Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 76 (2003) 335–342
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

# Chronic D1 agonist and ethanol coadministration facilitate ethanol-mediated behaviors

Manoranjan S. D'Souza, Aiko Ikegami, Christopher M. Olsen, Christine L. Duvauchelle*

*Division of Pharmacology and Toxicology, College of Pharmacy, University of Texas, 1 University Station A1915, Austin, TX 78712-0125, USA*
*Waggoner Center for Alcohol and Addiction Research, University of Texas, 1 University Station A1915, Austin, TX 78712-0125, USA*

Received 29 January 2003; received in revised form 8 August 2003; accepted 19 August 2003

**Abstract**

Separate lines of evidence suggest that neuroadaptations associated with ethanol (EtOH) reinforcement can be initiated by chronic EtOH preexposure and a signaling pathway activated by dopamine (DA) D1 receptor stimulation. We have previously shown that rewarding and locomotor effects of EtOH alone [Pharmacol. Biochem. Behav. 72 (2002) 787] are enhanced after chronic exposure to self-administered EtOH/cocaine combinations. To determine the importance of chronic EtOH exposure, dopamine D1 receptor activation and mode of drug administration in EtOH reward, animals were given daily intravenous infusions of experimenter-administered saline, EtOH (2.0 g/kg), the DA D1 receptor agonist, SKF81297 (0.2 mg/kg), or EtOH + SKF81297 over a 4-week period. Compared to other groups, animals preexposed to EtOH + SKF81297 self-administered significantly greater amounts of intravenous EtOH and showed greater enhancement and less suppression of locomotor activity in response to a range of intravenous EtOH dosages (0.125, 0.25, 0.5, 1.0 and 1.5 g/kg). Since chronic treatment with EtOH alone did not enhance EtOH-induced reinforcement or locomotor activity, it is unlikely that these effects were due to EtOH tolerance. These findings suggest that chronic D1 receptor activation combined with EtOH administration alters neural responsiveness to EtOH and support the notion that D1 activation is important to EtOH reward.


*Keywords:* Intravenous ethanol self-administration; SKF81297; Ethanol-induced locomotor activity; Chronic ethanol

## 1. Introduction

Similar to other drugs of abuse, neural changes that promote the rewarding qualities of EtOH are thought to involve the mesoaccumbal dopaminergic (DA) system (Wise, 1987; Koob, 1992; Samson et al., 1992). In addition to reports showing that nucleus accumbens (NAcc) DA levels increase after systemic administration of EtOH (Yoshimoto et al., 1992; Yim et al., 1998; Yim and Gonzales, 2000), there is also a positive correlation between NAcc DA activation and behaviors indicative of EtOH reinforcement. For instance, NAcc DA increases during oral EtOH self-administration (Weiss et al., 1993; Gonzales and Weiss, 1998) and NAcc-applied DA receptor agonists and antagonists alter EtOH intake (Hodge et al., 1992, 1997; Samson et al., 1993). In addition, coadministered intravenous EtOH significantly enhances cocaine self-administration (Ikegami et al., 2002).

The signaling pathway by which DA stimulates the phosphorylation of the DA and cyclic adenosine 3′,5′ monophosphate-regulated phosphoprotein 32 kDa (DARPP-32) has become the topic of an increasing number of reports (Lewis et al., 1990; Snyder et al., 1998; Calabresi et al., 2000; Flores-Hernandez et al., 2000; Gurden et al., 2000; Dunah and Standaet, 2001; Risinger et al., 2001; Edwards et al., 2002). This biochemical cascade of events is initiated by dopamine D1 receptor activation and is hypothesized to encompass critical components of EtOH reinforcement (Risinger et al., 2001; Maldve et al., 2002). For instance, since DARPP-32 knockout mice show a lack of EtOH-induced drinking and conditioned reward (Risinger et al., 2001), it is conceivable that, under natural circumstances, DA D1 receptor activation sets off the process by which DARPP-32 phosphoprotein becomes a crucial component of EtOH reward.

* Corresponding author. Division of Pharmacology and Toxicology, College of Pharmacy, University of Texas, 1 University Station A1915, Austin, TX 78712-0125, USA. Tel.: +1-512-471-1090; fax: +1-512-475-6088.
*E-mail address:* duvauchelle@mail.utexas.edu (C.L. Duvauchelle).

0091-3057/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2003.08.004

Behavioral studies indicate that chronic EtOH exposure produces a myriad of changes within the brain that may be associated with increased EtOH reward. For instance, a direct relationship between EtOH pretreatment and EtOH self-administration has been shown in mice (Camarini et al., 2000). In addition, chronic injections of EtOH facilitate place preference in laboratory animals (Holloway et al., 1992; Bienkowski et al., 1995; Biala and Kotlinska, 1999) and EtOH drinking is increased after prolonged access to EtOH/sucrose mixtures (Samson, 1986). Work carried out in our laboratory has demonstrated enhanced reinforcing and locomotor effects of intravenous EtOH alone when cocaine and EtOH combinations had been previously self-administered, but not when cocaine had been self-administered without the EtOH component (Ikegami et al., 2002). Taken together, these findings suggest a link between certain conditions of EtOH preexposure and ensuing behaviors demonstrating rewarding EtOH effects.

Without interference from orosensory factors, such as aversive taste or smell, the study of EtOH reinforcement is facilitated by the use of intravenous EtOH self-administration techniques. Indeed, intravenous EtOH self-administration in our prior study (Ikegami et al., 2002) resulted in higher blood alcohol levels than has been reported after voluntary intake through any route of administration. However, the high intake of self-administered intravenous EtOH and EtOH-induced hyperlocomotion we previously observed may be attributed to several factors, including chronic EtOH exposure, DA D1 receptor activation, self-administration and environmental conditioning. The present experiment was performed to determine the relative importance of these factors. In this study, animals were given daily intravenous injections of saline (Control), the D1 full agonist, SKF81297, EtOH (10%) or a combination of EtOH + SKF81297 over a 4-week period. Preexposure treatments were experimenter-administered in a home cage environment. These conditions were selected for a number of reasons. First, our earlier study indicated that EtOH alone would not be self-administered in EtOH-naive animals, thus, experimenter-administered treatment would be the only way to ensure consistent preexposure to EtOH alone. Also, using this mode of administration, it could be determined whether drug pretreatment is needed to be self-administered for EtOH reward and/or locomotor enhancement to occur. Finally, daily injections given in a home cage environment can rule out environmental conditioning as a factor contributing to reward-associated behaviors.

The effects of the drug pretreatments on EtOH reinforcement were subsequently determined by assessing operant responding during intravenous EtOH self-administration sessions. After 1 week of behavioral extinction sessions (intravenous saline availability) in the operant chambers, animals underwent an additional week of preexposure treatment (same groups as described above) in their home cages. Baseline and EtOH-induced locomotor activity assessments across the different pretreatment groups were then performed over the next 12 days.

## 2. Materials and methods

### 2.1. Animals

Thirty-two Sprague–Dawley rats weighing 250–300 g were started in the experiment. The rats were housed individually in polypropylene cages in a temperature and humidity controlled vivarium on a reversed 12-h light/dark cycle (off 7 a.m./on 7 p.m.). Animals were handled daily for 10 days prior to the start of the experiment. Food and water were available ad libitum except during operant conditioning sessions. All training and experimental sessions were conducted during the dark phase. The experimental protocol for this study was approved by the University of Texas Institutional Animal Care and Use Review Committee.

### 2.2. Apparatus

Operant and locomotor activity sessions were conducted in one-lever operant chambers (28 × 22 × 21 cm) made of Plexiglas and metal (Med-Associates, St. Albans, VT). The operant chambers had a single retractable lever located on the right wall with a stimulus light located above the retractable lever and a house light located on the opposite wall. The operant chambers were located within sound attenuating boxes (63 × 44 × 58 cm) with a front-opening door and peephole to enable viewing of the animal during the test session. Three pairs of photocells to record the locomotor activity were located in the center and 5 cm from each sidewall of the chamber. Self-administered solutions were infused through a single swivel, mounted on a counterbalanced arm, at the top of each chamber. One end of the swivel was connected via polyethylene tubing to a syringe (10 ml), mounted on a syringe pump (Razel Scientific Instruments, Model A) that delivered the drug over a programmed time period. Self-administering animals were on a FR1 schedule of reinforcement. There was a 20-s timeout after each infusion, during which time the houselights remained off and the lever was retracted. Self-administration sessions were conducted 5 days/week (drug-free weekends) and continued for a total of 2 weeks (1 week of EtOH availability and 1 week saline/extinction). Subsequent locomotor activity assessment was performed over a total of 12 sessions (two sessions to determine baseline activity levels and two sessions for each of five EtOH dosages). The number of lever responses and locomotor activity units was recorded with using MED-PC software.

### 2.3. Food training

After the handling phase, the animals were food restricted and trained in operant chambers to lever press for food

on a FR1 schedule of reinforcement. After lever press acquisition, they had daily 10-min food reinforced operant sessions (FR1) for 6 days. During these sessions, the animals received a food pellet (45 mg) each time they pressed the extended lever. To maintain this operant behavior during the preexposure phase, animals participated in 10-min food reinforced sessions once a week (e.g., total of four sessions).

### 2.4. Surgery

After the initial food-reinforced operant training, animals underwent jugular catheterization surgery. Sodium pentobarbital (50 mg/kg ip) supplemented with chloral hydrate (80 mg/kg ip) were utilized as surgical anesthetics. Atropine sulfate (250 μg/rat sc) was administered prophylactically to reduce respiratory secretions. A Silastic catheter (0.625 mm od) was inserted into the right external jugular vein. The free end of the catheter, fused with a modified cannula termination (C313G, Plastics One), was run subcutaneously along the side of the neck and out an incision in the skin at the top of the skull. Details of surgery have been previously reported (Duvauchelle et al., 1998). Animals were allowed to recover from surgery for 1 week before drug preexposure treatments. After surgery, the catheters were flushed with 0.1 ml of 1.0 mg streptokinase plus 100 mg Timentin in 2.5 ml of heparinized saline for 1 week. Thereafter, catheters were flushed with 0.1 ml of streptokinase solution (1.0 mg streptokinase/2.5 ml heparinized saline) every day until the end of the experiment.

### 2.5. Drug preexposure treatment

#### 2.5.1. Groups

One week after the surgery the animals were divided into four groups that received one of the following intravenous treatments daily for the next 4 weeks: (1) EtOH: 2.0 g/kg of 10% EtOH; (2) saline: 0.9% physiological saline; (3) the D1 agonist, SKF81297: 0.2 mg/kg; or (4) EtOH + SKF81297: 2.0 g/kg 10% EtOH + 0.2 mg/kg SKF81297. The EtOH concentration of 10% was held constant and volume of infusion was calculated based on the weight of the animal (e.g., for a 400 g rat, 1.0 g/kg EtOH = 2.0 ml 10% EtOH). Saline injections were equal in volume to EtOH infusions. Daily drug infusions were performed two times per day separated by an interval of 45 min (e.g., 1/2 of total drug treatment at each infusion). To maintain comparable levels of infusion volumes across groups, the SKF81297 alone group was given the D1 agonist in addition to a saline injection of equal volume to the EtOH infusion of the EtOH + SKF81297 group.

#### 2.5.2. Drugs

SKF81297 (Sigma, St Louis, MO), a D1 full agonist was dissolved in sterile physiological saline and injected intravenously at a dose of 0.1 mg/kg just before each infusion of EtOH or saline (total dose of 0.2 mg/kg/day of SKF81297). The 10% EtOH solution was made by diluting 95% EtOH (AAPER Alcohol and Chemical, Shelbyville, KY) with physiological saline and adding NaCl to the mixture to produce an isotonic solution.

#### 2.5.3. Preexposure procedure

The animals were transported from the animal colony into the lab in home cage environments ($45 \times 23.75 \times 20$ cm polypropylene cages). After flushing the catheters with the streptokinase solution (see Surgery above) and weight determination, they were returned to their transport cage and infused with the first of their injections through the use of an infusion pump (Razel Scientific Instruments, Model A). Solutions were delivered at a fixed rate of 1.1 ml/min. Depending upon the animal's weight, infusion durations ranged from approximately 2 min 20 s to 4 min. Forty-five min later, animals received an identical infusion. This procedure was carried out for 5 days/week for the next 4 weeks. Preexposure treatment conditions were repeated for an additional 5 days after saline extinction trials, just prior to EtOH locomotor activity testing.

### 2.6. Blood alcohol levels (BAL)

Blood was collected for BAL analyses during the preexposure phase to determine BAL levels in animals receiving EtOH during preexposure treatments (e.g., EtOH alone and EtOH + SKF81297 groups). Blood was collected from each animal on two separate occasions, with an interval of 1 day between the two samplings. The first set of BALs was determined after animals had received 1.0 g/kg of the intravenous EtOH dosage. The next set of BALs was determined after the second daily infusion of 1.0 g/kg EtOH (45 min after the first 1.0 g/kg infusion; total of 2.0 g/kg). Blood samples were collected in the following manner: After the intravenous EtOH infusion, intravenous catheters were flushed with heparinized saline (0.1 ml = 3 times the catheter volume). Two minutes elapsed prior to blood withdrawal, and the first 0.1 ml of blood withdrawn was discarded. The next 0.1 ml was collected in chromatography vials and analyzed using gas chromatography as previously described (Crippens et al., 1999). Samples were compared to EtOH blood standards ranging from 0 to 80 mM EtOH.

### 2.7. Self-administration phase

After the 4-week preexposure period, animals were placed in operant chambers and had the opportunity to self-administer intravenous 10% EtOH (0.25 g/kg/injection) during 1-h operant sessions. Intravenous EtOH was available during these sessions for the next 5 days. To extinguish responding in the operant chamber, intravenous saline was substituted for EtOH during five subsequent operant sessions.

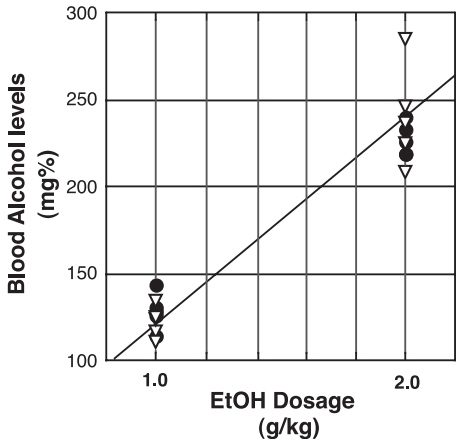


Fig. 1. BALs during preexposure: Blood sampled immediately after first and second daily intravenous infusion for individual rats in the EtOH alone (●; $n=5$) and EtOH + SKF81297 (▽; $n=5$) pretreatment groups. Blood sampling for 1.0 and 2.0 g/kg tests occurred on separate occasions.

### 2.8. Locomotor activity assessment

After saline extinction, animals were once again given the same drug treatment received during the preexposure phase for an additional 5 days. Locomotor activity was then assessed after various doses of experimenter-administered intravenous EtOH (0.0, 0.125, 0.25, 0.5, 1.0, and 1.5 g/kg) over the next 2 weeks. Animals received EtOH treatments in their transport cages and were then immediately placed into the operant chambers. Locomotor activity was measured for 1 h. During this session, operant chambers remained completely dark and the lever was retracted. Dosages were given in random order, and each dose was given on two separate occasions. Animals were tested at one dosage/day.

### 2.9. Statistical analyses

A two-way ANOVA (Group × Session Day) were used to determine treatment effects on the number of EtOH-reinforced responses made across the five 1-h self-administration sessions. The total number of responses during the five sessions across groups was also compared using a one-way ANOVA. A two-way ANOVA (Group × Dose) was used to assess the effects of EtOH on locomotor activity. Locomotor activity for each dose was determined as the average of the two treatments. Saline level locomotor activity differed across pretreatment groups (see Results). Therefore, EtOH-induced changes in activity were also calculated as the percent of baseline within each group. Least Significant Differences tests (Fishers LSD) were performed to determine specific group differences when ANOVA results were significant. Due to the development of leakages in implanted catheters during the preexposure or self-administration phase, self-administration data could not be collected from nine animals. Locomotor activity data from an additional animal in the saline control group could not be collected due to ill health.

## 3. Results

### 3.1. Effects of preexposure condition

#### 3.1.1. Blood alcohol levels (BALs)

During preexposure blood alcohol levels in the EtOH alone and EtOH + SKF81297 groups after 1.0 g/kg and 2.0 g/kg of 10% EtOH preexposure were comparable (see Fig. 1). After infusion of 1.0 g/kg of 10% EtOH, BALs in the EtOH alone and EtOH + SKF81297 groups were 128.8 + 10.33 and 121.8 + 8.8 mg%, respectively. After exposure to 2.0 g/kg of EtOH, BALs in the EtOH and EtOH + SKF81297 group were 229 + 8.8 and 224.1 + 17.6 mg%, respectively.

#### 3.1.2. Intravenous EtOH self-administration

A two-way ANOVA (Group × Session Day) showed significant group effects on the number of reinforced responses for EtOH [$F(3,19)=3.13$; $P=.04$] and significant session effects [$F(4,76)=8.3$; $P<0.0001$], but no significant interaction effects [$F(12,76)=0.86$; *ns*]. As can be seen in Fig. 2. Control (saline preexposed) animals and those chronically exposed to EtOH alone self-administered approximately equal amounts of EtOH throughout the five sessions, while the EtOH + SKF81297 group had the most

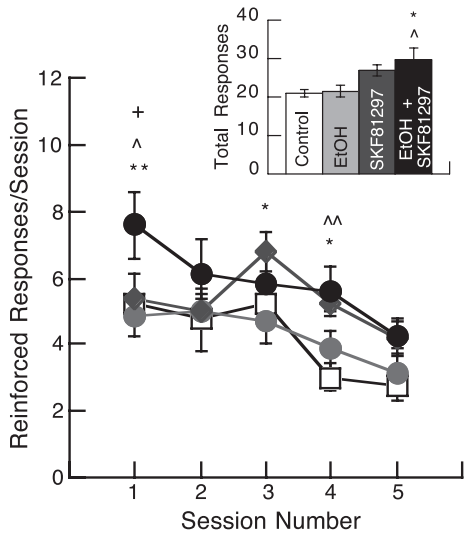


Fig. 2. Lever presses for intravenous EtOH after preexposure: mean ± S.E.M. Reinforced responses/session: control rats (□; $n=4$), EtOH alone (●; $n=7$), SKF81297 alone (◆; $n=5$) and EtOH + SKF81297 (●; $n=7$). Post hoc tests (Fisher LSD) revealed that during Sessions 1, 3 and 4, rats preexposed to EtOH + SKF81297 self-administered significantly more intravenous EtOH compared to other groups. In addition, animals pretreated with SKF81297 alone showed higher response rates than Control and EtOH alone pretreated animals on Days 3 and 4. Total Responses: The total number of responses from the EtOH + SKF81297 group were significantly greater than the control and EtOH alone groups. Cumulative responses of animals pretreated with SKF81297 alone did not significantly differ from any other group. ^, ^^; *, ** and $^{+}$ depicts significant difference at $P<.05$ and .01 compared to Control, EtOH alone and SKF81297 alone groups, respectively.

instances of significantly greater response rates. A one-way ANOVA performed on the 5-day response totals confirmed significant group effects [$F(3,19) = 3.69$; $P = .03$], with post hoc tests revealing that the EtOH + SKF81297 animals responded significantly more than animals in the saline and EtOH groups ($P = .05$).

*3.1.3. Locomotor activity during intravenous EtOH sessions*

A two-way ANOVA (Group × Session Day) was performed on locomotor activity occurring during EtOH self-administration sessions to determine whether the motor response of lever pressing for EtOH might have been facilitated by EtOH-mediated increases in activity. No significant group [$F(3, 19) = 0.8$; *ns*], session [$F(4,76) = 0.84$; *ns*] or interaction effects [$F(12,76) = 0.86$; *ns*] were detected.

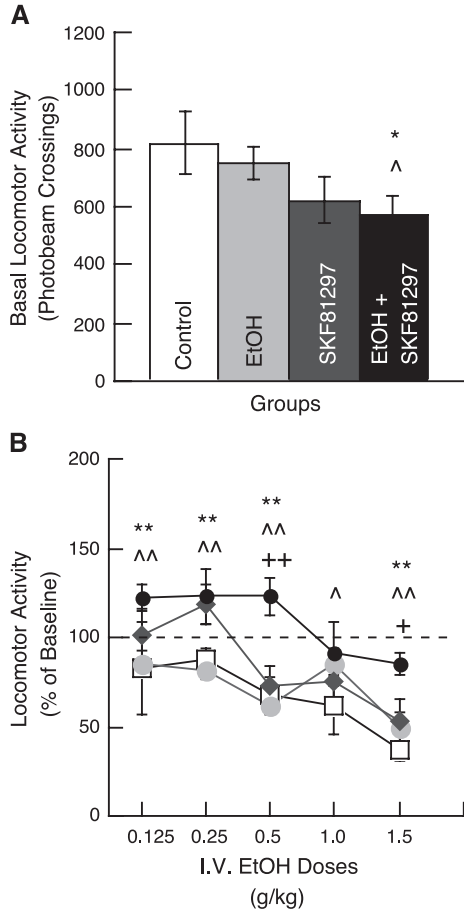


Fig. 3. (A) Baseline locomotor activity after preexposure: mean ± S.E.M. Animals chronically treated with EtOH + SKF81297 showed significantly less locomotor activity after saline treatment compared to control and EtOH alone pretreated groups. *, ^ Significantly less than control and EtOH alone groups, respectively, at $P = .05$. (B) EtOH-induced changes in locomotor activity after preexposure: data shown as percent of baseline locomotor activity. Rats pretreated with EtOH + SKF81297 (●) showed the most instances of EtOH-induced locomotor activity enhancement or least suppression. ^, ^^; *, **; $^{+}$, $^{+++}$ depicts significant difference at $P < .05$ and .01 compared to control (□), EtOH alone(●) and SKF81297 alone (◆) groups, respectively.

*3.1.4. Basal and ethanol-induced locomotor activity*

A two-way ANOVA performed on locomotor activity (Group × Dose) at three dosage levels (0.0, 0.5 and 1.5 g/kg) showed significant effects of Dose [$F(2,38) = 22.13$; $P \leq .001$] and a Group × Dose interaction effect [$F(6,38) = 2.9$; $P = .019$], but no overall Group effects [$F(3,19) = 1.56$; *ns*]. Post hoc analyses showed that basal level (saline-treated) locomotor activity in the EtOH + SKF81297 group was significantly less than observed in the Control and EtOH alone groups ($P = .05$). Within-group comparisons revealed at the highest dose (1.5 g/kg), locomotor activity was significantly suppressed in all but the EtOH + SKF81297 group. The EtOH alone group also showed significant suppression of locomotor activity from saline levels at the 0.5 g/kg dose. Due to group differences in saline level activity, locomotor activity was also analyzed using percentages of baseline activity within groups. A two-way ANOVA using these data showed significant Group [$F(3,19) = 6.76$; $P = .0027$] and Dose differences [$F(4,76) = 16.752$; $P < .0001$], but no significant interaction effects [$F(12,76) = 1.44$; *ns*]. Post hoc analyses showed that locomotor activity in the EtOH + SKF81297 group was significantly greater than saline- and EtOH-pretreated groups for the 0.125, 0.250, 0.5 and 1.5 g/kg dosages ($P < .01$), and saline at the 1.0 g/kg dose ($P < .05$). The EtOH + SKF81297 group was significantly different from the SKF81297 group at 0.5 ($P < .01$) and 1.5 g/kg ($P < .05$) doses (see Fig. 3B).

## 4. Discussion

In the present study, animals preexposed to EtOH + SKF81297 showed enhancement of behaviors associated with EtOH reinforcement. Animals preexposed to EtOH + SKF81297 self-administered significantly greater amounts of EtOH and showed locomotor responses to EtOH injections that were strikingly different than other treatment groups. For example, intravenous EtOH injections did not increase activity from baseline levels in the control (saline pretreated) and EtOH alone groups at any EtOH dose tested. However, at low to moderate EtOH doses (0.125, 0.25 and 0.5 g/kg), the EtOH + SKF81297 group showed significant activity enhancement compared to other groups. In addition, at the highest EtOH dose tested (1.5 g/kg), locomotor activity was suppressed below saline activity levels in all but the EtOH + SKF81297 group.

Higher response rates for intravenous EtOH reinforcement in the EtOH + SKF81297 group was not due to general activity enhancement during self-administration sessions. An examination of locomotor activity during self-administration sessions revealed no overall differences in activity between groups. Additional post hoc comparisons confirmed no instance of significantly higher activity levels between the EtOH + SKF81297 group and any other group on any given session day. Also unlikely is the attribution of these effects to EtOH tolerance. Chronic treatment with

EtOH alone did not enhance EtOH-induced reinforcement or locomotor activity. Also, animals that did not receive EtOH during the preexposure period responded for intravenous EtOH at higher response rates than the control or EtOH alone during two self-administration sessions.

By definition, self-administration behavior requires motor output to demonstrate reinforcing effects of administered substances. While self-administration procedures are the only means to assess drug reward through voluntary intake, the motor requirements of this task make observed results subject to interpretation. As noted above, in the present study, locomotor activity measures taken during EtOH self-administration sessions reveal that the EtOH + SKF81297-treated group did not show enhanced levels of nonspecific locomotor activity in the presence of higher lever response rates. Though these data support the notion that EtOH + SKF81297 preexposure did not increase nonspecific motor activity in general, the possibility of effects on other broad-spectrum behaviors cannot be entirely ruled out. For example, goal-directed activities, as a general class of behaviors, may have been affected by EtOH + SKF81297 treatments. In this scenario, the higher intake of intravenous EtOH shown in these animals may have been due to a common increase in reward-seeking behaviors, rather than the specific enhancement of EtOH reward. This alternate explanation of our findings cannot be excluded using our current reported methods. However, future studies may address such potential confounds by employing additional measures of reward. For example, the use of a two-lever choice procedure for different kinds of reinforcement could indicate specific effects on ethanol reinforcement vs. general effects on various rewarding substances. Also, place conditioning procedures as an adjunct reward measure can determine the presence of rewarding drug effects and circumvent the problems of motor output requirements during the test session.

EtOH-stimulated locomotor activity is thought to reflect rewarding effects of EtOH (Wise and Bozarth, 1987; Phillips and Shen, 1996; Colombo et al., 1998), though the exact mechanism for EtOH-induced locomotor activity is not clearly understood. However, it is widely believed that EtOH produces locomotor activity by activating the dopaminergic system (Friedman et al., 1980; Liljequist et al., 1981; Cohen et al., 1997; Itzhak and Martin, 1999). Investigations into the behavioral functions of D1 and D2 dopamine receptors revealed both receptor subtypes may play a role in mediating EtOH-stimulated motor activity (Cohen et al., 1997; Lê et al., 1997; Souza-Formigoni et al., 1999). Therefore, it may seem curious that, in the present study, the EtOH preexposed group did not exhibit EtOH-mediated locomotor activity enhancement or increased response rates for intravenous EtOH reinforcement, yet the EtOH + SKF81297 group did. The most parsimonious explanation is that the daily dosage of 2.0 g/kg EtOH alone was not enough to induce observable behavioral changes. However, in combination with chronic D1 receptor stimulation, the continued perturbation of multiple neural systems was sufficient to alter the responsiveness to EtOH. Another possibility is that, since many studies administer EtOH via intraperitoneal injections, the physiological effects associated with this mode of administration vary from those present during intravenous injections (e.g., as utilized in this study). For instance, drug treatments via surgically implanted intravenous catheters circumvent stressful effects of restraint and needle puncture inherent to intraperitoneal injections. Since drugs of abuse and stress invoke similar changes in dopamine neuronal activity (Saal et al., 2003), it is reasonable to consider that the stressful event of an intraperitoneal injection might potentiate the DA response to EtOH. Thus, the chronic absence of the stress component during intravenous injections may lead to different long-term physiological effects compared to the intraperitoneal route of administration.

Though significantly greater than previous studies of intravenous EtOH administration (Hyytia et al., 1996; Kuzmin et al., 1999), EtOH self-administration in EtOH + SKF81297 pretreated groups in the present report was not as robust as our prior report when animals self-administered EtOH + cocaine prior to EtOH-reinforced sessions (Ikegami et al., 2002). An obvious difference in these studies is that, through enhanced extracellular concentrations of DA, cocaine administration results in stimulation of all available DA receptors, while SKF81297 is a specific agonist of the D1 receptor. Since D2 receptor stimulation has been shown to potentiate the rewarding effects of cocaine (Caine et al., 1999), the involvement of D2 activation during cocaine treatment may account for discrepancies between the previous and present studies. In addition, intentional methodological differences utilized in this experiment may also account for disparities. Aside from different coadministered agents (e.g., cocaine vs. SKF81297), the present study used a different mode and context of pretreatment drug delivery. For example, preexposure treatment (e.g., cocaine/EtOH combinations) in the previous study was self-administered rather than delivered by the experimenter. Therefore, due to "practicing" self-administration responding daily over a lengthy preexposure period, animals were very facile in lever-pressing behavior. Conversely, animals in the present study engaged in food-reinforced sessions only once per week during the preexposure phase to maintain the learned response. In either case, it reasonable to imagine that differences in operant task skills could directly impact upon EtOH intake levels independent of the rewarding valence of the self-administered EtOH. Also, in the present study, since preexposure treatments were received in a home cage environment, there was no possibility of conditioning-induced reward enhancement in the operant chamber. Nonetheless, as measured by voluntary EtOH intake, the findings shown here indicate that chronic preexposure to EtOH + SKF81297 increase the rewarding value of intravenous EtOH.

DA D1 receptors have been shown to be involved in the rewarding effects of brain stimulation (Renaldi and Beninger, 1994) and the reinforcing effects of drugs of

abuse, including EtOH (Dyr et al., 1993; Ng and George, 1994; Hodge et al., 1997; Matsuzawa et al., 1999). In addition, disruption of D1 receptor gene expression has shown to attenuate EtOH-seeking behavior (El-Ghundi et al., 1998). Recently, it has been suggested that D1 activation may help in EtOH reinforcement by decreasing EtOH-mediated NMDA receptor inhibition via DARPP-32 protein (Maldve et al., 2002). In the present study, rats chronically exposed to EtOH alone did not show enhanced intravenous self-administration of EtOH, but animals preexposed to EtOH + SKF81297 did. Though the acute effects of D1 receptor stimulation were not tested here, EtOH reward enhancement in the present study may be due to D1 receptor sensitization acquired through chronic D1 activation. In any case, this study provides evidence that a history of D1 activation in combination with EtOH can subsequently result in neural changes that engender enhanced EtOH reinforcement. Understanding mechanisms by which EtOH can elicit enhanced reward and locomotor activation will assist efforts to target and treat alcohol abuse and addiction.

## Acknowledgements

This study was supported by NIH grant AA12903 (CLD), Fred Murphy Jones Fellowship from the University of Texas Waggoner Center For Alcohol and Addiction Research (A.I.), and NIH Training Grant AA07471 (CMO).

## References

Biala G, Kotlinska J. Blockade of the acquisition of ethanol-induced conditioned place preference by *N*-methyl-D-aspartate receptor antagonists. Alcohol Alcohol 1999;342:175–82.

Bienkowski P, Kuca P, Kostowski W. Conditioned place preference after prolonged pre-exposure to ethanol. Pol J Pharmacol 1995;472:189–91.

Caine SB, Negus SS, Mello NK, Bergman J. Effects of dopamine D1-like and D2-like agonists in rats that self-administer cocaine. J Pharmacol Exp Ther 1999;2911:353–60.

Calabresi P, Gubellini P, Centonze D, Picconi B, Bernardi G, Chergui K, et al. Dopamine and cAMP-regulated phosphoprotein 32 kDa controls both striatal long-term depression and long-term potentiation, opposing forms of synaptic plasticity. J Neurosci 2000;2022:8443–51.

Camarini R, Mehmert KK, Hodge CW. Effects of ethanol pretreatment on ethanol self-administration in DBA/2J and C57BL/6 mice. Alcohol Clin Exp Res (Suppl) 2000;24:16A.

Cohen C, Perrault G, Sanger DJ. Evidence for the involvement of dopamine receptors in ethanol-induced hyperactivity in mice. Neuropharmacology 1997;368:1099–108.

Colombo G, Agabio R, Lobina C, Reali R, Vacca G, Gessa G. Stimulation of locomotor activity by voluntarily consumed ethanol in Sardinian alcohol-preferring rats. Eur J Pharmacol 1998;3572–3573:109–13.

Crippens D, White ML, George MA, Jaworski JN, Brunner LJ, Lancaster FE, et al. Gender differences in blood levels, but not brain levels, of ethanol in rats. Alcohol Clin Exp Res 1999;233:414–20.

Dunah AW, Standaert DG. Dopamine D1 receptor-dependent trafficking of striatal NMDA glutamate receptors to the postsynaptic membrane. J Neurosci 2001;2115:5546–58.

Duvauchelle CL, Sapoznik T, Kornetsky C. The synergistic effects of combining cocaine and heroin (''speedball'') using a progressive ratio schedule of drug reinforcement. Pharmacol Biochem Behav 1998; 613:297–302.

Dyr W, McBride W, Lumeng L, Li T, Murphy J. Effects of D1 and D2 dopamine receptor agents on ethanol consumption in the high-alcohol-drinking (HAD) line of rats. Alcohol 1993;103:207–12.

Edwards S, Simmons DL, Galindo DG, Doherty JM, Scott AM, Hughes PD, et al. Antagonistic effects of dopaminergic signaling and ethanol on protein kinase A-mediated phosphorylation of DARPP-32 and the NR1 subunit of the NMDA receptor. Alcohol Clin Exp Res 2002;262: 173–80.

El-Ghundi M, George S, Drago J, Fletcher P, Fan T, Nguyen T, et al. Disruption of dopamine D1 receptor gene expression attenuates alcohol-seeking behavior. Eur J Pharmacol 1998;3532–3533:149–58.

Flores-Hernandez J, Hernandez S, Snyder GL, Yan Z, Feinberg AA, Moss SJ, et al. D(1) dopamine receptor activation reduces GABA(A) receptor currents in neostriatal neurons through a PKA/DARPP-32/PP1 signaling cascade. J Neurophysiol 2000;835:2996–3004.

Friedman HJ, Carpenter JA, Lester D, Randall CL. Effect of alpha-methyl-*p*-tyrosine on dose-dependent mouse strain differences in locomotor activity after ethanol. J Stud Alcohol 1980;411:1–7.

Gonzales RA, Weiss F. Suppression of ethanol-reinforced behavior by naltrexone is associated with attenuation of the ethanol-induced increase in dialysate dopamine levels in the nucleus accumbens. J Neurosci 1998;1824:10663–71.

Gurden H, Takita M, Jay TM. Essential role of D1 but not D2 receptors in the NMDA receptor-dependent long-term potentiation at hippocampal-prefrontal cortex synapse in vivo. J Neurosci (Online) 2000;20022: RC106.

Hodge CW, Samson HH, Haraguchi M. Microinjections of dopamine agonists in the nucleus accumbens increase ethanol-reinforced responding. Pharmacol Biochem Behav 1992;431:249–54.

Hodge CW, Samson HH, Chappelle AM. Alcohol self-administration: further examination of the role of dopamine receptors in the nucleus accumbens. Alcohol Clin Exp Res 1997;216:1083–91.

Holloway FA, King DA, Bedingfield JB, Gauvin DV. Role of context in ethanol tolerance and subsequent hedonic effects. Alcohol 1992;92: 109–16.

Hyytia P, Schulteis G, Koob GF. Intravenous heroin and ethanol self-administration by alcohol-preferring AA and alcohol-avoiding ANA rats. Psychopharmacology 1996;125:248–54.

Ikegami A, Olsen CM, Fleming SM, Guerra EE, Bittner MA, Wagner J, et al. Intravenous ethanol/cocaine self-administration initiates high intake of intravenous ethanol alone. Pharmacol Biochem Behav 2002; 72:787–94.

Itzhak Y, Martin JL. Effects of cocaine, nicotine, dizocipline and alcohol on mice locomotor activity: cocaine-alcohol cross-sensitization involves up-regulation of striatal dopamine transporter binding sites. Brain Res 1999;8182:204–11.

Koob GF. Neural mechanisms of drug reinforcement. Ann NY Acad Sci 1992;654:171–91.

Kuzmin A, Semenova S, Zvartau E, De Vry J. Effects of calcium channel blockade on intravenous self-administration of ethanol in rats. Eur Neuropsychopharmacol 1999;9:197–203.

Lê AD, Tomkins D, Higgins G, Quan B, Sellers EM. Effects of 5-HT3, D1 and D2 receptor antagonists on ethanol- and cocaine-induced locomotion. Pharmacol Biochem Behav 1997;571–572:325–32.

Lewis RM, Levari I, Ihrig B, Zigmond MJ. In vivo stimulation of D1 receptors increases the phosphorylation of proteins in the striatum. J Neurochem 1990;55:1071–4.

Liljequist S, Berggren U, Engel J. The effect of catecholamine receptor antagonists on ethanol-induced locomotor stimulation. J Neural Transm 1981;501:57–67.

Maldve RE, Zhang TL, Ferrani-Kile K, Lippmann M, Schreiber SS, Snyder GL, et al. The DARPP-32 cascade and regulation of the ethanol sensitivity of NMDA receptors in the nucleus accumbens. Nat Neurosci 2002;5:641–8.

Matsuzawa S, Suzuki T, Misawa M, Nagase H. Involvement of dopamine D(1) and D(2) receptors in the ethanol-associated place preference in rats exposed to conditioned fear stress. Brain Res 1999;8352:298–305.

Ng G, George S. Dopamine receptor agonist reduces ethanol self-administration in the ethanol-preferring C57BL/6J inbred mouse. Eur J Pharmacol 1994;2693:365–74.

Phillips TJ, Shen EH. Neurochemical bases of locomotion and ethanol stimulant effects. Int Rev Neurobiol 1996;39:243–82.

Renaldi R, Beninger RJ. The effects of systemic and intracerebral injections of D1 and D2 agonists on brain stimulation reward. Brain Res 1994; 651:283–92.

Risinger FO, Freeman PA, Greengard P, Feinberg AA. Motivational effects of ethanol in DARPP-32 knock-out mice. J Neurosci 2001;211:340–8.

Saal D, Dong Y, Bonci A, Malenka RC. Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. Neuron 2003;374: 577–82.

Samson HH. Initiation of ethanol reinforcement using a sucrose-substitution procedure in food- and water-sated rats. Alcoholism, Clinical and Experimental Research 1986;104:436–42.

Samson HH, Tolliver GA, Haraguchi M, Hodge CW. Alcohol self-administration: role of mesolimbic dopamine. Ann NY Acad Sci 1992;654: 242–53.

Samson HH, Hodge CW, Tolliver GA, Haraguchi M. Effect of dopamine agonists and antagonists on ethanol-reinforced behavior: the involvement of the nucleus accumbens. Brain Res Bull 1993;301–302:133–41.

Snyder GL, Feinberg AA, Huganir RL, Greengard P. A dopamine/D1 receptor/protein kinase A/dopamine- and cAMP-regulated phosphoprotein (Mr 32kDA)/protein phosphatase-1 pathway regulates dephosphorylation of the NMDA receptor. J Neurosci 1998;1824:10297–303.

Souza-Formigoni M, De Lucca E, Hipólide D, Enns S, Oliveira M, Nobrega J. Sensitization to ethanol's stimulant effect is associated with region-specific increases in brain D2 receptor binding. Psychopharmacology (Berl) 1999;1463:262–7.

Weiss F, Lorang MT, Bloom FE, Koob GF. Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants. J Pharmacol Exp Ther 1993;2671:250–8.

Wise RA. The role of reward pathways in the development of drug dependence. Pharmacol Ther 1987;351–352:227–63.

Wise RA, Bozarth MA. A psychomotor stimulant theory of addiction. Psychological Review 1987;944:469–92.

Yim H, Gonzales R. Ethanol-induced increases in dopamine extracellular concentration in rat nucleus accumbens are accounted for by increased release and not uptake inhibition. Alcohol 2000;222:107–15.

Yim HJ, Schallert T, Randall PK, Gonzales RA. Comparison of local and systemic ethanol effects on extracellular dopamine concentration in rat nucleus accumbens by microdialysis. Alcoholism, Clinical and Experimental Research 1998;222:367–74.

Yoshimoto K, McBride WJ, Lumeng L, Li TK. Alcohol stimulates the release of dopamine and serotonin in the nucleus accumbens. Alcohol 1992;91:17–22.