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Role of nucleus accumbens dopamine receptor subtypes in the learning and expression of alcohol-seeking behavior

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Abstract

These studies examined the roles of dopamine D1- and D2-like receptors within the nucleus accumbens (Acb) in the acquisition and expression of ethanol-induced (2 g/kg) conditioned place preference (CPP) in adult male DBA/2J mice. Bilateral intra-Acb infusions of the D1-like dopamine receptor antagonist SCH23390 (0.05, 0.5 µg/side) or the D2-like dopamine receptor antagonist raclopride (0.5–5.0 µg/side) were administered 30 min before each ethanol conditioning trial (acquisition studies) or before preference tests (expression studies). CPP was conditioned to tactile cues using an unbiased apparatus and procedure. Intra-Acb infusion of SCH23390 prevented CPP acquisition, whereas intra-Acb infusion of raclopride did not. Intra-Acb infusion of both antagonists, however, dose-dependently reduced ethanol-stimulated locomotor activity during conditioning. In contrast, intra-Acb antagonist infusion had no effect on ethanol CPP expression, suggesting that dopamine's role in the Acb is limited to neurobiological processes engaged during the learning of the relationship between contextual cues and ethanol reward. Control experiments showed that intra-Acb injection of SCH23390 alone produced no place conditioning and did not interfere with the acquisition of conditioned place aversion induced by lithium chloride, suggesting that the antagonist's effect on ethanol CPP was not due to a more general detrimental effect on associative learning. Overall, these data suggest that D1-like (but not D2-like) dopamine Acb receptors play an important role in the learning of context-ethanol associations, either by modulating the magnitude of ethanol reward or the rate of learning about ethanol reward.

Keywords

ethanol; reward; conditioned place preference; Pavlovian conditioning; locomotor activity; inbred mice (DBA/2J)

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Conflict of Interest

The authors declare no conflict of interest.

1. Introduction¹

The mesolimbic dopamine system is thought to influence both learning and the rewarding effects of abused drugs (Berridge, 2012; Hyman, Malenka & Nestler, 2006). The dopamine projection from the ventral tegmental area (VTA) to the nucleus accumbens (Acb) is believed to be of particular importance and many studies have shown that interference with dopamine signaling via that projection (e.g., lesions, antagonists) can influence the learning or performance of behaviors based on drug reward (Koob & LeMoal, 2006). One Pavlovian conditioning procedure, conditioned place preference (CPP), has become an especially popular tool for evaluating the role of this pathway in learned drug-seeking behavior. CPP offers a convenient way to examine effects of treatments applied during the acquisition of context-drug associations separately from effects of treatments applied later during the expression of context-approach behaviors based on those associations (Cunningham, Groblewski & Voorhees, 2011). Thus, it is possible to distinguish between treatments that alter the unconditioned effect of an abused drug (or learning based on that effect) and treatments that alter the memory or performance of behaviors induced by previous context-drug learning. This feature of CPP is a particularly important consideration when evaluating dopamine system manipulations that have significant effects on motor behavior.

Over the last two decades, several studies have used the CPP procedure to determine the specific roles played by Acb dopamine D1-like vs. D2-like receptor subtypes during the acquisition or expression of this form of drug-seeking behavior (see reviews by Tzschentke, 1998, 2007). Using microinjections of selective dopamine receptor antagonists directly into Acb, these studies have shown that one or both receptor subtypes are sometimes involved in either the acquisition or expression of drug-induced CPP, although the effects vary depending on the type of conditioning drug, the target subregion of Acb (i.e., core vs. shell) and species. For example, in separate studies from the same laboratory, D1-receptor blockade in Acb shell interfered with the acquisition of both morphine- and nicotine-induced CPP in rats, but D2-receptor blockade reduced only morphine-induced CPP (Fenu, Spina, Rivas, Longoni & DiChiara, 2006; Spina, Fenu, Longoni, Rivas & DiChiara, 2006). In these same studies, D1-receptor blockade in Acb core had no effect on CPP acquisition with either drug. However, D2-receptor blockade in Acb core interfered with the acquisition of morphine- but not nicotine-induced CPP. In studies examining CPP expression, one early study found that either D1- or D2-receptor blockade in Acb reduced expression of amphetamine-induced CPP in rats (Hiroi & White, 1991). However, later studies showed that only D1-receptor blockade in Acb was able to interfere with amphetamine-induced CPP in prairie voles (Liu, Young, Curtis, Aragona & Wang, 2011) or with morphine-induced CPP in rats (Shippenberg, Bals-Kubik & Herz, 1993); subregions of Acb were not characterized in these studies. Overall, these studies underscore the importance of delineating the effects of Acb dopamine receptor blockade across a wide range of conditioning drugs and species before drawing any general conclusions about the role that a specific receptor subtype might play in the learning or performance of drug-seeking behavior.

Effects of selective Acb dopamine receptor blockade have previously been studied in only a few recent CPP studies in mice. In one study, intra-Acb infusion of a siRNA-expressing lentiviral vector that knocked down D1 receptors blocked acquisition, but had no effect on expression of ethanol-induced CPP in C57BL/6 mice; no manipulation targeting D2 receptors was tested (Bahi & Dreyer, 2012). In another study, intra-Acb injection of either a D1- or a D2-receptor antagonist dose-dependently reduced the acquisition of

¹Abbreviations: Acb, nucleus accumbens; CPP, conditioned place preference; LiCl, lithium chloride; siRNA, small interfering RNA; VTA, ventral tegmental area

methamphetamine-induced CPP in ddY mice; effects on CPP expression were not tested (Kurokawa, Mizuno & Ohkuma, 2012). Thus, like all the rat studies cited above, both mouse studies supported a role for Acb D1 receptors in CPP acquisition. Although one study also suggested a possible role for Acb D2 receptors during acquisition (see also Naughton, Thirtamara-Rajamani, Wang, During & Gu, 2012), the generality of this effect across drugs and during expression remains to be tested in mice.

The present studies focused on effects of D1- or D2-receptor blockade during the acquisition or expression of ethanol-induced CPP in mice. Although the smaller brain of the mouse can complicate differentiation of shell vs. core effects, mice were used because they are generally more sensitive than rats to ethanol's rewarding effect in the CPP procedure (Cunningham, Niehus & Noble, 1993; Fidler, Bakner & Cunningham, 2004; Tzschentke, 1998, 2007). Experiments 1 and 2 examined the effects of intra-Acb injection of the D1-receptor antagonist SCH23390 or the D2-receptor antagonist raclopride, respectively, on the acquisition of ethanol-induced CPP in DBA/2J mice, an inbred strain that is known to be especially sensitive to ethanol reward in this procedure (Cunningham, Niehus, Malott, & Prather, 1992). On the basis of previous studies we predicted that Acb D1-receptor blockade would interfere with CPP acquisition, but we were uncertain about effects of Acb D2-receptor blockade since previous CPP studies have shown both positive (Fenu et al., 2006; Liao, 2008; Kurokawa et al., 2012; Taslimi, Arezoomandan, Omranifard, Ghalandari-Shamami, Riahi, Vafaei, Rashidy-Pour & Haghparast, 2012) and negative (Baker, Khroyan, O'Dell, Fuchs & Neisewander, 1996; Liu et al., 2011; Spina et al., 2006) effects. Experiments 3 and 4 examined the effects of D1- and D2-receptor blockade on the expression of ethanol-induced CPP. Although previous rat studies offer a conflicting pattern of findings, with some studies showing effects on expression (Hiroi & White, 1991; Shippenberg et al., 1993) while others do not (Fenu et al., 2006; Spina et al., 2006), we expected that selective Acb dopamine receptor blockade would have no effect since a previous study had shown that non-selective Acb dopamine receptor blockade did not alter expression of ethanol-induced CPP in DBA/2J mice (Gremel & Cunningham, 2009).

2. Methods and Materials

2.1. Subjects

DBA/2J adult male mice (n = 461) arrived from the Jackson Laboratory (Bar Harbor, ME) at 6 weeks of age and surgeries were performed at 7–8 weeks. Mice were initially housed in groups of four in a colony room maintained at 20–24°C on a normal 12-hr light dark cycle (lights on at 0700). After surgery, mice were housed two to a cage to minimize cannula damage. Food and water were freely available in the home cage. These procedures were conducted in accordance with The National Institute of Health (NIH) "Principles of Laboratory Animal Care" and the Oregon Health & Science University IACUC approved the protocol.

2.2. Surgery

Isoflurane (2–5% in air, flow rate 1L/min) was used to anesthetize mice throughout the cannulation procedure. Bilateral guide cannulae (10 mm, 25 gauge) aimed at the nucleus accumbens (Acb) core (coordinates from Bregma: AP +1.40; ML $\pm$ 1.26; DV –4.20 mm) were implanted using stereotaxic guidance (Model No. 1900; Kopf Instruments, Tujunga, CA). Cannulae were positioned 2.0 mm above the Acb and held in place with stainless-steel screws and carboxylate cement (Durelon™, 3M, St Paul, MN). Stainless-steel stylets (32-ga) were placed into cannulae to maintain patency. The NSAID analgesic Meloxicam (0.2 mg/kg SC) was used to reduce post-operative pain. Procedures began after 3–7 days of recovery (counterbalanced within infusion groups).

2.3. Apparatus

Twelve identical place-conditioning chambers (30 × 15 × 15 cm), constructed with clear acrylic walls and aluminum end panels, were used to assess CPP. Each box was enclosed in a sound- and light-attenuating chamber. Infrared emitters and detectors (mounted 2.2 cm above the floor at 5-cm intervals) were used to measure general activity (beam crosses) and time spent on each side of the box. Two distinct interchangeable floor halves were used as tactile conditioned stimuli (CSs). The grid floor consisted of stainless steel rods (2.3 mm diameter) mounted 6.4 mm apart in acrylic rails. The hole floor was made from perforated 16-gauge stainless steel with 6.4-mm round holes on 9.5-mm staggered centers. Matching floor halves were used during conditioning trials whereas different floor halves were presented on each side during the test session. These two textures have repeatedly been shown to produce approximately equal preference for each tactile floor cue, allowing for unbiased detection of CPP (Cunningham, Ferree & Howard, 2003). A more detailed description of the apparatus and procedure has been published (Cunningham, Gremel & Groblewski, 2006).

2.4. General Procedure

All experiments included 4 phases: piercing (1 session), habituation (1 session), conditioning (8 sessions) and testing (1 session). The acquisition experiments (Exp. 1–2) were run on consecutive days, whereas the expression experiments (Exp. 3–4) were conducted on consecutive days with a 48-hr break between the first four and last four conditioning sessions and a 72-hr break before the preference test.

2.4.1. Piercing—A 12-mm stylet was lowered into the injection site 24–48 hrs before the first intracranial infusion in all experiments to minimize direct behavioral effects of initial injector lowering (Gremel & Cunningham, 2009). This was done 24 hrs before the habituation session in the acquisition experiments (Exp. 1–2) and 24 hrs before the preference test in the expression experiments (Exp. 3–4).

2.4.2. Habituation—This session was designed to reduce the novelty and stress associated with handling, injection, and exposure to the apparatus. A sham infusion procedure (described below) was also implemented during habituation in acquisition experiments to familiarize mice with the microinjection procedure and to reduce nonspecific interference with CPP learning. All mice then received an IP injection of saline 30 min after the sham infusion and were placed on a smooth paper floor for 5 min. Since previous research suggested that pre-exposure to sham infusions is not necessary in expression studies (Gremel & Cunningham, 2009), mice in Exp. 3–4 received only an IP injection of saline immediately before 5 min placement on a smooth paper floor.

2.4.3. Conditioning—Mice were randomly assigned to one of several infusion groups described separately for each experiment below (Experimental Design). Within each infusion group, mice were then randomly assigned to one of two conditioning subgroups, Grid+ or Grid–, and exposed to an unbiased one-compartment CPP procedure (Cunningham et al., 2003, 2006). Mice in the Grid+ conditioning subgroups received ethanol paired with the grid floor (CS+) and saline paired with the hole floor (CS–). Conversely, mice in the Grid– conditioning subgroups received ethanol paired with the hole floor (CS+) and saline paired with the grid floor (CS–). Mice in Exp. 1A, 2, 3 and 4 received an injection of either saline (CS– trials) or ethanol (CS+ trials) immediately before placement in the apparatus. Mice in Exp. 1B received saline immediately before both types of trials whereas mice in Exp. 1C received lithium chloride (LiCl) instead of ethanol before CS+ trials. In all experiments, mice received four conditioning trials of each type on alternating days across an 8-day period (counterbalanced order within groups). Based on previous studies, the

conditioning trial duration was 5-min (Cunningham & Prather, 1992) for all but the LiCl study (Exp. 1C), which used a 30-min trial duration (Risinger & Cunningham, 2000).

2.4.4. Place preference test—In all experiments, mice received a saline injection (12.5 ml/kg) immediately before being placed into the conditioning chambers for 30 min. The floor was half hole and half grid and the left-right position of the floors was counterbalanced within groups.

2.5. Experimental Design

Experiments 1 and 2 examined the effects of intra-Acb infusion of dopamine receptor antagonists on acquisition of CPP. Experiments 3 and 4 examined the effects of antagonist infusion on CPP expression.

2.5.1. Experiments 1A–C: Dopamine D1-like receptor antagonist effects on

CPP acquisition—Mice ($n = 124$) in the first study (Experiment 1A) were randomly assigned to receive infusions of the selective D1 receptor antagonist SCH23390 (0.05 μg or 0.5 $\mu\text{g}/0.1\mu\text{l}$ on each side) or an equal volume of the vehicle (saline) before CS+ trials. On the basis of those findings, we conducted two follow-up experiments. Experiment 1B examined whether SCH23390 has rewarding or aversive effects when given alone. All mice ($n = 29$) received intra-Acb infusions of SCH23390 (0.5 $\mu\text{g}/0.1\mu\text{l}$) before CS+ trials, but mice received IP injections of saline instead of ethanol. Thus, the development of preference or aversion to CS+ would only reflect conditioning induced by the antagonist itself. To determine whether intra-Acb SCH23390 disrupted CPP by more generally interfering with associative learning (i.e., instead of interfering with ethanol reward), we conducted another study to examine the effect of this antagonist on place conditioning produced by a different drug, LiCl. Thus, mice ($n = 34$) in Experiment 1C were randomly assigned to receive SCH23390 (0 [saline], 0.05 or 0.5 $\mu\text{g}/0.1\mu\text{l}$) before LiCl (CS+) trials to assess the effects of SCH23390 on LiCl-induced conditioned place aversion (CPA). In all of these experiments, a sham infusion (described below) was given 30 min before each CS– trial.

2.5.2 Experiment 2: Dopamine D2-like receptor antagonist effects on CPP

acquisition—Experiment 2 examined the effects of the selective D2 antagonist raclopride on acquisition of ethanol-induced CPP. Mice ($n = 91$) were randomly assigned to one of four raclopride dose groups (0 [saline], 0.5, 2.0, or 5.0 $\mu\text{g}/0.1\mu\text{l}$ on each side). The procedure was otherwise identical to that used in Experiment 1A.

2.5.3. Experiments 3–4: Dopamine receptor antagonist effects on CPP

expression—These studies examined the effects of intra-Acb SCH23390 or intra-Acb raclopride microinfusions on expression of ethanol-induced CPP. Mice in Exp. 3 ($n = 62$) were randomly assigned to one of three groups that received SCH23390 before preference testing (0 [saline], 0.05 or 0.5 $\mu\text{g}/0.1\mu\text{l}$ on each side). Mice in Exp. 4 ($n = 60$) were randomly assigned to one of four groups (0 [saline], 0.5, 2.0, or 5.0 $\mu\text{g}/0.1\mu\text{l}$ on each side) that received raclopride before preference testing.

2.6. Drugs

Ethanol (20% v/v, saline vehicle) was prepared from a 95% stock solution and administered IP at a dose of 2 g/kg (12.5 ml/kg). These parameters were chosen on the basis of many studies reporting robust CPP in DBA/2J mice at this dose and concentration (e.g., Cunningham et al., 2003). LiCl was dissolved in sterile water and administered IP at a dose of 3 mEq/kg (20 ml/kg), which has previously been shown to produce reliable LiCl-induced CPA in DBA/2J mice (Risinger & Cunningham, 2000).

The dopamine D1 receptor antagonist SCH23390 hydrochloride (Tocris Bioscience, Ellisville, MO) and the D2 receptor antagonist raclopride (Sigma, Buchs, Switzerland) were dissolved in sterile saline and infused bilaterally in a volume of 0.1 μ l/side. Doses for each drug were chosen based on the literature (Couppis & Kennedy, 2008; Krzyzosiak, Szyszka-Niagolov, Wietrzyk, Gobaille, Muramatsu & Krezel, 2010; Samson & Chappell, 2003), pilot work and results from initial cohorts that tested effects of a 0.5 μ g/side injection of each antagonist (vs. vehicle) on ethanol CPP acquisition. For the antagonist that produced a positive effect (SCH23390), after determining that the antagonist had no motivational effect of its own, our goal was to identify at least one other (lower) dose that altered CPP but had little or no impact on ethanol-stimulated activity. For the antagonist that had no effect (raclopride), we tested two higher doses and stopped when we reached a dose that produced about 50% suppression of ethanol-stimulated activity without effect on CPP acquisition. The doses used for the expression studies were the same as those used in the acquisition study for each antagonist.

2.7. Microinfusion Procedure

Microinjections consisted of gently scruffing the animal, removing the stylet, and inserting a 12 mm injector (32-ga) into each cannula. Simultaneous infusions were delivered by a microinfusion pump (Model A-74900-10; Cole Parmer, Vernon Hills, IL) over 60 sec; injectors were kept in place for an additional 30 sec to ensure diffusion. To minimize Acb damage, a 10-mm injector was inserted into each guide cannula for an equivalent period of time during sham infusions (acquisition studies); no fluid was infused.

A 30-min delay was inserted between microinfusions (or sham infusions) and subsequent place conditioning in the acquisition experiments (1–2) because pilot studies suggested that the handling required to give microinfusions interfered with CPP acquisition in vehicle controls at shorter delays. To match the time delay used in the acquisition experiments, we also used a 30-min delay in the expression experiments (3–4). Mice were always returned to the home cage rack during the 30-min delay.

2.8. Histological verification

Mice were euthanized within 24 hrs of testing and brains were removed for histological analysis. Brains were postfixed in 2% (w/v) paraformaldehyde in isotonic sodium phosphate buffered saline (PBS) and then cryoprotected in 20% sucrose and 30% sucrose in PBS and 0.1% NaN₃ until saturation. A freezing cryostat (–20°C) was used to collect 40- μ m thick coronal sections of the entire infusion site. Slices were directly mounted onto slides and thionin stained.

Mice given microinfusions outside of the Acb core or the core/shell border were excluded from all analyses (n = 11). Data were also excluded in cases of procedural error (n = 4) or where the microinfusion site could not be determined (e.g., due to tissue damage during histology, lost headmounts, or infection; n = 46). Injector inclusion area criteria are shown in Figure 1A. Although our infusions were aimed primarily at the Acb core, we conservatively decided to not make a core vs. shell distinction given the small size and proximity of these structures in mice. An example photomicrograph of bilateral injection sites after four microinfusions is shown in Figure 1B. Out of the initial 461 mice, 400 mice were used in the final analyses; final group sizes are indicated in the table and figure captions.

3. Results

3.1. Preference tests

Time spent on the grid floor during the CPP test served as the primary dependent variable. Data were evaluated using two-way analysis of variance (ANOVA) using antagonist dose and conditioning subgroup as between-group factors. Post-hoc group comparisons were Bonferroni-corrected to control overall alpha level within each experiment; alpha was set at .05.

3.1.1. Experiment 1A: Intra-Acb D1 receptor antagonism interferes with acquisition of ethanol-induced CPP—Blocking D1 receptors in the Acb with SCH23390 during acquisition prevented development of a significant ethanol-induced conditioned place preference at both doses tested (Figure 2A). This observation was supported by a significant dose $\times$ conditioning subgroup interaction [$F(2, 118) = 6.4, p < .003$]. Pairwise comparisons between conditioning subgroups (Grid+ vs Grid-) revealed a significant CPP in saline control mice (Bonferroni-corrected $p < .0003$), but not in SCH23390-infused mice.

3.1.2. Experiment 1B: Intra-Acb D1 receptor antagonism does not produce place conditioning—There was no evidence of place conditioning in either direction when SCH23390 was given alone [$F(1,27) < 1, p > .6$], suggesting that intra-Acb SCH23390 is neither rewarding nor aversive at this dose (Figure 2B).

3.1.3. Experiment 1C: Intra-Acb D1 receptor antagonism does not affect acquisition of LiCl-induced CPA—As can be seen in Figure 2C, LiCl produced a significant CPA in all three groups [conditioning group main effect: $F(1,28) = 158.8, p < .0001$], but intra-Acb pretreatment infusions of SCH23390 on CS+ trials had no effect on CPA (as indicated by the absence of a significant main effect of SCH23390 dose or interaction [p 's $> .15$]). Thus, these data suggest that the antagonist blockade of CPP in Exp. 1A is specific to ethanol rather than a more general impairment of learning or memory.

3.1.4. Experiment 2: Intra-Acb D2 receptor antagonism does not affect acquisition of ethanol-induced CPP—Acquisition of ethanol CPP was not altered by intra-Acb raclopride treatment before CS+ trials (Figure 3) as shown by the development of significant CPP [conditioning subgroup main effect: $F(1,83) = 44.0, p < .0001$] that did not vary across raclopride dose groups [interaction: $F(3,83) < 1, p > .79$].

3.1.5. Experiments 3 and 4: Intra-Acb D1 or D2 receptor antagonism does not affect expression of ethanol-induced CPP—When given before the preference test, neither SCH23390 (Figure 4A) nor raclopride (Figure 4B) affected the expression of ethanol-induced CPP at any dose. Two-way (dose $\times$ conditioning subgroup) ANOVAs yielded only significant main effects of conditioning subgroup, confirming the development of place conditioning in all groups [Exp. 3: $F(1,56) = 131.1, p < .0001$; Exp. 4: $F(1,52) = 53.1, p < .0001$]. The interaction was not significant in either experiment [Exp. 3: $F(2,56) = 2.1, p > .12$; Exp. 4: $F(3,52) < 1, p > .47$].

3.2. Locomotor Activity

Group means and statistical comparisons for conditioning and test activity in all experiments are listed in Table 1. For ease of presentation, mean activity counts/min were averaged across the four CS+ (ethanol) and four CS- (saline) trials for all experiments. The means for CS+ trials in Experiment 1B reflect the activity reducing effects of intra-Acb SCH23390 when given alone, whereas the means for CS+ trials in Experiment 1C show the effects of

intra-Acb SCH23390 on LiCl-induced changes in activity. Conditioning activity data were analyzed with a mixed ANOVA using dose as a between group factor, and trial type (CS+ vs. CS-) as a within group factor. Activity during place preference tests was examined by one-way ANOVA using dose as the factor.

3.2.1. Acquisition experiments—Intra-Acb infusion of both SCH23390 (Exp. 1A) and raclopride (Exp. 2) dose-dependently reduced ethanol's locomotor activating effect during CS+ trials in the acquisition studies (Table 1). When given without ethanol, SCH23390 reduced activity relative to saline trials (Exp. 1B). Intra-Acb SCH23390 also dose dependently enhanced the suppressant effect of LiCl on activity (Exp. 1C). Finally, exposure to intra-Acb dopamine antagonists during conditioning trials had no residual effect on activity measured during later preference testing.

3.2.1. Expression experiments—Because dopamine antagonists were not administered before conditioning trials in the expression experiments (Exp. 3–4), conditioning trial activity was influenced only by the presence of ethanol (CS+ trials) or saline (CS- trials). As expected for DBA/2J mice (Cunningham et al. 1993), activity was higher on CS+ trials than on CS- trials (Table 1). During preference tests, pretreatment intra-Acb infusions of both antagonists dose-dependently reduced activity relative to vehicle infusions.

4. Discussion

4.1. Role of Acb dopamine receptor subtypes in ethanol CPP and ethanol-seeking behavior

These are the first studies to examine the effects of selective pharmacological blockade of Acb D1-like and D2-like receptors on the acquisition and expression of ethanol-induced CPP in mice. Our findings show that blockade of Acb D1-like (Exp. 1A) but not D2-like (Exp. 1B) receptors interferes with ethanol CPP acquisition, but that neither class of Acb dopamine receptors is involved in ethanol CPP expression (Exp. 3–4). The effect of Acb D1 receptor blockade cannot be attributed to direct aversive effects of the antagonist since intra-Acb injections SCH23390 without ethanol produced no place conditioning (Exp. 1B). Thus, these findings suggest that dopamine activation of Acb D1-like dopamine receptors is normally involved in the acquisition of this form of ethanol-seeking behavior.

The outcome of Exp. 1A complements and extends the recent finding that Acb D1 receptor knockdown interferes with ethanol CPP acquisition in C57BL/6 mice (Bahi & Dreyer, 2012) by showing a similar effect of pharmacological receptor blockade in a different inbred mouse strain (DBA/2J). The interfering effect of Acb D1 receptor blockade during acquisition is consistent with recent studies involving systemic D1 antagonist administration in both C57BL/6 (Bahi & Dreyer, 2012) and DBA/2J mice (Pina & Cunningham, submitted), implicating the Acb in the effect of systemic D1 receptor blockade on ethanol CPP acquisition. Exp. 2 also extends previous research by showing that Acb D2 receptor blockade has no effect on ethanol CPP acquisition, an observation consistent with previous studies that used systemic D2 antagonist administration (Risinger, Dickinson & Cunningham, 1992; Pina & Cunningham, submitted). Finally, the findings that blockade of neither Acb receptor subtype affected ethanol CPP expression (Exp. 3–4) are congruent with previous mouse studies showing no effect of intra-Acb blockade using a non-selective dopamine receptor antagonist (Gremel & Cunningham, 2009) and no effect of systemically administered selective dopamine receptor antagonists (Cunningham, Malott, Dickinson & Risinger, 1992; Dickinson, Lee, Rindal & Cunningham, 2003).

One possible limitation on conclusions about the greater sensitivity of ethanol CPP acquisition vs. expression to Acb dopamine receptor blockade is that the magnitude of CPP in vehicle controls was greater in our expression studies (Exp. 3–4) than in our acquisition

studies (Exp. 1–2), despite using identical conditioning parameters (compare the 0 groups in Figs. 2A and 3 with the 0 groups in Fig. 4). Thus, Acb D1-like receptor blockade might have had a greater effect on acquisition than on expression simply because CPP magnitude was in a lower part of the response scale (Grobowski, Bax & Cunningham, 2009). Based on preliminary studies that tested shorter time delays between the intracranial microinfusion and trial onset, we believe the reduced CPP seen in the acquisition study 0 groups reflects a time-dependent, nonspecific interfering effect of the microinfusion procedure on CPP learning. In contrast, the expression of CPP appears to be unaffected by the microinfusion procedure as suggested by a previous study that reported a similarly strong ethanol CPP whether or not mice received pre-test exposure to a handling procedure identical to that used for microinfusions (Bechtholt, Gremel & Cunningham, 2004). Since the basis for the handling-related interference with CPP acquisition is unknown, caution must be exercised when comparing effects of microinfusions during acquisition studies with effects during expression studies.

The most direct way to address the importance of CPP magnitude for detecting effects of Acb dopamine receptor blockade would be to vary the ethanol conditioning dose, using doses that are lower or higher than the 2 g/kg dose used here. Such studies would address the limitations imposed by testing only a single conditioning dose by determining whether the D1 antagonist would affect CPP expression when a lower ethanol dose is used to induce CPP in the same range as that seen in vehicle mice in the acquisition. Another approach would be to conduct the expression tests after fewer conditioning trials, which would be expected to produce a weaker CPP (Pina & Cunningham, submitted). However, given the nonspecific interfering effect of microinfusions on CPP acquisition and the fact that 2 g/kg is already near the asymptote of the CPP dose effect curve (Grobowski et al., 2008), it is not clear that a higher conditioning dose would produce an increase in CPP large enough to test whether the D1 antagonist effect on acquisition could be detected in the upper part of the response scale.

The effects of Acb dopamine D1 and D2 receptor antagonism in the CPP model of ethanol seeking can be contrasted with those from the most commonly studied animal model of ethanol seeking, operant oral self-administration (see review by Gonzales, 2004). Such studies have shown reductions in self-administration by rats after both Acb D1- and D2-receptor blockade (e.g., Hodge, Samson & Chappelle, 1997; Samson, Hodge, Tolliver & Haraguchi, 1993). Although effects of Acb D1-receptor antagonism on operant responding for ethanol might be viewed as consistent with Exp. 1A, the response-suppressing effect of Acb D2-receptor antagonism appears at odds with the null outcomes observed in Exp. 2 and 4. One difficulty in comparing these studies, however, is that the manipulations of Acb D1 or D2 receptors were made after self-administration was well established, not during the acquisition of self-administration. Thus, it is difficult to draw conclusions about effects on acquisition vs. expression. However, a recent study that explicitly examined Acb D1 receptor blockade during initial ethanol self-administration sessions found a dose-dependent reduction in responding during the appetitive interval before ethanol consumption (Doherty & Gonzales, 2012), an outcome analogous to our finding in Exp. 1A; effects of Acb D2 receptor blockade during acquisition of self-administration were not reported.

An early study showing that post-training 6-OHDA depletion of Acb dopamine (93%) did not reduce ethanol self-administration in rats suggested that dopamine signaling in the Acb was not required for maintaining an established self-administration response (Rassnick, Stinus & Koob, 1993). Although that outcome might be viewed as consistent with the lack of Acb dopamine antagonist effects in our CPP expression studies, recent antagonist studies have shown that Acb dopamine receptors are normally involved in the expression of ethanol seeking established by self-administration, even when ethanol reinforcement is not available

during testing. For example, in a self-administration procedure that allowed assessment of ethanol seeking in the absence of consumed ethanol, intra-Acb administration of the D2-like receptor antagonist raclopride significantly suppressed ethanol-seeking behavior (Czachowski, Chappell & Samson, 2001). In another self-administration study, context-induced reinstatement of ethanol responding after extinction was reduced by intra-Acb (either core or shell) administration of the D1-like receptor antagonist SCH23390 (Chaudhri, Sahuque & Janak, 2009). Overall, such findings suggest that both families of Acb dopamine receptor subtypes may be more involved in the expression of ethanol seeking established by response-outcome contingencies than in the expression of ethanol seeking established by stimulus-outcome contingencies.

4.2. Role of Acb dopamine receptor subtypes in basal activity and ethanol's locomotor stimulant effects

Although not their primary focus, the present studies also provide new information on the roles played by Acb dopamine D1- and D2-like receptors in locomotor behavior in mice. More specifically, blockade of both families of Acb dopamine receptor subtypes dose-dependently reduced activity on ethanol (CS+) trials during the acquisition studies and during testing in the expression studies (see Table 1), supporting the conclusion that both families of receptor subtypes within Acb are normally involved in basal activity and ethanol's locomotor activating effects. Our finding that Acb D1-like receptor blockade reduces the locomotor stimulant effect of ethanol is consistent with other recent studies showing similar effects of both pharmacological blockade of the Acb D1 receptor (Abraham, Quadros & Souza-Formigoni, 2011) and virally-induced knockdown of Acb D1 receptor mRNA (Bahi & Dreyer, 2012). Exp. 2 extends those studies by showing a role for Acb D2-like receptors in this behavior. Finally, our studies suggest that the Acb was likely an important site of action in previous studies reporting suppression of ethanol's locomotor stimulant effect in mice that received systemic administration of dopamine receptor antagonists (e.g., Shen, Crabbe & Phillips, 1995).

These effects of intra-Acb antagonists on locomotor activity raise more general questions about the relationships between antagonist effects on activity and their effects on the acquisition or expression of ethanol-induced CPP. For example, one might ask whether the effect of Acb D1-like receptor blockade on CPP acquisition is actually due to antagonist-induced activity suppression on CS+ conditioning trials (Exp. 1A). Perhaps the strongest argument against this interpretation is our finding that a similarly strong suppression of CS+ trial activity by raclopride had no impact on CPP (Exp. 2). The conclusion that activity level on CS+ trials is unrelated to CPP magnitude is also consistent with findings from a previous ethanol CPP study involving systemic antagonist administration (Risinger et al., 1992) and with studies showing no genetic correlation between ethanol-stimulated locomotor activation and ethanol-induced CPP (Cunningham, 1995; Risinger, Malott, Prather, Niehus & Cunningham, 1994).

Another important question is whether antagonist-induced suppression of basal activity during testing complicates interpretation of the expression studies (Exp. 3–4). Previous studies have shown an inverse relationship between basal activity and CPP expression (e.g., Cunningham, 1995; Gremel & Cunningham, 2007). Thus, mice with low basal activity would be expected to show higher CPP. However, despite seeing significant group differences in test activity in our expression studies (Table 1), there were no group differences in CPP. The Pearson correlation between test session activity and percent time on the ethanol paired floor in each of the dose groups was significant only in the vehicle treated group in Exp. 3 ($r = -.60$, $n = 19$, $p < .01$), although the direction of the relationship was negative in all other groups in both studies ($-.14 < r < -.37$). Overall, these data suggest

that antagonist-induced differences in test activity had relatively little impact on magnitude of CPP within the range of activity levels seen here.

4.3. Role of Acb dopamine receptor subtypes in drug reward, learning and the expression of drug-seeking behavior

Our findings that Acb D1- but not D2-receptor blockade interfered with the acquisition of ethanol CPP in mice are generally similar to those previously reported for CPP induced by cocaine (Baker, Fuchs, Specio, Khroyan & Neisewander, 1998; Baker et al., 1996) or nicotine (Spina et al., 2006) in rats and by amphetamine in prairie voles (Liu et al., 2011). In contrast, other studies have reported that blockade of both families of dopamine receptor subtypes in Acb interfered with acquisition of CPP induced by morphine (Fenu et al., 2006), amphetamine (Liao, 2008) or intra-VTA orexin A (Taslimi et al., 2012) in rats and by methamphetamine in mice (Kurokawa et al., 2012). Thus, the overall pattern of findings suggests that the role of Acb D2-like dopamine receptors during CPP acquisition varies depending on the conditioning drug and species. In contrast, the consistent interfering effect of Acb D1-receptor blockade on CPP acquisition across many different abused drugs in three species raises the possibility that Acb D1-like dopamine receptors are universally involved in mediating the rewarding effects of abused drugs or in the learning of associations based on drug reward.

Our finding that Acb D1-receptor blockade does not interfere with the acquisition of CPP induced by LiCl (Exp. 1C) argues against the idea that these receptors are involved in all forms of drug-induced associative learning. However, it does not eliminate the possibility that Acb D1 receptors are more generally involved in reward-based (appetitive) associative learning. One difficulty in comparing the outcomes of Exp. 1A vs. 1C is that the context-LiCl association might have been stronger than the context-ethanol association, making it more resistant to interference by Acb D1 receptor blockade. Future studies could address this issue by examining effects of Acb D1 receptor blockade on CPA induced by lower doses of LiCl.

Our findings that expression of ethanol CPP was not altered by intra-Acb blockade of either family of dopamine receptor subtypes (Exp. 3–4) are similar to those reported for CPP induced by morphine (Fenu et al., 2006) and nicotine (Spina et al., 2006) in rats. However, these results are at odds with other reports indicating that blockade of both Acb D1- and D2-like receptors reduced expression of amphetamine CPP in rats (Hiroi & White, 1991) or that blockade of Acb D1- but not D2-like receptors reduced expression of morphine CPP in rats (Shippenberg et al., 1993). Given the many differences across these studies (species, rat strain, drugs, drug doses, apparatus, conditioning parameters, etc.), it is difficult to reconcile these disparate results. Suffice it to say that the literature does not yet provide a clear and consistent pattern of findings related to the roles that Acb D1-like and D2-like receptors play in the expression of drug-induced CPP, encouraging additional research.

4.4. Neural circuits underlying ethanol-induced CPP

Previous studies in our lab have focused primarily on the brain structures and neurotransmitter systems involved in the expression of ethanol-induced CPP. Using site-specific infusions of various agonists or antagonists, these studies have implicated opioid and GABA_B receptors in the VTA (Bechtholt & Cunningham, 2005), opioid receptors in the anterior cingulate cortex (Gremel, Young & Cunningham, 2011), dopamine receptors in the basolateral amygdala and NMDA receptors in the Acb (Gremel & Cunningham, 2009) in the expression of ethanol CPP in mice. In contrast, these studies have shown no effects on CPP expression of antagonizing Acb opioid (Bechtholt & Cunningham, 2005), Acb dopamine or central nucleus of the amygdala dopamine (Gremel & Cunningham, 2009) receptors.

The working hypothesis for our expression studies has been that these structures and transmitter systems are involved either in the retrieval of the context-ethanol association or in the conditioned motivational state activated by retrieval of that association. One possibility, suggested by studies showing that systemic administration of the non-selective opioid antagonist naloxone facilitated extinction of ethanol CPP in mice (Cunningham, Dickinson & Okorn, 1995; Cunningham, Henderson & Bormann, 1998), is that CPP expression is normally maintained by conditioned release of endogenous opioids that bind to receptors in the VTA and anterior cingulate cortex. Opioid binding within VTA would be expected to increase dopamine release in several terminal areas, including the Acb, amygdala and prefrontal cortex (Fields, Hjelmstad, Margolis & Nicola, 2007). Our studies suggest that dopaminergic signaling in the basolateral amygdala plays a more important role in ethanol CPP expression than dopaminergic signaling in the Acb (Gremel & Cunningham, 2009). Furthermore, we speculate that dopamine release in basolateral amygdala activates a glutamate projection to Acb that is involved in ethanol CPP expression, although we cannot eliminate the contributions of other potential sources of increased glutamatergic signaling in Acb (Gremel & Cunningham, 2010).

The present studies add to this growing body of knowledge about the neural circuitry underlying ethanol-induced CPP in mice by examining, for the first time, specific receptors that might be involved in the acquisition of ethanol CPP. Our tentative hypothesis is that exposure to ethanol on CS+ conditioning trials increases dopamine release in Acb either through direct effects on VTA dopamine neurons (Brodie, Pesold & Appel, 1999), interactions with any of several possible VTA neurotransmitter receptors (e.g., GABA, NMDA, 5-HT₃) or effects on other brain structures that send projections to VTA (e.g., laterodorsal tegmental nucleus, pedunculo pontine tegmental nucleus, lateral hypothalamus). Presumably, the increased binding of dopamine to Acb D1 receptors is critical to formation of the context-ethanol association, although the underlying molecular mechanisms remain to be determined. Future research should address those mechanisms as well as the roles played by other Acb receptors and other brain structures that might be involved in the acquisition of ethanol CPP.

4.5. Learning Theory Perspective

Basic learning theory underlies our approach to these studies as well as our interpretation of the findings. The experimental strategy of distinguishing between mechanisms that influence acquisition vs. expression clearly has its roots in the learning-performance distinction formalized many decades ago in the theories of Hull (1943) and Tolman (1955). Learning theory also informed the follow-up approach to the significant detrimental effect of the D1 antagonist on ethanol CPP acquisition by distinguishing between several different learning-based interpretations of that finding. One possibility is that the antagonist simply functioned as another unconditioned stimulus (US) that, by virtue of its paired relationship to the CS+, established a conditioned response that interfered with or reversed (counterconditioned) the response conditioned by the ethanol US. By that account, expression of CPP would be diminished by the simultaneous elicitation of conflicting conditioned responses (CRs) at the time of testing. However, the outcome of Exp. 1B allowed us to dismiss that possibility by showing that pairings of the CS and antagonist without ethanol produced no place conditioning. A second interpretation is that antagonist administration interfered with learning per se, i.e., reducing the increment in associative strength produced by each CS-US pairing. In its broadest form, this interpretation predicts that intra-Acb infusion of the antagonist should interfere with associative learning induced by non-ethanol USs. That prediction was not supported by the outcome of Exp. 1C, which showed no interference with the development of CPA induced by LiCl, although this study did not eliminate more narrowly framed versions of this interpretation (e.g., interference with appetitive learning).

The third possible interpretation is that intra-Acb infusion of the antagonist more directly interfered the unconditioned response (“reward”) evoked by the ethanol US, in essence producing a reduction in US intensity.

As is often the case in research informed by learning theory, tentative interpretations are chosen on the basis of eliminating more easily tested alternative interpretations. In this case, our follow-up studies suggested that a functional reduction in US intensity offered the most plausible interpretation, i.e., intra-Acb infusion of the D1 antagonist reduced CPP by making ethanol less rewarding on each conditioning trial. However, as noted earlier, we cannot dismiss a narrower version of the interference-with-learning interpretation (e.g., interference with appetitive learning). Furthermore, there is no reason to believe that the reduced-US-intensity interpretation applies uniquely to the unconditioned rewarding effect produced by ethanol. Indeed, previous studies showing detrimental effects of intra-Acb D1 antagonism on acquisition of CPP induced by other abused drugs (e.g., Baker et al., 1998) could also be explained by either of these interpretations.

4.6. Conclusions

Using intracranial microinfusion of selective D1- and D2-like antagonists in mice, these studies show that although both Acb D1- and D2-like receptors are normally involved in basal activity and ethanol’s locomotor activating effects, neither family of Acb receptor subtype is involved in ethanol CPP expression. Furthermore, only the Acb D1-like receptor is involved in ethanol CPP acquisition. Control experiments do not support alternative interpretations of the interfering effect of Acb D1 receptor blockade based on direct aversive effects of the antagonist or non-specific interference with drug-induced associative learning, lending support to the conclusion that dopamine activation of Acb D1-like dopamine receptors is involved in the development of ethanol-seeking behavior as indexed by the CPP procedure. When considered together with similar findings from studies involving several different drugs and species, these data support the hypothesis that Acb D1-like dopamine receptors are more generally involved in mediating the rewarding effects of abused drugs or in learning about those effects.

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References

- Abrahao KP, Quadros IMH, Souza-Formigoni MLO. Nucleus accumbens dopamine D1 receptors regulate the expression of ethanol-induced behavioural sensitization. *Int J Neuropsychopharmacol.* 2011; 14:175–185. [PubMed: 20426882]
- Bahi A, Dreyer J-L. Involvement of nucleus accumbens dopamine D1 receptors in ethanol drinking, ethanol-induced conditioned place preference, and ethanol-induced psychomotor sensitization in mice. *Psychopharmacology.* 2012; 222:141–153. [PubMed: 22222864]
- Baker DA, Fuchs RA, Specio SE, Khroyan TV, Neisewander JL. Effects of intraaccumbens administration of SCH-23390 on cocaine-induced locomotion and conditioned place preference. *Synapse.* 1998; 30:181–193. [PubMed: 9723788]
- Baker DA, Khroyan TV, O'Dell LE, Fuchs RA, Neisewander JL. Differential effects of intra-accumbens sulpiride on cocaine-induced locomotion and conditioned place preference. *J Pharmacol Exp Ther.* 1996; 279:392–401. [PubMed: 8859018]

- Bechtholt AJ, Cunningham CL. Ethanol-induced conditioned place preference is expressed through a ventral tegmental area dependent mechanism. *Behav Neurosci.* 2005; 119:213–223. [PubMed: 15727526]
- Bechtholt A, Gremel C, Cunningham C. Handling blocks expression of conditioned place aversion but not conditioned place preference produced by ethanol in mice. *Pharmacol Biochem Behav.* 2004; 79:739–744. [PubMed: 15582682]
- Berridge KC. From prediction error to incentive salience: mesolimbic computation of reward motivation. *European Journal of Neuroscience.* 2012; 35:1124–1143. [PubMed: 22487042]
- Brodie MS, Pesold C, Appel SB. Ethanol directly excites dopaminergic ventral tegmental area reward neurons. *Alcoholism: Clinical and Experimental Research.* 1999; 23:1848–1852.
- Chaudhri N, Sahuque LL, Janak PH. Ethanol seeking triggered by environmental context is attenuated by blocking dopamine D1 receptors in the nucleus accumbens core and shell in rats. *Psychopharmacology (Berl).* 2009; 207:303–314. [PubMed: 19779700]
- Couppis MH, Kennedy CH. The rewarding effect of aggression is reduced by nucleus accumbens dopamine receptor antagonism in mice. *Psychopharmacology.* 2008; 197:449–456. [PubMed: 18193405]
- Cunningham C, Malott D, Dickinson S, Risinger F. Haloperidol does not alter expression of ethanol-induced conditioned place preference. *Behav Brain Res.* 1992; 50:1–5. [PubMed: 1449638]
- Cunningham C, Niehus J, Noble D. Species difference in sensitivity to ethanol's hedonic effects. *Alcohol.* 1993; 10:97–102. [PubMed: 8442898]
- Cunningham C, Prather L. Conditioning trial duration affects ethanol-induced conditioned place preference in mice. *Animal Learning & Behavior.* 1992; 20:187–194.
- Cunningham CL. Localization of genes influencing ethanol-induced conditioned place preference and locomotor activity in BXD recombinant inbred mice. *Psychopharmacology.* 1995; 120:28–41. [PubMed: 7480533]
- Cunningham CL, Dickinson SD, Okorn DM. Naloxone facilitates extinction but does not affect acquisition or expression of ethanol-induced conditioned place preference. *Experimental and Clinical Psychopharmacology.* 1995; 3:330–343.
- Cunningham CL, Ferree NK, Howard MA. Apparatus bias and place conditioning with ethanol in mice. *Psychopharmacology (Berl).* 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.; Groblewski, PA.; Voorhees, CM. Place conditioning. In: Olmstead, MC., editor. *Animal Models of Drug Addiction, Neuromethods.* Vol. Vol. 53. Totowa, NJ: Humana Press; 2011. p. 167–189.
- Cunningham CL, Henderson CM, Bormann NM. Extinction of ethanol-induced conditioned place preference and conditioned place aversion: effects of naloxone. *Psychopharmacology.* 1998; 139:62–70. [PubMed: 9768543]
- Cunningham CL, Niehus DR, Malott DH, Prather LK. Genetic differences in the rewarding and activating effects of morphine and ethanol. *Psychopharmacology (Berl).* 1992; 107:385–393. [PubMed: 1352057]
- Czachowski CL, Chappell AM, Samson HH. Effects of raclopride in the nucleus accumbens on ethanol seeking and consumption. *Alcoholism: Clinical and Experimental Research.* 2001; 25:1431–1440.
- Dickinson SD, Lee EL, Rindal K, Cunningham CL. Lack of effect of dopamine receptor blockade on expression of ethanol-induced conditioned place preference in mice. *Psychopharmacology (Berl).* 2003; 165:238–244. [PubMed: 12451436]
- Doherty, JM.; Gonzales, RA. Neuroscience Meeting Planner. New Orleans, LA: Society for Neuroscience; 2012. Consumption of ethanol during operant self-administration is not attenuated by a dopamine D1 receptor antagonist in nucleus accumbens of adult male Long-Evans rats. Program No. 454.22. 2012. Online.
- Fenu S, Spina L, Rivas E, Longoni R, Di Chiara G. Morphine-conditioned single-trial place preference: role of nucleus accumbens shell dopamine receptors in acquisition, but not expression. *Psychopharmacology.* 2006; 187:143–153. [PubMed: 16724186]

- Fidler TL, Bakner L, Cunningham CL. Conditioned place aversion induced by intragastric administration of ethanol in rats. *Pharmacol Biochem Behav.* 2004; 77:731–743. [PubMed: 15099918]
- Fields HL, Hjelmstad GO, Margolis EB, Nicola SM. Ventral tegmental area neurons in learned appetitive behavior and positive reinforcement. *Annu Rev Neurosci.* 2007; 30:289–316. [PubMed: 17376009]
- Gonzales RA, Job MO, Doyon WM. The role of mesolimbic dopamine in the development and maintenance of ethanol reinforcement. *Pharmacol Ther.* 2004; 103:121–146. [PubMed: 15369680]
- Gremel CM, Cunningham CL. Role of test activity in ethanol-induced disruption of place preference expression in mice. *Psychopharmacology (Berl).* 2007; 191:195–202. [PubMed: 17216157]
- Gremel CM, Cunningham CL. Involvement of amygdala dopamine and nucleus accumbens NMDA receptors in ethanol-seeking behavior in mice. *Neuropsychopharmacology.* 2009; 34:1443–1453. [PubMed: 18830237]
- Gremel CM, Cunningham CL. Effects of disconnection of amygdala dopamine and nucleus accumbens N-methyl-D-aspartate receptors on ethanol-seeking behavior in mice. *Eur J Neurosci.* 2010; 31:148–155. [PubMed: 20092560]
- Gremel CM, Young EA, Cunningham CL. Blockade of opioid receptors in anterior cingulate cortex disrupts ethanol-seeking behavior in mice. *Behav Brain Res.* 2011; 219:358–362. [PubMed: 21219940]
- Groblewski PA, Bax LS, Cunningham CL. Reference-dose place conditioning with ethanol in mice: empirical and theoretical analysis. *Psychopharmacology (Berl).* 2008; 201(1):97–106. [PubMed: 18633601]
- Hiroi N, White NM. The amphetamine conditioned place preference: differential involvement of dopamine receptor subtypes and two dopaminergic terminal areas. *Brain Research.* 1991; 552:141–152. [PubMed: 1833032]
- Hodge CW, Samson HH, Chappell AM. Alcohol self-administration: further examination of the role of dopamine receptors in the nucleus accumbens. *Alcoholism: Clinical and Experimental Research.* 1997; 21:1083–1091.
- Hull, CL. *Principles of behavior: An introduction to behavior theory.* New York, London: Appleton-Century; 1943.
- Hyman SE, Malenka RC, Nestler EJ. Neural mechanisms of addiction: the role of reward-related learning and memory. *Annu Rev Neurosci.* 2006; 29:565–598. [PubMed: 16776597]
- Koob, GF.; LeMoal, M. *Neurobiology of addiction.* London: Academic Press; 2006.
- Krzyzosiak A, Szyszka-Niagolov M, Wietrzyk M, Gobaille S, Muramatsu S-i, Krezel W. Retinoid $\times$ receptor gamma control of affective behaviors involves dopaminergic signaling in mice. *Neuron.* 2010; 66:908–920. [PubMed: 20620876]
- Kurokawa K, Mizuno K, Ohkuma S. Possible involvement of type 1 inositol 1,4,5-trisphosphate receptors up-regulated by dopamine D1 and D2 receptors in mouse nucleus accumbens neurons in the development of methamphetamine-induced place preference. *Neuroscience.* 2012; 227:22–29. [PubMed: 23000618]
- Liao R-M. Development of conditioned place preference induced by intra-accumbens infusion of amphetamine is attenuated by co-infusion of dopamine D1 and D2 receptor antagonists. *Pharmacol Biochem Behav.* 2008; 89:367–373. [PubMed: 18275994]
- Liu Y, Young KA, Curtis JT, Aragona BJ, Wang Z. Social bonding decreases the rewarding properties of amphetamine through a dopamine D1 receptor-mediated mechanism. *J Neurosci.* 2011; 31:7960–7966. [PubMed: 21632917]
- Naughton BJ, Thirumara-Rajamani K, Wang C, During MJ, Gu HH. Specific knockdown of the D2 long dopamine receptor variant. *Neuroreport.* 2012; 23:1–5. [PubMed: 22082989]
- Paxinos, G.; Franklin, K. *The mouse brain in stereotaxic coordinates.* San Diego: Academic Press; 2001.
- Pina MM, Cunningham CL. Effects of dopamine receptor antagonists on the acquisition of ethanol-induced conditioned place preference. (submitted).

- Rassnick S, Stinus L, Koob GF. The effects of 6-hydroxydopamine lesions of the nucleus accumbens and the mesolimbic dopamine system on oral self-administration of ethanol in the rat. *Brain Research*. 1993; 623:16–24. [PubMed: 8221085]
- Risinger F, Cunningham C. DBA/2J mice develop stronger lithium chloride-induced conditioned taste and place aversions than C57BL/6J mice. *Pharmacol Biochem Behav*. 2000; 67:17–24. [PubMed: 11113479]
- Risinger FO, Dickinson SD, Cunningham CL. Haloperidol reduces ethanol-induced motor activity stimulation but not conditioned place preference. *Psychopharmacology*. 1992; 107:453–456. [PubMed: 1615143]
- Risinger FO, Malott DH, Prather LK, Niehus DR, Cunningham CL. Motivational properties of ethanol in mice selectively bred for ethanol-induced locomotor differences. *Psychopharmacology*. 1994; 116:207–216. [PubMed: 7862950]
- Samson H, Hodge C, Tolliver G, Haraguchi M. Effect of dopamine agonists and antagonists on ethanol-reinforced behavior: the involvement of the nucleus accumbens. *Brain Res Bull*. 1993; 30:133–141. [PubMed: 8093596]
- Samson HH, Chappell A. Dopaminergic involvement in medial prefrontal cortex and core of the nucleus accumbens in the regulation of ethanol self-administration: a dual-site microinjection study in the rat. *Physiology & Behavior*. 2003; 79:581–590. [PubMed: 12954398]
- Shen EH, Crabbe JC, Phillips TJ. Dopamine antagonist effects on locomotor activity in naive and ethanol-treated FAST and SLOW selected lines of mice. *Psychopharmacology*. 1995; 118:28–36. [PubMed: 7597119]
- Shippenberg TS, Bals-Kubik R, Herz A. Examination of the neurochemical substrates mediating the motivational effects of opioids: role of the mesolimbic dopamine system and D-1 vs. D-2 dopamine receptors. *J Pharmacol Exp Ther*. 1993; 265:53–59. [PubMed: 8386244]
- Spina L, Fenu S, Longoni R, Rivas E, Di Chiara G. Nicotine-conditioned single-trial place preference: selective role of nucleus accumbens shell dopamine D1 receptors in acquisition. *Psychopharmacology*. 2006; 184:447–455. [PubMed: 16341849]
- Taslimi Z, Arezoomandan R, Omranifard A, Ghalandari-Shamami M, Riahi E, Vafaei AA, et al. Orexin A in the ventral tegmental area induces conditioned place preference in a dose-dependent manner: involvement of D1/D2 receptors in the nucleus accumbens. *Peptides*. 2012; 37:225–232. [PubMed: 22885169]
- Tolman EC. Principles of performance. *Psychol Rev*. 1955; 62:315–326. [PubMed: 13254969]
- Tzschentke TM. Measuring reward with the conditioned place preference paradigm: A comprehensive review of drug effects, recent progress and new issues. *Progress in Neurobiology*. 1998; 56:613–672. [PubMed: 9871940]
- Tzschentke TM. Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. *Addict Biol*. 2007; 12:227–462. [PubMed: 17678505]

Research Highlights

- Ethanol was used to establish conditioned place preference (CPP) in mice.
- Selective dopamine receptor antagonists were infused into the nucleus accumbens.
- CPP acquisition was reduced by the D1- but not by the D2-receptor antagonist.
- Neither antagonist affected CPP expression, but both reduced activity.
- D1 receptors in accumbens influence learning of context-ethanol associations.

Figure 1.

A. Diagram of Acb injector placements. Shaded regions depict injector tract inclusion area within the Acb. Numbers indicate the distance from Bregma in mm (Paxino & Franklin, 2001). **B.** Photomicrograph showing a thionin-stained coronal slice of bilateral intra-Acb injection sites from a representative mouse after receiving four microinfusions in an acquisition experiment.

Figure 2.

A. Intra-Acb SCH23390 during conditioning trials blocked development of ethanol CPP. Mean time (s/min; +SEM) spent on the grid floor during the 30-min preference test. Mice in the Grid+ conditioning groups were given ethanol paired with the grid floor (0 μg SCH23390 [vehicle], n = 29; 0.05 μg SCH23390, n = 12; 0.5 μg SCH23390, n = 24). Mice in the Grid- conditioning groups were given ethanol paired with the hole floor (vehicle, n = 26; 0.05 μg SCH23390 n = 11; 0.5 μg SCH23390, n = 22). Significant difference between Grid+ and Grid- conditioning subgroups, * $p < .0003$ Bonferroni-corrected. **B.** Intra-Acb SCH23390 during conditioning trials did not produce significant place conditioning on its own. Grid+ mice had 0.5 μg intra-Acb SCH23390 paired with the grid floor (n = 15). Grid- mice had 0.5 μg intra-Acb SCH23390 paired with the hole floor (n = 14). **C.** Intra-Acb SCH23390 during conditioning had no effect on development of LiCl-induced CPA. Mice in the Grid+ conditioning groups were given LiCl paired with the grid floor (0 μg SCH23390 [vehicle], n = 6; 0.05 μg SCH23390, n = 5; 0.5 μg SCH23390, n = 5). Mice in the Grid- conditioning groups were given LiCl paired with the hole floor (vehicle, n = 6; 0.05 μg SCH23390, n = 6; 0.5 μg SCH23390, n = 6). Significant difference between Grid+ and Grid- conditioning subgroups, * $p < .0001$ Bonferroni-corrected.

Figure 3.

Intra-Acb raclopride during conditioning trials did not alter development of ethanol CPP. Mean time (s/min; +SEM) spent on the grid floor during the 30-min preference test. Mice in the Grid+ conditioning groups were given ethanol paired with the grid floor (0 µg raclopride [vehicle], n = 17; 0.5 µg raclopride, n = 7; 2 µg raclopride, n = 10; 5 µg raclopride, n=10). Mice in the Grid- conditioning groups were given ethanol paired with the hole floor (vehicle, n = 20; 0.5 µg raclopride, n=8; 2 µg raclopride, n = 9; 5 µg raclopride, n = 10). A conditioned place preference was shown in all groups by a significant difference between the Grid+ and Grid- conditioning subgroups, *p's <.05, Bonferroni-corrected.

Figure 4.

A. Intra-Acb SCH23390 before the expression test had no affect on ethanol CPP. Mean time (s/min; +SEM) spent on the grid floor during the 30-min preference test. Mice in the Grid+ conditioning groups were given ethanol paired with the grid floor (0 μg SCH23390 [vehicle], n = 10; 0.05 μg SCH23390, n = 11; 0.5 μg SCH23390, n = 9). Mice in the Grid- conditioning groups were given ethanol paired with the hole floor (vehicle, n = 9; 0.05 μg SCH23390, n = 11; 0.5 μg SCH23390, n = 12). A conditioned place preference was shown in all groups by a significant difference between the Grid+ and Grid- conditioning subgroups. *p's < .05, Bonferroni-corrected. **B.** Intra-Acb raclopride before the expression test did not alter ethanol CPP. Mice in the Grid+ conditioning groups were given ethanol paired with the grid floor (0 μg raclopride [vehicle], n = 7; 0.5 μg raclopride, n = 9; 2 μg raclopride, n = 8; 5 μg raclopride, n = 6.) Mice in the Grid- conditioning groups were given ethanol paired with the hole floor (vehicle, n = 8; 0.5 μg raclopride, n = 6; 2 μg raclopride, n = 8; 5 μg raclopride, n = 8). A conditioned place preference was shown in all groups by a significant difference between the Grid+ and Grid- conditioning subgroups, *p's < .05, Bonferroni-corrected.

Table 1

Locomotor Activity (mean counts/min $\pm$ SEM)

Experiment	Dose (μ g/side)	n	CS+ trials	CS- trials	Test activity
1A: Acquisition (SCH23390)	0	55	213.8 $\pm$ 4.2	82.2 $\pm$ 2.4	53.9 $\pm$ 1.8
	0.05	23	200.7 $\pm$ 7.2	85.9 $\pm$ 3.6	50.2 $\pm$ 2.4
	0.5	46	139.8 $\pm$ 3.4 ^{a,b}	79.0 $\pm$ 1.9	53.8 $\pm$ 1.9
			Dose: F(2,121) = 57.5 ^{*****} Trial type: F(1,121) = 1423.2 ^{*****} Interaction: F(2,121) = 81.7 ^{*****}		Dose: F(2,121) = 0.8
1B: Acquisition (SCH23390)	0.5	29	39.1 $\pm$ 2.0	74.0 $\pm$ 2.5	54.7 $\pm$ 1.8
			Trial type: F(1,28) = 511.8 ^{*****}		
1C: Acquisition (SCH23390)	0	12	13.4 $\pm$ 1.0	40.5 $\pm$ 2.7	33.4 $\pm$ 2.7
	0.05	11	11.5 $\pm$ 1.0	40.7 $\pm$ 2.3	38.6 $\pm$ 2.2
	0.5	11	8.1 $\pm$ 0.7	43.2 $\pm$ 2.2	39.8 $\pm$ 1.8
			Dose: F(2,31) = 0.2 Trial type: F(1,31) = 458.3 ^{*****} Interaction: F(2,31) = 2.8		Dose: F(2,31) = 2.2
2: Acquisition (Raclopride)	0	37	211.9 $\pm$ 4.8	87.3 $\pm$ 3.2	54.3 $\pm$ 1.7
	0.5	15	188.1 $\pm$ 9.8	93.4 $\pm$ 7.0	56.9 $\pm$ 4.3
	2	20	152.8 $\pm$ 9.1 ^{a,d}	78.8 $\pm$ 3.0	52.3 $\pm$ 1.9
	5	19	108.4 $\pm$ 10.0 ^{a,c,e}	89.5 $\pm$ 3.5	57.8 $\pm$ 2.4
3: Expression (SCH23390)			Dose: F(3,87) = 24.5 ^{*****} Trial type: F(1,87) = 378.6 ^{*****} Interaction: F(3,87) = 36.8 ^{*****}		Dose: F(3,87) = 1.0
	0	19	204.6 $\pm$ 7.8	74.4 $\pm$ 4.1	41.6 $\pm$ 2.6

Experiment	Dose ($\mu\text{g/side}$)	n	CS+ trials	CS- trials	Test activity
4: Expression (Raclopride)	0.05	22	211.7 $\pm$ 7.2	72.0 $\pm$ 3.0	28.5 $\pm$ 2.2 ^a
	0.5	21	200.1 $\pm$ 7.6	71.4 $\pm$ 2.9	17.0 $\pm$ 1.6 ^{a,b}
			Dose: F(2,59) = 0.5		Dose: F(2,59) = 32.3 ^{*****}
			Trial type: F(1,59) = 1110.7 ^{*****}		
			Interaction: F(2,59) = 0.8		
	0	15	238.9 $\pm$ 5.3	71.1 $\pm$ 3.3	46.6 $\pm$ 2.7
	0.5	15	222.2 $\pm$ 10.3	76.1 $\pm$ 4.9	47.8 $\pm$ 2.3
	2	16	218.3 $\pm$ 8.3	73.8 $\pm$ 3.8	32.4 $\pm$ 1.5 ^{a,c}
	5	14	214.3 $\pm$ 6.1	71.7 $\pm$ 5.0	23.3 $\pm$ 2.2 ^{a,c,f}
			Dose: F(3,56) = 1.0		Dose: F(3,56) = 27.6 ^{*****}
		Trial type: F(1,56) = 1697.9 ^{*****}			
			Interaction: F(3,56) = 2.6		

** p< .01,
***** p< .0001

^a significantly different from 0 µg per side group p < .001

^b significantly different from 0.05 µg per side group p < .001

^c significantly different from 0.5 µg per side group p < .0001

^d significantly different from 0.5 µg per side group p < .05

^e significantly different from 2 µg per side group p < .002

^f significantly different from 2 µg per side group p < .05

Note: p values for 2-group comparisons are Bonferroni-corrected within each experiment