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Effects of Dopamine Receptor Antagonists on the Acquisition of Ethanol-Induced Conditioned Place Preference in Mice

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Abstract

Rationale—Studies support differential roles of dopamine receptor subfamilies in the rewarding and reinforcing properties of drugs of abuse, including ethanol. However, the roles these receptor subfamilies play in ethanol reward are not fully delineated.

Objective—To examine the roles of dopamine receptor subfamilies in the acquisition of ethanol-induced conditioned place preference (CPP), we pretreated animals systemically with antagonist drugs selective for dopamine D1- (SCH-23390) and D2-like (raclopride) receptors prior to ethanol conditioning trials.

Methods—Effects of raclopride (0–1.2 mg/kg) and SCH-23390 (0–0.3 mg/kg) on the acquisition of ethanol-induced CPP were examined in DBA/2J mice (experiments 1 and 2). Based on significant effects of SCH-23390, we then determined if SCH-23390 (0.3 mg/kg) produced a place preference on its own (experiment 3). To evaluate whether SCH-23390 impaired learning, we used a conditioned place aversion (CPA) paradigm and pretreated animals with SCH-23390 (0–0.3 mg/kg) before conditioning sessions with LiCl (experiment 4).

Results—Whereas raclopride (0–1.2 mg/kg) did not affect acquisition, SCH-23390 (0.1–0.3 mg/kg) impaired the development of ethanol-induced CPP. SCH-23390 (0.3 mg/kg) did not produce place preference when tested alone and SCH-23390 (0.1–0.3 mg/kg) did not perturb the acquisition of LiCl-induced CPA.

Conclusions—Our results support a role for dopamine D1-like but not D2-like receptors in ethanol's unconditioned rewarding effect as indexed by CPP. Blockade of D1-like receptors did not affect aversive learning in this procedure.

Keywords

alcohol; ethanol; SCH-23390; raclopride; locomotor activity; inbred mice; DBA/2J; place conditioning

Introduction

The mesolimbic system plays a central role in the motivational properties of abused drugs. Dopamine (DA) is a key catecholaminergic neurotransmitter of this pathway that supports drug reward and reinforcement (Wise and Rompre 1989). Within mesolimbic-striatal brain regions, DA acts on two main subfamilies of receptors, D1 and D2. These subfamilies can be further divided into subtypes, with the D1-like subfamily including dopamine D1 and D5 receptors and the D2-like subfamily including dopamine D2, D3, and D4 receptors (Neve et al. 2004). Though both DA receptor subfamilies are G protein-coupled, they are hypothesized to serve differential roles in neurobiological processes, as D1-like receptors are positively coupled and D2-like receptors are negatively coupled to adenylyl cyclase (Neve 2009).

Numerous studies using DA antagonists have indicated dopamine D1- and D2-like receptor activation supports the rewarding effects of psychostimulants and opiates. Of note, systemic administration of the highly selective dopamine D1-like receptor antagonist SCH-23390 impaired the acquisition of conditioned preference for an environment previously paired with amphetamine, cocaine, methylphenidate, nicotine, or morphine in rodents (Bardo et al. 1999; Hoffman and Beninger 1989; Leone and Di Chiara 1987; Liu et al. 2003; Meririnne et al. 2001; Nazarian et al. 2004; Shershen et al. 2010; Shippenberg and Herz 1988; Zarrindast et al. 2007). Dopamine D2-like receptors also appear to be involved in drug reward, with studies showing that pretreatment with the antagonist haloperidol blocks the development of place conditioning with amphetamine (Banasikowski et al. 2012; Hoffman and Donovan 1995; Mithani et al. 1986; Spyraiki et al. 1982), methylphenidate (Mithani et al. 1986), heroin (Spyraiki et al. 1983) and morphine (Manzanedo et al. 2001). More highly selective dopamine D2-like receptor antagonists like raclopride have also been shown to attenuate amphetamine- and morphine-induced conditioned place preference (CPP) acquisition (Hoffman and Donovan 1995; Manzanedo et al. 2001).

Multiple lines of evidence have provided a role for mesolimbic DA in the primary reinforcing effects of ethanol (for review see Gonzales et al. 2004). As with other psychoactive drugs, ethanol has been shown to increase DA release in the nucleus accumbens (NAc; Imperato and Di Chiara 1986) by increasing firing of dopaminergic cells in the ventral tegmental area (VTA; Brodie et al. 1990; Gessa et al. 1985). Excitation of DA cells in the VTA by ethanol has been strongly correlated with the rewarding effect of this drug and is supported by experiments showing that rats will self-administer ethanol directly into the VTA (Gatto et al. 1994; Rodd et al. 2004). Although it is clear DA transmission plays a fundamental role in the primary reinforcing effect of drugs of abuse, the contribution of specific DA receptor subfamilies remains less delineated.

Despite evidence of DA's modulatory role in ethanol reward, only a small number of studies have examined the contributions of specific DA receptor subfamilies to the unconditioned or primary rewarding effects of this drug. Even fewer studies have employed systemic pharmacological blockade selective for D1- and D2-like receptors during the acquisition of context-ethanol associations. From these studies, a small body of literature exists showing that D1-like receptor blockade by SCH-23390 impairs the acquisition of an ethanol-induced

conditioned taste aversion (CTA; Risinger et al. 1999) and reduces ethanol drinking (Dyr et al. 1993) and self-administration (Hodge et al. 1997). Recently, a study by Bahi and Dreyer (2012) illustrated that viral-mediated knockdown (LV-siD1R) in the NAc and systemic pharmacological blockade (SCH-39166) of D1-like receptors reduced the acquisition but not expression of an ethanol-induced conditioned place preference in mice.

Similarly, blockade of dopamine D2-like receptors has been shown to reduce ethanol seeking and drinking (Czachowski et al. 2001; Kaczmarek and Kiefer 2000; Samson and Chappell 2004). Results from experiments using dopamine D2 receptor-deficient mice have shown reductions in ethanol-induced CPP (Cunningham et al. 2000) as well as a lack of operant ethanol self-administration (Risinger et al. 2000). In contrast, systemic administration of haloperidol had no effect on acquisition of ethanol-induced CPP (Risinger et al. 1992). It is possible that this null finding may have been due to a lack of sensitivity in the CPP parameters used by Risinger et al. instead of a lack of effect of D2-like receptor antagonism on ethanol-induced CPP. Hence, in the current study, we chose to test the effects of D2-like receptor blockade on ethanol-induced CPP using raclopride, a more highly specific D2/D3 receptor antagonist. We also implemented procedural modifications (detailed below) that we believed would enhance the sensitivity of our CPP protocol and remove the possibility that ceiling effects obscured detection of changes in CPP induced by D2-like receptor blockade.

Though previous studies implicate dopamine D1- and D2-like receptors in the unconditioned effect of ethanol, the findings are mainly restricted to studies using self-administration procedures. Considering that most ethanol self-administration procedures require oral intake and testing in the presence of drug, further characterization of the contributions of DA receptor subfamilies in the primary rewarding effect of ethanol can be inferred by our use of a Pavlovian conditioning paradigm. Notably, our place preference model did not require extensive training and enabled us to employ noncontingent administration of a fixed ethanol dose and test in the absence of drug. Furthermore, there is some difficulty in using self-administration procedures to examine the effects of treatments administered during the acquisition of context-drug associations as opposed to those administered during the expression of context-approach behaviors after context-drug associations have been established (Cunningham et al. 2011). By using CPP in the current studies, we were able to more precisely target the acquisition phase to determine whether the antagonist treatments alter the unconditioned effect of ethanol (acquisition) as opposed to the context-ethanol memory or behaviors induced by previous context-drug learning (expression).

In the present study, we examined the contributions of dopamine D1- and D2-like receptors on the acquisition of ethanol-induced CPP using systemic administration of the highly selective DA receptor antagonists, SCH-23390 and raclopride. Notably, we used a modified procedure from that of Risinger et al. (1992), which found no effect of haloperidol on the acquisition of ethanol-induced CPP. In order to detect changes in CPP of low magnitude, we decreased the number of conditioning trials animals experienced before their first preference test (Grobowski et al. 2008). This approach reduces the possibility that ceiling effects would obscure the detection of drug manipulations. By using these procedures, we anticipated that

we would detect impairments in ethanol-induced CPP acquisition by both SCH-23390 and raclopride.

Materials and Methods

Animals

Adult male DBA/2J mice were acquired from the Jackson Laboratory (Sacramento, CA, USA) at 6 weeks of age and allowed to acclimate to the colony for 2–3 weeks. Subjects were group housed (four per cage) with food and water on a normal 12:12 light-dark cycle. All procedures were conducted in accordance with the *NIH Guide for the Care and Use of Laboratory Animals* and approved by the Institutional Animal Care and Use Committee.

Apparatus

Infrared detectors within acrylic and aluminum chambers (30 × 15 × 15 cm) enclosed in individual ventilated, light- and sound-attenuating boxes (Coulbourn Instruments Model E10-20) were used to record locomotor activity and amount of time spent on each side of the chamber (detailed fully in Cunningham et al. 2006). Chamber floors consisted of interchangeable halves characterized by a grid (stainless steel rods) or hole (perforated stainless steel sheets) design that are equally preferred by experimentally naïve DBA/2J mice (Cunningham et al. 2003).

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 to produce a robust CPP when administered before 5-min CS exposure (Cunningham et al. 1997). S(–)-Raclopride (+)-tartrate salt (dopamine D2-like receptor antagonist) was prepared daily by dissolution in a 0.9% saline vehicle and injected IP in a volume of 10 ml/kg at doses of 0.3, 0.6, and 1.2 mg/kg. Doses and pretreatment interval were chosen based on previous studies (Dickinson et al. 2003; Manzanedo et al. 2001). R(+)-SCH-23390 hydrochloride (dopamine D1-like receptor antagonist) was prepared daily in a vehicle of 0.9% saline and injected IP in a volume of 10 ml/kg at doses of 0.01, 0.03, 0.10, 0.20, and 0.30 mg/kg. Doses and pretreatment interval were chosen based on previous studies (Dickinson et al. 2003; Scibelli and Phillips 2009). Lithium chloride (LiCl) was dissolved in sterile distilled water and administered IP at a dose of 3 mEq (127.2 mg/kg) in a 20 ml/kg volume (Risinger and Cunningham 2000).

General Procedures for Place Conditioning

The place conditioning procedure involved three phases: habituation (5 min), conditioning (5 or 30 min), and place preference testing (30 min). Procedures and session durations are based on parameters established by our laboratory that have been reliably shown to produce CPP or conditioned place aversion (CPA), depending on the conditioning drug (Cunningham et al. 1997, 2006; Risinger and Cunningham 2000). Within each experiment, mice were randomly assigned to dose groups, then further subdivided into counterbalanced subgroups (n = 12/subgroup) by conditioning (Grid+ or Grid–), trial order (+/– or –/+), and test day floor position (GH or HG). For animals in the Grid+ conditioning subgroup, the

conditioning drug was paired with the grid floor and saline was paired with the hole floor; in the Grid- subgroup, the conditioning drug was paired with the hole floor and saline with the grid floor. Mice received injections of a conditioning drug (ethanol or LiCl) on CS+ trial days, and saline injections on CS- days. Conditioning drug injections were always given immediately before exposure to the tactile floor cue (grid or hole). Preference tests began 24 h after the first two conditioning sessions of each type (CS+ and CS-) and again 24 h after two additional conditioning sessions of each type. Animals were drug-free during preference tests.

Experiment 1 – Raclopride effects on CPP acquisition

Mice (n = 96) were pretreated in the home cage 20 min prior to CS+ (ethanol) conditioning trials with saline or raclopride (0.30, 0.60, or 1.20 mg/kg). On CS- (saline) conditioning trials, animals received saline injections in place of raclopride.

Experiment 2 - SCH-23390 effects on CPP acquisition

Experiment 2A—Mice (n = 96) were pretreated in the home cage 15 min prior to CS+ (ethanol) conditioning trials with saline or SCH-23390 (0.01, 0.03, or 0.10 mg/kg). On CS- (saline) conditioning trials, animals received saline injections in place of SCH-23390.

Experiment 2B—After observing a trend toward impaired ethanol-induced CPP at the highest dose of SCH-23390, experiment 2A was replicated using the highest dose of SCH-23390 administered previously (0.1 mg/kg) and two higher doses (0.2 and 0.3 mg/kg).

Experiment 3 – Motivational effects of SCH-23390 alone

To determine whether the perturbed development of ethanol-induced CPP (experiment 2) was due to SCH-23390 producing aversive effects, the drug's motivational effects were assessed within the parameters of our CPP procedure. Mice (n = 36) were injected with saline or 0.3 mg/kg SCH-23390 15 min prior to CS+ conditioning trials. On CS- trials, animals received saline injections in place of SCH-23390. As SCH-23390 alone served as the conditioning drug, no ethanol was given in this experiment.

Experiment 4 - SCH-23390 effects on LiCl-CPA acquisition

To address the possibility that SCH-23390 interfered with learning, the antagonist was tested using a different conditioning drug, LiCl. On CS+ trials, LiCl was injected immediately before placement on the CS+ floor cue and the effect of SCH-23390 pretreatment on LiCl-induced CPA acquisition was assessed. The protocol for this experiment followed that of experiment 2 except for the use of 30-min conditioning sessions and LiCl as the conditioning drug. The trial duration was chosen based on previous experiments (Risinger and Cunningham 2000).

Statistical Analysis

Preference Tests—Time spent on the grid floor served as the primary dependent variable on all CPP tests. The recorded time (in second) spent on the grid side of the apparatus was divided by the test duration (30 min), yielding time spent on the grid floor in second per

minute, where 0 s/min indicated complete aversion to and 60 s/min indicated complete preference for the grid floor. Preference data from each test were analyzed separately using two-way ANOVA (dose $\times$ conditioning subgroup), where dose refers to antagonist dose groups and conditioning subgroup represents the assigned conditioning subgroup (Grid+/Grid-). Bonferroni-corrected pairwise comparisons were used to follow-up significant interactions. Preference test activity was analyzed by one-way ANOVA (dose).

Conditioning Activity—Conditioning activity data were collapsed across all four trials of each type (CS+ and CS-) then analyzed using two-way mixed-factor ANOVA (dose $\times$ trial type), where “trial type” corresponds to CS+ and CS- trials.

Results

Preference Tests

Experiment 1 – Raclopride does not affect acquisition of ethanol-induced CPP

—As shown in Figure 1, ethanol-induced CPP developed in all groups [conditioning main effect on tests 1 and 2: both $F(3,88) = 49.7$, $p < 0.001$] and was not affected by raclopride as indicated by a lack of significant main effect of raclopride dose or interaction [p 's > 0.05].

Experiment 2 - SCH-23390 interferes with acquisition of ethanol-induced CPP

—After confirming there were no significant differences in activity or grid times between experiments 2A and 2B in the groups receiving 0.1 mg/kg SCH-23390 and saline, all data from both experiments were combined and analyzed as a whole. Evidence of significant place preference was found on both tests [conditioning subgroup main effect on tests 1 and 2: both $F(1,180) = 69.6$, $p < 0.001$]. However, blockade of D1-like receptors with SCH-23390 impaired the acquisition of ethanol-induced CPP at the three highest doses (0.1 – 0.3 mg/kg) on Test 1 but not on Test 2 (Figure 2). This was confirmed by a significant dose $\times$ conditioning subgroup interaction, which was found for Test 1 [$F(5,180) = 3.7$, $p < 0.01$] but not Test 2 [$F(5,180) < 1$, $p > 0.5$] and the absence of a significant main effect of dose on both tests. Pairwise comparisons between conditioning subgroups on Test 1 showed a significant CPP only in groups that received saline or SCH-23390 at doses of 0.01 mg/kg and 0.03 mg/kg (Bonferroni-corrected p 's < 0.007).

Experiment 3 – SCH-23390 does not produce place conditioning—There was no evidence that CPP or CPA developed when SCH-23390 was administered alone (Figure 3). This was supported by the absence of a significant main effect of dose or dose $\times$ conditioning subgroup interaction on both tests [p 's > 0.1]. This finding indicated that no motivational effect of SCH-23390 was detected with this protocol.

Experiment 4 - SCH-23390 does not affect acquisition of LiCl-induced CPA

Acquisition of LiCl-induced place aversion was not impaired by SCH-23390 pretreatment (Figure 4), as shown by significant CPP in all groups during both tests [conditioning subgroup main effect on tests 1 and 2: both $F(1,88) = 79.5$, $p < 0.001$; dose main effect and interaction on tests 1 and 2: $p > 0.05$].

Locomotor Activity

Preference Tests—Table 1 shows mean ($\pm$ SEM) activity counts per min during tests 1 and 2 for all experiments. There was no significant difference in test activity among raclopride or SCH-23390 dose groups in Exps. 1–3, as confirmed by the absence of a main effect of dose at both tests [p 's > 0.05]. Increased locomotor activity among SCH-23390 dose groups was observed at Test 2 but not Test 1 of experiment 4. This was confirmed by a significant main effect of dose on Test 2 [$F(3,92) = 3.3$, $p < 0.03$] but not Test 1 [$p > 0.1$]. Pairwise comparisons revealed a significant difference in activity between the saline control and 0.3 mg/kg SCH-23390 dose groups (Bonferroni-corrected $p < 0.03$) during Test 2.

Conditioning Activity—Systemic administration of both raclopride (experiment 1) and SCH-23390 (experiment 2) dose-dependently reduced the locomotor activating effects of ethanol during CS+ trials (Table 2). When given in conjunction with LiCl, SCH-23390 dose-dependently enhanced LiCl-induced reductions in locomotor activity (experiment 4). When given alone, SCH-23390 suppressed locomotor activity relative to saline trials (experiment 3).

Discussion

These experiments implicate dopamine D1-like but not D2-like receptors in the unconditioned rewarding effect of ethanol as indexed by CPP. Although blockade of dopamine D1-like receptors by the highly selective antagonist SCH-23390 impaired the development of ethanol-induced CPP (experiment 2), blockade of dopamine D2-like receptors by raclopride failed to produce an effect on ethanol-induced CPP acquisition (experiment 1). Follow-up studies suggest that the effects of SCH-23390 on ethanol-induced CPP were due to an attenuation of ethanol reward and not a function of its aversive effects (experiment 3) or general impairments in learning (experiment 4).

This impairment of ethanol-induced CPP development by SCH-23390 (0.1–0.3 mg/kg) was only present during preference Test 1, after two conditioning sessions, and was not detected on the final preference test, after animals received a total of four conditioning sessions. This outcome suggests that dopamine D1-like receptor antagonism by SCH-23390 slowed but did not block ethanol-induced CPP acquisition. It is also possible that after receiving four conditioning sessions performance levels had reached a ceiling that obscured the detection of differences in CPP magnitude produced by SCH-23390 (Grobowski et al. 2008). However, given the complex interactions between D1 and D2 receptors (for review see Neve 2009), it is possible that our effects were limited by another mechanism. Moreover, we cannot rule out the possibility that another neurotransmitter system or mechanism of action contributed to these effects, as we used only one D1-like antagonist drug and its effects were observed only at high doses. For example, it has been suggested that SCH-23390 binds serotonin receptors (Millan et al. 2001), raising the possibility that activation of these receptors influenced CPP acquisition at higher doses.

Interestingly, conditioning data show that SCH-23390 did not significantly reduce ethanol-stimulated locomotor activity until doses reached 0.1 mg/kg and higher. This may be due to these doses providing too low an available brain concentration of SCH-23390 after IP

administration, resulting in inadequate blockade of dopamine D1-like receptor signaling. This would also explain why impairments in ethanol-induced CPP were found only in the higher SCH-23390 dose groups.

Our results are generally in agreement with findings from self-administration studies that suggest dopamine D1-like receptors are involved in ethanol intake and reward in rats (e.g., Dyr et al. 1993; El-Ghundi et al. 1998). However, a definitive role still remains unclear for D1-like receptor involvement in ethanol drinking procedures, as other studies have shown no decrease in ethanol intake after D1-like receptor antagonism by SCH-23390 (Linseman 1990; Silvestre et al. 1996). Notably, our results are consistent with recent work in mice showing that systemic blockade of D1-like receptors using SCH-39166 impairs ethanol-induced CPP acquisition (Bahi and Dreyer 2012) and further extends those findings to an additional mouse strain and antagonist drug. Recent work using site-specific D1-like receptor manipulations suggests the systemic effect of SCH-23390 might be due to its effects on D1-like receptors in the NAc, as focal infusions of this antagonist into NAc reduced acquisition of ethanol-induced CPP (Young et al. 2013). Our finding that SCH-23390 did not significantly impact the development of LiCl-induced CPA acquisition suggests that dopamine D1-like receptors do not mediate aversive motivational learning in our procedure. These findings are in contrast to those of Risinger et al. (1999) who found that SCH-23390 impaired ethanol-induced CTA, thereby providing an example of SCH-23390 producing an effect on associative learning in a procedure using ethanol. Our lack of a similar finding may be due to our use of a procedure that involves drug-induced contextual learning and suggests that SCH-23390 may impact ethanol-induced learning in other procedures. However, we feel that the outcomes of the current experiments support our conclusion that the impairments seen in the acquisition of ethanol-induced CPP in experiment 2 are likely due to dopamine D1-like receptor modulation of ethanol's primary rewarding effect.

The lack of effect of dopamine D2-like receptor blockade by raclopride on ethanol-induced CPP (experiment 1) suggests this subfamily of DA receptors plays little to no role in ethanol reward when assessed within the parameters of our protocol. This is consistent with previous findings from our laboratory (Risinger et al. 1992), which showed no effect of haloperidol on ethanol-induced CPP acquisition after four CS+ (ethanol) exposures. Despite enhancing the sensitivity of our measure by testing after two CS+ (ethanol) exposures, we found that dopamine D2-like receptor blockade did not reduce ethanol-induced CPP acquisition. Although these findings agree with studies showing that dopamine D2-like receptors play only a minor role in ethanol reward (e.g., Cunningham et al. 1992; Risinger et al. 1992), they oppose others that have shown reduced ethanol-induced CPP in D2 receptor-deficient mice (Cunningham et al. 2000) and a robust positive correlation between expression of the dopamine D2 receptor gene (*Drd2*) and ethanol-induced CPP magnitude (Hitzemann et al. 2003).

Similar to the locomotor effects found with high doses of SCH-23390, D2-like receptor blockade by raclopride produced robust reductions in ethanol-stimulated activity. These dose-dependent reductions in activity were consistent across all four ethanol-conditioning trials in experiment 1. Despite these significant decreases in ethanol-stimulated activity,

animals given raclopride developed a significant place preference. This finding suggests that ethanol-induced CPP is independent of ethanol-induced locomotor stimulation. These results are consistent with previous studies that have consistently shown no association between ethanol-stimulated locomotor activation and ethanol reward (e.g., Chester and Cunningham 1999; Risinger et al. 1994, 1992).

Mixed results from studies on ethanol reward using self-administration procedures may be due to the difficulty of using such procedures to distinguish between the primary reinforcing effect of ethanol and the conditioned motivational or conditioned reinforcing effects of cues previously associated with ethanol's effects. Considering the training procedures that are required to induce ethanol self-administration and the use of ethanol intake as the primary dependent variable, it is difficult to separate acquisition from maintenance and other phases of intake. Moreover, pharmacological manipulations that suppress motor behavior can greatly impair performance on tasks that require operant responding, such as in self-administration procedures. By using CPP, we are able to minimize these potential confounds and interpretation issues that arise as a result of performance deficits during testing, as animals are tested in a drug-free state. The present study showed that there were no differences in activity levels at the time of testing except on the second test of experiment 4 in the 0.3-mg/kg SCH-23390 dose group. Despite this small difference in activity, we found no difference in CPP among groups. Our CPP procedure also enabled us to restrict our pharmacological manipulations to the acquisition phase of place conditioning thereby allowing us to more precisely examine the effects of DA receptor antagonism on unconditioned ethanol reward.

Another possibility for discrepancies between self-administration and CPP studies is the use of different rodent species (i.e., rats versus mice) and different strains. Studies examining receptor density among mouse strains have shown that differences exist by strain not just in the density of dopamine D1- and D2-like receptors, but in the distribution of these receptors throughout the brain (Kanes et al. 1993; Qian et al. 1992). Hence, differential responding to antagonism of DA receptor subfamilies may be a function of natural variations in DA receptor signaling among murine strains. This also suggests the possibility that our results may be explained by differences in the density of D1- and D2-like receptors in brain regions involved in ethanol reward. Though several studies have examined differences in density of DA receptors among murine strains, they do not directly compare the regional differences in density of DA receptor subtypes within strains (e.g., Ng et al. 1994; Michaluk et al. 1982). Given the lack of information on differential distribution of D1- and D2-like receptors within the DBA/2J strain and the fact that increased receptor density does not necessarily confer increased function, we are hesitant to more seriously consider this as an alternative interpretation of our findings. Additionally, the sensitivity of CPP to D1-like receptor antagonists appears consistent across mouse strains as our findings in DBA/2J mice are similar to those of Bahi and Dreyer (2012), where C57BL/6 mice were used.

In these studies, we did not evaluate the effects of DA subfamily selective antagonism on the expression of ethanol-induced CPP. Therefore, it is possible that D1- and D2-like receptors are also involved in the conditioned effects of cues associated with past ethanol exposure. However, this seems unlikely given previous studies showing that the expression

of ethanol-induced CPP is not attenuated by SCH-23390, raclopride, U99194A (Dickinson et al. 2003) or haloperidol (Cunningham et al. 1992). Such evidence suggests that although initially important for the unconditioned or primary rewarding effect of ethanol, the role of DA receptor activation becomes less critical once the context-ethanol association has been established.

Summary and Conclusions

Taken together, results from our experiments support the oft-reported dichotomous role of dopamine D1-like and D2-like receptors in modulating ethanol reward. Though behavioral studies have provided mixed results, by and large they demonstrate that dopamine D1-like receptors are importantly involved in the unconditioned and conditioned rewarding effects of abused drugs (Beninger and Miller 1998). Our finding that systemic antagonism of dopamine D1-like receptors impairs the development of ethanol-induced CPP is consistent with this observation and likely indicates this DA receptor subfamily modulates ethanol reward during initial exposures.

In contrast, self-administration procedures have shown that dopamine D2-like receptor blockade produces non-specific effects on intake and consumption, as shown by general decreases in intake and responding for food and water as well as blockade of reinstatement of natural reward seeking (e.g., Chausmer and Ettenberg 1997; Duarte et al. 2003; Silvestre et al. 1996). Other experiments suggest a greater role for dopamine D2-like receptors in information processing of drug-associated stimuli and goal-directed behavior (Samson and Chappell 2004). These findings are generally consistent with our lack of ethanol-induced CPP attenuation by dopamine D2-like receptor blockade and suggest that this subfamily of receptors may play a lesser role in the unconditioned rewarding effects of ethanol. Nevertheless, D2-like receptors have frequently been implicated in reward produced by other abused drugs such as cocaine (Volkow et al. 1993).

Whereas CPP expression studies are useful for assessing interventions that may reduce craving and the risk of relapse, acquisition studies are valuable in evaluating interventions that may reduce the risk of continued use after an initial relapse by reducing the rewarding effects of a drug. While we are not proposing the use of DA antagonist drugs to treat alcoholism given the adverse side effects produced by these medications, it may be possible to manipulate DA transmission through other means such as GABAergic, glutamatergic, opioidergic, and cholinergic receptor systems or other downstream signaling cascades. Future studies will be needed to determine whether these mechanisms are effective in altering DA transmission or signaling and if they are sufficient to impair alcohol reward/abuse.

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Figure 1.

Mean time on the grid floor (s/min + SEM) after two trials (left panel) and after four trials (right panel) for each raclopride dose group in experiment 1. Black bars represent animals that received ethanol on the grid floor (Grid+), whereas gray bars represent animals that received ethanol on the hole floor (Grid-) (n = 12/conditioning subgroup).

Figure 2.

Mean time on the grid floor (s/min + SEM) after two trials (left panel) and after four trials (right panel) for each SCH-23390 dose group in experiment 2. Black bars represent animals that received ethanol on the grid floor (Grid+), whereas gray bars represent animals that received ethanol on the hole floor (Grid-) (n = 12/conditioning subgroup). n.s. No significant difference between Grid+ and Grid-

Figure 3.

Mean time on the grid floor (s/min + SEM) after two trials (left panel) and after four trials (right panel) for SCH-23390 (n = 12/conditioning subgroup) and saline groups (n = 6/conditioning subgroup) in experiment 3. Black bars represent animals that received SCH-23390 on the grid floor (Grid+), whereas gray bars represent animals that received SCH-23390 on the hole floor (Grid-)

Figure 4.

Mean time on the grid floor (s/min + SEM) after two trials (left panel) and after four trials (right panel) for each SCH-23390 dose group in experiment 4. Black bars represent animals that received LiCl on the grid floor (Grid+), whereas gray bars represent animals that received LiCl on the hole floor (Grid-) (n = 12/conditioning subgroup).

Table 1Mean Activity Counts per Minute ($\pm$ SEM) on Preference Test Days

	Dose Group	Test after 2 trials	Test after 4 trials
<i>Experiment 1</i>			
<i>Raclopride EtOH CPP</i>	Saline	33.1 $\pm$ 1.5	33.5 $\pm$ 1.6
	0.3 mg/kg	34.8 $\pm$ 1.4	31.7 $\pm$ 1.5
	0.6 mg/kg	37.0 $\pm$ 1.4	36.2 $\pm$ 1.6
	1.2 mg/kg	35.5 $\pm$ 1.4	33.5 $\pm$ 1.5
<i>Experiment 2</i>			
<i>SCH-23390 EtOH CPP</i>	Saline	34.3 $\pm$ 1.0	33.1 $\pm$ 1.3
	0.01 mg/kg	33.6 $\pm$ 1.7	34.9 $\pm$ 1.4
	0.03 mg/kg	34.5 $\pm$ 1.5	33.9 $\pm$ 2.0
	0.1 mg/kg	36.0 $\pm$ 1.1	33.7 $\pm$ 1.5
	0.2 mg/kg	34.3 $\pm$ 1.6	31.7 $\pm$ 1.8
	0.3 mg/kg	35.3 $\pm$ 1.9	34.2 $\pm$ 1.9
<i>Experiment 3</i>			
<i>SCH-23390 alone</i>	Saline	34.9 $\pm$ 2.2	31.6 $\pm$ 1.2
	0.3 mg/kg	38.9 $\pm$ 1.2	35.9 $\pm$ 1.4
<i>Experiment 4</i>			
<i>SCH-23390 LiCl CPA</i>	Saline	32.9 $\pm$ 1.7	29.9 $\pm$ 1.0
	0.1 mg/kg	35.9 $\pm$ 1.2	33.2 $\pm$ 1.2
	0.2 mg/kg	36.4 $\pm$ 1.2	34.3 $\pm$ 1.4
	0.3 mg/kg	36.3 $\pm$ 1.2	35.0 $\pm$ 1.3 ^a
Dose Group F(3,88) = 3.2*			

* p<0.05 (p-values for all group comparisons are Bonferroni-corrected)

^a Differs from Saline Group, p<0.05.

Table 2Mean Activity Counts per Minute ($\pm$ SEM) during Conditioning Trials

	Dose Group	CS+ (Ethanol or LiCl) Trials	CS- (Saline) Trials
<i>Experiment 1</i>			
<i>Raclopride EtOH CPP</i>	Saline	200.2 $\pm$ 3.8	57.9 $\pm$ 2.1
	0.3 mg/kg	97.1 $\pm$ 4.5 ^a	52.0 $\pm$ 2.1
	0.6 mg/kg	66.0 $\pm$ 5.2 ^{ab}	57.7 $\pm$ 2.7
	1.2 mg/kg	45.0 $\pm$ 3.2 ^{abc}	57.6 $\pm$ 1.9
<i>Experiment 2</i>			
<i>SCH-23390 EtOH CPP</i>	Saline	192.9 $\pm$ 3.6	58.6 $\pm$ 1.8
	0.01 mg/kg	184.4 $\pm$ 5.3	53.5 $\pm$ 1.8
	0.03 mg/kg	185.8 $\pm$ 5.2	57.0 $\pm$ 2.4
	0.1 mg/kg	134.4 $\pm$ 4.5 ^a	57.4 $\pm$ 1.6
	0.2 mg/kg	92.3 $\pm$ 6.7 ^{ad}	55.7 $\pm$ 2.2
	0.3 mg/kg	75.0 $\pm$ 5.0 ^{ad}	55.7 $\pm$ 3.2
<i>Experiment 3</i>			
<i>SCH-23390 alone</i>	Saline	52.1 $\pm$ 2.1	48.5 $\pm$ 2.5
	0.3 mg/kg	15.0 $\pm$ 1.1 ^a	58.4 $\pm$ 1.5 ^a
<i>Experiment 4</i>			
<i>SCH-23390 LiCl CPA</i>	Saline	18.1 $\pm$ 1.2	31.6 $\pm$ 1.3
	0.1 mg/kg	13.2 $\pm$ 0.7 ^a	33.3 $\pm$ 1.5
	0.2 mg/kg	8.3 $\pm$ 0.9 ^{ad}	34.8 $\pm$ 1.1
	0.3 mg/kg	7.8 $\pm$ 0.9 ^{ad}	33.0 $\pm$ 1.3

^aDiffers from Saline Group, $p < 0.01$ ^bDiffers from 0.3 mg/kg Raclopride Dose Group, $p < 0.001$ ^cDiffers from 0.6 mg/kg Raclopride Dose Group, $p < 0.01$ ^dDiffers from 0.1 mg/kg SCH-23390 Dose Group, $p < 0.01$