Pharmacology Biochemistry and Behavior, Vol. 58, No. 4, pp. 975–982, 1997

0091-3057/97 $17.00 + .00

ELSEVIER

PII S0091-3057(97)00304-3

The Effects of Naloxone on Expression and Acquisition of Ethanol Place Conditioning in Rats

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Received 12 September 1996; Revised 23 February 1997; Accepted 4 March 1997

BORMANN, N. M. AND C. L. CUNNINGHAM. *The effects of naloxone on expression and acquisition of ethanol place conditioning in rats.* PHARMACOL BIOCHEM BEHAV **58**(4) 975–982, 1997—Naloxone has been shown to facilitate extinction of ethanol-induced conditioned place preference (CPP) in mice. The present-study extended these findings by examining naloxone's effect on the expression (Experiment 1) and acquisition (Experiment 2) of place conditioning with ethanol in rats. In Experiment 1, after place conditioning with ethanol (1.8 g/kg, IP), groups N0, N1.5, and N10 received 0, 1.5, or 10 mg/kg naloxone before testing. As expected, ethanol produced a robust conditioned place aversion (CPA). However, naloxone had no effect on expression of CPA. In contrast to studies with mice, the endogenous opioid system does not appear to be involved in the conditioned motivational effects of ethanol in rats. In Experiment 2, groups SE1 and SE2, NS(1.5), NE(1.5), and NE(10), received ethanol alone (1.2 g/kg), naloxone alone (1.5 mg/kg), naloxone 1.5 mg/kg plus ethanol, and naloxone 10 mg/kg plus ethanol during acquisition, respectively. All naloxone-treated groups exhibited CPA. Moreover, group NE(1.5) showed a stronger CPA than group NS(1.5). The CPA produced by coadministration of naloxone and ethanol was attributed to naloxone's effects on the neural processes underlying ethanol's unconditioned aversive effects, or to other nonspecific effects on ethanol's motivational properties. © 1997 Elsevier Science Inc.

Place conditioning Aversion Rats Ethanol Naloxone Opioid antagonist Conditioned place aversion Locomotor activity

THERE is evidence that ethanol's effects may be partially mediated by the activation of endogenous opioid systems through changes in the synthesis, release, processing and/or binding properties of opioid peptides (18,19). With respect to ethanol's rewarding effects, the nonselective opiate antagonists naloxone and naltrexone have been shown to decrease ethanol self-administration in animals in several behavioral paradigms [see (9) and (15) for reviews]. Moreover, clinical trials using naltrexone as a possible pharmacotherapy for the treatment of alcoholism (in conjunction with behavior therapy in one study) have been relatively successful, in that alcohol-dependent humans given naltrexone reported significantly less craving and relapse to excessive alcohol drinking than placebo controls (27,28,44,45).

One problem with studying the effects of opioid antagonists on the rewarding properties of ethanol using an oral self-administration procedure is that these antagonists have been shown to have nonspecific depressant effects on food and water intake [e.g., (13)]. Indeed, in several previous experiments, naloxone decreased water intake in conjunction with decreases in ethanol intake [e.g., (14,32)]. To study the role of the endogenous opioid system in the rewarding effects of ethanol, while eliminating the confounding effects of naloxone on ingestive responses, Cunningham et al. (9) performed experiments that examined the effect of naloxone on ethanol-induced conditioned place preference in mice.

In their first experiment, naloxone (1.5 or 10 mg/kg) was administered to mice before ethanol on CS+ conditioning trials to determine whether naloxone would block the unconditioned motivational effects of ethanol. The results showed that neither dose of naloxone had an effect on the acquisition of an ethanol-induced conditioned place preference, even though naloxone alone produced a conditioned place aversion. The magnitude of the conditioned preference in groups

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that received naloxone in combination with ethanol during conditioning was equal to that of saline control groups. This result strongly suggests that the unconditioned rewarding effects of ethanol in mice are not mediated through the activation of endogenous opioid systems. In a different experiment, naloxone (0, 0.15, 1.5, 3.0, or 10 mg/kg) was administered 15 min before a 60 min ethanol-free test session to determine whether it would block expression of the conditioned place preference. During the first 10 min of the test session, all naloxone-treated groups showed a conditioned place preference comparable in magnitude to saline controls. However, the conditioned preference in naloxone groups (doses of 1.5 and higher) gradually extinguished over the course of the test session, such that it was completely extinguished within 30–60 min. In contrast, saline controls exhibited a strong place preference throughout two 60-min test sessions. Hence, naloxone did not interfere with the initial expression, but disrupted the maintenance of the conditioned preference, leading to a facilitation of extinction.

The authors hypothesized that blocking the endogenous opioid system with naloxone facilitated extinction of place preference because either: 1) naloxone facilitated the inhibitory conditioning processes that underlie extinction; or, 2) naloxone blocked the conditioned positive motivational response produced by the CS+ that would normally be responsible for maintaining conditioned place preference (9). In other words, naloxone either had an effect on general learning processes responsible for maintenance of spatial choice behavior in the place conditioning procedure, or a specific effect on the conditioned positive motivational effects of ethanol.

One way to further our understanding of naloxone's influence on ethanol-induced place conditioning is to examine its effect under conditions where ethanol normally produces the opposite motivational effect, i.e., conditioned place aversion. Because most general process learning theories assume that a common inhibitory learning process underlies extinction of both appetitively and aversively motivated behavior [e.g., (29)], an antagonist that generally interfered with extinction would be expected to have that effect regardless of the direction of the motivational effect produced by ethanol. However, if the antagonist selectively altered ethanol's positive conditioned motivational effects, one might not expect to see an effect on conditioned aversion produced by ethanol. The present experiments tested these predictions by examining effects of naloxone on the expression (Experiment 1) and acquisition (Experiment 2) of ethanol-induced conditioned place aversion in rats. In contrast to mice, rats appear relatively insensitive to the rewarding effects of ethanol in the place conditioning paradigm, and usually show no conditioning or shown place aversion to ethanol-paired cues (3,4,6,7,10,11,16,17,30, 31,33,36–38,40,42).

EXPERIMENT 1

The purpose of Experiment 1 was to study the effects of naloxone (1.5 and 10 mg/kg) on the expression of an ethanol-induced conditioned place aversion in rats. After place conditioning with ethanol, groups N0, N1.5, and N10 received naloxone 0, 1.5, and 10 mg/kg, respectively, 15 min before a 60 min test session. Ethanol was not administered during the test. The dose of ethanol (1.8 g/kg) was selected to produce a strong place aversion so that possible decreases in magnitude of aversion by naloxone could be detected. The doses of naloxone were chosen because both were effective at facilitating extinction in the Cunningham et al. (9) study.

METHOD

Subjects

Sixty male Holtzman albino rats weighing approximately 380–450 g were obtained from Harlan–Holtzman. All rats were housed individually in stainless steel wire mesh hanging cages and were maintained on a 12L:12D cycle (lights on at 0700 h). Lab chow and water were available at all times in the home cage.

Apparatus

The apparatus consisted of eight place conditioning boxes each enclosed in a 71 × 58.8 × 68.4 cm (internal dimension) sound attenuating chamber (Kalt, Portland, OR). The place conditioning boxes were composed of 47.5 × 15.5 × 18 cm clear acrylic and aluminum chambers with five sets of infrared light sources and photobeam detectors on the long walls of each box. The detectors were placed 5 cm above the floor with one set of photodetectors placed in the center of the walls and two additional photodetectors 7 cm apart on each side of center. Occlusion of the infrared beams was used to measure general activity and side position (left vs. right) in each box. A rat was considered to have switched sides when both of the outer photobeams on one side were released and at least one of the outer photobeams on the other side was occluded. Activity and amount of time spent on both sides of the box were collected and analyzed by microcomputer (10 ms resolution).

The floors of each box were composed of interchangeable halves of two floor types: grid and hole. The "grid" floor consisted of 2.3-mm stainless steel rods mounted 13 mm apart in an acrylic frame. The "hole" floor was made of perforated stainless steel with 13-mm round holes on 19-mm staggered centers. The grid and hole floors were selected as CSs on the basis of previous pilot experiments in which rats showed approximately equal preference between grid and hole floor types. The floors were cleaned and the litter paper was changed after each animal.

Ethanol was prepared for injection by diluting 95% ethanol in isotonic saline to yield a concentration of 15% (v/v). The dose of ethanol was 1.8 g/kg (15 ml/kg, IP). Naloxone was prepared by diluting naloxone hydrochloride in saline at concentrations of 1.5 mg/ml and 10 mg/ml. Naloxone injection volumes were 1 ml/kg (IP).

Procedure

The general design and procedure were similar to those previously reported (11). All experiments consisted of three phases: habituation, conditioning, and test. Training occurred 7 days per week.

Habituation (3 days). On habituation days, subjects were weighed and then placed in the sound attenuating chambers in their home cages for 3 hr (four cages per chamber) per day. Subjects were placed in the chambers in their home cages so as to maximize the amount of time that the 60 rats could be exposed to a limited number of chambers (eight chambers). The purpose of these sessions was to latently inhibit general box and handling cues so that they would acquire little associative strength during subsequent conditioning. Exposure to box cues before conditioning has been shown to increase aversive conditioning to floor cues in this procedure with rats (Cunningham and Niehus, unpublished data).

Conditioning (8 days). Subjects within each of three groups (groups N0, N1.5, and N10; n = 20/group) were randomly

assigned to one of two conditioning subgroups (Grid+ or Grid−). Subjects were exposed to a differential Pavlovian conditioning procedure in which they received four CS+ and four CS− trials, with CS+ and CS− trials occurring on alternate days, and with the order of CS+ and CS− presentation counterbalanced within conditioning subgroup. Subjects had access to the entire floor of the conditioning compartment with both halves of the floor being either hole or grid. Grid+ subgroups received ethanol paired with the grid floor and saline paired with the hole floor. Grid− subgroups received ethanol paired with the hole floor and saline paired with the grid floor. All groups received ethanol or saline injections immediately before placement on the appropriate floor for 60 min.

Test (1 day). Groups N0, N1.5, and N10 received injections of 0, 1.5, and 10 mg/kg naloxone, respectively, 15 min before a saline injection. Immediately after the saline injection, subjects were placed into the conditioning box for 60 min with access to both grid and hole floor types. Position of grid and hole floors was counterbalanced within each subgroup.

RESULTS

Data in all experiments were analyzed using analysis of variance (ANOVA) with the alpha level set at 0.05. One rat in group N0 (Grid− subgroup) died before the end of the experiment; its data were excluded from all analyses.

Conditioning Trials

Preliminary statistical analysis showed a general decrease in activity across successive conditioning trials on both ethanol and saline days in all groups. This decrease across trials most likely reflects habituation to the novelty/stress of the apparatus, handling, and injection. In addition, as shown in Table 1 (Experiment 1), ethanol had a depressant effect on locomotor activity compared to saline activity levels during conditioning trials. This result is generally consistent with other rat place conditioning studies (4,11). Activity data were collapsed across conditioning trials, and a two-way [group (3) × CS type (2)] analysis of variance (ANOVA) was performed on mean activity counts per min for groups N0, N1.5, and N10. This analysis revealed a significant main effect of CS type, $F(1, 56) = 50.40$, $p < 0.001$, confirming that ethanol produced lower levels of activity. No other effects were significant.

TABLE 1

MEAN ACTIVITY COUNTS PER MINUTE (± SEM) AVERAGED ACROSS ETHANOL AND SALINE CONDITIONING TRIALS DURING EXPERIMENTS 1 (TOP) AND 2 (BOTTOM)

Experiment 1		
Group	Ethanol	Saline
N0 (n = 19)	5.8 ± 0.35	7.0 ± 0.50
N1.5 (n = 20)	5.1 ± 0.34	7.8 ± 0.44
N10 (n = 20)	5.6 ± 0.25	7.5 ± 0.34
Experiment 2		
Group	Drug	Saline
SE1 (n = 19)	5.0 + 0.34	6.9 + 0.43
SE2 (n = 19)	5.4 + 0.27	7.1 + 0.34
NS(1.5) (n = 19)	4.5 + 0.30	6.1 + 0.32
NE(1.5) (n = 37)	4.7 + 0.22	7.4 + 0.37
NE(10) (n = 19)	4.2 + 0.32	6.8 + 0.36

Place Preference Test

Preference. Preliminary analyses of preference data showed that the magnitude of the conditioned aversion in each group varied across the 60-min test session. Hence, as in the Cunningham et al. report (9), preference data were analyzed in three separate time intervals: min 1–10, 11–30, and 31–60. Mean sec/min minutes spent on the grid floor for all groups during min 1–10, 11–30, and 31–60 are shown in the top, middle, and bottom left panels, respectively, of Fig. 1. As can be seen, subjects in all groups exposed to ethanol on the grid floor (Grid+) spent less time on the grid floor than subjects exposed to saline on the grid floor (Grid−). Differences between Grid+ and Grid− conditioning groups reflect a significant place aversion to ethanol-paired floors. Left panels in Fig. 1 also show

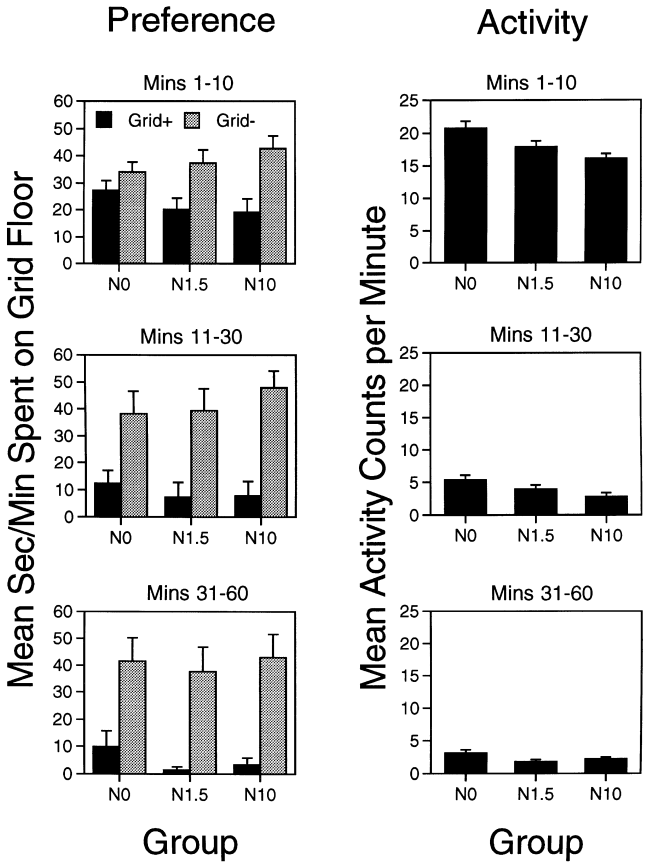

FIG. 1. Top, middle, and bottom left panels show mean (±SEM) sec/min spent on the grid floor during Min 1–10, Min 11–30, and Min 31–60 of the preference test in Experiment 1, respectively. Grid+ and Grid− refer to the conditioning subgroups within each group that had previously received either the grid floor (Grid+) or hole floor (Grid−) and ethanol on CS+ conditioning trials. These subgroups were exposed to the opposite floor type and saline on CS− conditioning trials. Right panels show mean (±SEM) activity counts per min during Min 1–10, Min 11–30, and Min 31–60 of the test session. Groups N0, N1.5, and N10 received 0, 1.5, or 10 mg/kg naloxone 15 min before test sessions, respectively.

that the magnitude of conditioned aversion increased in all groups across the 60-min test session. In addition, it appears that both doses of naloxone produced a slight enhancement of the expression of place aversion in groups N1.5 and N10 compared to Group N0.

Separate two-way ANOVAs [group (3) × conditioning subgroup (2)] were performed on mean sec/min spent on the grid floor for all groups during min 1–10, 11–30, and 31–60. The ANOVAs revealed a significant main effect of conditioning subgroup at each time interval, min 1–10, $F(1, 53) = 19.50$, $p < 0.001$; min 11–30, $F(1, 53) = 38.71$, $p < 0.001$; min 31–60, $F(1, 53) = 42.57$, $p < 0.001$. However, there were no significant group main effects or group × conditioning subgroup interactions at any of the time intervals. Thus, naloxone did not have a significant effect on expression of place aversion.

Activity. The top, middle, and bottom right panels of Fig. 1 show mean activity counts per minute for each group during min 1–10, min 11–30, and min 31–60, respectively. As can be seen in the figure, naloxone produced a dose-dependent decrease in locomotor activity throughout the test session. Three separate one-way ANOVAs performed on test session activity data revealed significant group main effects during each time interval, min 1–10, $F(2, 56) = 3.21$, $p < 0.05$; min 11–30, $F(2, 56) = 3.96$, $p < 0.05$; min 31–60, $F(2, 56) = 3.18$, $p < 0.05$. Pairwise comparisons between groups revealed that activity in group N10 was significantly below that of group N0 during min 11–30 only (Bonferroni-corrected $p < 0.02$). During min 1–10, the difference between groups N0 and N10 just missed significance (Bonferroni-corrected $p = 0.06$). Activity counts were not significantly different between pairs of groups during the remainder of the test session once alpha levels were adjusted for multiple comparisons (Bonferroni-corrected $ps > 0.05$).

DISCUSSION

Naloxone did not have a significant effect on initial expression or short-term maintenance of a conditioned place aversion to ethanol-paired cues in rats. Hence, unlike the Cunningham et al. study with mice (9), naloxone did not facilitate extinction of the conditioned response. These data suggest that activation of naloxone-sensitive endogenous opioid receptors is not necessary for the expression of ethanol-induced conditioned place aversion. Furthermore, they suggest that the facilitation of extinction of conditioned place preference by naloxone in mice is probably not due to a general facilitatory effect on inhibitory learning processes, but rather to a disruption of the conditioned reinforcing effects produced by the CS+, which are necessary for maintenance of conditioned preference. It seems likely that the conditioned positive and negative motivational effects of ethanol are affected by different neurochemical systems. The data of Cunnmingham et al. suggest that expression of ethanol's positive conditioned effects in the place conditioning paradigm is modulated by the endogenous opioid system, while the present data suggest that expression of ethanol's negative conditioned effects is controlled by some other, yet unidentified, neurochemical system.

An alternative explanation might be that mice and rats differ in the neurochemical mechanisms responsible for the inhibitory processes that underlie extinction of place conditioning. This is a distinct possibility, because mice and rats differ in the direction of conditioned responses produced by ethanol in the place conditioning paradigm. Mice consistently exhibit a conditioned preference for ethanol paired cues, while rats typically show place aversion [e.g., (4,11)]. In this scenario, the endogenous opioid system would mediate learning mechanisms responsible for the maintenance of place conditioning in mice, but not in rats. If this is indeed the case, then naloxone could differentially affect extinction in the two species, without necessarily affecting the positive and negative conditioned motivational effects elicited by the CS+.

Decreases in locomotor activity produced by naloxone may have been partially responsible for the nonsignificant trend toward enhancement of ethanol-induced conditioned aversion. Moreover, it is possible that disruption of activity by naloxone could have interfered with observing a facilitation of extinction of place aversion. In the place conditioning literature, several findings indicate that higher magnitude preferences are correlated with lower levels of test-session activity (8,25,43). In the present experiment, locomotor activity decreased across the 60-min test session in all groups (even in group N0, which did not receive naloxone), most probably due to within-session habituation to the place conditioning boxes. In all groups, as locomotor activity decreased, magnitude of place aversion increased. However, this outcome seems unlikely since Cunningham et al. (9) found that naloxone facilitated extinction of place preference (or decreased the magnitude of the CR), despite the fact that it also produced a significant reduction in locomotor activity.

EXPERIMENT 2

Experiment 1 showed that naloxone did not affect the expression of a conditioned place aversion, suggesting that the endogenous opioid system does not play a role in the conditioned aversive motivational effects of ethanol in rats. Although naloxone had no effect on the acquisition of ethanol conditioned place preference in mice (9), it is possible that naloxone might affect the *unconditioned* aversive motivational effects of ethanol in rats. A few studies in the taste aversion paradigm have implicated a role for the endogenous opioid system in the negative motivational effects of ethanol. There are reports that coadministration of nonspecific opiate receptor antagonists with ethanol during conditioning both augmented (5,22) and attenuated (26) ethanol-induced conditioned taste aversion.

The purpose of Experiment 2 was to determine whether naloxone would affect the unconditioned motivational effects of ethanol by administering naloxone during acquisition of ethanol place conditioning in rats. Groups SE1 and SE2, NS(1.5), NE(1.5), and NE(10), received ethanol alone (1.2 g/kg), naloxone alone (1.5 mg/kg), naloxone 1.5 g/kg plus ethanol, and naloxone 10 mg/kg plus ethanol, respectively, during conditioning. The dose of ethanol was reduced to 1.2 g/kg to decrease the magnitude of conditioned place aversion and provide room to observe a possible enhancement of aversion by naloxone (5,22).

METHOD

Subjects

One hundred twenty naive adult Holtzman albino rats weighing approximately 355–470 g were obtained from Harlan–Holtzman. All rats were housed individually in stainless steel wire cages, maintained on a 12L:12D cycle, and received free access to food and water in the home cage.

Apparatus and Procedures

The apparatus and basic procedures were the same as in Experiment 1 except: 1) the dose of ethanol used during con-

ditioning was 1.2 g/kg, instead of 1.8 g/kg; and, 2) naloxone was administered before conditioning trials instead of before the preference test. The study was run as two separate experiments, with groups SE1, NE1(1.5), and NE(10) trained at a different time than groups SE2, NS(1.5), and NE2(1.5). All training for all groups was conducted within a 2-month period by the same experimenter.

During the conditioning phase, all subjects received two injections each day. The first injection was given 15 min before the second injection, with subjects placed back in their home cages between injections. After the second injection, subjects were placed on CS+ or CS− floors. Subjects in six groups [groups SE1, SE2, NS(1.5), NE1 (1.5), NE2(1.5), and NE(10)] of 20 rats were assigned to Grid+ and Grid− conditioning subgroups. Groups NE1(1.5) and NE2(1.5) received 1.5 mg/kg naloxone 15 min before ethanol injection (1.2 g/kg) on CS+ days. Group NS(1.5) received 1.5 mg/kg naloxone 15 min before saline injection on CS+ days. Group NE(10) received 10 mg/kg naloxone 15 min before ethanol injection (1.2 g/kg) on CS+ days. Groups SE1 and SE2 received saline (1 ml/kg) 15 min before ethanol (1.2 g/kg) on CS+ days. On CS− days all groups received two saline injections 1 ml/kg and 10 ml/kg.

During test sessions, subjects in each group received two saline injections (1 ml/kg and 10 ml/kg) 15 min apart before placement into conditioning boxes for 60 min with access to both grid and hole floor types.

RESULTS

One subject from each of groups SE2 (Grid+) and NE2(1.5) (Grid−) were removed from the experiment because of health-related problems. Due to experimenter errors, the data from one subject in each of groups SE1, NE(10), and NS(1.5), and two subjects from group NE(1.5) were excluded from analyses.

Analyses were performed to determine whether data from the two pairs of replicate groups [SE and NE(1.5) groups] could be combined. No significant differences between groups NE1(1.5) and NE2(1.5) in mean sec/min spent on grid floor (preference data) or activity were detected, so data from these two groups were combined. However, a two-way ANOVA on test preference data revealed a significant group × conditioning subgroup interaction between group SE1 and SE2, $F(1, 34) = 4.2$, $p < 0.05$. For this reason, data from groups SE1 and SE2 are presented separately.

Conditioning Trials

Table 1 (Experiment 2) shows mean activity counts per min for all groups during CS+ and CS− conditioning trials. As can be seen in the table, ethanol alone, naloxone alone, and ethanol plus naloxone (1.5 or 10 mg/kg) all had depressant effects on locomotor activity. However, in general, locomotor activity was not systematically affected by the addition of naloxone in groups NE(1.5) and NE(10). A three-way ANOVA [group (5) × CS type (2)] performed on mean activity counts/min for all groups on CS+ and CS− days showed a significant main effect of CS type, $F(1, 108) = 163.68$, $p < 0.001$, but no effects involving group.

Preference Test

Preference. Mean sec/min spent on the grid floor during the preference test is plotted for each group in Fig. 2. The figure shows that groups NS(1.5), NE(1.5), and NE(10) developed a conditioned aversion for the ethanol-paired floor, i.e., rats that received drug on the grid floor (Grid+) spent significantly less time on the grid floor than subjects that received drug on the hole floor (Grid−). Unexpectedly, groups SE1 and SE2 did not show a significant aversion to the ethanol paired floor. A two-way ANOVA [group (5) × conditioning subgroup (2)] performed on mean time spent on grid floor revealed a significant conditioning subgroup main effect, $F(1, 103) = 43.83$, $p < 0.001$, and a significant group × conditioning subgroup interaction, $F(4, 103) = 9.31$, $p < 0.001$. Comparisons of Grid+ vs. Grid− subgroups within each group (to determine whether conditioning occurred) revealed significant differences between subgroups in groups NS(1.5) ($p < 0.03$), NE(1.5) ($p < 0.001$), and NE(10) ($p < 0.001$), but not groups SE1 and SE2 ($ps > 0.05$) (Bonferroni-corrected p-values). Thus, all of the naloxone-treated groups showed a significant conditioned place aversion, but neither of the ethanol-alone (SE) groups developed an aversion. A follow-up two-way ANOVA (group (2) × conditioning subgroup (2)] comparing groups NS(1.5) and NE(1.5) confirmed a significant conditioning subgroup main effect, $F(1, 52) = 68.27$, $p < 0.001$, and a significant group × conditioning subgroup interaction, $F(1, 52) = 5.56$, $p < 0.02$. The interaction confirms that conditioned place aversion in the group that received ethanol and naloxone (NE(1.5)) was greater than that in the group receiving naloxone, but no ethanol (NS(1.5)).

Activity. No group differences in locomotor activity were found between groups during the test session, $F(1, 108) = 1.6$, $p < 0.05$. Mean activity counts per minute (±SEM) were 5.2 ± 0.4, 5.7 ± 0.4, and 5.9 ± 0.4, 5.5 ± 0.3, and 4.6 ± 0.5 in groups SE1, SE2, NS(1.5), NE(1.5), and NE(10), respectively.

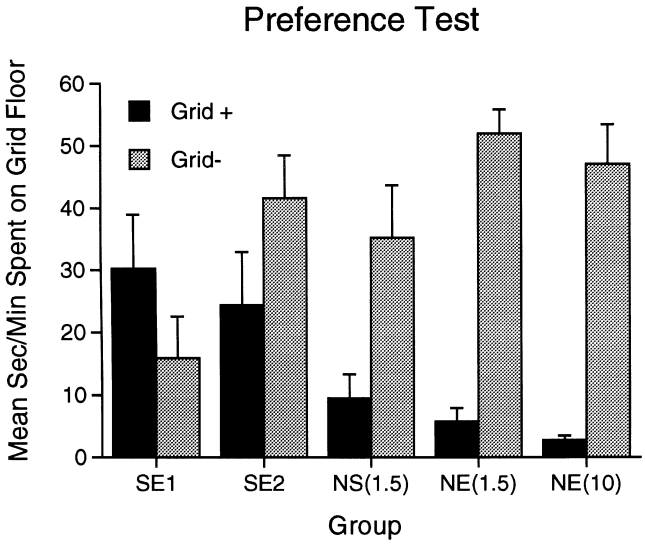

FIG. 2. The figure shows mean (±SEM) sec/min spent on the grid floor during the preference test of Experiment 2. Grid+ and Grid− refer to the conditioning subgroups within each group that had previously received either the grid floor (Grid+) or hole floor (Grid−) and ethanol on CS+ conditioning trials. These subgroups were exposed to the opposite floor type and saline on CS− conditioning trials. Groups SE1 and SE2, NS(1.5), NE(1.5), and NE(10), received ethanol alone (1.2 g/kg), naloxone alone (1.5 mg/kg), naloxone 1.5 mg/kg plus ethanol, and naloxone 10 mg/kg plus ethanol during acquisition, respectively.

DISCUSSION

Subjects that received naloxone alone [group NS(1.5)] and naloxone in combination with ethanol [groups NE(1.5) and NE(10)] during conditioning exhibited a significant conditioned place aversion, with group NE(1.5) exhibiting significantly greater aversion than group NS(1.5). Conditioned place aversion was not observed in either of the ethanol alone groups (groups SE1 and SE2). The fact that naloxone alone produced a significant place aversion in group NS(1.5) is consistent with prior reports that naloxone possesses aversive motivational properties. Several studies have demonstrated that naloxone alone is capable of producing both conditioned taste aversion (20,23,39) and place aversion in rats (1,2,12,23, 24,34,41).

Unexpectedly, ethanol alone in Groups SE1 and SE2 did not produce a significant conditioned place aversion in Experiment 2. The 1.2 g/kg dose of ethanol should have been effective at producing aversion, because ethanol doses of 1 g/kg and higher have usually been shown to produce place aversion (4,6,7,10,11,16,17,30,31,33,36–40,42). In fact, there is one prior report from this laboratory (10) of place aversion in rats using the same parameters employed in Experiment 2 (a 1.2 g/kg ethanol dose and a 60-min CS duration). The only known procedural difference between Experiment 2 and the previous study was that rats in SE groups in the present experiment were injected with saline 15 min before ethanol injection and exposure to CS+ floors. It is possible that the double injection procedure in some way interfered with the ability of the 1.2 g/kg dose of ethanol to produce a significant place aversion in the SE groups. An experiment employing the same conditioning parameters, comparing saline preinjection vs. no injection would answer this question.

Coadministration of naloxone and ethanol in group NE(1.5) produced a stronger place aversion than either naloxone or ethanol alone. The most parsimonious explanation of this result is that a subthreshold aversive effect of ethanol combined with an above-threshold aversive effect of naloxone to produce a larger place aversion than either of the two drugs alone. The suggestion that ethanol had a subthreshold aversive effect at this dose is consistent with a large number of rat studies showing ethanol-induced conditioned taste aversion or conditioned place aversion at similar or slightly higher doses [e.g., (4,6,7,10,11,22,26,38)]. The interpretation offered here does not involve any assumptions about opioid modulation of ethanol's effects. That is, one can explain the greater aversion in the naloxone–ethanol group without arguing that naloxone affected the neurochemical mechanisms underlying ethanol's unconditioned aversive effects.

As in the present study, Cunningham et al. (12) found that naloxone alone also produced a conditioned place aversion in mice. However, the aversive effects of naloxone did not summate with the rewarding effects of ethanol to produce a decrease in preference compared to ethanol alone. Rather, mice given naloxone + ethanol showed a conditioned place preference equal in magnitude to that shown by mice given ethanol alone. The authors explained naloxone's failure to affect acquisition of ethanol preference in terms of the two drugs producing two distinct unconditioned stimulus effects. Presumably, naloxone-alone mice, given naloxone 15 min before saline injection and exposure to CS+, associated the aversive effects of naloxone with CS+. However, naloxone + ethanol mice were exposed to the aversive effects of naloxone first followed (15 min later) by the rewarding effects of ethanol and CS+ exposure. It was hypothesized that these mice only associated ethanol's motivational effects with CS+ because ethanol's temporal relationship with the CS was more optimal for conditioning than naloxone's (12). In the present experiment with rats, the motivational effects of naloxone and ethanol were most probably indiscriminable to subjects as two separate USs because both drugs produced aversive motivational effects. Presumably, the magnitude of the aversive US produced by the naloxone and ethanol combination was greater, and thus, produced stronger conditioning than that produced by naloxone or ethanol alone.

GENERAL DISCUSSION

The results of the expression study (Experiment 1) suggest that activation of the endogenous opioid system is not involved in the conditioned aversive motivational effects of ethanol in rats as measured by the place conditioning paradigm. Unlike the Cunningham et al. study with mice (9), naloxone did not facilitate extinction of conditioned place aversion in rats. If facilitation of extinction in the Cunningham et al. study was produced by naloxone through a facilitation of general inhibitory conditioning processes, independent of ethanol's motivational effects, then we should have observed facilitation of extinction of conditioned place aversion in rats in Experiment 1. Hence, the results of Experiment 1 are important, in part, because they lend support for the interpretation that activation of the endogenous opioid system is necessary for expression of the conditioned positive reinforcing (but not aversive) effects of ethanol by the CS+ in mice (9).

The fact that activation of endogenous opioid systems may be necessary for maintenance of the conditioned reinforcing effects of environmental stimuli paired with ethanol is significant, because conditioned positive motivational responses are thought to underlie "craving", and possibly contribute to relapse to drinking in abstinent alcoholics (35). Cunningham et al. (9) proposed that naltrexone was successful at decreasing craving and relapse to excessive drinking in human studies (27,28,44,45) because it facilitated extinction of the conditioned positive motivational effects of ethanol that underlie craving (9). A better understanding of the specific opioid receptor systems mediating this effect could lead to the development of effective pharmacotherapies to prevent relapse to excessive alcohol drinking in abstinent alcoholics.

The acquisition study (Experiment 2) showed that coadministration of naloxone and ethanol produced a significant conditioned place aversion that was stronger than that produced by either ethanol or naloxone alone. We interpret the increase in magnitude of place aversion produced by the two drugs in combination to have been due to the summation of a subthreshold aversive effect of ethanol with the above-threshold aversive effect of naloxone. This analysis is consistent with a study by Miceli et al. (22), in which coadministration of naloxone and ethanol produced a significantly larger taste aversion than the two drugs alone, with naloxone not producing a significant taste aversion. Although their results would appear to support a role for the endogenous opioid system in the aversive effects of ethanol, Miceli et al. found that naloxone also enhanced conditioned taste aversion produced by lithium chloride. In line with the interpretation of Experiment 2, one would predict that coadministration of naloxone with any other drug known to produce conditioned place aversion (e.g., lithium chloride) would also produce an increase in conditioned aversion.

Experiment 2 confirms and extends the results of a report by Marglin and Reid (21), which demonstrated that coadmin-

istration of naloxone and ethanol produced a conditioned place aversion. Although the authors concluded that their results supported the notion that the endogenous opioid system mediates the motivational properties of ethanol, their findings are equivocal for several reasons. First, groups that received naloxone in combination with ethanol before exposure to the CS+ also received naloxone prior to experiencing the CS−. Because naloxone alone may have produced aversion to CS−, interpretation of performance during the preference test is complicated. Second, Marglin and Reid did not include a naloxone-alone control group in their study, so there is no information regarding the magnitude of naloxone's aversive effects using their procedures. Moreover, there was no evidence of conditioned preference or aversion in rats given ethanol alone. Finally, subjects were exposed to an unequal number of CS+ (nine trials) and CS− (three trials) conditioning trials. Hence, one cannot rule out the possibility that rats were approaching the CS− side, in part, because they had less exposure to these cues, and were exploring a relatively novel environment. The present experiment demonstrates more clearly that an ethanol + naloxone combination produces stronger place aversion than ethanol or naloxone alone. This effect was replicated in two independent groups, NE1(1.5) and NE2(1.5).

In conclusion, the present set of experiments provide new information on the neuropharmacological mechanisms underlying the aversive motivational effects of ethanol in rats. Specifically, Experiment 1 demonstrates that the endogenous opioid system is not involved in the *conditioned* aversive effects of ethanol. Experiment 2 does not support, but also does not rule out a role for the endogenous opioid system in the *unconditioned* aversive motivational effects of ethanol. Further place conditioning studies with naloxone in rats are needed to clarify the role of the endogenous opioid system in the unconditioned aversive effects of ethanol.

ACKNOWLEDGEMENTS

This research was supported in part by NIAAA Grants AA07702 and AA07468. Portions of these data were presented at the annual meeting of the Research Society on Alcoholism (Washington, D.C., June 1996).

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