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Effects of naltrexone and LY255582 on ethanol maintenance, seeking, and relapse responding by alcohol-preferring (P) rats

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Abstract

Research indicates opioid antagonists can reduce alcohol drinking in rodents. However, tests examining the effects of opioid antagonists on ethanol seeking and relapse behavior have been limited. The present study examined the effects of two opioid antagonists on ethanol maintenance, seeking, and relapse responding by alcohol-preferring (P) rats. Adult P rats were self-trained in two-lever operant chambers to self-administer 15% (vol/vol) ethanol on a fixed-ratio 5 (FR5) versus water on a FR1 concurrent schedule of reinforcement in daily 1-h sessions. After 10 weeks, rats underwent extinction training, followed by 2 weeks in their home cages. Rats were then returned to the operant chambers without ethanol or water to measure responses on the ethanol and water levers for four sessions. After a subsequent 2 weeks in the home cage, without access to ethanol, rats were returned to the operant chambers with ethanol and water available. Effects of antagonists on maintenance responding were tested after several weeks of daily 1-h sessions. Naltrexone (NAL; 1–10 mg/kg, subcutaneously [s.c.]; *n* = 8/dose), LY255582 (LY; 0.03–1 mg/kg, s.c.; *n* = 8/dose), or vehicle were injected 30 min before the first session (in the absence of ethanol), following 2 weeks in their home cages, and for four consecutive sessions of ethanol self-administration under maintenance and relapse conditions. Both NAL and LY reduced responses on the ethanol lever without any fluids present, and ethanol self-administration under relapse and on-going drinking conditions, with LY being more potent than NAL. Both NAL and LY were less effective in reducing responding in the absence of ethanol than in reducing ethanol self-administration. Overall, the results indicate that the opioid system is involved in mediating ethanol

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seeking, and ethanol self-administration under relapse and on-going alcohol drinking, but that different neurocircuits may underlie these behaviors.

Keywords

Ethanol reinforcement; Pavlovian Spontaneous Recovery; Alcohol deprivation effect; Alcohol seeking; Operant; Alcohol relapse

Introduction

Preclinical and clinical data implicate the endogenous opioid system in alcohol dependence (Modesto-Lowe and Fritz, 2005; Oswald and Wand, 2004). Preclinically, naltrexone (NAL), an opioid receptor subtype nonspecific antagonist, has been shown to decrease alcohol consumption in nonhuman primates (Boyle et al., 1998; Kornet et al., 1991; Myers et al., 1986; Williams et al., 1998), in rat lines selectively bred for high-alcohol consumption (Gilpin et al., 2008; Koistinen et al., 2001; Rezvani et al., 2007; Sabino et al., 2006; Sable et al., 2006; Zalewska-Kaszubska et al., 2008), in rat lines selectively bred for characteristics other than alcohol consumption (Pellicano and Sadile, 2006), in nonselected genetically heterogenous rats (Czachowski and Delory, 2009; Ji et al., 2008; Kiefer et al., 2005; Kuzmin et al., 2008; Mhatre et al., 2004; Oliva and Manzanares, 2007; Walker and Koob, 2008), and in inbred C57BL/6J (B6) mice (Escher and Mittleman, 2006; Grahame et al., 2000; Kamdar et al., 2007; Kim et al., 2004). Among most double-blind controlled clinical trials, NAL has demonstrated a consistent yet modest effect in reducing heavy alcohol consumption in both alcohol-dependent (Assanangkornchai and Srisurapanont, 2007; Krishnan-Sarin et al., 2007; Pettinati et al., 2006) and nondependent (Tidey et al., 2008) individuals. In addition to the reduction of heavy drinking, NAL was found to facilitate the maintenance of abstinence, and the prevention of relapse to heavy drinking (O'Malley et al., 1992; Rösner et al., 2008; Spanagel and Kiefer, 2008).

Operationally, ethanol-seeking behavior is a result of a prior history of chronic ethanol drinking that is expressed in the absence of alcohol, in response to ethanol-associated cues, a priming dose of ethanol, or stress. In several studies, NAL has blocked or decreased seeking induced by ethanol-associated cues, and by a priming dose of ethanol, but not by stress. Cue-induced ethanol-seeking behavior of Long-Evans rats was blocked with administration of 10 mg/kg dose of NAL (Williams and Schimmel, 2008), and reduced with a 0.3 mg/kg dose of NAL (Burattini et al., 2006). In Wistar rats, a 1-mg/kg dose of NAL was sufficient to block cue-induced ethanol-seeking behavior (Ciccocioppo et al., 2003; Dayas et al., 2007; Liu and Weiss, 2002). In Wistar rats, NAL blocked reinstatement of ethanol responding induced by a priming dose of ethanol, but not by stress (Lê et al., 1999; Liu and Weiss, 2002). Bäckström and Hyytia (2004) indicated that NAL administered to Long-Evans rats, decreased reinstatement induced by a priming dose of ethanol. Overall, the data indicate that the opioid system is involved in cue-induced and ethanol-induced alcohol-seeking behavior, as measured in a reinstatement model using nonselected lines of rats.

Relapse can be defined as a return to drug use or drinking after a period of abstinence. It has been demonstrated that NAL decreased ethanol-consuming behaviors in animal models of

alcohol relapse. The alcohol deprivation effect (ADE) is a commonly used animal model of relapse behavior (Rodd et al., 2004). The ADE is defined as a temporary increase in the voluntary intake of ethanol solutions when ethanol is restored following a period of alcohol deprivation (Sinclair and Senter, 1967, 1968). The ADE can be blocked by NAL in Wistar rats (Heyser et al., 2003; Hölter and Spanagel, 1999; Kuzmin et al., 2007), in Fawn Hooded rats (Cowen et al., 1999), in high ethanol-preferring rats (Mormede et al., 2004), and in rhesus monkeys (Kornet et al., 1991). These findings indicate that the opioid system is involved in ethanol relapse drinking and self-administration.

Although several studies have been carried out to determine the effect of NAL on ethanol seeking, none have been carried out using Pavlovian Spontaneous Recovery (PSR) as a model of ethanol-seeking behavior. PSR is the recovery of responding in the absence of the previously trained reward that is observed following a period of rest after extinction (Domjan and Burkhard, 1982; Macintosh, 1977). One beneficial aspect of the PSR procedure is that the spontaneous responding operant behavior occurs in the absence of drug administration following prolonged periods of abstincence. Because the behavior occurs within the environment previously associated with drug availability in the absence of drug reinforcement, and following a prolonged drug-free period, all responses are thought to be intrinsically motivated (Pavlov, 1927). Thus, the persistence of PSR in the absence of reward can be conceived as a suitable paradigm to assess drug-seeking behavior in animals (Rodd et al., 2004), and in modeling the compulsive nature of drug abuse in humans (Anton, 1999). The present study is the first to determine the effect of NAL or LY255582 (LY) on the robust PSR observed in alcohol-preferring (P) rats.

Although studies have been carried out to determine the role of the opioid system in alcohol-seeking and relapse behaviors (Burattini et al., 2006; Ciccocioppo et al., 2002, 2003; Marinelli et al., 2007), no opioid antagonist studies have been carried out with P rats using the PSR test. With respect to relapse, one study carried out in selectively bred P rats (Badia-Elder et al., 1999) reported a reduction with naloxone, whereas the other study carried out in the inbred P rat strain (Rezvani et al., 2009) reported no effect with NAL. However, neither study examined the effects of the opioid antagonist under operant conditions.

The objectives of the present study were to determine the effects of NAL on robust alcohol-seeking and relapse behaviors following prolonged deprivation, and assess their effect on maintenance of alcohol self-administration under operant conditions and to compare these effects with a novel nonspecific opioid antagonist, LY.

Materials and methods

Animals

Adult female selectively bred P rats from the 53rd to 54th generations weighing 250–325 g at the start of the experiment were used. P rats were obtained from breeding colonies on campus. Female P rats were used throughout the study because females were available when the study was initiated. Although estrous cycle was not monitored, previous studies carried out with female P rats, in which ethanol motivated responding (ethanol consumption and intracranial ethanol self-administration) was monitored over a 2–4-week period, indicated no

significant day-to-day alterations in responding, suggesting little influence of the estrous cycle on motivated responding by female P rats (Rodd et al., 2005; Sable et al., 2006; Toalston et al., 2008). Rats were maintained on a 12-h reversed light-edark cycle (lights off at 0900 h). Food and water were available ad libitum throughout the experiment, except during operant testing. The animals used in these experiments were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. All research protocols were approved by the Institutional Animal Care and Use Committee (Indiana University School of Medicine) and are in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute on Drug Abuse, National Institutes of Health, and the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council 1996).

Drug preparation

LY was provided by the Eli Lilly Company (Indianapolis, IN). LY was dissolved in lactic acid and pH adjusted to 5.00 with 1 N NaOH. NAL HCL was obtained from Sigma (St. Louis, MO) and was dissolved in saline. NAL and LY were administered subcutaneously 30 min prior to the test session. NAL was given in doses of 1, 5, or 10 mg/kg; LY was given in doses of 0.03, 0.1, 0.3, or 1.0 mg/kg. Doses for NAL were chosen based on background studies carried out in adolescent and adult P rats showing that doses as high as 20 and 30 mg/kg were needed to decrease ethanol consumption. These doses did not alter water and food intake. In fact, in the adolescent rats, there was a compensatory increase in water intake, indicating a decreased preference for ethanol with the total volume of fluid consumed remaining unaffected (Sable et al., 2006). The rationale for using the doses for LY that were used stem from studies indicating that doses as high as 1 mg/kg (the highest dose used in our study) did not decrease food intake in obese zucker rats (Statnick et al., 2003).

Operant apparatus

Ethanol self-administration (in 60-min sessions) was conducted in standard two-lever experimental chambers (Coulbourn Instruments) contained within ventilated, sound-attenuated enclosures as previously described (Rodd-Henricks et al., 2002a, 2002b).

Operant training

Without any prior training, exposure to the experimental setup, or access to ethanol, rats were placed into the operant chambers. The ethanol (15% vol/vol) and water levers were maintained on a fixed-ratio 1 (FR1) schedule of reinforcement for the first 5 weeks (Fig. 1). Subsequently, the reinforcement schedule on the ethanol lever was increased to FR3 in weeks 6 and 7 and to FR5 in weeks 8–10 (Fig. 1). The response requirement was increased to demonstrate that ethanol was a more potent reinforcer than water, and was also increased so as to have a high baseline level of responding to allow for determining dose-dependent reductions in responding. Water was always reinforced on an FR1 schedule. The FR1 schedule was maintained for water because increasing the requirement would result in a further reduction in the low level of responding for water.

Extinction

After the P rats had established stable levels of responding, rats were placed into the operant chambers during the 60-min period, but neither water nor ethanol was available. The delivery system operated exactly as during acquisition; rats still received the auditory stimulus of the dipper raising and the visual cue of the small light being illuminated above the dipper trough. Rats were exposed to the extinction sessions for seven consecutive sessions (Fig. 1). Similar methodology was used by Rodd et al. (2006).

PSR testing

After extinction training, all rats were maintained in their home cages for 14 days (Fig. 1). Following this 2-week period, rats were returned to the operant chambers for PSR testing. Similar to the extinction phase of the experiment, both the ethanol and water troughs were empty. Except for the absence of fluids, the delivery system operated exactly as during acquisition. Rats were exposed to the PSR testing for 4 consecutive days (Fig. 1). Similar methodology was used by Rodd et al. (2006).

Relapse

Following the PSR phase of the experiment, all rats were again maintained in their home cages for 14 days (Fig. 1). Rats were then transferred to the operant chambers with both ethanol and water available for the 60-min sessions. The ethanol lever was maintained on an FR5 schedule and the water lever on an FR1 schedule.

Maintenance

For the NAL study, a separate group of rats were processed through the same experimental technique but only received NAL during the maintenance period.

For the LY study, following 30 consecutive daily sessions of operant access to 15% ethanol and water (Fig. 1), the effects of LY on ethanol maintenance responding were tested. This number of sessions was carried out to establish on-going ethanol self-administration and minimize any possible carryover effect of LY.

NAL administration on ethanol PSR/ADE/maintenance responding

Following acquisition and extinction training, 32 adult P female rats were randomly assigned to one of four groups. Rats received NAL or saline 30 min prior to the all four PSR test sessions. These same rats were also used to test the effects of NAL during relapse responding, using a counterbalanced design (i.e., rats that were administered NAL or saline during the PSR test sessions were randomly assigned to separate groups that received NAL or saline during relapse).

The separate group of rats in the NAL study that were tested only during maintenance were also taken through extinction, PSR testing, and relapse drinking. Following 30 consecutive operant sessions after relapse testing, rats received NAL or saline 30 min prior to four consecutive operant sessions (Fig. 1). Rats then received 8 consecutive sessions of ethanol operant access to assess carryover effects of NAL (Fig. 1).

LY administration on ethanol PSR/ADE/maintenance responding

Following acquisition and extinction training, 40 adult female P rats were randomly assigned to one of five groups. Rats received LY or vehicle 30 min prior to the all four PSR test session. These same rats were also used to test the effects of LY during relapse and maintenance responding, using a counterbalanced design (i.e., rats that were administered LY or vehicle during the PSR test sessions were randomly assigned to separate groups that received LY or vehicle during relapse, with doses counterbalanced similarly for the maintenance phase as well). For relapse testing, rats received LY or vehicle 30 min prior to the initial four reinstatement sessions. Following 30 consecutive operant sessions, the rats received LY or vehicle 30 min prior to four consecutive operant sessions (Fig. 1). Rats then received 4 consecutive sessions of ethanol operant access to assess for carryover effects of LY (Fig. 1).

Statistical analyses

Overall operant responding (60 min) data were analyzed with a mixed factorial analysis of variance (ANOVA) with a between-subject factor of “dose” and a repeated measure of “session.” For the PSR experiments, the baseline measure for the factor of “session” was the average number of responses on the ethanol lever for the last three extinction sessions. For the deprivation studies, the baseline measure for the factor of “session” was the average number of responses on the ethanol lever for the three sessions immediately prior to the extinction phase. Baseline measure for the maintenance experiment was the three sessions immediately prior to NAL or LY testing.

Results

Effects of NAL administration on ethanol PSR/relapse/maintenance responding

For the PSR test, responses on the ethanol lever were lower in the NAL-treated rats than in the saline group (Fig. 2). Examining the number of responses on the lever previously associated with the delivery of ethanol indicated a significant effect of “session” ($F[4, 25] = 42.2$; $P < .001$), “dose” ($F[3, 28] = 4.7$; $P = .009$), and a “session” by “dose” interaction ($F[12, 81] = 2.9$; $P < .001$). Decomposing the interaction term by performing individual ANOVAs on each session indicated that there was a significant effect of “dose” during the initial PSR session ($F[3, 28] = 12.7$; $P < .001$). Post hoc comparisons indicated that saline-treated rats responded more than all other groups, and that rats treated with 1 or 5 mg/kg responded more than rats treated with 10 mg/kg NAL. Additionally, compared with extinction baseline levels, the amount of responding on the lever associated with the delivery of ethanol was significantly higher during the initial PSR test session in rats administered saline, 1 or 5 mg/kg NAL (P values $< .001$). Compared with saline levels, NAL reduced responses on the ethanol lever by 39% at 1 mg/kg, 37% at 5 mg/kg, and 70% at 10 mg/kg during the first PSR session. During PSR sessions 2–4, responses on the ethanol lever for all groups (saline and NAL) were similar and not significantly different than extinction baseline (Fig. 2). Responding on the lever previously associated with water was not significantly different between extinction (7.6 ± 1.3 responses/session) and PSR testing (9.3 ± 2.2: Session $F[4, 25] = 2.6$, $P = .066$; Session × Group $F[12, 81] = 1.3$, $P = .23$; Group $F[3, 28] = 0.4$, $P = .77$).

For the relapse test (Fig. 3), responses on the ethanol lever were lower for the NAL-treated rats than for the saline group. There was no evidence that previous treatment with NAL influenced subsequent treatment with NAL (carryover effect; all *P* values > .45); therefore, this variable was not included in the final analysis. Examining the number of responses on the ethanol lever indicated a significant effect of "session" ($F[4, 25] = 23.3$; $P < .001$) "dose" ($F[3, 28] = 45.4$; $P < .001$), and a "session" by "dose" interaction ($F[12, 81] = 2.9$; $P = .002$). Individual ANOVAs performed on each of the four injection/relapse days revealed that NAL reduced responding for ethanol across all four sessions ("dose"—*F* values [3, 28] > 16.8; *P* values < .001). Post hoc comparisons indicate that saline-treated rats responded more than all other groups during each relapse session tested. Additionally, saline-treated rats responded more for ethanol during the first relapse session compared with baseline values ($P = .004$). During subsequent sessions, responses on the ethanol lever by the saline group were not different from baseline (Fig. 3). Responding on the water lever was not significantly different between baseline (8.2 ± 1.1) and relapse testing (9.3 ± 0.8: Session $F[4, 25] = 1.2$, $P = .46$; Session × Group $F[12, 81] = 1.4$, $P = .17$; Group $F[3, 28] = 2.4$, $P = .088$). After treatment, responding on the ethanol lever for the NAL groups returned to control levels (Fig. 3).

NAL also reduced responding on the ethanol lever under maintenance conditions (Fig. 4). There was a significant effect of "session" ($F[4, 52] = 61.1$; $P < .001$), "dose" ($F[3, 28] = 19.8$; $P < .001$), and a "session" by "dose" interaction ($F[12, 81] = 3.7$; $P < 001$). Individual ANOVAs performed on each of the four treatment days revealed that NAL reduced responding for ethanol across all four sessions ("dose"—*F* values [3, 28] > 6.3; *P* values < .002). Post hoc comparisons indicated that saline-treated rats responded more than all other groups during the first, second, and third injection sessions. During the fourth injection session, saline-treated rats responded more than rats treated with the two highest doses of NAL. Additionally, the 1 and 5 mg/kg NAL-treated rats had higher responding on the ethanol lever than the 10 mg/kg NAL groups in the fourth injection session. There were no group differences for ethanol responding during the postinjection sessions (*P* values > .51). Responding on the water lever was low (14.5 ± 2.3 responses/session), and was not significantly altered by NAL during maintenance testing (10.9 ± 2.7: data not shown, *P* values > .66).

Effects of LY administration on ethanol PSR/relapse/maintenance responding

Overall, LY reduced or completely inhibited responding on the ethanol lever during the PSR test (Fig. 5) Examining the number of responses on the lever previously associated with the delivery of ethanol indicated a significant effect of "session" ($F[4, 32] = 6.0$; $P < .001$), "dose" ($F[4, 35] = 6.9$; $P < .001$), and a "session" by "dose" interaction ($F[16, 140] = 3.7$; $P < .001$). Decomposing the interaction term by performing individual ANOVAs on each session indicated that there was a significant effect of "dose" during the initial PSR session ($F[4, 35] = 19.3$; $P < .001$). Post hoc comparisons indicated that rats administered vehicle or 0.03 mg/kg LY responded more during the initial PSR test session compared with extinction baseline values than rats administered 0.1, 0.3, or 1 mg/kg LY (*P* values < .001). Responding on the lever previously associated with water was not significantly altered between extinction baseline (12.9 ± 2.1 responses/session) and PSR testing (10.0 ± 1.4).

Statistically, there was no effect of Session (F[4, 32] = 1.33; P = .28), Group (F[4, 35] = 1.26; P = .31), or Session × Group interaction (F[16, 140] = 1.3; P = .21).

LY reduced responding on the ethanol lever by P rats across all four sessions under relapse conditions (Fig. 6). There was no statistical evidence that previous treatment with LY influenced subsequent treatment with LY (carryover effect; all P values > .52). Therefore, this variable was not included in the final analysis. Examining the number of responses on the ethanol lever (Fig. 6) indicated a significant effect of "session" (F[4, 32] = 42.5; P < .001), "dose" (F[4, 35] = 23.5; P < .001), and a "session" by "dose" interaction (F[16, 140] = 3.8; P < .001). Individual ANOVAs performed on each of the four injection/reinstatement days revealed that LY reduced responding for ethanol across all four sessions ("dose"—F values [4, 35] > 5.7; P values < .001). Post hoc comparisons indicated that vehicle-treated rats responded more than all other groups for the first three relapse sessions, and more than rats treated with 0.1, 0.3, or 1 mg/kg LY during the fourth relapse session. Individual ANOVAs performed on the three postinjection sessions indicated no significant group differences (P values > .08). Responding on the water lever was not significantly different between baseline (10.7 ± 3.7 responses/session) and relapse levels (16.7 ± 4.2).

LY reduced responding on the ethanol lever under maintenance conditions also (Fig. 7). There was an effect of "session" (F[4, 32] = 31.8; P < .001), "dose" (F[4, 35] = 21.5; P < .001), and a "session" by "dose" interaction (F[16, 140] = 2.9; P < .001). Individual ANOVAs performed on each of the four injection days revealed that LY dose dependently reduced responding for ethanol across all four sessions ("dose"—F values [4, 35] > 9.3; P values < .001). Post hoc comparisons indicated that vehicle-treated rats responded more than all LY groups during the first three injection sessions and responded more than the 0.1, 0.3, and 1.0 mg/kg LY groups in the fourth injection session. There were no group differences for ethanol responding during the postinjection sessions (P values > .12). Responding on the water was not significantly altered between baseline (11.2 ±1.2 responses/session) and maintenance testing (9.5 ± 1.7). Statistically, there was no effect of Session (F[4, 32] = 0.85; P = .5), Group (F[4, 35] = 1.6; P = .19), or Session × Group interaction (F[16, 140] = 1.5; P = .11).

Discussion

The current data indicate that the opioid antagonists, NAL and LY, decrease seeking, relapse, and maintenance responding for ethanol in adult female P rats, with LY being 100 times more potent than NAL at blocking seeking behavior. For both drugs, the lowest dose was twice as effective at reducing relapse responding (80–90% for NAL and 70% for LY) than seeking (40% for NAL and 35% for LY), suggesting that the opioid mechanisms underlying seeking and relapse are different. LY was three times more effective at reducing relapse than maintenance, suggesting a different mechanism underlying these two behaviors. Overall, these results suggest that opioid receptors are involved in ethanol reinforcement, and that opioid mechanisms underlying seeking, relapse, and maintenance responding may be different.

It should be noted that the doses of NAL that were necessary to block PSR in P rats in our experiment were much higher than the doses necessary to block seeking in other rat models. For example, a 1 mg/kg dose of NAL was sufficient to block cue-induced ethanol-seeking behavior in Wistar rats (Ciccocioppo et al., 2003; Dayas et al., 2007; Liu and Weiss, 2002), and only 0.2 mg/kg dose of NAL was necessary to block ethanol priming-induced reinstatement of ethanol self-administration in Wistar rats (Lê et al., 1999). In another model of ethanol seeking in Wistar rats, where ethanol-associated cues induced an increase in ethanol consumption, this increase was blocked with a 1 mg/kg dose of NAL (Pickering and Liljequist, 2003). Reinstatement of ethanol-seeking behavior induced by an exposure to ethanol-associated cues was blocked with administration of 10 mg/kg of NAL in Long-Evans rats (Williams and Schimmel, 2008). In our rat model of alcohol seeking, a 10 mg/kg dose of NAL was necessary to completely block the ethanol-seeking behavior. Although 1 and 5 mg/kg doses of NAL resulted in a decrease in ethanol seeking, these doses did not completely block the ethanol-seeking behavior. The reason why NAL needed to be at a higher dose to block seeking behavior in P rats than Wistar rats might either have to do with the nature of the behavioral test (i.e., reinstatement vs. PSR), with a difference between the two rat strains in the affinity of the opioid receptors for NAL, and/or a result of ethanol being a stronger reinforcer in P rats than in Wistar rats. Unlike NAL, LY was capable of completely blocking ethanol-seeking behavior at 0.1 mg/kg. The results indicate that PSR in the P rat is a robust model of ethanol-seeking behavior, and LY is over 100 times more potent than NAL at blocking this seeking behavior.

It should be pointed out that built into the design of the relapse experiments, there is over a 5-week period of abstinence in which the rats do not consume alcohol. Although the possibility exists that what appears to be an increase in consumption as a result of deprivation is in fact merely an alteration in baseline consumption over time, several studies (reviewed in Rodd et al., 2004) indicate that baseline consumption, in similar operant conditions as the present study, is stable and does not increase over a period of several weeks (Toalston et al., 2008). With regard to the effect of treatment on baseline self-administration, because the saline-treated rats increase ethanol self-administration from baseline and the treatments reduced self-administration compared with saline, it can be concluded that NAL and LY are inhibiting ethanol self-administration under relapse conditions.

It was necessary to use high doses of NAL to observe effects under the present conditions. However, it is possible that, at these doses, there might be some nonspecific effects of the drugs on other behaviors, such as aversion or general motor activity. Although the responses on the water lever were low (10–15 responses/session), the finding that NAL or LY did not reduce responses suggest that the high doses of NAL or LY were not having a major effect on gross motor activity. There have been studies indicating that NAL can induce a conditioned taste aversion (CTA) in nonhuman primates at a dose of 0.32 mg/kg (Williams and Woods, 1999), and a conditioned place aversion (CPA) at a dose of 10 mg/kg in albino CFW mice (Bespalov et al., 1999) and Sprague–Dawley rats (Parker and Rennie, 1992). In an elegant study, Mitchell et al. (2009) demonstrated that Lewis rats with experience with ethanol, when given a subcutaneous injection of 3 mg/kg NAL, showed a CPA to NAL that correlated positively with the amount of ethanol consumed. Rats that showed a strong CPA,

consumed the highest level of ethanol. Rats that consumed moderate amounts of ethanol showed no aversion, and rats that showed low amounts of ethanol consumption, demonstrated a slight conditioned place preference. Interestingly, this correlation was only present if the rats had experience with consuming ethanol prior to the CPA testing. Rats that were ethanol naïve prior to CPA testing showed no correlation between level of aversion to NAL and amount of ethanol consumed; In addition, the level of aversion was slight in the majority of rats. It was also shown in this study that passive administration of two different doses of ethanol (in two separate groups) 1.5 h prior to NAL injection (given immediately before NAL aversion training) did not correlate with level of CPA seen in these rats, indicating that the high level of aversion seen in the high ethanol consuming rats is not purely due to the pharmacological effects of ethanol.

Several additional studies have shown that NAL, at certain doses in certain strains of rat in certain conditions, does not produce a CPA. For example, administration of NAL at doses as high as 3 mg/kg did not induce CPA in either Wistar rats (Häggkvist and Lindholm, 2009) or Spraguee–Dawley rats (White et al., 2005). In the latter study (White et al., 2005), NAL induced CPA only if administered after passive intraperitoneal administration of morphine. To date, there have been no studies to determine if NAL given to P rats is aversive at a dose of 10 mg/kg as measured by CPA or CTA. There are data, however, indicating that P rats given doses of NAL as high as 30 mg/kg did not decrease food or water consumption (Sable et al., 2006), thus suggesting that NAL does not induce gross aversion at doses as high as 30 mg/kg.

It should be pointed out that most of the background studies cited in the Introductory section used male rats. To control for the possibility that the estrous cycle may have an effect on ethanol-associated behaviors, female rats were housed in the same colony room as male rats ensuring that the estrous cycle among females was not synchronized, thus helping to control for effects of estrous cycle on behavior. One study by Sable et al. (2006) carried out in both adolescent and adult male and female P rats, demonstrated no difference in the effect of NAL on alcohol consumption levels between the sexes. Thus, it can be concluded that the results presented in this article may be applicable to both sexes.

LY is a nonspecific opioid receptor antagonist belonging to a family of antagonists referred to as the 3,4-dimethyl-4-(3-hydroxyphenyl)piperidines (Emmerson et al., 2004). It has been shown that there is no LY binding in mouse brain sections incubated in the presence of NAL (Gackenheimer et al., 2005), suggesting common binding sites between the two drugs, which would explain the similarities in the results observed in the present study. In a series of experiments carried out by Emmerson et al. (2004), it was determined that the presence of Na+ had an effect on LY binding affinity to the opioid receptors, but had no effect on NAL binding affinity to these receptors. Addition of Na+ increased the binding affinity of LY by sixfold at the mu-opioid receptor from a Ki of 0.32 ± 0.03 to 0.051 ± 0.006 nM, 12-fold at the kappa-opioid receptor from a Ki of 10.3 ± 2.9 to 0.82 ± 0.14 nM, and 34-fold at the delta-opioid receptor from 151 ± 47 to 4.44 ± 0.52 nM. Comparison of the rank order of opioid receptor antagonist affinity under low Na+ conditions established that LY was equipotent with NAL at the mu-opioid receptor and 10-fold less potent at the kappa- and delta-opioid receptors. In the presence of Na+, LY was 10-fold more potent than NAL at the

mu-opioid receptor and twofold more potent at the kappa- and delta-opioid receptors. Such differences in mu-, delta-, and kappa-opioid receptor binding affinities might explain the difference seen between NAL and LY regarding potency in blocking ethanol seeking.

Differences between the pharmacokinetics of NAL and LY are small, with LY having a half-life of 1.5 h (Swanson et al., 1995) and NAL having a half-life of 1 h (Akala et al., 2008). Thus, it can be stated that the differences seen in the behavioral effects of these drugs on seeking, relapse, and maintenance cannot be explained in terms of pharmacokinetic differences.

Besides differences in opioid receptor affinity, another important difference between NAL and LY that might explain some of the behavioral differences are that LY has inverse agonist activity in conditions that NAL does not (Emmerson et al., 2004). This was indicated by a decrease in GTPγS binding induced by LY that was not induced by NAL (Emmerson et al., 2004). However, under certain conditions, NAL does exhibit inverse agonist activity. For example, NAL behaved as a neutral antagonist only in membranes from vehicle-treated cells and mice, but acted as an inverse agonist in membranes from morphine-and ethanol-treated cells in vitro, and morphine-treated mice in vivo (Marczak et al., 2007; Wang et al., 2001, 2007). Overall, it can be concluded that the conditions under which LY and NAL act as inverse agonists differ. Thus, it is possible that the differences seen between the drugs with respect to potency may be due to differences in inverse agonist activity.

Naloxone, a nonselective opioid antagonist, has also been shown to reduce 2-h limited access ethanol intake in P rats under relapse conditions (i.e., after 2 weeks of ethanol deprivation) (Badia-Elder et al., 1999). This result is consistent with the present study that, NAL blocked relapse in P rats, induced by 2 weeks of ethanol deprivation, at doses as low as 1 mg/kg. A recent study conflicted with our own, for it found that a 20 mg/kg dose of NAL did not block relapse in the inbred P rat (Rezvani et al., 2009). The reason for the discrepancy may be due to a number of factors. For instance, in our study, the rats drank in a 1-h limited access operant procedure, and in the study by Rezvani et al. (2009), the rats were exposed to alcohol for 24 h in a free-bottle choice procedure. Another major difference between the two studies was in the amount of time given for ethanol deprivation. In our study, 2 weeks of deprivations was given, while in the study by Rezvani et al., 1 day was given.

As summarized in the introduction, NAL has been reported to reduce alcohol drinking in humans, nonhuman primates, rat lines selectively bred for high-alcohol consumption, in genetically heterogenous rats, and in B6 mice. These results are consistent with those found in the present study, which showed that NAL decreased the maintenance of ethanol self-administration in the P rat. Results from the present study also indicated that LY decreased the maintenance of ethanol self-administration, which is consistent with other studies in the literature, indicating that other nonselective opioid antagonists also have a suppressant effect on ethanol consumption. One of the differences between NAL and LY could be seen on the first day of drug administration during maintenance self-administration, where the highest dose of NAL used (10 mg/kg) decreased self-administration, whereas the highest dose of LY used (1 mg/kg) completely blocked ethanol self-administration. Another difference between

NAL and LY was that 1 mg/kg NAL lost effectiveness on the fourth day of treatment, whereas 0.1 mg/kg LY maintained its effectiveness. P rats have shown the development of tolerance to the effect of NAL over days (Rezvani et al., 2007; Sable et al., 2006). On the whole, the results indicate that the opioid receptor system is involved in maintaining alcohol consumption and self-administration behaviors in both limited and free-access procedures.

In conclusion, our findings indicate that the opioid system is involved in alcohol seeking, relapse, and maintenance responding in the P rat, as indicated by the ability of the opioid antagonists NAL and LY to decrease these three behaviors. However, LY was more potent than NAL at reducing responding during seeking, relapse, and maintenance.

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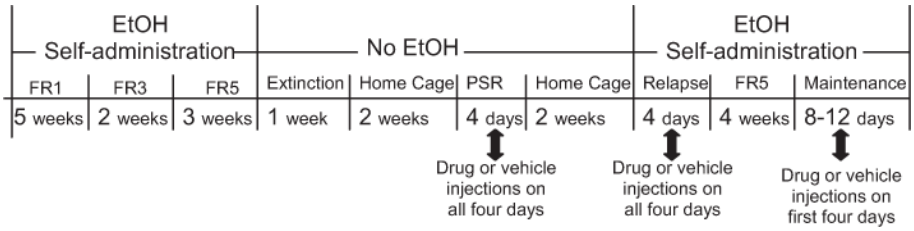

Fig. 1.

Depicts the timeline in which the rats proceed through the Pavlovian Spontaneous Recovery (PSR), relapse, and maintenance procedures.

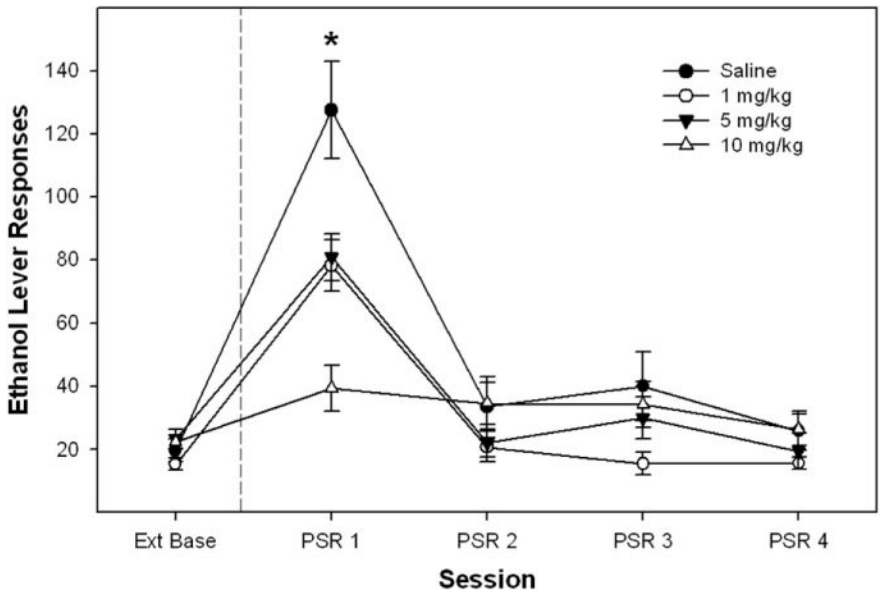

Fig. 2.

Depicts the mean (±standard error of the mean) responses/session on the lever previously associated with the delivery of ethanol in alcohol-preferring (P) rats (*n* = 8/group) given 0, 1, 5, or 10 mg/kg naltrexone (NAL) subcutaneously 30 min prior to the four Pavlovian Spontaneous Recovery (PSR) session. *Indicates that saline and 1 or 5 mg/kg NAL-treated rats responded significantly more on the ethanol lever during the first PSR session compared with baseline levels.

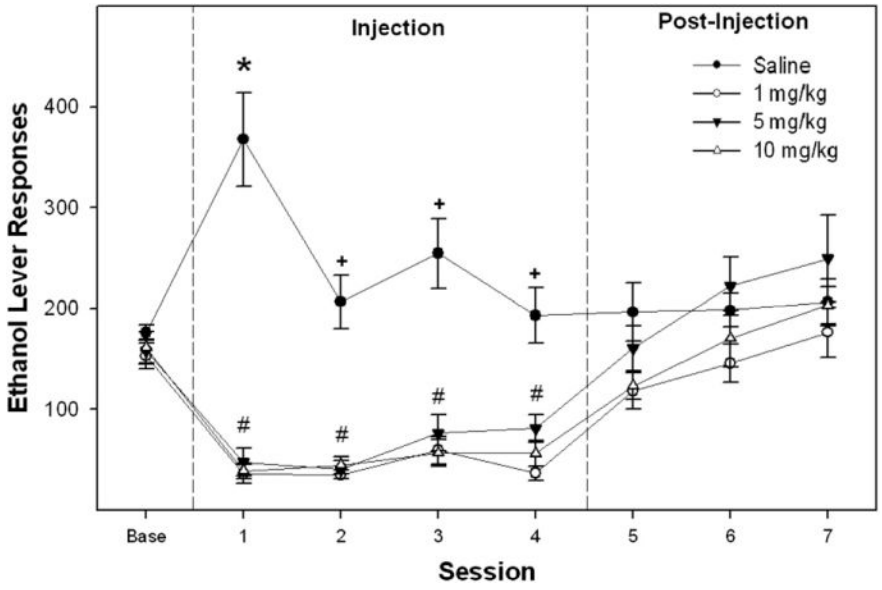

Fig. 3.

Depicts the mean (±standard error of the mean) responses/session on the ethanol lever by alcohol-preferring (P) rats (*n* = 8/group) given 0, 1, 5, or 10 mg/kg naltrexone (NAL) 30 min prior to each of the initial four ethanol reexposure session. *Indicates that saline-treated rats responded significantly more on the ethanol lever during the first relapse session compared with baseline levels, and is significantly higher than all other groups. +Indicate that saline-treated rats responded for ethanol more than all other groups, but was not different than baseline. #Indicates that responses for all NAL doses were lower than baseline.

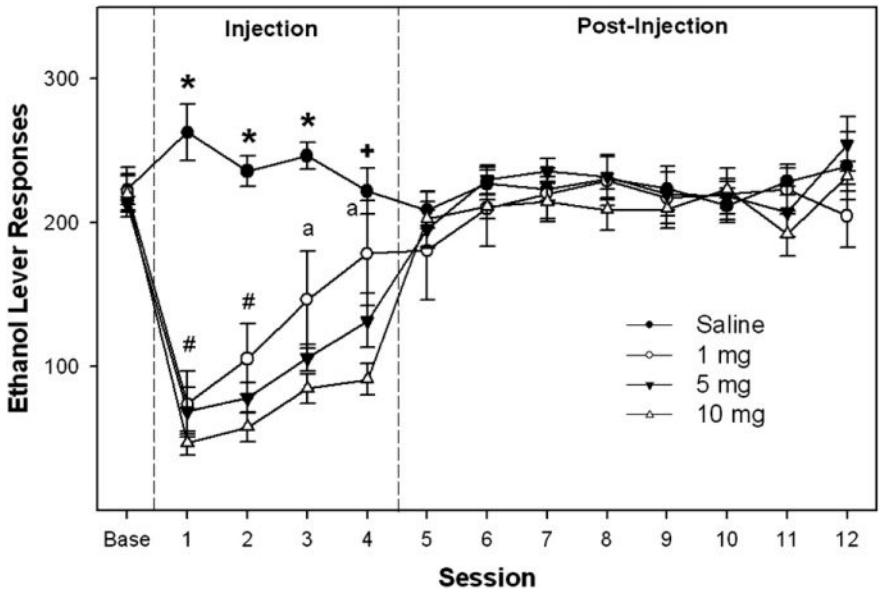

Fig. 4.

Depicts the mean (±standard error of the mean) responses/session on the ethanol lever by alcohol-preferring (P) rats (*n* = 8/group) given 0, 1, 5, or 10 mg/kg naltrexone (NAL) 30 min during four consecutive maintenance test sessions. *Indicates that saline-treated rats responded significantly more on the ethanol lever than all other groups. +Indicates that saline-treated rats responded more for ethanol than did the 5, or 10 mg/kg NAL-treated rats. #Indicates responses for all NAL doses were lower than baseline. [a]Indicates responses for the 5 and 10 mg/kg doses are still lower than baseline.

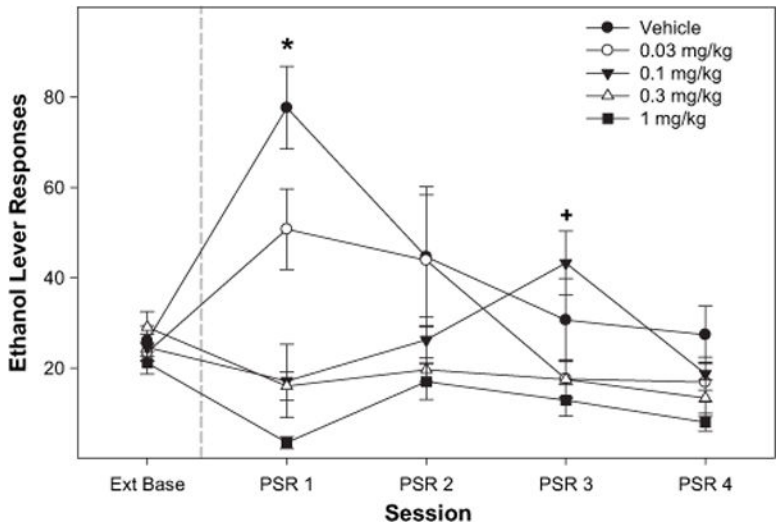

Fig. 5.

Depicts the mean (±standard error of the mean) responses/session on the lever previously associated with the delivery of ethanol in alcohol-preferring (P) rats (*n* = 8/group) given 0, 0.03, 0.1, 0.3, or 1 mg/kg LY255582 (LY) 30 min prior to each of the four Pavlovian Spontaneous Recovery (PSR) sessions. *Indicates that vehicle and 0.03 mg/kg LY rats responded significantly more on the ethanol lever during the first PSR session compared with baseline levels. +Indicates that 0.1 mg/kg group responded significantly more on the ethanol lever during the first PSR session compared with baseline levels.

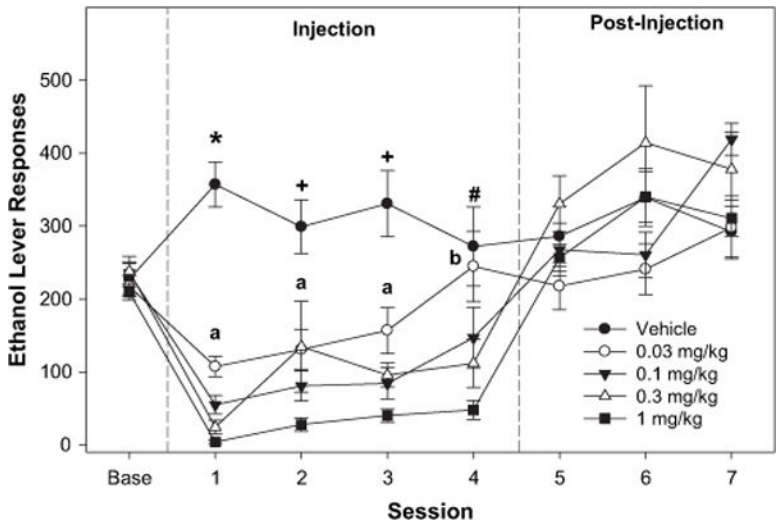

Fig. 6.

Depicts the mean (±standard error of the mean) responses/session on the ethanol lever under relapse conditions by alcohol-preferring (P) rats (n = 8/group) given 0, 0.03, 0.1, 0.3, or 1 mg/kg LY255582 (LY) 30 min prior to each of the initial four reexposure sessions. *Indicates that rats administered vehicle responded significantly more on the ethanol lever during the first reinstatement session compared with baseline levels and significantly more than all LY-treated rats. +Indicates that vehicle-treated rats responded more on the ethanol lever than all LY-treated rats, but responding was different than baseline. #Indicates that vehicle-treated rats responded more than 0.1, 0.3, or 1 mg/kg LY-treated rats. [a]Indicates that all LY were lower than baseline. [b]Indicates that 0.1, 0.3, and 1.0 LY doses were lower than baseline.

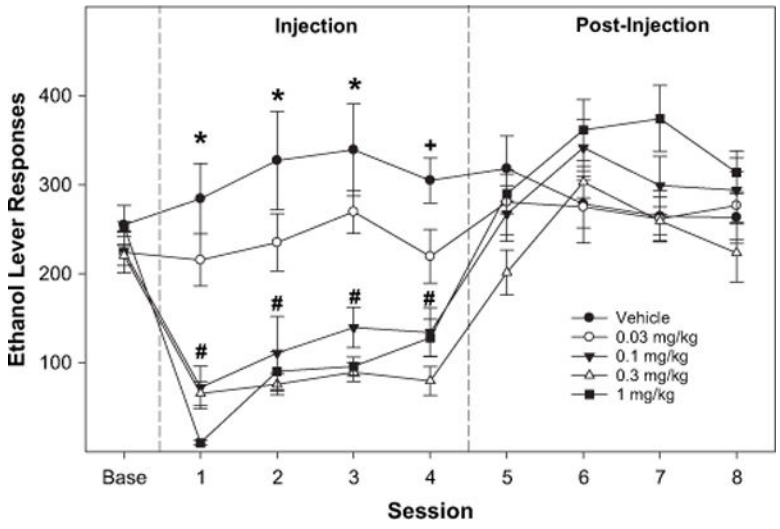

Fig. 7.

Depicts the mean (±standard error of the mean) responses/session on the ethanol lever under maintenance conditions by alcohol-preferring (P) rats (n = 8/group) given 0, 0.03, 0.1, 0.3, or 1 mg/kg LY255582 (LY) 30 min prior to maintenance test sessions. *Indicates that rats administered vehicle or 0.03 mg/kg LY responded significantly more on the ethanol lever than other LY-treated rats. +Indicates that vehicle-treated rats responded more than all LY-treated rats, and that 0.03 mg/kg LY-treated rats responded more than all other LY-treated rats. #Indicates that 0.1, 0.3, and 1.0 LY groups had lower responses than baseline.