagonist 2-(2-chlorophenyl)-3-(4-chlorophenyl)-7-(2,2-difluoropropyl)-6,7-dihydro-2H-pyrazolo[3,4-f][1,4]oxazepin-8(5H)-one (PF 514273) was administered 30 min before place preference conditioning with a fixed dose of ethanol (acquisition). To evaluate the role of CB1 receptors in ethanol-conditioned reward, PF 514273 was administered 30 min before place preference testing (expression). Although PF 514273 reduced ethanol-stimulated and basal locomotor activity, it did not perturb the acquisition or expression of ethanol-induced CPP. Results from the present study appear inconsistent with other studies that have demonstrated a role for CB1 antagonism in ethanol reward using oral administration paradigms. Our findings suggest that CB1 antagonism may have greater involvement in consummatory behavior than ethanol reward.

Keywords

ethanol; reward; place preference; locomotor activity; CB1; PF 514273; rimonabant; SR141716; inbred mice; DBA/2J

Introduction

Cannabinoid neurotransmission appears to serve a modulatory role in the mesolimbic dopamine (DA) system. Activation of the centrally expressed CB1 receptor has been shown to stimulate ventral tegmental area (VTA) DA neurons and increase DA release in the nucleus accumbens (NAc) (Gessa, Melis, Muntoni, & Diana, 1998), implicating the

cannabinoid system in neural processes that underlie reward. Indeed, studies have shown the cannabinoid system plays a role in the rewarding properties of many abused drugs (Heilig & Egli, 2006; Le Foll & Goldberg, 2005; Lupica & Riegel, 2005). Much of this research has focused on the role of the centrally expressed cannabinoid receptor CB1 in ethanol-mediated reward behaviors in animal models such as drinking preference, voluntary intake/consumption, and operant self-administration (for review see Basavarajappa & Hungund, 2005). Selective CB1 antagonists such as SR141716 (rimonabant) have been shown to reduce ethanol intake in Warsaw High-Preferring rats (Dyr, Ligieza, & Kostowski, 2008), suppress oral operant self-administration of ethanol in Long-Evans rats (Freedland, Sharpe, Samson, & Porrino, 2001), and decrease voluntary oral consumption of ethanol in C57BL/6 mice (Arnone et al., 1997) and CB1 knockout and heterozygous mice (Thanos, Dimitrakakis, Rice, Gifford, & Volkow, 2005). Although these studies lend support to the idea that CB1 receptors modulate the motivational effect of ethanol, they are complicated by evidence that CB1 antagonism reduces general consummatory behaviors (Houri & Pratley, 2005). Of note, SR141716 has been used clinically to treat obesity due to its high anorectic liability (Fong & Heymsfield, 2009).

Studies using the conditioned place preference (CPP) procedure have not been as widely used to assess the effect of CB1 antagonism on ethanol reward. The few published studies that evaluated the role of CB1 receptors in ethanol-induced CPP have used CB1 knockout mice. These studies support a role of CB1 receptors in the rewarding properties of ethanol by showing that lifelong depletion of CB1 receptors attenuates ethanol-induced CPP (Houchi et al., 2005; Thanos et al., 2005). While these studies illustrate CB1 receptor involvement in ethanol-mediated reward behavior that is independent from consumption, they are difficult to interpret because of their use of genetic knockouts, which are known to result in developmental compensation. Furthermore, the use of animals with lifelong deletion of the CB1 receptor makes it difficult to isolate the role these receptors play in the primary rewarding effects (acquisition) versus the conditioned rewarding effects (expression) of ethanol.

The present study investigated the effects of pretreatment with the novel CB1 antagonist drug 2-(2-chlorophenyl)-3-(4-chlorophenyl)-7-(2,2-difluoropropyl)-6,7-dihydro-2H-pyrazolo[3,4-f][1,4]oxazepin-8(5H)-one (PF 514273) on the acquisition and expression of ethanol-induced CPP. PF 514273 is a highly potent and selective bicyclic lactam-based CB1 receptor antagonist (Dow et al., 2009). To our knowledge, this is the first study to test the effect of this novel compound on ethanol or drug reward *in vivo*. Based on the ability of SR141716 to disrupt ethanol-mediated reward behaviors in previous studies, we hypothesized that pretreatment with PF 514273 at both low and high doses would interfere with the acquisition and expression of ethanol-induced CPP by reducing primary and conditioned ethanol reward.

Materials and Methods

Subjects

Male DBA/2J mice ($n = 144$) acquired from the Jackson Laboratory (Sacramento, CA, USA) at 6 weeks of age were allowed to acclimate to the colony for 2–3 weeks prior to

training. Mice were housed in groups of 4 at an ambient temperature of 21 ± 1 °C on a 12:12 light-dark cycle with lights on at 07:00 AM. Food and water were available *ad libitum* in home cages.

Apparatus

Twelve identical acrylic and aluminum chambers ($30 \times 15 \times 15$ cm) enclosed in individual ventilated, light- and sound-attenuating boxes (Coulbourn Instruments Model E10-20) were used to record activity and amount of time spent on each side of the chamber. Within each apparatus, activity and grid time were detected by six sets of infrared photodetectors mounted at 5-cm intervals, 2.2 cm above the floor along the front and rear sides of each inner chamber and recorded by computer (detailed fully in Cunningham, Gremel, & Groblewski, 2006).

Chamber floors consisted of grid (2.3-mm stainless steel rods mounted 6.4 mm apart in an acrylic frame) or hole (16-gauge stainless steel sheets perforated with 6.4-mm diameter holes on 9.5-mm staggered centers) interchangeable halves that are equally preferred by experimentally naïve DBA/2J mice (Cunningham, Ferree, & Howard, 2003). Floors and chambers were wiped with a damp sponge between animals.

Drugs

Ethanol (95%) was prepared 20% v/v in a solution of 0.9% saline and administered intraperitoneally (IP) in a 12.5 mL/kg volume at a dose of 2 g/kg. This dose has been shown previously to produce a robust CPP when administered before 5-min CS exposure (Cunningham, Okorn, & Howard, 1997).

PF 514273 (Tocris Bioscience, Ellisville, Mo., USA) was prepared in a vehicle of 50% dH₂O and 50% DMSO and injected IP in a 5-mL/kg volume at 1- and 5-mg/kg doses. Injections were given 30 min before conditioning sessions (Exp. 1 – acquisition) or place preference testing (Exp. 2 – expression). A stock solution of PF 514273 was made before conditioning trials and frozen at -20 °C. Upon removal from the freezer, PF 514273 stock was warmed by hand and vortexed for 3 min in order to ensure the drug remained in solution.

General Procedures for Place Conditioning

The place conditioning procedure involved three phases: habituation (one 30-min session), conditioning (eight 5-min sessions), and place preference tests (two 30-min sessions). Session durations were based on temporal parameters established by our laboratory that have been reliably shown to produce ethanol-induced CPP (Cunningham et al., 2006; Cunningham et al., 1997). In both experiments, mice were randomly assigned to one of three drug pretreatment groups ($n = 24$ each): vehicle, PF-1 (1 mg/kg PF 514273), and PF-5 (5 mg/kg PF 514273). Each treatment group was further subdivided into counterbalanced subgroups ($n = 12$ /subgroup) by conditioning (Grid+ or Grid–), trial order (+/– or –/+), and test day floor position (GH or HG). During conditioning sessions, ethanol was paired with and given immediately before exposure to a distinct tactile floor cue (grid or hole). Animals in the Grid+ conditioning subgroups received ethanol on the grid floor and saline on the hole

floor, whereas animals in the Grid– subgroups received ethanol on the hole floor and saline on the grid floor. In both experiments, mice received ethanol on CS+ trials, whereas saline injections were given in place of ethanol on CS– trials. Tests began 24 h after the first two conditioning sessions of each type (CS+ and CS–) and again 24 h after conducting two additional conditioning sessions of each type. Both floor cues were presented during preference tests. No ethanol was administered on test days; instead, saline was given immediately before each preference test. The procedures for each experiment and conditioning subgroup are outlined in the timeline presented in Table 1.

Experiment 1 – Effects of PF 514273 on CPP acquisition

Mice ($n = 72$) were pretreated in the home cage 30 min prior to CS+ (ethanol) conditioning trials with vehicle or PF 514273 at doses of 1 or 5 mg/kg. On CS– (saline) conditioning trials, animals received saline injections in place of PF 514273.

Experiment 2 – Effects of PF 514273 on CPP expression

Mice ($n = 72$) were pretreated in the home cage 30 min prior to place preference testing with vehicle or PF 514273 at doses of 1 or 5 mg/kg.

Statistical Analysis

Preference Tests

Time spent on the grid floor served as the primary dependent variable for CPP tests. This measure was derived from the recorded time (in sec) spent on the grid side of the apparatus divided by the test duration (30 min). This transformation yielded a dependent variable of time spent on the grid floor in units of sec/min, where 0 sec/min indicated complete aversion to and 60 sec/min indicated complete preference for the grid floor. Preference data from each test were analyzed separately using 2-way ANOVA (dose $\times$ conditioning subgroup), where dose refers to PF 514273 dose groups and conditioning subgroup represents the assigned conditioning group (Grid+/Grid–).

Test Activity

Activity during preference testing was analyzed by 1-way ANOVA (dose).

Conditioning Activity

Conditioning activity during CS+ (ethanol) and CS– (saline) trials was analyzed separately using 2-way mixed-factor ANOVA (dose $\times$ trial), where trial refers to the 4 conditioning trials.

Results

Six subjects were removed from analyses due to death ($n = 4$) or experimental error ($n = 2$). One additional subject was removed from conditioning trial analyses due to equipment error.

Place Preference Tests

Experiment 1 – PF 514273 does not impair the acquisition of ethanol-induced CPP

Blocking CB1 receptors with PF 514273 during acquisition did not interfere with the development of a significant ethanol-induced CPP at either test (Fig. 1). This finding was supported by a main effect of conditioning subgroup [Test 1 $F(1,61) = 31.9, p < 0.001$; Test 2 $F(1,61) = 111.2, p < 0.001$] and the absence of a dose $\times$ conditioning subgroup interaction (p 's > 0.05) or main effect of dose (p 's > 0.10) at either test. There were no group differences in activity during preference tests (Table 2), as evidenced by the lack of a main effect of dose (p 's > 0.3 for both tests).

Experiment 2 – PF 514273 does not impair the expression of ethanol-induced CPP

Blocking CB1 receptors with PF 514273 during preference tests did not affect the expression of ethanol-induced CPP (Fig. 2). This finding was supported by the absence of a significant dose $\times$ conditioning subgroup interaction (p 's > 0.05) and the presence of a conditioning subgroup main effect at both tests [Test 1 $F(1,66) = 39.9, p < 0.001$; Test 2 $F(1,66) = 93.3, p < 0.001$]. During Test 2, there was a significant main effect of dose [$F(2,66) = 4.6, p < 0.02$], as animals that received a 5 mg/kg dose of PF 514273 spent more time on the grid floor, regardless of their conditioning subgroup, as compared to animals that received vehicle injections ($p < 0.02$).

PF 514273 produced decreases in test activity (Table 2). This observation was supported by a main effect of dose on Test 1 [$F(2,69) = 14.4, p < 0.001$] and Test 2 [$F(2,69) = 13.8, p < 0.001$]. Significant activity differences were found between all dose groups (p 's < 0.05) on Test 1. During Test 2, activity did not significantly differ between PF-1 and PF-5 groups, though both groups continued to show significantly lower activity levels when compared to vehicle ($p < 0.001$).

Conditioning Activity

Activity was higher on CS+ trials than on CS– trials (Fig. 3), as is expected for DBA/2J mice (Cunningham, Niehus, Malott, & Prather, 1992). In Experiment 1, PF 514273 (Exp. 1) reduced ethanol's locomotor activating effect during CS+ (ethanol) trials 3 and 4 (Fig. 3). This was confirmed by a significant main effect of trial [$F(3,207) = 121.1, p < 0.001$] and trial $\times$ group interaction [$F(6,207) = 2.3, p < 0.04$]. No main effect of group was found ($p > 0.5$). Because PF 514273 was not administered before conditioning trials in Experiment 2, CS+ trial activity was influenced only by the presence of ethanol and all groups showed similar levels of ethanol-induced activation.

Discussion

In the present study, the cannabinoid antagonist PF 514273 did not perturb the acquisition or expression of ethanol-induced CPP. In Experiment 1, this conclusion was supported by data showing that all dose groups developed a place preference for the ethanol-paired floor cue (grid or hole) after 2 and 4 conditioning trials. In Experiment 2, animals exhibited a place

preference after 2 and 4 ethanol conditioning trials, and administration of PF 514273 during the CPP test did not impair the expression of this preference.

Interestingly, CB1 antagonism appeared to mildly interfere with the normal pattern of ethanol-induced locomotor sensitization, as animals receiving 1- or 5-mg/kg doses of PF 514273 displayed a trend toward lower ethanol-stimulated locomotor activity during later conditioning trials (trials 3 and 4) as compared to vehicle-group animals. This finding follows that of a recent study where the CB1 antagonist, AM251, reversed locomotor sensitization to nicotine (Bhatti et al., 2009) and suggests that CB1 antagonism may influence sensitization to the stimulating effects of ethanol and other drugs of abuse. It is also possible that repeated exposure to PF 514273 is required before some of its behavioral effects can be seen.

Outcomes from both Experiments 1 and 2 were not expected based on ethanol self-administration studies where systemic CB1 receptor antagonists were given. These studies have shown that peripheral injections of the CB1 antagonist SR141716A reduced ethanol intake, maintenance, and stress- and conditioned cue-induced reinstatement of ethanol seeking (Arnone et al., 1997; Cippitelli et al., 2005; Dyr et al., 2008; Economidou et al., 2006; Freedland et al., 2001). However, some of these studies report that in addition to decreasing ethanol intake, SR141716A also decreased sucrose intake (Arnone et al., 1997; Freedland et al., 2001). This effect suggests that CB1 antagonism may non-selectively inhibit ethanol intake and decrease consummatory behavior, in general. An additional factor to consider is that these self-administration studies were performed using rats or strains of mice (e.g., C57BL/6) that differ from those used in the present study (DBA/2J mice). Studies have routinely shown there are differences in receptor localization, density, and binding between species and strain. Notably, Vinod et al. (2008) reported that C57BL/6 mice exhibit greater CB1 receptor-mediated G protein activation than DBA/2J mice. Therefore, it is possible that CB1 receptors differ functionally between strains and this may be reflected by their differences in sensitivity to CB1 receptor manipulations. Another interpretation is that CB1 receptors are less important for mediating reward-related behaviors in DBA/2J mice versus C57BL/6 mice. It is also possible that the effects of CB1 antagonism noted in earlier studies are due to the anorectic liability of CB1 antagonist drugs, as these studies employed paradigms requiring oral or ingestive routes of ethanol administration. Notably, in a clinical trial SR141716 failed to prevent relapse to drinking in recently abstinent alcohol-dependent patients but did cause weight loss (Soyka et al., 2008). More studies will be needed to determine the clinical utility of CB1 antagonist drugs in treating alcohol use disorders.

While our results also appear inconsistent with studies that have reported reduced ethanol CPP in CB1 knockout mice (Houchi et al., 2005; Thanos et al., 2005), it is important to consider the developmental compensation that is possible when employing lifelong genetic receptor deletion. Indeed, increases in striatal dopamine D2 receptor expression in CB1 knockout mice have been reported and correlated with reduced ethanol reward (Houchi et al., 2005). Therefore, it is possible that reduced ethanol reward in these transgenic mice is conferred by changes in another receptor system or synergistic effects between CB1 receptor depletion and adaptations in another receptor system. It is also difficult to compare results

from the present study to findings obtained from CB1 receptor knockout studies, as CB1 receptor knockout mice used in those ethanol CPP studies are on a CD1 background as originally described by Ledent et al. (1999).

Finally, consideration must be given to the relative novelty of PF 514273 use *in vivo*. There is currently only one published study using PF 514273 *in vivo* and this report is that of the compound's discovery and ability to reduce food intake in rats (Dow et al., 2009). That study showed a 40% reduction in food intake after oral administration of 1 mg/kg. Thus, it was difficult to determine dosing in the present study, leaving the possibility that PF 514273 doses above 5 mg/kg may have resulted in a detectable effect on ethanol CPP. While SR141716 and PF 514273 are structurally similar and most likely act through a shared mechanism, we also cannot rule out the possibility that our results differ from those in studies using SR141716 due to differences between these compounds. For example, SR141716 has been shown to act at cannabinoid receptors that have been more recently discovered (e.g., Bátakai, Pacher, Járαι, Wagner, & Kunos, 2004; Cinar & Szűcs, 2009), yet it remains unclear whether other CB1 antagonists such as PF 514273 or AM251 act similarly at these receptors.

When viewed collectively, our findings and those reported in the literature may suggest that CB1 receptors play a more important role in mediating oral intake as opposed to ethanol reward. In the present study, we administered the novel CB1 receptor antagonist drug PF 514273 during ethanol CPP conditioning (acquisition) and testing (expression). This compound did not reduce the acquisition or expression of ethanol CPP, which was unexpected. Our outcomes differ from CPP studies in CB1 knockouts and drinking studies where the more commonly used CB1 antagonist SR141716A was administered. Additional studies using this novel compound in a variety of ethanol-reward paradigms would help clarify the impact of CB1 receptor antagonism on ethanol reward and intake.

Acknowledgments

Research reported in this paper was supported by the National Institutes of Health under award numbers R01AA007702 and T32DA007262. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. A Scholar Award from the ARCS Foundation Portland Chapter and a Graduate Scholarship from the Vertex Pharmaceutical Company provided additional support (MMP). Experiments reported here comply with the current laws of the United States.

References

- Arnone M, Maruani J, Chaperon F, Thiébot MH, Poncelet M, Soubrié P, et al. Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB1) receptors. *Psychopharmacology*. 1997; 132:104–106. [PubMed: 9272766]
- Basavarajappa BS, Hungund BL. Role of the endocannabinoid system in the development of tolerance to alcohol. *Alcohol and Alcoholism*. 2005; 40:15–24. [PubMed: 15550443]
- Bátakai S, Pacher P, Járαι Z, Wagner JA, Kunos G. Cannabinoid antagonist SR-141716 inhibits endotoxic hypotension by a cardiac mechanism not involving CB1 or CB2 receptors. *American Journal of Physiology. Heart and Circulatory Physiology*. 2004; 287:H595–H600. [PubMed: 15059774]
- Bhatti AS, Aydin C, Oztan O, Ma Z, Hall P, Tao R, et al. Effects of a cannabinoid receptor (CB) 1 antagonist AM251 on behavioral sensitization to nicotine in a rat model of novelty-seeking

- behavior: correlation with hippocampal 5HT. *Psychopharmacology*. 2009; 203:23–32. [PubMed: 18936914]
- Cinar R, Szűcs M. CB1 receptor-independent actions of SR141716 on G-protein signaling: coapplication with the mu-opioid agonist Tyr-D-Ala-Gly-(NMe)Phe-Gly-ol unmasks novel, pertussis toxin-insensitive opioid signaling in mu-opioid receptor-Chinese hamster ovary cells. *The Journal of Pharmacology and Experimental Therapeutics*. 2009; 330:567–574. [PubMed: 19448142]
- Cippitelli A, Bilbao A, Hansson AC, del Arco I, Sommer W, Heilig M, et al. Cannabinoid CB1 receptor antagonism reduces conditioned reinstatement of ethanol-seeking behavior in rats. *The European Journal of Neuroscience*. 2005; 21:2243–2251. [PubMed: 15869521]
- Cunningham CL, Ferree NK, Howard MA. Apparatus bias and place conditioning with ethanol in mice. *Psychopharmacology*. 2003; 170:409–422. [PubMed: 12955296]
- Cunningham CL, Gremel CM, Groblewski PA. Drug-induced conditioned place preference and aversion in mice. *Nature Protocols*. 2006; 1:1662–1670.
- Cunningham CL, Niehus DR, Malott DH, Prather LK. Genetic differences in the rewarding and activating effects of morphine and ethanol. *Psychopharmacology*. 1992; 107:385–393. [PubMed: 1352057]
- Cunningham CL, Okorn DM, Howard CE. Interstimulus interval determines whether ethanol produces conditioned place preference or aversion in mice. *Animal Learning Behavior*. 1997; 25:31–42.
- Dow RL, Carpino PA, Hadcock JR, Black SC, Iredale PA, DaSilva-Jardine P, et al. Discovery of 2-(2-chlorophenyl)-3-(4-chlorophenyl)-7-(2,2-difluoropropyl)-6,7-dihydro-2H-pyrazolo[3,4-f][1,4]oxazepin-8(5H)-one (PF-514273), a novel, bicyclic lactam-based cannabinoid-1 receptor antagonist for the treatment of obesity. *Journal of Medicinal Chemistry*. 2009; 52:2652–2655. [PubMed: 19351113]
- Dyr W, Ligieja J, Kostowski W. The effect of cannabinoid CB(1) receptor antagonist rimonabant (SR-141716) on ethanol drinking in high-preferring rats. *Alcohol*. 2008; 42:509–512. [PubMed: 18504086]
- Economidou D, Mattioli L, Cifani C, Perfumi M, Massi M, Cuomo V, et al. Effect of the cannabinoid CB1 receptor antagonist SR-141716A on ethanol selfadministration and ethanol-seeking behaviour in rats. *Psychopharmacology*. 2006; 183:394–403. [PubMed: 16261315]
- Fong TM, Heymsfield SB. Cannabinoid-1 receptor inverse agonists: current understanding of mechanism of action and unanswered questions. *International Journal of Obesity*. 2009; 33:947–955. [PubMed: 19597516]
- Freedland CS, Sharpe AL, Samson HH, Porrino LJ. Effects of SR141716A on ethanol and sucrose self-administration. *Alcoholism: Clinical and Experimental Research*. 2001; 25:277–282.
- Gessa GL, Melis M, Muntoni AL, Diana M. Cannabinoids activate mesolimbic dopamine neurons by an action on cannabinoid CB1 receptors. *European Journal of Pharmacology*. 1998; 341:39–44. [PubMed: 9489854]
- Heilig M, Egli M. Pharmacological treatment of alcohol dependence: target symptoms and target mechanisms. *Pharmacology & Therapeutics*. 2006; 111:855–876. [PubMed: 16545872]
- Houchi H, Babovic D, Pierrefiche O, Ledent C, Daoust M, Naassila M. CB1 receptor knockout mice display reduced ethanol-induced conditioned place preference and increased striatal dopamine D2 receptors. *Neuropsychopharmacology*. 2005; 30:339–349. [PubMed: 15383833]
- Houri M, Pratley RE. Rimnabant: a novel treatment for obesity and the metabolic syndrome. *Current Diabetes Reports*. 2005; 5:43–44.
- Ledent C, Valverde O, Cossu G, Petit F, Aubert JF, Beslot F, et al. Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. *Science*. 1999; 283:401–404. [PubMed: 9888857]
- Le Foll B, Goldberg SR. Cannabinoid CB1 receptor antagonists as promising new medications for drug dependence. *The Journal of Pharmacology and Experimental Therapeutics*. 2005; 312:875–883. [PubMed: 15525797]
- Lupica CR, Riegel AC. Endocannabinoid release from midbrain dopamine neurons: a potential substrate for cannabinoid receptor antagonist treatment of addiction. *Neuropharmacology*. 2005; 48:1105–1116. [PubMed: 15878779]

- Soyka M, Koller G, Schmidt P, Lesch OM, Leweke M, Fehr C, et al. Cannabinoid receptor 1 blocker rimonabant (SR 141716) for treatment of alcohol dependence: results from a placebo-controlled, double-blind trial. *Journal of Clinical Psychopharmacology*. 2008; 28:317–324. [PubMed: 18480689]
- Thanos PK, Dimitrakakis ES, Rice O, Gifford A, Volkow ND. Ethanol self-administration and ethanol conditioned place preference are reduced in mice lacking cannabinoid CB1 receptors. *Behavioural Brain Research*. 2005; 164:206–213. [PubMed: 16140402]
- Vinod KY, Yalamanchili R, Thanos PK, Vadasz C, Cooper TB, Volkow ND, et al. Genetic and pharmacological manipulations of the CB(1) receptor alter ethanol preference and dependence in ethanol preferring and nonpreferring mice. *Synapse*. 2008; 62:574–581. [PubMed: 18509854]

Figure 1.

Mean time on the grid floor (sec/min + SEM) after 2 trials (left panel) and after 4 trials (right panel) for each dose group in Experiment 1. For animals in the Grid+ conditioning subgroup, ethanol (2 g/kg) was given immediately before 5-min placement on the grid floor and saline was paired with the hole floor, whereas for animals in the Grid- subgroup ethanol was paired with the hole floor and saline was paired with the grid floor. On CS+ (ethanol) days, mice were pretreated with their assigned dose of PF 514273 (0, 1, or 5 mg/kg) 30 min before receiving ethanol ($n = 10-12$ /conditioning subgroup). A conditioned place preference was shown in all groups as indicated by the significant difference between the Grid+ and Grid- conditioning subgroups. $*p < 0.05$

Figure 2.

Mean time on the grid floor (sec/min + SEM) after 2 trials (left panel) and after 4 trials (right panel) for each dose group in Experiment 2. For animals in the Grid+ conditioning subgroup, ethanol (2 g/kg) was given immediately before 5-min placement on the grid floor and saline was paired with the hole floor, whereas for animals in the Grid- subgroup ethanol was paired with the hole floor and saline was paired with the grid floor. During Test 1 and Test 2, mice were pretreated with their assigned dose of PF 514273 (0, 1, or 5 mg/kg) 30 min before being placed in the apparatus with both floor cues (grid and hole) present ($n = 11-12$ /conditioning subgroup). A conditioned place preference was shown in all groups as indicated by the significant difference between the Grid+ and Grid- conditioning subgroups.

* $p < 0.05$

Figure 3.

Mean ($\pm$ SEM) activity counts per min for CS+ and CS- conditioning trials in vehicle, PF1 (1 mg/kg PF 514273), and PF5 (5 mg/kg PF 514273) dose groups. Closed symbols represent CS+ trials, where animals received ethanol immediately before 5-min placement in the conditioning apparatus. Open symbols represent CS- trials, where saline only was administered. Animals were pretreated with their assigned doses of PF 514273 on CS+ trials only. In Experiment 1 (left panel), PF 514273 was administered 30 min before CS+ conditioning sessions, whereas PF 514273 was not given during conditioning in Experiment 2 (right panel).

Table 1

Timeline of procedures for each experiment and conditioning subgroup

Day	1	2	3	4	5	6	7	8	9	10
	Trial 1		Trial 2		Test 1	Trial 3		Trial 4		Test 2
	CS+	CS-	CS+	CS-		CS+	CS-	CS+	CS-	
Conditioning Subgroups	Grid+	G	H	G	H	G	H	G	H	G vs H
	Grid-	H	G	H	G	H	G	H	G	G vs H
Conditioning Drug	EtOH	+	-	+	-	+	-	+	-	
Acquisition (Exp. 1)	PF/V	+	-	+	-	+	-	+	-	-
Expression (Exp. 2)	PF/V	-	-	-	+	-	-	-	-	+

G = grid floor; H = hole floor; EtOH = ethanol; PF/V = PF 514273 or vehicle
+ = drug administered, - = saline administered

Note: order of exposure to CS+ and CS- was counterbalanced within each conditioning subgroup; positions (left vs. right) of the G and H floors were also counterbalanced; see text for additional details

Table 2Mean Activity Counts per Minute ($\pm$ SEM) on Preference Test Days

	PF 514273 Dose	Test after 2 trials	Test after 4 trials
<i>Experiment 1</i>			
<i>EtOH CPP Acquisition</i>	0 mg/kg	32.5 $\pm$ 1.8	29.1 $\pm$ 1.5
	1 mg/kg	31.2 $\pm$ 2.1	30.9 $\pm$ 2.0
	5 mg/kg	33.2 $\pm$ 2.0	33.0 $\pm$ 2.1
<i>Experiment 2</i>			
<i>EtOH CPP Expression</i>	0 mg/kg	28.6 $\pm$ 1.5	28.3 $\pm$ 1.9
	1 mg/kg	22.6 $\pm$ 1.6 ^b	18.7 $\pm$ 1.3 ^a
	5 mg/kg	17.5 $\pm$ 1.2 ^{ac}	18.7 $\pm$ 1.3 ^a
		Dose $F(2,69) = 14.4^*$	Dose $F(2,69) = 13.8^*$

*
 $p < 0.001$ ^a Differs from 0 mg/kg, $p < 0.001$ ^b Differs from 0 mg/kg, $p < 0.02$ ^c Differs from 1 mg/kg PF 514273, $p < 0.05$