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Ethanol does not affect discriminative-stimulus effects of nicotine in rats ☆

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Abstract

The effects of ethanol were evaluated in rats trained to discriminate 0.4 mg/kg of nicotine from saline under a fixed-ratio 10 schedule of food delivery. Ethanol (0.1–1 g/kg, i.p.) did not produce any nicotine-like discriminative effects and did not produce any shift in the dose–response curve for nicotine discrimination. Thus, the ability to discriminate nicotine's effects does not appear to be altered by ethanol administration. However, the high dose of 1 g/kg ethanol, given either alone or in combination with nicotine, markedly depressed food-maintained responding. This later effect was associated in some rats with an attenuation of the discriminative-stimulus effects of the training dose of nicotine. This suggests that previous reports of increased tobacco smoking following ethanol consumption in humans are connected, in some way, with an increase in motivation to consume nicotine that is produced by ethanol, rather than with a decrease in the subjective response to nicotine.

Keywords: Subjective effect; Drug discrimination; Reward; Nicotine; Ethanol; (Rat)

1. Introduction

There is a strong association between drinking alcohol and smoking cigarettes (Istvan and Matarazzo, 1984; Larsson and Engel, 2004; Le Foll et al., in press-b). This positive correlation between alcohol and tobacco consumption applies across various demographic variables (age, race, and socioeconomic status) and has been replicated in different countries (Zacny, 1990). Although a very large majority of alcoholics smoke cigarettes and although alcoholism is far more frequent among smokers than non-smokers (DiFranza and Guerrera, 1990), little is known of the ways in which these drugs interact in the brain (Collins, 1990; Larsson and Engel, 2004; Zacny, 1990).

Experimental studies in humans suggest that consumption of one substance may have an effect on consumption of the other substance. Notably, volunteer studies have shown that consumption of alcohol increases the number of cigarettes smoked (Griffiths et al., 1976; Henningfield et al., 1983, 1984) and alters smoking topography by increasing puff size (Mintz et al., 1985; Nil et al., 1984). Several mechanisms may be involved in the ability of ethanol to increase tobacco smoking (Zacny, 1990). A likely explanation is that alcohol and nicotine, like other drugs of abuse, interact with the same brain reward systems that trigger the desire to take drug (Imperato and Di Chiara, 1986; Imperato et al., 1986). This idea is supported by the fact that abused drugs have the common effect of producing elevation of dopamine levels in the nucleus accumbens (Di Chiara and Imperato, 1988; Koob, 1992) and that similar neurobiological circuits may underlie the reinforcing effects of drugs from different pharmacological classes (Le Foll and Goldberg, 2005a,b). Since elevations in dopamine levels in the nucleus accumbens

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are implicated in the initiation of drug-seeking behavior (Phillips et al., 2003), nicotine and ethanol exposure may trigger the desire to consume drug. This desire to consume drug has been shown to be triggered in both animals and humans by drug exposure, stressful events or presentation of drug-associated stimuli (Le Foll and Goldberg, 2005b, in press; Shalev et al., 2002). Another explanation is that ethanol, which possess central nervous system depressant effects (Koelega, 1995), may reduce the subjective effects of nicotine and therefore produce a compensatory increase in puff size and cigarette intake.

The subjective effects of drugs are most frequently assessed in humans through the use of subject-rating scales but may be evaluated in experimental animals using two-lever choice drug-discrimination procedures (Le Foll and Goldberg, 2004). For this purpose, rats can be trained under a schedule of food delivery to respond on one lever during sessions following an injection of a particular dose of drug and on another lever during sessions following an injection of saline. This procedure allows assessment of 'drug-like' subjective responses by determining whether different drugs or different doses of the training drug result in the animal preferentially pressing the drug-associated lever. This procedure also allows assessment of the effects of drug treatments on the animal's ability to discriminate the effects of administered nicotine. Here, we used the two-lever drug discrimination choice procedure as an animal model for assessing ethanol's ability to alter the psychomotor and subjective effects of nicotine.

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats ($n=24$; 230–260g) were obtained from Charles River (Wilmington, MA) and housed in a temperature- and humidity-controlled room. Animals used in this study were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC) and all experiments were conducted in accordance with the guidelines of the Institutional Care and Use Committee of the Intramural Research Program, National Institute on Drug Abuse (NIDA), National Institutes of Health and the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council, 2003). Experiments were conducted during the light phase of a 12-h/12-h light/dark cycle (lights on at 0700 hours). Rats were housed individually and water was available ad libitum. A diet restriction was maintained throughout the study (3 pellets/day).

2.2. Drugs

Nicotine [(−)-nicotine hydrogen tartrate] was purchased from Sigma Chemical Company (St Louis, MO, USA). The pH of the nicotine solution was adjusted to 7.0 with dilute NaOH. Nicotine was administered subcutaneously (s.c.) in a volume of 1.0 ml/kg. Ethanol and nicotine were diluted in saline. A 0.1 g/ml ethanol solution was used and injected intraperitoneally (i.p.) for the tests involving ethanol doses of 0.1 g/ml and higher. All doses are expressed as milligrams of free base per kilogram body weight.

2.3. Nicotine discrimination

2.3.1. Apparatus

Twelve standard operant-conditioning chambers (Coulbourn Instruments, Lehigh Valley, PA) were used. Each chamber contained a white house light and two levers, separated by a recessed tray into which a pellet dispenser could deliver 45 mg of food pellets (F0021, Bioserv, Frenchtown, NJ). Each press of a lever with a force of 0.4 N or more through 1 mm was recorded as a response and was accompanied by an audible click. The operant-conditioning chambers were controlled by microcomputers using the MED Associates MED-PC software package (MED Associates Inc., East Fairfield, VT).

2.3.2. Procedure

Training of the rats have been previously reported (Le Foll and Goldberg, 2004; Le Foll et al., 2005). Rats were trained, as described previously, under a discrete-trial schedule of food pellet delivery to respond on one lever after an injection of a training dose of 0.4 mg/kg nicotine and on the other lever after an injection of 1 ml/kg of saline vehicle ($n=24$). Injections of nicotine or saline were given subcutaneously 10 min before the start of the session. At the start of the session, a white house light was turned on and in its presence the rats were required to make ten consecutive responses (fixed-ratio 10 schedule of food delivery) on the lever appropriate to the pre-session treatment. The completion of ten consecutive responses on the appropriate lever produced delivery of 45 mg food pellet and initiated a 45-s time-out during which lever-press responses had no programmed consequences and the chamber was dark. Responses on the incorrect lever had no programmed consequences other than to reset the fixed-ratio requirement on the correct lever. After each time-out, the white house light was again turned on and the next trial began. Each session ended after completion of 20 fixed-ratio trials or after 30 min elapsed, whichever occurred first. Discrimination-training sessions were conducted 5 days per week under a double alternation schedule (i.e. DDSSDDSS, etc., D=drug; S=saline). Training continued until there were eight consecutive sessions during which rats completed at least 90% of their responses during the session on the appropriate lever and no more than four responses occurred on the inappropriate lever during the first trial. Test sessions with other doses of nicotine and with ethanol were then initiated.

2.3.3. Test session

A range of doses of ethanol were substituted for the training dose of nicotine. Ethanol was also administered together with various doses of nicotine to assess possible alteration of the dose–response curve for nicotine discrimination. Test sessions were identical to training sessions, with the exception that both levers were active and ten consecutive responses on either one of the two levers resulted in the delivery of a food pellet. Switching responding from one lever to the other lever resets the ratio requirement. In a test phase, a single alternation

schedule was introduced and test sessions were usually conducted on Tuesdays and Fridays. Thus, a 2-week sequence starting on Monday was DTSDTSTDST (T=test). In this way, test sessions occurred with equal probability after saline and drug sessions. Test sessions were conducted only if the criterion of 90% accuracy and not more than 4 incorrect responses during the first trial was maintained in the two preceding training sessions.

2.3.4. Data analysis

Two independent measures of behavior were collected in the nicotine-discrimination study: a measure of discrimination performance expressed as the percentage of nicotine-appropriate responses and a measure of motor performance expressed as response rate. The percentage of nicotine-appropriate responses during each session (training or test) reflected the percentage of the number of responses emitted on the nicotine-appropriate

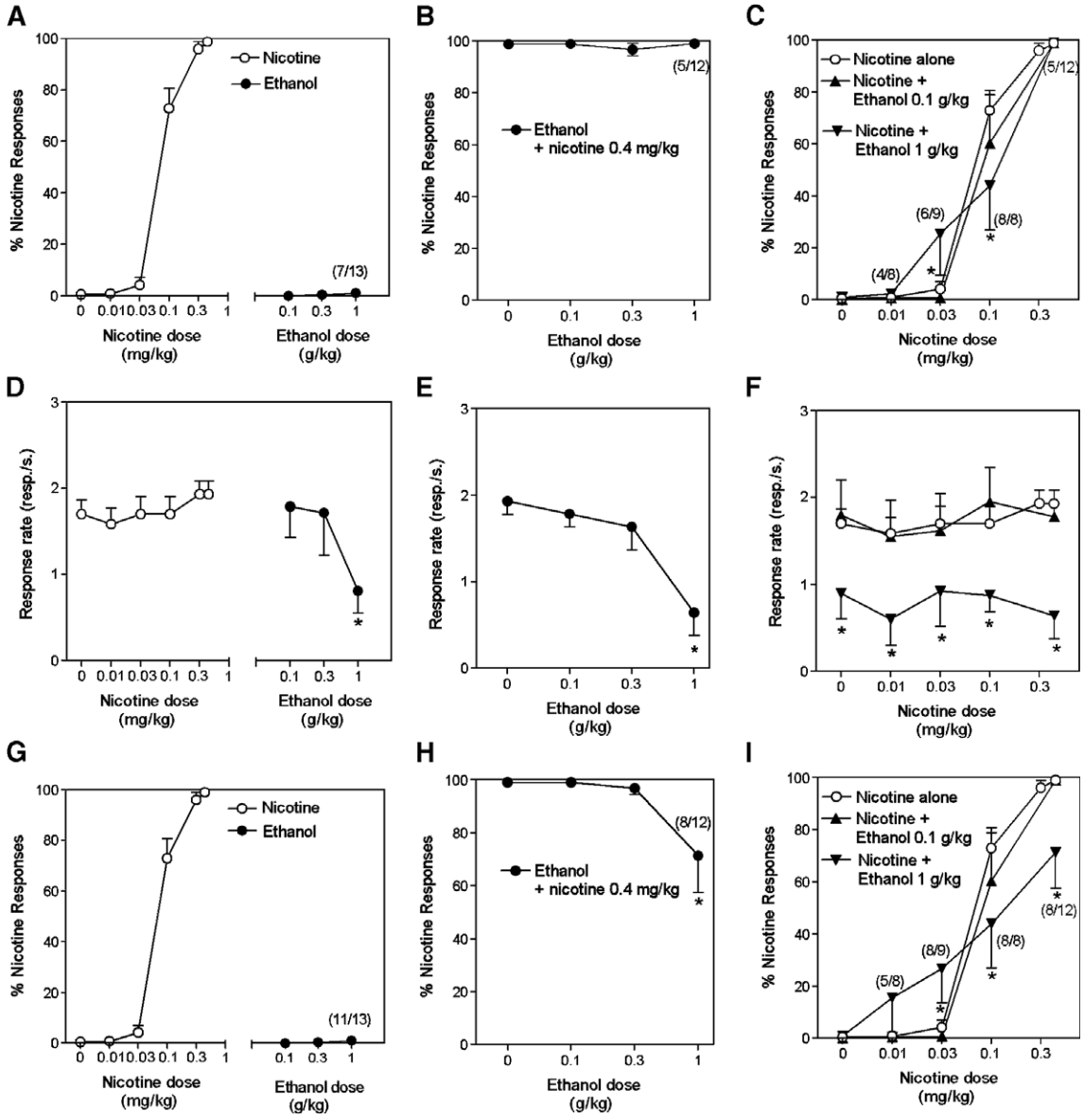

Fig. 1. No effect of ethanol on nicotine discrimination. Data are means ± S.E.M of the percentage of responses on the lever associated with nicotine administration (upper and lower panels) or rates of responding (middle panels) during the session. The discrimination performance of the rats is indicated in both the upper panels (A, B, and C; results analyzed with the first criteria based on exclusion of the rats with response rates below 0.5 responses/s) and the lower panels (G, H, and I; results analyzed with the second criteria based on exclusion of only the rats that did not complete at least one FR10). Left panels: Ethanol administered alone did not produce nicotine-like discriminative effects (A and G), but 1 g/kg of ethanol significantly depressed rates of responding (D). Middle panels: When ethanol was administered in combination with the training dose of 0.4 mg/kg nicotine, it did not significantly reduce nicotine discrimination of rats presenting response rates above 0.5 responses/s during the test session (B). However, 1 mg/kg ethanol significantly depressed rates of responding (E) and the discriminative-stimulus effect of the training dose of nicotine in rats that did complete at least one FR10 ratio during the test session (H). Right panels: Doses of 0.1 and 1 g/kg ethanol did not shift the dose–response curve for nicotine discrimination (C and I). ED_{50} values with 95% CI for these dose–response curves and for the experiments evaluating the effects of 0.3 g/kg ethanol are given in Table 1. The high dose of 1 g/kg ethanol significantly decreased rates of responding of rats when given alone or in combination with nicotine (F).

lever relative to the total number of responses emitted on both levers during a session. Response rate (responses/s) during each session was calculated by dividing the total number of responses emitted on both levers during a session by the total session length. Response rate and percentage of nicotine-appropriate responses were individually calculated for each rat and then expressed as a group mean (±S.E.M.).

Two criteria were chosen to analyze the discrimination performance of the rats. Following the first criteria, nicotine-associated lever selection data were excluded from analysis if the response rate of the rat was below 0.5 responses/s or if the rat emitted fewer than ten responses during the test session (results are shown in Fig. 1A, B and C). For the second criteria, only rats which completed at least one FR10 on either vehicle or nicotine lever were considered when nicotine-appropriate responding was calculated (results are shown in Fig. 1G, H and I). Data from all rats were included in statistical analysis of response rates (results are shown in Fig. 1D, E and F). No generalization to the nicotine training stimulus was defined as 20% or lower responding on the nicotine-appropriate lever. The dose of nicotine predicted to produce 50% of responses on the nicotine-appropriate lever were calculated by a linear regression analysis of the log dose–effect function in generalization tests when different doses of nicotine were tested in combination with ethanol. These doses were expressed as ED_{50} values (in mg/kg) with 95% confidence interval (CI).

Analysis of variance (ANOVA) was used to analyze experimental data from the nicotine-discrimination study. Post-hoc analysis was performed using Dunnett's test following detection of a significant main effect (i.e. a significant effect of drug's dose for within-group comparisons) by one-way ANOVA. Statistical analyses were performed on raw (rate of responding) or transformed (percentage of nicotine-appropriate lever selection) data. Data were considered statistically significant at $P<0.05$. Two ED_{50} values were considered statistically different if their 95% confidence limits did not overlap.

3. Results

3.1. Establishment of a nicotine discrimination baseline

Reaching the final level of accuracy (eight consecutive sessions with at least 90% of the responses on the correct lever and no more than four incorrect responses during the first trial) required 18 to 70 sessions with a mean value (±S.E.M.) of 36.6±3.0 sessions. Once the training criterion was reached, performance during training sessions was maintained with a high degree of accuracy (95–100% responding on the appropriate lever). Rates of responding during training sessions were stable across sessions during the whole study and were slightly higher after nicotine than after saline pretreatment, as was observed in previous studies using the same 0.4 mg/kg training dose of nicotine (Le Foll and Goldberg, 2004; Le Foll et al., 2005; Shoaib et al., 1997). When the dose of nicotine was varied, there was a dose-dependent increase in drug-lever selection with maximal selection at the training dose of nicotine (one-way ANOVA for repeated measures: $F5,138=92.9$, $P<0.0001$, Fig. 1, left upper panel). The nicotine dose–response curve remained stable throughout the study.

3.2. Generalization tests (left panels)

The left panels of Fig. 1 show the percentage of responses made on the drug lever (Fig. 1A and G) and overall rates of responding (Fig. 1D) obtained during sessions when different doses of ethanol were tested for their ability to substitute for the training dose 0.4 mg/kg of nicotine. Ethanol failed to generalize to the nicotine training stimulus over a large range of doses (less than 5% of responses emitted on the nicotine-associated lever with doses of ethanol ranging from 0.1 to 1 g/kg). The criteria chosen to analyze the results of the discrimination performance of the rats had no influence on the results (see Fig. 1A and G). One-way ANOVA indicated a significant effect of ethanol pretreatment on rate of responding ($F3,49=3.8$, $P=0.02$) and post-hoc analysis indicated that only the 1 g/kg dose of ethanol significantly depressed rates of responding ($P<0.05$, Fig. 1D) and this rate of responding totally suppressed responding in 2 of the 13 rats tested (Fig. 1G).

3.3. Effect of ethanol on discrimination of the training dose of nicotine (middle panels)

The middle panels of Fig. 1 shows the percentage of responses made on the drug-associated lever (Fig. 1B and H) and overall rates of responding (Fig. 1E) during sessions when different doses of ethanol were tested for their ability to alter the discriminative-stimulus effects of the 0.4 mg/kg training dose of nicotine. Ethanol given in combination with nicotine significantly depressed rate of responding ($F3,55=8.0$, $P<0.01$; Fig. 1E), as it did when it was given alone (Fig. 1D), and post-hoc analysis indicated that only rats treated with the highest dose of ethanol (1 g/kg) had a significant disruption of responding ($P<0.01$). In contrast, injection of the same volume of saline (10 ml/kg i.p.) had no significant effect on rates of responding of the rats (mean of rates of responding after 10 ml/kg saline injection=1.3±0.2, $n=12$).

Analysis of the discrimination performance of the rats with the first criteria (exclusion of the rats with response rates below 0.5 responses/s) indicates that ethanol did not alter the discriminative-stimulus effects of the 0.4 mg/kg training dose of nicotine, even at the highest ethanol dose tested ($F3,48=0.5$, $P=0.7$; Fig. 1B). However, analysis of the discrimination performance of the rats with the second criteria (exclusion of only the rats that did not complete at least one FR10) indicates that 1 g/kg ethanol significantly alter the discriminative-stimulus effects of the 0.4 mg/kg training dose of nicotine ($F3,51=3.9$, $P<0.05$; Fig. 1H).

3.4. Effects of ethanol on discrimination of various doses of nicotine (right panels)

The right panels of Fig. 1 show the effects of 0.1 and 1 g/kg ethanol on the dose–response curve for nicotine discrimination (Fig. 1C and I) and on overall rates of responding (Fig. 1F).

When 1 g/kg of ethanol was given alone or in combination with nicotine, it significantly depressed response rates in the rats (Fig. 1F). A two-way ANOVA analysis of results indicated a significant effect of ethanol ($F2,117=17.5$, $P<0.001$), no significant effect of nicotine dose ($F4,117=0.3$, $P=0.9$), and no significant interaction between ethanol pretreatment and nicotine dose ($F8,117=0.1$, $P=0.9$). Post-hoc analysis indicated that rats receiving 1 g/kg ethanol alone or in combination with nicotine significantly reduced rates of responding (Fig. 1F, all $P<0.05$).

Table 1
ED_{50} values (95% CI) for percentage of drug-lever selection when nicotine was administered alone and with various doses of ethanol

	ED_{50} (95% CI) as mg/kg	
	(First criteria)	(Second criteria)
Nicotine alone	0.07 (0.06–0.08)	0.07 (0.06–0.08)
Nicotine+0.1 g/kg ethanol	0.08 (0.06–0.12)[a]	0.08 (0.06–0.12)[a]
Nicotine+0.3 g/kg ethanol	0.05 (0.02–0.07)[a]	0.05 (0.02–0.07)[a]
Nicotine+1 g/kg ethanol	0.09 (0.04–0.28)[a]	0.04 (0.01–0.06)[a]

The discrimination performances of the rats have been analyzed with two distinct criteria based on the response rates of the rats. The first criteria excluded rats with response rates below 0.5 responses/s, whereas the second criteria excluded rats that did not complete at least one FR10 during the test session. [a]Overlapping 95% CI compared with the dose–response curves of nicotine alone.

ED_{50} values for drug-lever selection with 95% CI are shown in Table 1. The ED_{50} values overlap demonstrating that ethanol produced no significant shift of the dose–response curves for nicotine discrimination. This absence of shift of the dose–reponse curves for nicotine discrimination has been obtained regardless of the criteria chosen for the analysis of the results (see Table 1). This is supported by two-way ANOVA analysis of results. Analysis of the discrimination performance of the rats with the first criteria (exclusion of the rats with response rates below 0.5 responses/s) indicates a significant effect of nicotine dose ($F4,100=116.6$, $P<0.001$) but no significant effect of ethanol pretreatment on nicotine discrimination ($F2,100=0.5$, $P=0.6$). However, there was a significant interaction between ethanol pretreatment and nicotine dose ($F8,100=2.8$, $P<0.05$). Post-hoc analysis indicated that 1 g/kg ethanol enhanced the discriminative-stimulus effect of a low 0.03 mg/kg dose of nicotine but decreased the discriminative-stimulus effect of a higher 0.1 mg/kg dose of nicotine (all $P<0.05$). The 0.1 and 0.3 g/kg doses of ethanol had no significant effects on the discriminative-stimulus effects of any dose of nicotine (all $P>0.07$). Similarly, analysis of the discrimination performance of the rats with the second criteria (exclusion of only the rats that did not complete at least one FR10) indicates a significant effect of nicotine dose ($F4,104=80.3$, $P<0.001$) but no significant effect of ethanol pretreatment on nicotine discrimination ($F2,104=0.6$, $P=0.5$). However, there was a significant interaction between ethanol pretreatment and nicotine dose ($F8,104=3.3$, $P<0.05$). Post-hoc analysis indicated that 1 g/kg ethanol significantly enhanced the discriminative-stimulus effect of a low 0.03 mg/kg dose of nicotine ($P=0.02$) but significantly decreased the discriminative-stimulus effect of highest 0.1 and 0.4 mg/kg doses of nicotine (both $P<0.05$).

4. Discussion

In the present study, ethanol administered alone produced no nicotine-like discriminative effects in rats (Fig. 1A and G). Also, when ethanol was administered in combination with nicotine it did not produce a significant shift of the dose–response curves for nicotine discrimination (Table 1 and Fig. 1C and I). However, a high 1 g/kg dose of ethanol significantly decreased rates of responding when given alone or in combination with nicotine (Fig. 1D, E, and F). Lower 0.1 and 0.3 g/kg doses of ethanol had no significant effects on rates of responding of the rats. These low doses of ethanol had also no significant effects on the discriminative-stimulus effects of the 0.4 mg/kg training dose of nicotine. However, depending on the method chosen to analyze the discrimination performance of the rats, the high 1 g/kg dose of ethanol either produced no effect or significantly decreased the discriminative-stimulus effects of the 0.4 mg/kg training dose of nicotine (Fig. 1B and H).

In agreement with previous reports (Kim and Brioni, 1995; Korkosz et al., 2005; McMillan et al., 1999), ethanol did not produce any nicotine-like effects in rats trained to discriminate nicotine from saline (Fig. 1A and G). The discriminative-stimulus effects of nicotine are mainly mediated by neuronal nicotinic acetylcholine receptors (Kumar et al., 1987; Pratt et al., 1983; Shoaib et al., 2002; Stolerman et al., 1997, 1984), but dopaminergic receptors may be also involved (Desai et al., 2003; Le Foll et al., 2005). It is interesting to note that the dopaminergic component of the nicotine discriminative-stimulus effect is only partial and has been difficult to demonstrate (Corrigall and Coen, 1994; Le Foll et al., 2005). It is therefore not surprising that the dopamine-releasing effect of ethanol (Imperato and Di Chiara, 1986) was insufficient to produce any nicotine-like effect in our experiment. It has also been proposed that ethanol may produce dopamine release through activation of cholinergic afferents and release of acetylcholine in the ventral tegmental area (see (Larsson and Engel, 2004) for a review). The lack of nicotine-like effects induced by ethanol (Fig. 1A) suggests that acetylcholine release induced by ethanol is considerably less effective than nicotine in stimulating acetylcholine receptors.

Ethanol appears unable to alter the subjective effects of nicotine since, in the present experiments, ethanol did not significantly shift the dose–response curves for nicotine discrimination (Table 1). However, it has been previously suggested by several investigators that ethanol can reduce the discriminative-stimulus effects of nicotine in standard two-lever choice drug-discrimination procedures in rats (Kim and Brioni, 1995; Korkosz et al., 2005; McMillan et al., 1999). In the report by Kim and Brioni (1995), only the highest dose of ethanol tested (22 mmol/kg, equivalent to 1 g/kg) was able to significantly decrease the discriminative-stimulus effects of the training dose of 0.3 mg/kg nicotine, whereas a lower dose of ethanol (11 mmol/kg, equivalent to 0.5 g/kg) had no effect. Thus, blockade of the discriminative-stimulus effects of nicotine in this study occurred only after administration of a high dose of ethanol that markedly reduced rates of responding by rats in other drug-discrimination studies (present results, (Gatch et al., 2003; Korkosz et al., 2005). McMillan et al. (1999) also reported that 0.6 to 0.8 g/kg ethanol reduced the discriminative-stimulus effects of nicotine in alcohol-preferring rats. Unfortunately, response rates of the rats in these experiments were not reported. More recently, Korkosz et al.

(2005) reported that lower 0.25 and 0.5 g/kg doses of ethanol blocked the discriminative-stimulus effects of a 0.4 mg/kg training dose of nicotine in Wistar rats. In these experiments, however, the highest doses of ethanol (0.75–1 g/kg) administered in combination with the training dose of nicotine completely eliminated operant responding and the discrimination behavior could not be assessed. In agreement with these previous studies and using the same exclusion criteria than Korkosz et al. (exclusion of only the rats that did not complete at least one FR10), we found that a high 1 g/kg dose of ethanol significantly decreased the discriminative-stimulus effect of the training dose of nicotine in Sprague–Dawley rats (Fig. 1H). However, this effect seems related to the alteration of the discrimination performance in rats presenting a marked depression of response rates, since 1 mg/kg ethanol produced no effects on the discriminative-stimulus effect of the training dose of nicotine in rats presenting response rates above 0.5 responses/s during the test session (Fig. 1 B). Moreover, in the previous studies, the effects of ethanol were not evaluated against the entire dose–response curve for nicotine discrimination. The lack of significant effect of ethanol is clearly demonstrated in our experiments by the absence of any significant shift in the dose–response curve for nicotine discrimination by 0.1, 0.3 and 1 g/kg doses of ethanol (Table 1).

Although ethanol produced no significant shift of the dose–response curve for nicotine, the high 1 g/kg dose of ethanol significantly enhanced the discriminative-stimulus effect of 0.03 mg/kg nicotine and decreased the discriminative-stimulus effect of 0.1 mg/kg nicotine (regardless of the criteria chosen for analysis of discrimination performance, Fig. 1C and I). In contrast, lower 0.1 and 0.3 g/kg doses of ethanol had no significant effect on the discriminative-stimulus effects of any dose of nicotine (Fig. 1C; Table 1, results with 0.3 g/kg ethanol are not shown in Fig. 1). Interestingly, over a restricted range of doses, ethanol may potentiate the effects of nicotine on the firing of dopaminergic neurons (Clark and Little, 2004). This suggests that, under a restricted set of conditions, synergism between ethanol and nicotine effects may be present in the brain. In agreement, in a recent clinical study, Rose et al. found that ethanol did not decrease but instead tended to increase the subjective effects of tobacco in human smokers, increasing ratings of 'harsh' and of 'like cigarette's effects' (Rose et al., 2004).

Interestingly, there is no evidence for a relationship between the ability of a test drug to produce reinstatement of extinguished drug-seeking behavior and its ability to elicit drug-like, discriminative-stimulus effects (Spealman et al., 1999; Le Foll and Goldberg, in press). This dissociation has been illustrated recently with several distinct pharmacological ligands. For example, ligands blocking dopamine D_3 receptors are able to block the influence of nicotine-associated cues on behavior (De Vries and Schoffelmeer, in press; Le Foll et al., 2003a,b, 2005, in press-a; Le Foll and Goldberg, 2005b) without influencing the discriminative-stimulus effects of nicotine (Le Foll et al., 2005). Similarly, ligands blocking cannabinoid CB_1 receptors are also able to block the influence of these nicotine-associated cues (Cohen et al., 2005, 2002; Le Foll and Goldberg, 2004, 2005a) without affecting the discriminative-stimulus effects of nicotine (Cohen et al., 2002; Le Foll and Goldberg, 2004) (see Le Foll and Goldberg, 2005a,b; Le Foll et al., 2000; Newman et al., 2005; Sokoloff et al., in press for reviews on the role of dopamine D_3 and cannabinoid CB_1 receptors in drug addiction). Therefore, the inability of ethanol to block or enhance the discriminative-stimulus effects of nicotine is not in contradiction with its ability to enhance relapse and drug-seeking behavior in animals and humans.

In conclusion, the present findings provide evidence that ethanol does not possess any nicotine-like effects of its own and does not significantly reduce the subjective effects of nicotine assessed with drug-discrimination procedures. It is, therefore, unlikely that reported increases in cigarette consumption associated with ethanol consumption by smokers are related to an effect on the subjective effects of tobacco. It appears more likely that ethanol and nicotine may trigger a common neurological pathway involved in the control of drug consumption that is independent of nicotine's subjective effects.

References

Clark, A., Little, H.J., 2004. Interactions between low concentrations of ethanol and nicotine on firing rate of ventral tegmental dopamine neurones. Drug Alcohol Depend. 75, 199–206.

Cohen, C., Perrault, G., Voltz, C., Steinberg, R., Soubrie, P., 2002. SR141716, a central cannabinoid (CB(1)) receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats. Behav. Pharmacol. 13, 451–463.

Cohen, C., Perrault, G., Griebel, G., Soubrie, P., 2005. Nicotine-associated cues maintain nicotine-seeking behavior in rats several weeks after nicotine withdrawal: reversal by the cannabinoid (CB(1)) receptor antagonist, rimonabant (SR141716). Neuropsychopharmacology 30, 145–155.

Collins, A.C., 1990. Interactions of ethanol and nicotine at the receptor level. Recent Dev. Alcohol. 8, 221–231.

Corrigall, W.A., Coen, K.M., 1994. Dopamine mechanisms play at best a small role in the nicotine discriminative stimulus. Pharmacol. Biochem. Behav. 48, 817–820.

De Vries, T.J., Schoffelmeer, A.N., in press. Cannabinoid CB(1) receptors control conditioned dry seeking. Trends Pharmacol. Sci.

Desai, R.I., Barber, D.J., Terry, P., 2003. Dopaminergic and cholinergic involvement in the discriminative stimulus effects of nicotine and cocaine in rats. Psychopharmacology (Berl) 167, 335–343.

Di Chiara, G., Imperato, A., 1988. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. U. S. A. 85, 5274–5278.

DiFranza, J.R., Guerrera, M.P., 1990. Alcoholism and smoking. J. Stud. Alcohol 51, 130–135.

Gatch, M.B., Youngblood, B.D., Forster, M.J., 2003. Effects of ethanol on cocaine discrimination in rats. Pharmacol. Biochem. Behav. 75, 837–844.

Griffiths, R.R., Bigelow, G.E., Liebson, I., 1976. Facilitation of human tobacco self-administration by ethanol: a behavioral analysis. J. Exp. Anal. Behav. 25, 279–292.

Henningfield, J.E., Chait, L.D., Griffiths, R.R., 1983. Cigarette smoking and subjective response in alcoholics: effects of pentobarbital. Clin. Pharmacol. Ther. 33, 806–812.

Henningfield, J.E., Chait, L.D., Griffiths, R.R., 1984. Effects of ethanol on cigarette smoking by volunteers without histories of alcoholism. Psychopharmacology (Berl) 82, 1–5.

Imperato, A., Di Chiara, G., 1986. Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. J. Pharmacol. Exp. Ther. 239, 219–228.

Imperato, A., Mulas, A., Di Chiara, G., 1986. Nicotine preferentially stimulates dopamine release in the limbic system of freely moving rats. Eur. J. Pharmacol. 132, 337–338.

Istvan, J., Matarazzo, J.D., 1984. Tobacco, alcohol, and caffeine use: a review of their interrelationships. Psychol. Bull. 95, 301–326.

Kim, D.J.B., Brioni, J.D., 1995. Modulation of the discriminative stimulus properties of (−)-nicotine by diazepam and ethanol. Drug Dev. Res. 34, 47–54.

Koelega, H.S., 1995. Alcohol and vigilance performance: a review. Psychopharmacology (Berl) 118, 233–249.

Koob, G.F., 1992. Dopamine, addiction and reward. Semin. Neurosci. 4, 139–148.

Korkosz, A., Taracha, E., Plaznik, A., Wrobel, E., Kostowski, W., Bienkowski, P., 2005. Extended blockade of the discriminative stimulus effects of nicotine with low doses of ethanol. Eur. J. Pharmacol. 512, 165–172.

Kumar, R., Reavill, C., Stolerman, I.P., 1987. Nicotine cue in rats: effects of central administration of ganglion-blocking drugs. Br. J. Pharmacol. 90, 239–246.

Larsson, A., Engel, J.A., 2004. Neurochemical and behavioral studies on ethanol and nicotine interactions. Neurosci. Biobehav. Rev. 27, 713–720.

Le Foll, B., Goldberg, S.R., 2004. Rimonabant, a CB1 antagonist, blocks nicotine-conditioned place preferences. Neuroreport 15, 2139–2143.

Le Foll, B., Goldberg, S.R., 2005. Cannabinoid CB1 receptor antagonists as promising new medications for drug dependence. J. Pharmacol. Exp. Ther. 312, 875–883.

Le Foll, B., Goldberg, S.R., 2005. Control of the reinforcing effects of nicotine by associated environmental stimuli in animals and humans. Trends Pharmacol. Sci. 26, 287–293.

Le Foll, B., Goldberg, S.R., in press. Nicotine as a typical drug of abuse in experimental animals and humans, Psychopharmacology (Berl.).

Le Foll, B., Schwartz, J.-C., Sokoloff, P., 2000. Dopamine D3 receptor agents as potential new medications for drug addiction. Eur. Psychiatr. 15, 140–146.

Le Foll, B., Diaz, J., Sokoloff, P., 2003a. Increased dopamine D3 receptor expression accompanying behavioural sensitization to nicotine in rats. Synapse 47, 176–183.

Le Foll, B., Schwartz, J.-C., Sokoloff, P., 2003b. Disruption of nicotine conditioning by dopamine D3 receptor ligands. Mol. Psychiatry 8, 225–230.

Le Foll, B., Sokoloff, P., Stark, H., Goldberg, S.R., 2005. Dopamine D3 ligands block nicotine-induced conditioned place preferences through a mechanism that does not involve discriminative-stimulus or antidepressant-like effects. Neuropsychopharmacology 30, 720–730.

Le Foll, B., Goldberg, S.R., Sokoloff, P., in press-a. Dopamine D3 receptor and drug dependence: effect on reward or beyond? Neuropharmacology.

Le Foll, B., Melihan-Cheinin, P., Rostoker, G., Lagrue, G. for the working group of AFSSAPS, in press-b. Smoking cessation guidelines: evidence-based recommendations of the French Health Products Safety Agency, Eur. Psychiatry.

McMillan, D.E., Li, M., Shide, D.J., 1999. Differences between alcohol-preferring and alcohol-nonpreferring rats in ethanol generalization. Pharmacol. Biochem. Behav. 64, 415–419.

Mintz, J., Boyd, G., Rose, J.E., Charuvastra, V.C., Jarvik, M.E., 1985. Alcohol increases cigarette smoking: a laboratory demonstration. Addict. Behav. 10, 203–207.

National Research Council, 2003. Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research. The National Academy Press, Washington, DC, USA.

Newman, A.H., Grundt, P., Nader, M.A., 2005. Dopamine D3 receptor partial agonists and antagonists as potential drug abuse therapeutic agents. J. Med. Chem. 48, 3663–3679.

Nil, R., Buzzi, R., Battig, K., 1984. Effects of single doses of alcohol and caffeine on cigarette smoke puffing behavior. Pharmacol. Biochem. Behav. 20, 583–590.

Phillips, P.E., Stuber, G.D., Heien, M.L., Wightman, R.M., Carelli, R.M., 2003. Subsecond dopamine release promotes cocaine seeking. Nature 422, 614–618.

Pratt, J.A., Stolerman, I.P., Garcha, H.S., Giardini, V., Feyerabend, C., 1983. Discriminative stimulus properties of nicotine: further evidence for mediation at a cholinergic receptor. Psychopharmacology (Berl) 81, 54–60.

Rose, J.E., Braver, L.H., Behm, F.M., Cramblett, M., Calkins, K., Lawhon, D., 2004. Psychopharmacological interactions between nicotine and ethanol. Nicotine Tob. Res. 6, 133–144.

Shalev, U., Grimm, J.W., Shaham, Y., 2002. Neurobiology of relapse to heroin and cocaine seeking: a review. Pharmacol. Rev. 54, 1–42.

Shoaib, M., Thorndike, E., Schindler, C.W., Goldberg, S.R., 1997. Discriminative stimulus effects of nicotine and chronic tolerance. Pharmacol. Biochem. Behav. 56, 167–173.

Shoaib, M., Gommans, J., Morley, A., Stolerman, I.P., Grailhe, R., Changeux, J.P., 2002. The role of nicotinic receptor beta-2 subunits in nicotine discrimination and conditioned taste aversion. Neuropharmacology 42, 530–539.

Sokoloff, P., Diaz, J., Le Foll, B., Guillin, O., Leriche, L., Bezard, E., Gross, C., in press. The dopamine D3 receptor: a therapeutic target for the treatment of neuropsychiatric disorders, Current Drug Targets-CNS and Neurol. Disord.

Spealman, R.D., Barrett-Larimore, R.L., Rowlett, J.K., Platt, D.M., Khroyan, T.V., 1999. Pharmacological and environmental determinants of relapse to cocaine-seeking behavior. Pharmacol. Biochem. Behav. 64, 327–336.

Stolerman, I.P., Garcha, H.S., Pratt, J.A., Kumar, R., 1984. Role of training dose in discrimination of nicotine and related compounds by rats. Psychopharmacology (Berl) 84, 413–419.

Stolerman, I.P., Chandler, C.J., Garcha, H.S., Newton, J.M., 1997. Selective antagonism of behavioural effects of nicotine by dihydro-beta-erythroidine in rats. Psychopharmacology (Berl) 129, 390–397.

Zacny, J.P., 1990. Behavioral aspects of alcohol–tobacco interactions. Recent Dev. Alcohol. 8, 205–219.