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Ethanol Enhances Nicotine's Effects on DRL Performance in Rats

E. JON POPKE, CHARLES M. FOGLE AND MERLE G. PAULE

Division of Neurotoxicology, National Center for Toxicological Research, FDA, Jefferson, AR

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POPKE, E. J., C. M. FOGLE AND M. G. PAULE. *Ethanol potentiates nicotine's effects on DRL performance in rats.* PHARMACOL BIOCHEM BEHAV **66**(4) 819–826, 2000.—The present experiment examined effects of nicotine (0.0, 0.3, 0.56, and 1.0 mg/kg; IP) and ethanol (0.0, 0.5, 1.5, and 3.0 g/kg; IG) on operant behavior using a differential reinforcement of low response rate (DRL) schedule in rats. DRL schedules are sensitive to effects of nicotine and provide an assessment of the subject's ability to accurately estimate time and to inhibit schedule-controlled responding. When administered alone, nicotine shifted the mode of the interresponse time distribution to the left and reduced the percentage of reinforced responses. Nicotine also had an inverted U-shaped dose effect on the number of "bursting" responses. When administered after pretreatment with ethanol, nicotine's effects on the distribution of interresponse times and bursting were potentiated. These effects are consistent with previous reports and with the suggestion that ethanol pretreatment can potentiate effects of subsequently administered nicotine. Published by Elsevier Science Inc.

Nicotine Ethanol Operant behavior Rats DRL

A positive epidemiological relationship exists between the use of cigarettes and the use of alcohol. Drinkers are more likely to be smokers than are nondrinkers (55), and alcohol consumption poses a significant risk for relapse among people who are trying to quit smoking (7,17). Although the mechanisms that underlie this relationship are not known, reports that ethanol can potentiate responses to subsequently administered nicotine suggest an effect of these drugs which may contribute to the propensity of some smokers to smoke more when they drink. This effect may be particularly relevant when the initial effect of nicotine is experienced positively, and when no ceiling effect exists with respect to this experience. Ethanol has been shown to potentiate nicotine's effects on locomotor behavior and on intracranial self-stimulation in mice (52), and can enhance nicotine-induced rotation in rats (21). In humans, cigarettes and alcohol can act synergistically to improve performance on measures of motor performance and decision times (30). Because cognitive-behavioral effects of nicotine are known to contribute to the maintenance of the smoking habit (11,58,62), reports that ethanol can potentiate cognitive-behavioral effects of nicotine may suggest a mechanism by which alcohol consumption can lead to increases in smoking.

The present experiment extends previous research by examining nicotine's effects, with and without ethanol, on schedule-controlled behavior using a differential reinforcement of a low response-rate schedule (DRL) in rats. Previous experiments have examined effects of nicotine alone on DRL responding, and suggest that nicotine administration can increase response rates while concurrently reducing reinforcement rates (38). Subsequent experiments, including those conducted in our laboratory, indicate that this pattern of results reflects a shift in the distribution of responses in the direction of shorter interresponse times (44,46). Studies of ethanol's effects on DRL responding suggest that ethanol, like nicotine, reduces DRL reinforcement rate (16,22,53), but that ethanol does not consistently shift the mode of the interresponse time distribution (35,45).

In the present experiment, ethanol was administered 10 minutes prior to nicotine administration to assess the effects of ethanol pretreatment on subsequent nicotine-induced DRL performance. Because responding under DRL schedules is known to be sensitive to effects of nicotine (38,44,46), such schedules may provide a useful model to study ethanol's effects on nicotine-induced behavior. Further, DRL schedules generate behavior that is thought to reflect cognitive pro-

Requests for reprints should be addressed to E. Jon Popke, Division of Neurotoxicology, National Center for Toxicological Research, FDA, 3900 NCTR Road, Jefferson, AR 72079-9502.

cesses that cannot easily be measured using simple locomotion and reaction time measures (20,23,34,36,48,55).

METHOD

Subjects

Subjects in the present experiment were eight male Sprague–Dawley rats, approximately 12 months old, and weighing roughly 325 g (322.5 ± 18.4 g) at the start of testing. Subjects were housed in 35.6 × 15.2 × 20.3-cm Plexiglas cages with absorbent wood-chip bedding. Temperature and humidity in the housing room were maintained at 21°C and at 45–55%, respectively. Food was available ad lib from weaning (postnatal day 22) through postnatal day 70. Beginning on Postnatal day 70, subjects were gradually food deprived to 80–85% of their free-feeding body weight, and were maintained at this weight throughout the experiment. Water was available ad lib throughout.

Subjects in the present experiment had previously been trained to perform the DRL task and also had served in two earlier experiments to determine the effects of acute ethanol (0.5–3.0 g/kg ethanol administered twice weekly for 5 weeks) and acute nicotine (0.3–1.0 mg/kg administered twice weekly for 4 weeks) administration on operant performance (44,45). The time between the end of the previous experiments and the start of the present experiment was 31 days.

Drug Administration

Each dose of nicotine (0.0, 0.3, 0.56, and 1.0 mg/kg, prepared as base and administered IP) was administered alone, and after pretreatment with each of four doses of ethanol (0.0, 0.5, 1.5, and 3.0 g/kg; via orogastric gavage). These doses were selected based on previous experiments in our laboratory, which examined the separate acute effects of nicotine and ethanol on operant performance in rats (44,45). Each of the 16 possible dosing combinations was administered twice, in a randomized repeated measures design. Tap water was used to prepare the ethanol solutions from 95% ethanol (40% v/v), and was used as the ethanol control (vehicle) solution. Physiologic saline (0.9% NaCl) was used to prepare the nicotine solutions from nicotine hydrogen tartrate, and was used as the nicotine control (vehicle) solution. Volumes of the orogastric ethanol injections did not exceed 3.2 ml. Volumes of the IP nicotine injections did not exceed 0.37 ml.

Procedure

Behavior was assessed using one of 12 identical operant test chambers. Each 24.6 × 22.9 × 21.0-cm chamber was housed in a sound-attenuating box equipped with a ventilating fan. The test panel contained three retractable levers (Stoelting, Co., #26446) each positioned under an array of nine stimulus lights (3 × 3). Food reinforcers (45-mg dustless precision food pellets, Bioserve, Frenchtown, NJ) were delivered into a feeding trough located immediately beneath the middle retractable lever. Each operant test panel was interfaced with a microcomputer that administered the DRL schedule and that recorded the behavioral responses. Each operant chamber was equipped with a house light that remained on for the duration of each test session.

DRL Training

At the start of DRL training (training level 1), subjects were required to withhold responding to an extended operant lever for at least 0.5 s to receive reinforcement. After 40 reinforcements were earned at level 1, the duration that subjects were required to withhold responding was increased to 1.0 s (training level 2). After each 40 reinforcers were earned at level 2, the duration requirement was increased to 1.5, 2.0, 2.5 s, and so on, until subjects were required to withhold responding for the full 10 s.

DRL Testing

DRL testing was conducted for 30 min per day, and was followed by testing of a conditioned position responding and a progressive ratio task (data not shown). On Tuesdays and Fridays, subjects received one of four doses of ethanol, followed by one of four doses of nicotine, as previously described. Testing without prior injection was conducted on Mondays and Wednesdays, and vehicle(s) (saline and water) was administered on Thursdays. There were no behavioral test sessions conducted on Saturdays or Sundays. Ethanol was administered 10 min prior to nicotine administration, and nicotine was administered 15 min prior to the start of DRL testing.

At the start of each DRL test session, the center retractable lever was extended and the house light was illuminated. To receive reinforcement, subjects were required to withhold responding to this lever for at least 10, but not more than 14 s. The first response emitted within this 10–14-s window resulted in food delivery. Responses emitted outside of this 10–14-s window were not reinforced and resulted in the initiation of a new trial. At no time during the test session was the house light extinguished or the operant lever retracted. A maximum of 120 reinforcers could be earned during a 30-min DRL test session.

Statistical Analyses

Prior to analysis, data were combined for each dosing replicate to derive mean values for each subject under each dosing condition. Two subjects died during the experiment, and were removed from consideration during subsequent analyses. These deaths did not appear to be treatment related.

The percentage of reinforced responses was determined by dividing the number of correct responses by the total number of responses and multiplying by 100. The resulting scores were analyzed using one-way analysis of variance for repeated measures. Dunnett's a posteriori comparisons were used to compare each dosing condition to vehicle and to control.

To examine drug-induced changes in mode of the interresponse time distributions, DRL responses were first classified as either "targeted" responses or "bursting" responses. Targeted responses were defined as those having interresponse times of 3 s or more. Bursting responses were defined as those having interresponse times of less than 3 s. The total number of bursting responses was analyzed separately from targeted responses using one-way ANOVA for repeated measures. Dunnett's a posteriori comparisons were used to compare each dose condition to vehicle and to control.

To examine changes in the distribution of interresponse times, the modes of the distributions under each treatment condition were paired, and a Wilcoxon matched-pairs, signed-rank test was used (3,19) This analysis was used to maintain consistency between the present report and previous reports that have examined effects of nicotine and ethanol on the temporal distribution of operant responding (25,44,45).

RESULTS

Table 1 presents the mean percentage of responses that were reinforced under each of the 16 dosing conditions described above. When administered alone, nicotine signifi-

TABLE 1
EFFECTS OF NICOTINE AND ETHANOL ON THE PERCENTAGE OF REINFORCED RESPONSES

Ethanol dose	Nicotine dose			
	0.0 mg/kg	0.3 mg/kg	0.56 mg/kg	1.0 mg/kg
0.0 g/kg	39.4 ± 3.2	20.9 ± 4.0*	18.1 ± 2.0*	18.9 ± 4.1*
0.5 g/kg	39.3 ± 3.9	12.2 ± 2.3*†	12.2 ± 4.1*†	17.7 ± 4.2*†
1.5 g/kg	32.2 ± 1.1	8.8 ± 1.7*†	12.2 ± 2.8*†	11.8 ± 3.6*†
3.0 g/kg	21.6 ± 3.6	11.1 ± 2.3*	8.9 ± 2.6*†	11.6 ± 2.7*

Table 1 presents the percentage of responses reinforced in each of the 16 treatment conditions (mean ± SEM).

*Denotes significant difference from vehicle condition (saline/water).

†Indicates significant difference from the appropriate dose of ethanol administered without nicotine ($p < 0.05$).

cantly reduced the percentage of reinforced responses, with each dose of nicotine differing significantly from vehicle conditions, $F(3, 27) = 7.18$, $p < 0.01$. Similar effects were observed when subjects were pretreated with 0.5 g/kg ethanol, $F(4, 19) = 34.71$, $p < 0.0001$, 1.5 g/kg ethanol, $F(4, 34) = 32.74$, $p < 0.0001$, and 3.0 g/kg ethanol, $F(4, 39) = 23.24$, $p < 0.0001$. When administered without nicotine, ethanol reduced the percentage of reinforced responses with the 3.0 g/kg dose differing significantly from vehicle, $F(3, 23) = 5.361$, $p < 0.05$).

Figure 1 presents the distributions of interresponse times (averaged across subjects) for each of the 16 dosing conditions. Effects of nicotine alone are presented in Fig. 1a. Effects of nicotine following pretreatment with varying doses of ethanol are presented in Fig. 1b–d. When administered alone (Fig. 1a), 0.3 mg/kg nicotine and 0.56 mg/kg nicotine each shifted the mode of the interresponse time distribution to the left ($p < 0.05$ and $p < 0.01$, respectively). When administered after pretreatment with 1.5 g/kg ethanol (Fig. 1c), the effects of nicotine were potentiated in a manner consistent with a leftward shift in nicotine's dose effect. Direct comparisons made between the effects of nicotine alone and the effects of nicotine after ethanol pretreatment indicate that 0.3 mg/kg nicotine produced a significantly shorter modal interresponse time when administered following 1.5 g/kg ethanol than when administered without ethanol pretreatment ($p < 0.05$). Pretreatment with either 0.5 or 3.0 g/kg ethanol had a similar but less pronounced potentiating effects. Despite the apparent effect of 3.0 g/kg ethanol to shift the mode of the interresponse time distribution to the left when administered alone (Fig. 1d), there were no significant effects of any dose of ethanol to alter DRL responding when administered without nicotine. Modal interresponse times for individual subjects are presented in Fig. 2.

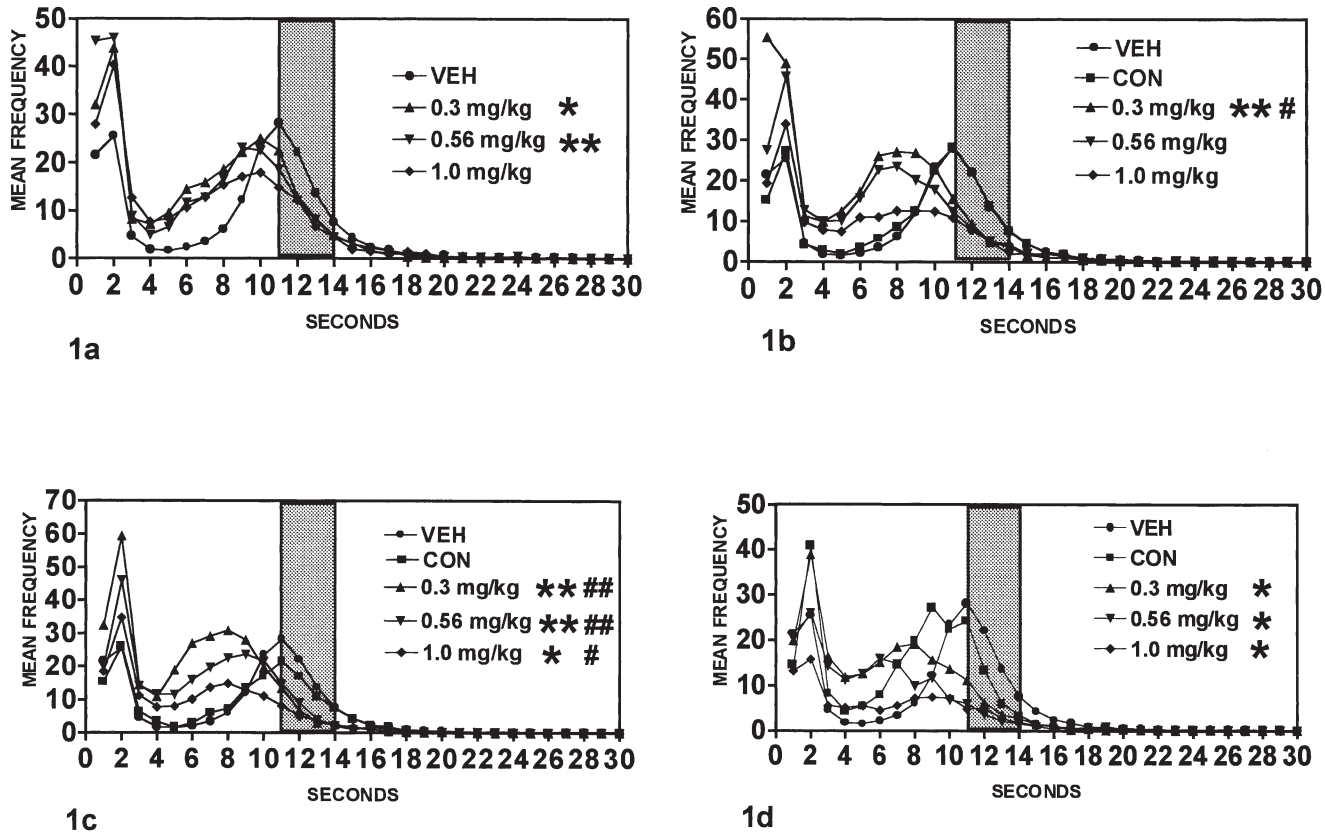

FIG. 1. (a) Presents effects of nicotine alone on DRL responding. (b–d) Presents effects of nicotine following pretreatment with 0.5, 1.5, and 3.0 g/kg ethanol, respectively. VEH values represent conditions in which subjects were treated with vehicle (water and saline) only. CON values represent control conditions in which subjects received the appropriate dose of ethanol without nicotine. *Denotes significant difference from VEH ($p < 0.05$). **Denotes significant difference from VEH ($p < 0.01$). #Denotes significant difference from CON ($p < 0.05$). ##Denotes significant difference from CON ($p < 0.01$). The shaded area represents the reinforced "window."

Figure 3 presents the effects of nicotine on the number of "bursting" responses measured with and without ethanol pretreatment. Bursting responses, defined as those responses with interresponse times of less than 3 s, are common to DRL schedules, and were exhibited by all subjects in the present experiment. When administered alone (Fig. 3a), nicotine had an inverted U-shaped dose effect on bursting with the effects of 0.56 mg/kg nicotine differing significantly from control, $F(3, 18) = 3.24$, $p < 0.05$. Pretreatment with 0.5 g/kg ethanol (Fig. 3b) produced an apparent shift in the low dose effect of nicotine, with the effects of 0.3 mg/kg nicotine differing significantly from control, $F(4, 20) = 4.79$, $p < 0.01$. A similar result is apparent following administration of 1.5 g/kg ethanol and 3.0 g/kg ethanol, $F(4, 20) = 3.58$, $p < 0.05$, but the individual dose effects did not achieve statistical significance ($p = 0.10$). There were no significant effects of any dose of ethanol on bursting responses when administered without nicotine.

DISCUSSION

The present experiment examined the effects of nicotine and ethanol on differential reinforcement of low response-rate (DRL) performance in rats. When administered alone, nicotine shifted the mode of the DRL interresponse time distribution to the left and reduced the percentage of reinforced responses. Nicotine also had an inverted U-shaped dose effect on bursting. When subjects were pretreated with ethanol, some of the effects of nicotine were potentiated. Specifically, ethanol pretreatment enhanced the leftward shift in the mode of the interresponse time distribution and enhanced the effect of low-dose nicotine on bursting. The highest dose of ethanol (3.0 g/kg) also reduced the percentage of reinforced responses when administered alone.

The results of the present experiment are consistent with previous reports of nicotine's effects on DRL responding (38,44,46) and with the suggestion that ethanol pretreatment can potentiate effects of subsequently administered nicotine (21,24,30,52). Further, the results of the present experiment appear largely consistent with previously reported effects of nicotine and alcohol on cognitive-behavioral performance in other paradigms. Lyon and colleagues (30) reported effects of ethanol on decision time and motor speed in deprived smokers, smokers who were not deprived, and in nonsmokers. Smokers who were not deprived exhibited faster motor and

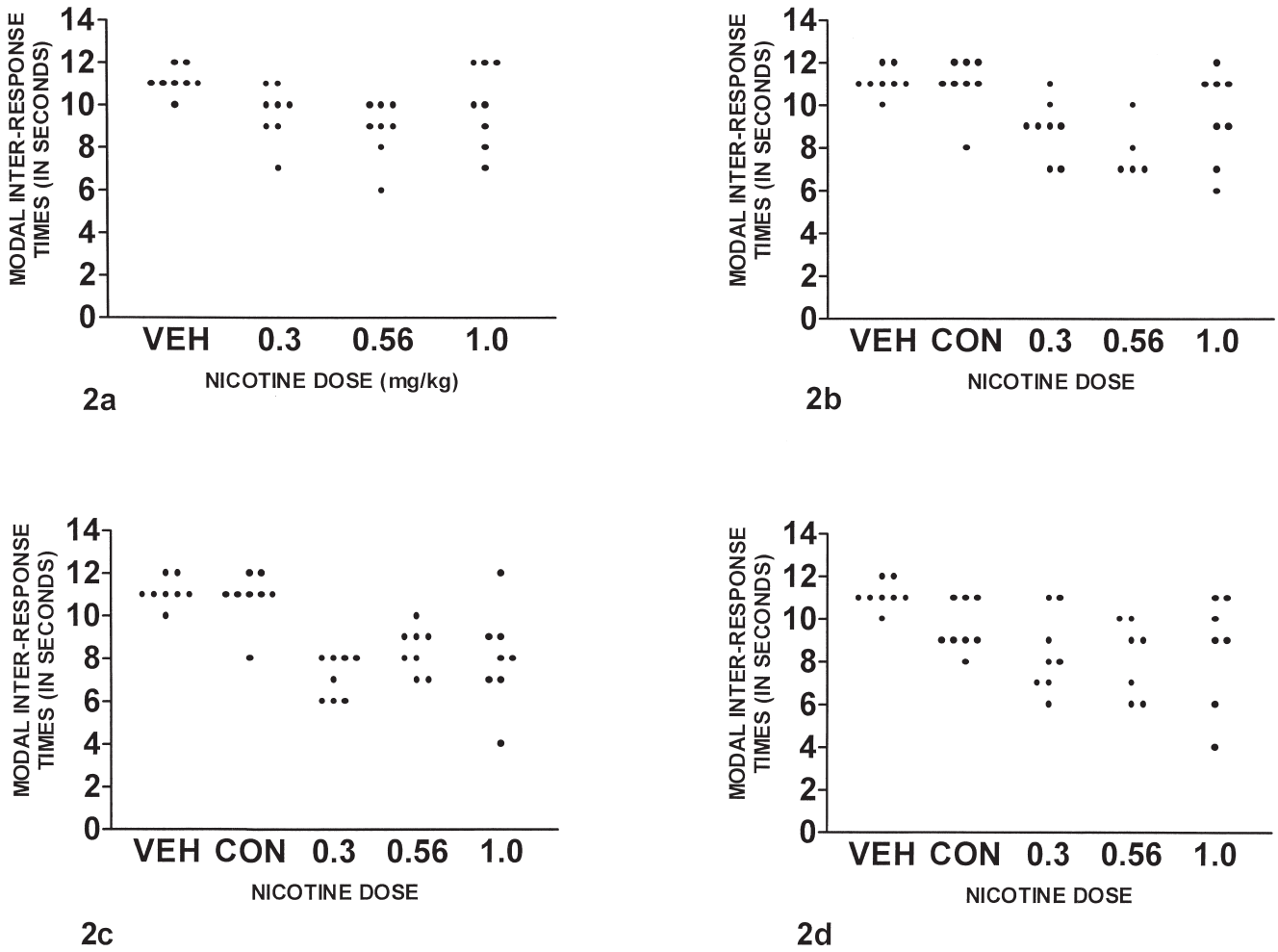

FIG. 2. Presents the modal interresponse times of each individual animal under each of the 16 treatment conditions. (a) Presents the effects of nicotine alone. (b–d) Presents effects of nicotine after pretreatment with 0.5, 1.5, and 3.0 g/kg ethanol, respectively. VEH values represent conditions in which subjects were treated with vehicle (water and saline) only. CON values represent control conditions in which subjects received the appropriate dose of ethanol without nicotine.

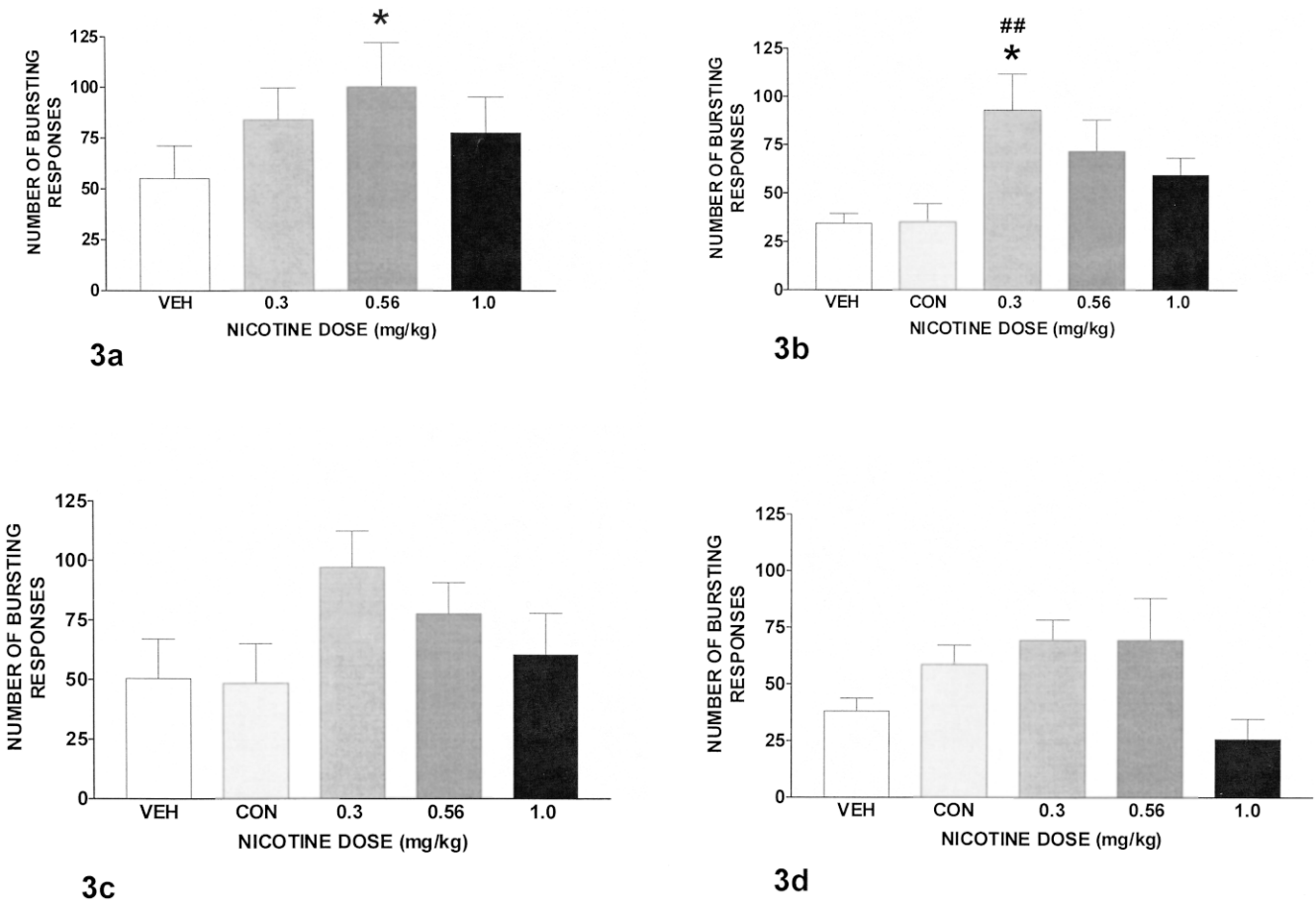

FIG. 3. (a) Presents effects of nicotine alone on bursting. (b–d) Presents effects of nicotine following pretreatment with 0.5, 1.5, and 3.0 g/kg ethanol, respectively. VEH values represent conditions in which subjects were treated with vehicle (water and saline) only. CON values represent control conditions in which subjects received the appropriate dose of ethanol without nicotine. *Denotes significant difference from VEH ($p < 0.05$). **Denotes significant difference from VEH ($p < 0.05$). #Denotes significant difference from CON ($p < 0.05$). ##Denotes significant difference from CON ($p < 0.01$).

decision times than did either of the other two groups, with the fastest times recorded in nondeprived smokers who had also received alcohol. Like the present results, this finding is consistent with the suggestion that ethanol pretreatment can potentiate the effects of subsequently administered nicotine. It is interesting to note, however, that this finding reflects a nicotine-induced enhancement of performance rather than a nicotine-induced impairment, as described presently. Although the reasons for this apparent discrepancy are unclear, it seems likely that differences in the experimental demands imposed by these behavioral procedures could result in different interpretations of their respective results. In the experiment by Lyon et al., for example, nicotine-induced increases in rapid responding could be viewed as an improvement in performance. Under conditions imposed by the present experiment, however, nicotine-induced increases in rapid responding result in increased bursting and reductions in the percentage of reinforced responses (i.e., impairments in performance). Future experiments, which address nicotine's effects on a variety of specific cognitive-behavioral abilities, will help to clarify nicotine's effects on performance.

Previous experiments examining effects of ethanol alone on DRL performance have yielded similarly unsettled results. Flynn and Harris (16), for example, reported effects of ethanol to reduce DRL reinforcement rates in a manner similar to those reported in subjects having received 3.0 g/kg ethanol presently. Because this report did not present the distribution of interresponse times, however, it is difficult to discern whether the effects resulted from a systematic shift in the mode of interresponse times or from a generalized disruption of responding. McMillan (35) reported ethanol-induced shifts in the mode of the DRL interresponse time distributions in humans, but only in those subjects that had received very specific feedback regarding their performance (referred to as the "high-feedback" group). In other words, only subjects that were told whether their responses were too early, too late, or correct (i.e., within a 5-s window) demonstrated discernable shifts in the mode of the interresponse time distributions after ethanol administration. Subjects that were told only when their responses were correct (the "low-feedback" group) and subjects that received no feedback at all (the "no-feedback" group) showed no consistent shifts in the distribution of their responses after ethanol. In the present experiment, rats were tested under conditions most similar to the low-feedback condition presented by McMillan, with feedback provided in the form of reinforcers only when the responses were correct.

Given this notable methodological similarity, it is not surprising that subjects in the present experiment behaved in a manner that was strikingly similar to that exhibited by McMillan's low-feedback subjects with reductions in the rate of reinforcement that were not accompanied by discernible shifts in the interresponse time distribution.

Although the present experiment does not directly address the mechanisms that may underlie ethanol's effect on nicotine-induced responding, a review of the relevant literature suggests several plausible hypotheses. Recent reports suggest that ethanol may potentiate agonist-induced ion currents mediated by the $\alpha3\beta4$, $\alpha3\beta2$, $\alpha4\beta2$, and $\alpha4\beta4$ subtypes of nicotinic receptors (1,10,12,32). Conversely, ethanol may inhibit agonist-induced ion currents mediated by the $\alpha7$ subtype of nicotinic-cholinergic receptor (1,10,12,13). Although the relative contribution of each of these nicotinic receptor subytpes to nicotine's effects on DRL responding are unknown, the effects of ethanol to alter currents mediated by these receptors suggest an effect of ethanol that may alter effects of subsequently administered nicotine. Alternatively, ethanol may alter nicotine-induced responding through its interaction with dopaminergic (4,61), serotonergic (29,63), GABAergic (2,37), opioid (4,47), or excitatory amino acid (60) systems. Dopaminergic and serotonergic function in particular, are known to influence DRL performance (6,28,31,49,56), and therefore, may provide a substrate for ethanol's effects on nicotine-induced responding (26,27). Although each of these hypotheses is consistent with published literature, it is important to emphasize that neither has been systematically investigated. Therefore, statements regarding mechanisms that may underlie behavioral interactions of nicotine with ethanol should be regarded cautiously.

As with any behavioral model, it is important to comment on the use of DRL procedures to study cognitive processes and to comment on the relationship between the present results and the positive relationship between the use of cigarettes and alcohol. Variations of the DRL schedule have been used to model a variety of cognitive functions including time estimation (34,50) impulsivity (36), and delay of reward (7). DRL schedules also have been used to screen compounds for potential antidepressant activity (31,48) and to characterize behavioral disorders in children (33,54) and adults (40). Although the relationship between each of these cognitive variables and the use of cigarettes and alcohol is unclear, the results of the present experiment suggest that a functional interaction exists between the effects of nicotine and ethanol that may be relevant to the propensity of some smokers to smoke more when they drink. Future experiments are required to define the effects of nicotine and ethanol on each of these cognitive functions and to identify their respective significance for the positive relationship between the use of cigarettes and alcohol.

Finally, it is relevant to note the effects of nicotine and ethanol on "bursting," and to discuss their possible implications for the positive relationship between the use of cigarettes and alcohol. Many of the drugs that increase bursting under DRL schedules such as diazepam (9,48), chlordiazepoxide (50,59), pentobarbitone (51), and nicotine (44) also demonstrate efficacy in behavioral models of anxiety (8,14,15,39,42). Similarly, drugs that do not increase bursting under DRL schedules such as *d*-fenfluramine and other amphetamine analogues (49) do not demonstrate efficacy in behavioral models of anxiety (18). The fact that the effects of drugs on bursting often correspond with anxiolytic activity in other behavioral paradigms (5,9,48) have led some authors to speculate that bursting under DRL schedules may be part of a general anxiolytic profile (48) and may reflect an "attenuation of the punishment effect of nonreward" (5). Because nicotine is known to be anxiolytic in humans (41,43), and because anxiolytic effects of smoking are known to be important factors in the maintenance of the smoking habit (43,57), effects of ethanol to potentiate effects of nicotine on bursting may reflect a potentiated anxiolytic effect that motivates some people to smoke more when they drink. This interpretation must be tempered somewhat by the fact that ethanol, which is known to exhibit anxiolytic effects in other paradigms, did not alter bursting in the present experiment. Future experiments that examine the effects of nicotine and ethanol in animal models of anxiety may help to address this hypothesis.

In summary, the present experiment examined effects of nicotine and ethanol, alone and in combination, on performance of a differential reinforcement of low response rates (DRL) schedule in rats. When administered alone, nicotine shifted the mode of the DRL interresponse time distribution to the left and reduced the percentage of reinforced responses. Nicotine also had an inverted U-shaped dose effect on bursting. When subjects were pretreated with ethanol, these effects of were potentiated. Ethanol pretreatment enhanced the leftward shift in the interresponse time distribution and enhanced the effect of low-dose nicotine on bursting. The highest dose of ethanol (3.0 g/kg) also reduced the percentage of reinforced responses when administered alone. Together, the results of the present experiment are consistent with previous reports (44,45) and with the suggestion that ethanol pretreatment can potentiate effects of subsequently administered nicotine (30,52).

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