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Nicotine Enhances the Locomotor Stimulating but Not the Conditioned Rewarding Effect of Ethanol in DBA/2J mice

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Abstract

Background—One hypothesis to explain the high rate of nicotine and alcohol co-abuse is that these drugs have enhanced rewarding effects when taken together. The goal of the present work was to use the conditioned place preference (CPP) procedure to determine if nicotine would enhance the development of ethanol-induced CPP.

Methods—The conditioned rewarding effects of nicotine (1 or 2 mg/kg of nicotine tartrate), ethanol (1 g/kg), and nicotine plus ethanol in combination were assessed using a well-established CPP procedure chosen specifically for examining alterations in the development of ethanol-induced CPP by nicotine. In addition, the reference dose procedure was used to directly compare the conditioned rewarding effect of ethanol versus nicotine plus ethanol. DBA/2J mice were used because they are an inbred strain that has repeatedly been shown to develop CPP to ethanol.

Results—Neither dose of nicotine alone produced CPP, whereas ethanol did, using the standard ethanol CPP procedure. The magnitude of ethanol-induced CPP was not affected by co-administration of 1 mg/kg nicotine, but 2 mg/kg nicotine interfered with the development of ethanol-induced CPP. Using the reference dose procedure, there was no significant preference or aversion for either nicotine + ethanol dose combination versus ethanol alone. However, combined nicotine and ethanol had a larger effect on locomotor activity, during the conditioning trials, compared to their additive effect when given alone, consistent with previous data.

Conclusions—These data do not support the hypothesis that nicotine enhances the conditioned rewarding effect of ethanol. This finding differs from the combined locomotor stimulant effects of nicotine and ethanol that were observed in this study and in our previously published work, and suggests that combined stimulant effects of nicotine and ethanol do not predict enhanced reward.

Keywords

Alcohol; CPP; conditioned place preference; locomotor activity; reward

Introduction

The use of nicotine-containing tobacco products and alcohol (ethanol) in excess remains a significant health risk with a high cost to society (Rehm et al. 2009; NIDA 2009; Danaei et al. 2009). Of particular concern is the fact that nicotine and alcohol share a high rate of co-abuse (Anthony & Echeagaray-Wagner 2000; Falk et al. 2006). One hypothesis to explain the high rate of co-abuse is that in combination they have an increased rewarding effect compared to the effect of either drug alone.

We have previously found that nicotine and ethanol have robust combined effects on behavioral traits relevant to addiction. Our previously published work found that doses of nicotine that had limited effects alone significantly enhanced ethanol-induced locomotor stimulation in genotypes of mice vulnerable to this ethanol effect (Gubner et al., 2013). Greater sensitivity to the stimulant effects of ethanol has been implicated as a risk factor for the development of ethanol dependence (Schuckit 1994; Holdstock et al., 2000; King et al., 2002). Locomotor stimulation can be used as a behavioral model to assess activation of brain reward systems (Wise & Bozarth 1987; Phillips & Shen, 1996).

One possible explanation of this finding is that nicotine and ethanol in combination have a greater rewarding effect compared to the rewarding effect of either drug alone. There are several human laboratory studies that provide support for this hypothesis. Ethanol was found to enhance some of the subjective rewarding effects of nicotine, including “smoking satisfaction” and “stimulation” (Glautier et al., 1996; Rose et al., 2004); nicotine administered as a transdermal patch was also found to enhance some of the subjective effects of ethanol, including “euphoria” and “feeling drunk” (Kouri et al., 2004); individuals also reported greater feelings of “pleasure” and being “buzzed” when tobacco and ethanol were co-administered (Piasecki et al., 2011). In the current work, we evaluated the hypothesis that nicotine enhances the rewarding effects of ethanol, using the conditioned place preference (CPP) procedure, a well-established model for assessing drug-conditioned rewarding effects by measuring preference for environmental stimuli that were previously paired with drug administration (Cunningham et al., 2006, 2011; Bardo & Bevins, 2000). We used a CPP procedure that has been well-established, specifically, for measuring the conditioned rewarding effects of ethanol. The use of an animal model allowed for a more controlled method of testing this hypothesis. Drug-associated cues have a strong role in addiction/relapse (Brennan et al., 2011). Drugs of abuse, including nicotine and alcohol, in certain genotypes of mice have been found to produce a preference for the drug-paired environment (Cunningham et al., 2006; Grabus et al., 2006). Mecamylamine, a nicotinic acetylcholine receptor (nAChR) antagonist was previously found to attenuate ethanol-induced CPP in mice (Bhutada et al., 2012), and ethanol-induced euphoria in humans (Chi & de Wit, 2003), indicating a role for nAChRs in mediating the conditioned rewarding effect of ethanol. Given that nicotine is a nAChR agonist, it is possible that nicotine may enhance ethanol reward and contribute to the high rate of tobacco and alcohol co-abuse.

The focus of the current studies was to determine if nicotine can enhance the rewarding effect of ethanol (enhance ethanol-induced CPP). While multiple drugs can induce a CPP, different CPP protocols or genotypes of mice may be more or less effective for detecting a

CPP for a given drug (Cunningham et al., 1992; Cunningham, 2014). The current studies used a CPP procedure that has been well characterized for ethanol, and the DBA/2J inbred strain that is sensitive to ethanol-induced CPP (Cunningham, 2014; Cunningham et al., 1992, 2003, 2006) and highly sensitive to the enhancing effect of nicotine on ethanol-induced locomotor stimulation (Gubner et al., 2013). In contrast, DBA/2J mice have been found to be less sensitive to nicotine-induced CPP (Grabus et al., 2006). However, for the current study, the goal was to determine if nicotine could enhance the development of ethanol-induced conditioned reward, in a similar way to which it enhanced ethanol-induced stimulation in our previous work (Gubner et al., 2013). It was hypothesized that nicotine alone would have limited to no conditioned rewarding effects alone, but would enhance ethanol-induced CPP,

One limitation of the standard CPP procedure is that it can sometimes lack sensitivity to dose-response effects (Cunningham et al., 1992, Bevins, 2005). The reference dose procedure was developed to compare the conditioned rewarding effects of different doses of a drug in the same animals (Grolewski et al., 2008; Bevins, 2005). In the current studies, we used a reference dose procedure in a slightly different way to directly compare the conditioned rewarding effect of nicotine plus ethanol to the conditioned rewarding effect of ethanol alone (see Font et al. 2006 for a similar approach using ethanol and D-penicillamine). An advantage of the reference dose procedure is that it allows for the determination of relative preference by comparing the conditioned rewarding effects of two different treatments that both produce CPP.

Overall, we evaluated the hypothesis that nicotine would enhance ethanol-induced CPP using a standard CPP and reference dose procedure in DBA/2J mice. We predicted that mice treated with nicotine and ethanol would show enhanced preference, compared to mice that received ethanol alone. If this hypothesis is correct, combined use of these drugs may increase vulnerability to develop dependence and provide an explanation for the high rates of nicotine and ethanol co-abuse.

Materials and Methods

Animals

Male DBA/2J mice were purchased from The Jackson Laboratory (Sacramento, CA) and housed 2-4 per cage. All mice were allowed to acclimate for at least 2 weeks after arrival before behavioral testing, which began when mice were 55-60 days old. All mice were maintained in standard mouse shoebox cages (28.5 L × 17.5 W × 12 H cm) lined with Bed-o'Cobs® bedding (The Andersons, Inc., Maumee, OH, USA) and had *ad libitum* access to water and food (LabDiet® 5001, PMI Nutrition International LLC, St. Louis, MO, USA) that was purchased from Animal Specialties Inc. (Hubbard, OR, USA). All mice were experiment- and drug-naïve prior to testing, and behavioral testing was conducted during the light phase of the 12:12 h light:dark cycle (lights on at 0600 h), between 0800 and 1600 h. All CPP conditioning and preference test sessions were run Monday through Friday.

Drugs

Ethyl alcohol was purchased from Decon Laboratories Inc. (King of Prussia, PA, USA). Nicotine tartrate salt was purchased from Sigma Aldrich (St. Louis, MO, USA). All drugs were prepared in physiological (0.9%) saline (Baxter Healthcare Corp., Deerfield, IL, USA) and administered as IP (intraperitoneal) injections in a volume of 20 ml/kg. Nicotine and ethanol combined doses were delivered together in a cocktail (wt/vol solution) matching our previously published work (Gubner et al., 2013). Doses of nicotine are expressed as mg/kg of the tartrate salt (1 mg nicotine tartrate = 0.33 mg freebase nicotine).

Procedures

Standard CPP

The effect of nicotine on the development of ethanol-induced CPP was measured using a standard ethanol CPP procedure (Cunningham et al., 1992; 2003; 2006). The CPP parameters used in these studies were chosen to maximize the detection of the rewarding effects of ethanol, as the goal of this research was to determine the effect of nicotine on ethanol-induced CPP, rather than to determine if there would be a summation of ethanol-reward and nicotine-reward. The CPP chambers (San Diego Instruments, San Diego, CA, USA) consisted of clear plastic walls 30 L × 15 W × 15 H cm equipped with exchangeable floor panels. Three different floor types were used: a solid black plastic acrylic floor; a “grid” floor constructed of 2.3 mm stainless steel rods mounted 6.4 mm apart; and a “hole” floor constructed of a stainless steel panel with 6.4 mm round holes aligned with 9.5 mm staggered centers. CPP chambers were housed in illuminated and ventilated sound attenuating chambers (AccuScan Instruments, Inc., Columbus, OH, USA). Activity and location of the mouse were measured by photocell beam interruptions recorded by a fully automated system. This experiment was designed so that mice received the same handling and injection procedures on all conditioning and test days. On all days, mice were injected immediately before placement in the CPP apparatus.

On day 1, to habituate the mice to handling and the CPP apparatus, all mice were injected with saline and immediately placed in the CPP chambers for a 5-min session with black plastic flooring on both sides of the chamber and free access to both sides of the chamber. This flooring was used to avoid exposing the mice to the floor types (grid and hole) that served as associative cues during subsequent conditioning sessions. On the next 4 alternating days, mice were conditioned with drug (ethanol, nicotine, or ethanol+nicotine; see Table 1 for dose groups and treatment) and saline, during 5-min sessions with a single floor type (grid or hole) on both sides of the chamber. The first preference test was run after these 4 sessions, and a second preference test was run after 4 additional conditioning sessions. There were a total of 4 ethanol conditioning and 4 saline conditioning sessions. The 1 g/kg dose of ethanol was used with 1 and 2 mg/kg nicotine because these were the doses used in our previous work showing greater than additive effects on locomotor activity (Gubner et al., 2013). In addition, it was desirable to use a lower dose of ethanol that would be less likely to induce maximal CPP on its own (Font & Cunningham, 2012). On the preference test days, mice were injected with saline (no drug on board) and placed in the CPP apparatus for 30-min with both floor types (grid and hole) present. There were two preference test days, each

occurring after 4 conditioning trials (2 drug and 2 saline). Groups were counterbalanced for floor type associated with ethanol, side on which the grid floor was placed during the preference test, and whether ethanol or saline was the first conditioning session.

Reference dose procedure

The reference dose CPP procedure was used to directly compare the conditioned rewarding effect of nicotine in combination with ethanol to the conditioned rewarding effect of ethanol alone. One floor type was paired with ethanol alone and the alternative floor type was paired with nicotine in combination with ethanol (either E1+N1 or E1+N2) during conditioning (see Table 1). Although E1+N1 and E1 groups showed comparable levels of preference in the standard procedure, we hypothesized that the reference dose procedure would be more sensitive to differences in reward and mice would prefer the E1+N1-paired floor over the E1-paired floor. However, because the E1+N2 dose did not induce a CPP in the standard procedure (unlike E1+N1 and E1 alone), we hypothesized that mice would show a greater preference for the E1-paired cues.

Data analysis

All statistical analyses were performed using Statistica 12 software (StatSoft, Tulsa, OK, USA). Data were analyzed by factorial ANOVA, with repeated measures (RM-ANOVA) when appropriate. Significant interactions involving multiple factors were followed by ANOVA including fewer factors to determine the sources of interaction. Two-way interactions were interpreted using simple main effects analysis and Newman-Keuls post-hoc mean comparisons when appropriate. Effects were considered significant at an alpha level of 0.05 or less. Consistent with previous studies for ethanol (Cunningham et al., 1992; 2003; 2006), the dependent variable used to determine the expression of a CPP was seconds spent on the grid floor divided by total duration of the test session in minutes (i.e. sec/min on the grid floor). The advantage of transforming the data from “total time” to “sec/min” on the grid floor is that it generates a full range of possible outcomes (e.g. 0 sec/min = no preference for grid floor; 60 sec/min = complete preference to grid floor). This transformation also creates a variable that is independent of the preference test duration making it easier to compare results between experiments using different preference test lengths (Cunningham et al., 2003). A CPP was determined to be present if mice from the G+ group (drug was paired with the grid floor) spent more time on the grid floor compared to the G- group (drug paired with the hole floor). This between group comparison allows for detection of CPP within each dose and controls for potential floor type bias. This analysis was run using an analysis of variance (ANOVA) with sec/min on the grid floor during the preference tests as the dependent variable and conditioning group (G+ or G-) and dose group as the independent variables.

Results

Effects of nicotine on the development of ethanol-induced CPP

Neither dose of nicotine (1 or 2 mg/kg nicotine tartrate, N1 or N2) produced a significant CPP when mice were tested after 2 or 4 drug-conditioning trials (Fig 1). This is consistent with published work examining nicotine CPP in DBA/2J mice, showing low sensitivity of

this strain (Grabus et al., 2006). Furthermore, there was no significant difference in locomotor activity between the N1 and N2 groups during either of the preference test sessions when mice were treated with saline prior to testing (Table 2).

For the E1 and E1+N1 groups, there was significant preference on the second but not the first preference test; a similar magnitude of preference was seen in these two groups. There was no significant CPP seen for the E1+N2 dose combination on either test day (Fig. 2). These results were supported by a significant interaction of dose group and grid group (G+ and G-) for sec/min on the grid floor for the second preference test ($F_{[2,90]} = 4.05$, $p < 0.05$), and follow-up analyses supporting a significant difference between grid groups for the E1 and E1+N1 groups, but not E1+N2 group. Thus, 1 mg/kg nicotine did not enhance the development of ethanol-induced CPP, and 2 mg/kg nicotine interfered with the development of ethanol-induced CPP. There were no significant differences between any of these groups for locomotor activity on either of the preference test days, when mice were treated with saline prior to testing (Table 2).

Locomotor activity effects during conditioning sessions in the standard CPP procedure

First, data were examined for drug treatment effects using repeated measures analysis to compare locomotor activity data between saline and drug conditioning trials for each drug group. There were no interactions with trial, but activity levels were higher during the drug conditioning trials, compared to the saline conditioning trials for all groups (all $p < 0.001$). Next, data were examined for differences among groups. There were significant differences in locomotor activity among the groups during the drug ($F_{[4,123]} = 41.76$, $p < 0.001$), but not saline conditioning trials. There were no significant changes in activity across trials. Overall, E1+N1 and E1+N2 group mice had greater locomotor activity levels compared to E1 group mice ($p < 0.001$). Groups treated with N1 and N2 had lower locomotor activity levels compared to E1 group mice ($p < 0.01$). Locomotor activity data are shown for each conditioning trial in Fig 3.

CPP results using the reference dose procedure

For the reference dose CPP procedure, no significant preference or aversion was detected for either dose combination (E1+N1 or E1+N2) versus E1 alone (Fig 4). Thus, the reference dose procedure did not distinguish a difference in conditioned rewarding effect of ethanol alone versus ethanol in combination with nicotine. There were no significant differences in locomotor activity among the groups on either of the preference test days, when mice were treated with saline prior to testing (Table 2).

Locomotor activity effects during conditioning sessions for the reference dose CPP procedure

For the reference dose groups, data were first examined for drug treatment effects using repeated measures analysis to compare locomotor activity data between E1 alone and nicotine plus ethanol conditioning trials. There were no interactions with trial, but activity levels were higher during the conditioning trials when mice received nicotine in combination with ethanol, compared to the ethanol alone conditioning trials (both $p < 0.001$). Next, data were examined for differences among groups. There were also significant

differences in locomotor activity between the two reference dose groups (E1+N1 and E1+N2) during the conditioning trials when mice received nicotine in combination with ethanol, but not during the conditioning trials when mice received ethanol alone (Fig 5). During the nicotine plus ethanol conditioning trials there was a significant interaction between dose group and trial ($F_{[3,186]} = 2.67, p < 0.05$). Mice treated with E1+N2 but not E1+N1 had significantly higher locomotor activity on the third and fourth conditioning trials compared to the first conditioning trial. This suggests mice expressed a sensitized locomotor response to E1+N2 after repeated exposure. This also differed from what was found in the standard CPP study, when mice were treated with E1+N2 and saline on alternating days.

Discussion

The goal of the present work was to determine if nicotine enhances the development of ethanol-induced CPP, first, using an established CPP procedure for ethanol with DBA/2J mice (Cunningham et al., 1992; 2003; 2006). Using the standard CPP procedure, a 1 mg/kg dose of nicotine tartrate did not significantly affect the conditioned rewarding effect of 1 g/kg ethanol; the E1+N1 and E1 alone groups both developed similar levels of CPP. However, the 2 mg/kg dose of nicotine tartrate interfered with the development of ethanol-induced CPP, so that no significant floor preference was seen for the E1+N2 group. These data do not support our original hypothesis that nicotine would enhance the conditioned rewarding effect of ethanol. Rather, they suggest that the higher 2 mg/kg dose of nicotine tartrate might have interfered with ethanol-induced CPP based on its own aversive motivational effect (Risinger & Oakes, 1995) or by interfering with the development of the cue-ethanol association (Gould & Wehner 1999). Nicotine did enhance the locomotor stimulant effects of ethanol during conditioning sessions, which is consistent with our previously published work in DBA/2J mice (Gubner et al., 2013). Thus, combined effects of nicotine and ethanol on locomotor activity did not correspond with amount of conditioned reward. Exemplifying this is the fact that the E1+N2 dose combination did not induce a CPP but did cause enhanced locomotor activity compared to E1 alone.

One limitation of the standard CPP procedure is that it does not consistently detect dose-dependent effects. The reference dose CPP procedure was used here to address this issue because previous studies have suggested that it increases sensitivity to dose-related effects of some drugs, including ethanol (Grolewski et al., 2008; Bevins 2005). In this case, the reference dose procedure was used to directly compare the conditioned rewarding effects of E1+N1 and E1+N2 versus ethanol alone; however, there was no significant preference or aversion for either dose combination (E1+N1 or E1+N2) versus E1 alone. Thus, these data do not support our hypothesis that the conditioned rewarding effect of nicotine combined with ethanol is greater than that of ethanol alone.

It could be argued that ceiling effects prevented the ability to detect an enhanced CPP by nicotine plus ethanol due to the fact that 1 g/kg ethanol alone produced a CPP. However, in the current study, 1 g/kg ethanol produced a significant CPP after 4, but not 2, drug conditioning trials (i.e. on Test 2, but not Test 1). Thus, there was no ceiling effect that prevented the detection of enhanced CPP on the first preference test. Further, similar studies using 2 g/kg ethanol have found significant CPP after only 2 pairings and a larger difference

between the Grid+ and Grid- groups after 4 pairings (Pina & Cunningham, 2014), compared to the difference seen in our study. This indicates that maximal CPP was not reached, using the lower 1 g/kg dose of ethanol.

The doses of nicotine used in the current study were based on our previously published work examining the combined effects of nicotine and ethanol in DBA/2J mice (Gubner et al., 2013); specifically doses with minimal effect alone, but that enhanced the locomotor stimulant response to ethanol. However, because the higher dose of nicotine interfered with the development of ethanol-induced CPP and there was no enhancement induced by 1 mg/kg nicotine, we cannot rule out the possibility that lower doses of nicotine could have a different effect. It is also important to point out that in the current study, neither dose of nicotine produced a CPP or aversion.

There was no significant difference between the dose groups for locomotor activity on the preference test day, when all mice were treated with saline. Locomotor activity during the preference test has been found to interfere with the expression of CPP (Cunningham, 1995; Gremel & Cunningham, 2007), but this was not a contributing factor in the current studies. However, nicotine and ethanol in combination enhanced locomotor activity during the conditioning trials, compared to ethanol alone. This finding was consistent with our previously published work (Gubner et al., 2013), and could have influenced the development of CPP. For example, DBA/2J mice have been found to be sensitive to trial duration for cocaine-induced CPP (Cunningham et al., 1999). That study found that DBA/2J mice were sensitive to cocaine-induced locomotor stimulation and longer conditioning trial durations produced CPP that was not observed with shorter trial durations, when cocaine stimulation was maximal; it is possible that stimulation interfered with conditioning. However, shorter trial durations, during the rising phase of the ethanol dose response curve, have been found to produce the most robust ethanol-induced CPP in DBA/2J mice (Cunningham & Prather, 1992). This time period corresponds with maximal stimulation by ethanol in DBA/2J mice (Scibelli & Phillips 2009), suggesting that locomotor stimulation does not always interfere with conditioning. In the current study, both the E1+N1 and E1+N2 groups had similar levels of higher locomotor activity during the conditioning trials, compared to the E1 group in the standard CPP study. However, the E1+N1, but not E1+N2, group showed a CPP. Furthermore, both the E1+N1 and E1 groups developed similar levels of CPP. Thus, there was no correspondence between amount of activation during conditioning and magnitude of preference. However, the possibility that the enhanced locomotor activity during conditioning may have blunted the development of maximal CPP in the E1+N1 group cannot be completely ruled out.

In the standard CPP procedure, there was no change in locomotor response across the locomotor activity sessions, indicating that mice did not develop significant drug-induced behavioral sensitization across trials. The 1 g/kg dose of ethanol used in that study is lower than the doses of 2 and 2.5 g/kg ethanol that have typically been used to induce behavioral sensitization in DBA/2J mice (Phillips et al., 1994; Meyers et al., 2005; Meyers & Phillips 2003), so this result was not unexpected. Data from the standard CPP experiment also suggested that nicotine combined with E1 did not induce sensitization. Interestingly, in the reference dose procedure, sensitization developed across E1+N2 treatment days, with a

larger locomotor response on the third and fourth conditioning trials, an effect not seen across E1+N1 treatment days. It is important to remember that for the reference dose groups, ethanol alone and ethanol plus nicotine were administered on alternating conditioning days so that the total ethanol exposure was greater in that study, compared to the standard CPP study. This could account for the sensitization seen across E1+N2 treatment days. This suggests that mice developed neuroadaptations that resulted in behavioral sensitization, only when the higher dose of nicotine was given with ethanol, combined with alternating days of ethanol alone.

The standard CPP procedure used in the current study is a well-established model for measuring ethanol-induced CPP in DBA/2J mice (Cunningham et al., 1992; 2003; 2006). However, nicotine CPP studies have primarily used C57BL/6J (Walters et al., 2006; Grabus, et al., 2006; Korkosz et al., 2006) or an outbred strain (Risinger & Oakes 1995). Further, the conditioned rewarding effect of nicotine is genotype-dependent, with nicotine-induced CPP found in C57BL/6J, but not DBA/2J, mice (Grabus et al., 2006). This strain difference is opposite to what is found for ethanol, with greater CPP in DBA/2J versus C57BL/6J mice (Cunningham 2014; Cunningham et al. 1992). DBA/2J mice were chosen for the current studies because it has been well established that they are sensitive to ethanol-induced CPP. However, DBA/2J mice do not readily drink ethanol, unlike C57BL/6J mice; in fact, they avoid it (Belknap et al., 1977). On the other hand, they will self-infuse ethanol when delivered intravenously (Grahame & Cunningham 1997) or intragastrically (Fidler et al., 2011). This makes DBA/2J mice better for modeling certain ethanol traits versus others and it is possible that greater sensitivity to the conditioned rewarding effect of nicotine combined with ethanol would be detected with a different genotype of mouse, such as C57BL/6J mice.

A conditioning trial duration of 5 min has been found to be the most effective at inducing a CPP to ethanol (Cunningham & Prather 1992). However, longer conditioning trial durations have been found to work better for other drugs like cocaine (Cunningham et al., 1999). Although, to our knowledge, this has not been extensively examined for nicotine, the conditioning trial durations that have been previously used to produce nicotine-induced CPP in mice were 15, 20, or 30 min (Walters et al., 2006; Grabus, et al., 2006; Risinger & Oakes 1995; Korkosz et al., 2006). Thus, it is possible that altering parameters of the CPP procedure, such as duration of the conditioning trials, would allow for greater sensitivity to detect the conditioned rewarding effect of nicotine and ethanol in combination. Clearly, the overall parameters are complicated. For example, as trial duration is adjusted to maximize the conditions for one drug, conditions for detecting reward may be non-ideal for the other. The focus of this work was on the effect of nicotine on ethanol reward and the procedures were designed to specifically maximize detection of the rewarding effects by presenting cues immediately after administering drug and presenting the cues for a relatively short period; for ethanol, this has been found to be associated with the highest probability of detecting a place preference.

Another factor to consider is that, in the current study, nicotine and ethanol were co-administered, rather than given sequentially. It is possible that pretreating mice with nicotine before ethanol or vice versa could produce different results. In humans, use of alcohol has been found to increase smoking behavior and urge to smoke (King & Epstein 2005; Epstein

et al., 2007; King et al., 2009), though the effect of nicotine on alcohol consumption and craving remains less clear. To our knowledge, only one CPP study in mice has been reported in which ethanol and nicotine treatment were temporally separated (Korkosz et al., 2006). In that study, C57BL/6J mice were pretreated with ethanol 5 min before an injection of nicotine on conditioning days. Overall, pretreatment with 1 g/kg ethanol resulted in a non-significant enhancement of nicotine-induced CPP, compared to that seen in animals conditioned with nicotine alone. It is possible that giving nicotine before alcohol, rather than simultaneously could produce different effects than those seen here.

This work evaluated the hypothesis that nicotine and alcohol are co-abused because enhanced ethanol reward occurs in the presence of nicotine. However, it is also possible that other reasons explain or contribute to this co-abuse. For example, nicotine may help to alleviate the sedating or motor incoordinating effects of ethanol or reduce cognitive impairments induced by chronic alcohol abuse. These hypotheses remain to be explored in future research.

Overall, the results of the current study suggest that nicotine does not enhance the development of ethanol-induced CPP in DBA/2J mice. This finding differs from the synergistic-like stimulant effects of combined nicotine and ethanol that were observed in this study and in our previously published work (Gubner et al., 2013). Sensitivity to the stimulant effects of ethanol has been identified as a genetically mediated factor associated with risk to develop alcohol dependence. However, our data suggest that the enhanced locomotor stimulant effects of nicotine and ethanol do not correspond with enhanced reward measured by CPP. Additional research is needed to determine the mechanism underlying the combined locomotor stimulant effects of nicotine and ethanol. Activation of the mesolimbic dopamine system appears to be a common mechanism for drug reward and stimulation/euphoria (Wise & Bozarth 1987; Soderpalm & Ericson 2013) and is an obvious mechanism to consider. However, that nicotine and ethanol have greater than additive (synergistic-like) effects on acute locomotor stimulation suggests the possibility that these drugs are acting via at least somewhat different mechanisms to produce their combined effect. Nicotine alone had limited to no effects on any of the behaviors measured in DBA/2J mice. This suggests that, rather than both drugs activating the mesolimbic dopamine system, the drugs in combination may have unique interactive effects (e.g., nicotine could accentuate the ability of alcohol to alter the activity of a relevant mechanism). Regardless of what the underlying mechanism may be, this nicotine enhancement of ethanol's stimulant effects may increase risk for ethanol dependence in individuals who co-morbidly smoke and drink. However, this may not be through an enhancement of ethanol's conditioned rewarding effects.

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Figure 1.

Neither dose of nicotine (1 or 2 mg/kg) produced a significant CPP during either Test 1 (A) or Test 2 (B) in the standard CPP procedure. Shown are means $\pm$ SEM sec/min spent on the grid floor during a 30-min preference test after saline treatment ($n=8$ /grid group/dose group). N1 and N2 = 1 or 2 mg/kg nicotine tartrate; G+ = nicotine paired with grid floor; G- = nicotine paired with hole floor.

Figure 2.

For the ethanol alone and ethanol plus nicotine groups, there was a significant dose by grid group interaction during Test 2 (B) in the standard CPP procedure [$F_{(2,90)}=4.05, p<0.05$]. Both E1 and E1+N1 produced significant CPP, whereas E1+N2 did not. No significant CPP was found for Test 1 (A). Shown are means $\pm$ SEM sec/min spent on the grid floor during a 30-min preference test after saline treatment ($n=16/\text{grid group/dose group}$). N1 and N2 = 1 or 2 mg/kg nicotine tartrate; E1 = 1 g/kg ethanol; G+ = drug paired with grid floor; G- = drug paired with hole floor. ** $p<0.01$.

Figure 3.

Locomotor activity levels are shown for the 4 CS+ (drug) conditioning trials (A) and for the 4 CS- (saline) conditioning trials (B), for the standard CPP procedure. Shown are means $\pm$ SEM total beam breaks during each 5 min conditioning session. During the drug (CS+) conditioning trials there was a significant effect of dose ($F_{[4,123]} = 41.76$, $p < 0.001$). Mice treated with E1+N1 and E1+N2 had greater locomotor activity compared to the E1 group ($P < 0.001$). Groups treated with N1 and N2 had lower locomotor activity compared to the E1 group ($P < 0.01$). There were no significant differences in level of locomotor activity between the dose groups during the saline (CS-) conditioning trials. For each group, conditioning sessions were alternated between E1 and E1+N2 or E1+N2 trials. Order of CS+ and CS- trials was counterbalanced among animals for each dose group. N1 and N2 = 1 or 2 mg/kg nicotine tartrate; E1 = 1 g/kg ethanol.

Figure 4.

For the reference procedure dose groups, there was not a significant preference or aversion for either E1+N1 or E1+N2 versus E1 alone during either Test 1 (A) or Test 2 (B). Shown are means $\pm$ SEM sec/min spent on the grid floor during a 30-min preference test after saline treatment ($n=16$ /grid group/dose group). N1 and N2 = 1 or 2 mg/kg nicotine tartrate; E1= 1 g/kg ethanol; G+ = E1+N1 or E1+N2 paired with grid floor and E1 paired with hole floor; G- = E1+N1 or E1+N2 paired with hole floor and E1 paired with grid floor.

Figure 5.

Locomotor activity levels for the reference dose CPP procedure are shown for the 4 nicotine plus ethanol (E1+N2 or E1+N2) conditioning trials (A), and for the 4 ethanol alone (E1) conditioning trials (B). Shown are means $\pm$ SEM total beam breaks during each 5 min conditioning session. During the nicotine plus ethanol conditioning trials, there was a significant dose group by trial interaction ($F_{[3,186]} = 2.67$, $p < 0.05$). The groups treated with E1+N2 but not E1+N1 had significantly higher locomotor activity on the third and fourth conditioning trials compared to the first conditioning trial (** $p < 0.01$). There were no significant group or trial effects during the E1 conditioning trials. For each dose group, conditioning sessions were alternated between E1 alone and E1+N2 or E1+N2 trials. Order of trials was counterbalanced among animals for each dose group. N1 and N2 = 1 or 2 mg/kg nicotine tartrate; E1 = 1 g/kg ethanol.

Table 1
Outline of experimental groups for the CPP studies

Dose group	Grid Group	Habituation	Conditioning	Preference Test
Standard CPP groups				
N1 versus SAL	G+ (n=8)	SAL	N1 or SAL	SAL
	G- (n=8)			
N2 versus SAL	G+ (n=8)	SAL	N2 or SAL	SAL
	G- (n=8)			
E1 versus SAL	G+ (n=16)	SAL	E1 or SAL	SAL
	G- (n=16)			
E1+N1 versus SAL	G+ (n=16)	SAL	E1+N1 or SAL	SAL
	G- (n=16)			
E1+N2 versus SAL	G+ (n=16)	SAL	E1+N2 or SAL	SAL
	G- (n=16)			
Reference dose CPP groups				
E1+N1 versus E1	G+ (n=16)	SAL	E1+N1 or E1	SAL
	G- (n=16)			
E1+N2 versus E1	G+ (n=16)	SAL	E1+N2or E1	SAL
	G- (n=16)			

“E1” = 1 g/kg ethanol. “SAL” = 0.9% Saline. “N1” and “N2” = 1 or 2 mg/kg of nicotine tartrate. “G+” = drug paired with the grid floor, saline paired with the hole floor. “G-” = drug paired with hole floor, saline paired with grid floor.

Table 2
Locomotor activity during the two preference tests

Dose group	1st Preference test	2nd Preference test
Standard CPP groups		
N1 versus SAL	1424.8 ± 138.6	1112.3 ± 78.0
N2 versus SAL	1496.3 ± 144.1	1215.3 ± 57.5
E1 versus SAL	1362.6 ± 60.8	1170.4 ± 45.1
E1+N1 versus SAL	1259.6 ± 48.9	1109.1 ± 36.5
E1+N2 versus SAL	1300.2 ± 53.9	1124.9 ± 38.7
Reference dose CPP groups		
E1+N1 versus E1	1229.8 ± 37.2	1199.8 ± 44.7
E1+N2 versus E1	1282.8 ± 44.7	1283.7 ± 44.3

Shown are beam breaks during the two 30 min preference tests (means ± SEM). "E1" = 1 g/kg ethanol. "SAL" = 0.9% Saline. "N1" and "N2" = 1 or 2 mg/kg of nicotine tartrate. Preference test 1 after 4 conditioning trials. Preference test 2 after 8 conditioning trials.