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Effects of naltrexone, duloxetine, and a corticotropin-releasing factor type 1 receptor antagonist on binge-like alcohol drinking in rats

Dong Ji[a], **Nicholas W. Gilpin**[b], **Heather N. Richardson**[b], **Catherine L. Rivier**[a], and **George F. Koob**[b]

a*Clayton Foundation Laboratories for Peptide Biology, Salk Institute, La Jolla, California, USA*

b*Committee on the Neurobiology of Addictive Disorders, The Scripps Research Institute, La Jolla, California, USA*

Abstract

A ‘binge’ is defined by National Institute on Alcohol Abuse and Alcoholism as an excessive pattern of alcohol drinking that produces blood–alcohol levels (BALs) greater than 0.08 g% within a 2-h period and may or may not be associated with dependence. The purpose of this investigation was to explore the effects of several neuropharmacological agents in an animal model in which outbred rats voluntarily and orally self-administer pharmacologically meaningful alcohol doses that produce BALs ≥ 0.08 g% in daily limited access two-bottle choice and operant drinking sessions. Rats were trained to self-administer either 10% (w/v) alcohol solution sweetened with ‘supersac’ (3% glucose + 0.125% saccharin) or supersac alone versus water in a two-bottle choice or operant situation during 30-min daily sessions. Rats were then injected systemically with multiple doses of duloxetine, naltrexone, and the corticotropin-releasing factor antagonist, MPZP, in Latin-square designs. Alcohol binge drinkers reliably consumed amounts of alcohol sufficient to produce BALs ≥ 0.08 g%. Duloxetine dose-dependently suppressed two-bottle choice alcohol binge drinking and operant alcohol responding as well as operant supersac responding, but did not affect two-bottle choice supersac drinking. Naltrexone-suppressed alcohol binge drinking at very low doses and suppressed supersac drinking at moderate-to-high doses. MPZP did not affect alcohol or supersac consumption. Different profiles for drugs that suppress binge-like alcohol drinking compared with dependence-induced drinking provide a heuristic foundation for future medications development.

Keywords

alcoholism; binge; corticotropin-releasing factor antagonist; duloxetine; MPZP; naltrexone; rat

Introduction

Like all patterns of alcohol abuse, binge drinking is problematic for the individual and for society. A substantial portion of the human population drinks alcohol in a binge pattern (Wechsler and Isaac, 1992; Wechsler *et al.*, 2000), and there are several adverse consequences associated with this alcohol abuse pattern, including the increased risk of developing alcohol dependence following binge-like drinking during adolescence (Hingson *et al.*, 2005, 2007;

Correspondence to Dr Nicholas W. Gilpin, PhD, Committee on Neurobiology of Addictive Disorders, The Scripps Research Institute, SP30-2400, 10550 N. Torrey Pines Road, La Jolla, CA 92037, USA, E-mail: nickg@scripps.edu.
Dong Ji and Nicholas W. Gilpin contributed equally to this manuscript.

Miller *et al.*, 2007). Billions of dollars in the United States alone are lost annually owing to problems that stem from alcoholism (Harwood *et al.*, 1998).

A 'binge' is defined by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) as a pattern of drinking that produces blood–alcohol levels (BALs) greater than 0.08 g% (i.e., 80 mg%; approved by NIAAA National Advisory Council, 2004). The pattern of drinking required to produce these BALs in the average human is generally accepted to be 4–5 drinks in a 2-h period (NIAAA, 2004). It is worth noting that a binge-drinking pattern is fundamentally different from NIAAA definitions of both 'risky' drinking (< 0.08 g% BALs) and a 'bender' (at least 2 days of sustained heavy drinking).

A procedure that has long been used to promote ethanol drinking by rats is the addition of sweeteners to the ethanol solution. This animal model, unlike many others, contains construct validity for the human condition because humans tend to drink alcohol in a sweetened form (Gilbert, 1978; Samson *et al.*, 1996), especially early in the development of alcohol abuse/alcohol dependence when consumption patterns are reflective of the NIAAA definition of binge drinking (NIAAA, 2004). The addition of sweeteners (usually sucrose or saccharin) to the ethanol solution produces increase in consumption by adult rats relative to ethanol alone in water (Samson, 1986; Rassnick *et al.*, 1992; Doremus *et al.*, 2005; Truxell *et al.*, 2007) and sweeteners (at specific concentrations) alone in water (Samson *et al.*, 1996). Historically, however, studies employing sweetened ethanol have also failed to reliably produce BALs (0.08 g%) determined by NIAAA to be the defining factor in binge alcohol drinking (e.g., Doremus *et al.*, 2005; Truxell *et al.*, 2007) or the studies have not measured BALs (e.g., Samson *et al.*, 1996).

The present investigation sought to test several neuropharmacological compounds, selected for their different mechanisms of action and behavioral profiles, in a rat model of binge-like alcohol drinking. Duloxetine is a mixed selective serotonin/norepineprhine reuptake inhibitor (SSNRI) that has powerful effects in facilitating serotonin neurotransmission (Westanmo *et al.*, 2005) and produces robust reductions in operant alcohol responding by both alcohol-dependent and nondependent rats (unpublished findings). Naltrexone is an opioid antagonist that is prescribed clinically as a treatment for alcoholism (Heilig and Egli, 2006). In rats, naltrexone suppresses alcohol drinking (e.g., Altshuler *et al.*, 1980; Reid and Hunter, 1984; Walker and Koob, 2007), and this effect is exaggerated in rats selectively bred for high alcohol preference [Sardinian alcohol-preferring (sP) rats; Sabino *et al.*, 2006]. Finally, a corticotropin-releasing factor type 1 (CRF_1) receptor antagonist, *N*,*N*-bis(2-methoxyethyl)-3-(4-methoxy-2-methylphenyl)-2,5-dimethyl-pyrazolo [1,5a]pyrimidin-7-amine (MPZP) was tested. CRF_1-receptor antagonists robustly suppress dependence-induced increases in alcohol drinking without affecting alcohol drinking by nondependent animals (Funk *et al.*, 2006, 2007). More specifically, dependence-induced increases in drinking are blocked by the particular antagonist (MPZP) used in this study (Richardson *et al.*, 2007).

The purpose of the present investigation was to explore pharmacological challenges in an animal model of excessive drinking with face validity for binge drinking in humans. Outbred rats achieve binge-like drinking without the use of food or water deprivation, and rats voluntarily and orally self-administer pharmacologically relevant alcohol doses capable of producing BALs that reliably exceed 0.08 g% in daily limited access two-bottle choice and operant drinking sessions. The overall hypothesis was that binge-like alcohol drinking (both operant and two-bottle choice) would be sensitive to compounds that suppress drinking motivated by the positive reinforcing effects of alcohol, but not compounds that suppress drinking motivated by alcohol dependence-related factors. It was hypothesized that (i) duloxetine would suppress binge-like drinking of alcohol but not a sweetened solution, (ii)

naltrexone would suppress binge-like drinking of alcohol and, possibly, a sweetened solution, and (iii) MPZP would not affect binge-like drinking of either alcohol or a sweetened solution.

Methods

Subjects

Twelve adult male Wistar rats obtained from Charles River (Kingston, New York, USA) were used in Experiment 1 and 24 adult male Wistar rats were used in Experiment 2. Animals weighed between 250 and 320 g at the start of the experiment and 522 and 567 g at the end of the experiment (body weights were not significantly different between the two groups at any point of the study; data not shown). Animals were single-housed in standard plastic cages with wood chip bedding under a 12 h light/12 h dark cycle (lights off at 10.00 h in Experiment 1; lights off at 08.00 h in Experiment 2). Animals were given free access to food and water throughout except during experimental drinking sessions. All procedures were conducted in the dark cycle and met the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Experiment 1

Two-bottle choice oral self-administration—Rats were trained to self-administer 'supersac' (3% glucose + 0.125% saccharin; Valenstein *et al.*, 1967) or 10% (w/v) ethanol solution sweetened with supersac, versus water in a two-bottle choice home cage situation during 30-min daily sessions that occurred 4 h into the dark cycle. Food was removed from the lid of the home cage and the rats were weighed immediately prior to drinking sessions. Bottles were attached to cage lids with stainless steel springs to reduce spilling. The positions of the bottles were alternated daily to avoid a possible side preference. All bottles were weighed immediately before and after 30-min drinking sessions. Differences in bottle weights were converted to volume intakes by accounting for solution densities as follows: supersac volume (ml) = weight (g)/1.0118; and 10% ethanol + supersac volume (ml) = weight (g)/0.9868.

Development of binge-like self-administration—Animals were divided into alcohol binge drinkers ($n = 6$) and supersac controls ($n = 6$). Prior to two-bottle choice training, all rats were given an initial 2-h training session during which they were allowed to drink supersac in a single-bottle situation. All rats acquired the behavior during this initial training session, and two-bottle choice training started the following day. Prior to pharmacological manipulations, animals in the alcohol binge group were allowed 30-min drinking sessions for 9 consecutive days whereas supersac controls were allowed 30-min drinking sessions for 8 consecutive days.

Duloxetine dose–response test—Duloxetine is highly absorbed and extensively metabolized in rats and it readily crosses the blood–brain barrier (Bymaster *et al.*, 2005). Rats were injected intraperitoneally (i.p.) with duloxetine (0, 2, 4, 8 mg/kg) 40 min before (doses and pretreatment time chosen based on preliminary data in which duloxetine effectively blocked ethanol responding by alcohol-dependent and nondependent rats) two-bottle choice test sessions in a within-subjects (rats) Latin-square design. Rats were allowed to self-administer every day during this time but were injected every other day. Rats were injected in the colony room and returned to the home cage immediately thereafter for the 40-min interim period.

Naltrexone dose–response test—Naltrexone (0, 16, 50, 150, 450 μg/kg), which readily crosses the blood–brain barrier, was injected subcutaneously into rats 30 min before (doses and pretreatment times chosen based on Sabino *et al.*, 2006) two-bottle choice test sessions in a within-subjects Latin-square design. Rats were allowed to self-administer every day during

this time but were injected every other day. Rats were injected in the colony room and returned to the home cage immediately thereafter for the 30-min interim period.

MPZP dose–response test—MPZP is a small molecule, non-peptide CRF_1-receptor selective antagonist that readily crosses the blood–brain barrier. Rats were injected ssubcutaneously with MPZP (0, 5, 10, 20 mg/kg) 60 min prior to two-bottle choice test sessions in a within-subjects (rats) Latin-square design. These doses and pretreatment times were chosen because these parameters were used in a study that showed the ability of MPZP to suppress anxiety-like behavior and dependence-induced alcohol drinking in rats (Richardson *et al.*, in press). Rats were allowed to self-administer every day during this time but were injected every other day. Rats were injected in the colony room and returned to the home cage immediately thereafter for the 30-min interim period.

Experiment 2

Operant ethanol self-administration—Operant ethanol self-administration was conducted in standard operant chambers (Coulbourn Instruments, Allentown, Pennsylvania, USA) housed in sound-attenuated ventilated cubicles. Animals were trained to orally self-administer ethanol or water in a concurrent, two-lever, free-choice contingency during daily 30-min sessions that occurred at the start of the dark cycle. Syringe pumps (Razel Scientific Instruments, Stamford, Connecticut, USA) dispensed ethanol or water into two stainless steel drinking cups mounted 4.0 cm above the grid floor in the middle of one side panel. Two retractable levers were located 4.5 cm to either side of the drinking cups. Fluid delivery and recording of operant self-administration were controlled by a standard PC computer. Lever presses were not recorded during the 0.5 s in which the pumps were active. A continuous reinforcement (fixed ratio-1) schedule was used such that each response resulted in delivery of 0.1 ml of fluid. Fluid delivery and recording of operant responding were controlled by a microcomputer. All rats were weighed prior to drinking sessions twice per week.

Development of operant binge-like self-administration—On a single initial training day, all rats were trained to press a lever for supersac in a single-lever situation for 2 h. Then all rats underwent 5 days of 30-min training sessions with two levers available (supersac versus water). All rats successfully learned the lever-pressing contingency during these training sessions. Rats were then divided into alcohol binge (n = 12) and supersac control (n = 12) groups, matched for responding during the operant training phase. Rats were then allowed 17 consecutive days of operant self-administration in which alcohol binge rats were allowed to press lever for sweetened ethanol (10% w/v ethanol in supersac) versus water, whereas supersac controls were still allowed to press lever for supersac versus water in a two-lever situation.

Duloxetine dose–response test—Rats were injected intraperitoneally with duloxetine (0, 2, 4, 8 mg/kg) 40 min before (pretreatment time chosen based on preliminary data) operant test sessions in a within-subjects Latin-square design. Rats were allowed to self-administer every day during this time but were injected every 3 to 4 days.

Naltrexone dose–response test—Rats were injected subcutaneously with naltrexone (0, 16, 50, 150, 450 μg/kg) 30 min before operant test sessions in a within-subjects Latin-square design. Rats were allowed to self-administer every day during this time but were injected every 3 to 4 days.

MPZP dose–response test—Rats were injected subcutaneously with MPZP (0, 5, 10, 20 mg/kg) 60 min before operant sessions in a within-subjects Latin-square design. Rats were allowed to self-administer every day during this time but were injected every 3 to 4 days.

Drugs

Duloxetine (LY248686) is a SSNRI. Naltrexone hydrochloride (Sigma-Aldrich, St Louis, Missouri, USA) is a nonselective opioid receptor antagonist. MPZP is an antagonist of CRF_1 receptors. All drugs were administered systemically. Duloxetine hydrochloride (Cymbalta, Eli lilly and Co., Indianapolis, Indiana, USA) was acquired in enteric-coated pellets that were crushed using a mortar and pestle, suspended in 1 mol/l HCl (10% of total volume), then diluted with 20% (w/v) 2-hydroxypropyl-β-cyclodextrin (Sigma-Aldrich) in distilled water (90% of total volume). MPZP was dissolved in 1 mol/l HCl (10% of total volume), then diluted with 20% (w/v) 2-hydroxypropyl-β-cyclodextrin in distilled water (90% of total volume). Solutions were then back-titrated with NaOH to pH 4.5 (Sabino *et al.*, 2006). Naltrexone HCl was dissolved in saline. The duloxetine suspension was injected intraperitoneally in a volume of 1 ml/kg body weight. Naltrexone hydrochloride solution was administered subcutaneously in a volume of 1 ml/kg body weight. MPZP solution was administered subcutaneously in a volume of 2 ml/kg body weight.

Blood–alcohol level determinations

Tail blood was sampled at the end of two representative baseline drinking sessions. Rats were gently restrained while the tip of the tail (2 mm) was cut with a clean razor blade. Tail blood (0.2 ml) was collected and centrifuged. Plasma (5μl) was used for the measurement of BALs using an Analox AM 1 analyzer (Analox Instruments Ltd, Lunenberg, Massachusetts, USA). The reaction is based on the oxidation of alcohol by alcohol oxidase in the presence of molecular oxygen (alcohol + O_2 → acetaldehyde + H_2O_2). The rate of oxygen consumption is directly proportional to the alcohol concentration. Single-point calibrations were made for each set of samples with reagents provided by Analox Instruments (0.025– 0.400 g%).

Statistical analysis

Solution consumption is expressed as mean ± SEM and normalized for body weight (i.e., g ethanol/kg body weight; ml, supersac/kg body weight). The effects of duloxetine, naltrexone, and MPZP on alcohol (g/kg) intake and supersac (ml/kg) intake, were separately analyzed using a series of one-way repeated-measures analyses of variance, with dose as a within-subjects factor. Post-hoc comparisons were made using the Student Newman–Keuls test. Statistical significance was set at $P < 0.05$.

Results

Experiment 1

Development of two-bottle choice binge-like self-administration—Average intake of sweetened alcohol solution (Fig. 1a) by animals in the alcohol binge group increased from 1.8 ± 0.5 ml (0.58 ± 0.15 g/kg) on day 1 of the baseline period to 5.5 ± 1.0 ml (1.53 ± 0.27 g/kg) on day 9 of the same period (Fig. 1b). Average supersac intake by supersac controls increased from 9.4 ± 1.0 ml on day 1 of the baseline period to 26.6 ± 1.9 ml on the final day of the same period (Fig. 1c). Tail blood samples were collected and BALs determined on two representative drinking days. Five of the six animals in the alcohol binge group achieved BALs of greater than 0.10 gm% on at least one of those 2 days (Fig. 1d). The mean BAL for animals in the alcohol binge group across 2 days was 0.079 g%, the approximate equivalent of the BAL criterion for binge drinking in humans.

Effects of duloxetine on two-bottle choice binge-like drinking—Duloxetine pretreatment dose-dependently reduced intake of sweetened alcohol solution by animals in the alcohol binge group, $F(3,15) = 8.48$, $P < 0.01$ (Fig. 2a; left panel). Post-hoc analysis indicated that all doses of duloxetine significantly suppressed binge-like alcohol intake ($P < 0.05$ in all

cases), whereas water intake was not affected (Table 1). Duloxetine administration did not affect supersac intake (Fig. 2a; right panel) or water intake (Table 1) by animals in the supersac group.

Effects of naltrexone on two-bottle choice binge-like drinking—Naltrexone pretreatment dose-dependently reduced intake of sweetened alcohol solution by animals in the alcohol binge group, $F(4,20) = 4.88$, $P < 0.01$ (Fig. 2b; left panel). Post-hoc analysis revealed that 50, 150, and 450 μg/kg significantly suppressed binge-like alcohol intake ($P < 0.05$ in all cases). Naltrexone also dose-dependently suppressed supersac intake by supersac controls $F(4,20) = 7.20$, $P < 0.01$. Post-hoc analysis revealed that 150 and 450 μg/kg suppressed supersac intake ($P < 0.05$ in both cases) in those rats (Fig. 2b; right panel). Water intake was not affected in either of the two groups (Table 1).

Effects of MPZP on two-bottle choice binge-like drinking—MPZP pretreatment did not affect consumption of sweetened alcohol solution, supersac, or water by either of the two groups (Fig. 2c and Table 1).

Experiment 2

Development of operant binge-like self-administration—Figure 3 (panels a and b) shows the mean number of daily lever presses and average ethanol intake by animals in the alcohol binge group. Figure 3c shows the average number of lever presses by the supersac control group. Tail blood samples were collected and BALs determined on days 13 and 16 of the baseline period. Eight of the 12 animals in the alcohol binge group achieved BALs of greater than 0.08 g% on at least one of those 2 days (Fig. 3d). The mean BAL for all 12 animals in the alcohol binge group across 2 days was 0.084 g%, which is higher than the BAL criterion for binge drinking in humans.

Effects of duloxetine on operant binge-like drinking—Duloxetine pretreatment dose-dependently reduced intake of sweetened alcohol solution by animals in the alcohol binge group, $F(3,33) = 12.62$, $P < 0.01$ (Fig. 4a; left panel). Post-hoc analysis revealed that all doses significantly suppressed binge-like alcohol intake ($P < 0.05$ in all cases). Duloxetine also suppressed supersac intake by controls $F(3,33) = 28.54$, $P < 0.01$. Post-hoc analysis revealed that all doses significantly suppressed supersac intake ($P < 0.01$ in all cases) in those rats (Fig. 4a; right panel). Water intake was not affected in either of the two groups (Table 2).

Effects of naltrexone on operant binge-like drinking—Naltrexone pretreatment dose-dependently reduced intake of sweetened alcohol solution by animals in the alcohol binge group, $F(4,40) = 7.95$, $P < 0.01$ (Fig. 4b; left panel). Post-hoc analysis revealed that 50, 150, and 450 μg/kg significantly suppressed binge-like alcohol intake ($P < 0.05$ in all cases). Naltrexone also suppressed supersac intake by controls $F(4,44) = 4.43$, $P < 0.01$, though only at the highest dose; post-hoc analysis revealed that only the 450 μg/kg dose suppressed supersac intake ($P < 0.05$) in those rats (Fig. 4b; right panel). Water intake was not affected in either of the two groups (Table 2).

Effects of MPZP on operant binge-like drinking—Analyses of variance revealed no effect of MPZP pretreatment on the consumption of sweetened alcohol solution, supersac or water by either of the two groups (Fig. 4c and Table 2). The analysis did, however, reveal a significant upward linear trend of MPZP dose (Fig. 4c) on binge-like alcohol drinking, $F(3,43) = 2.69$, $P < 0.01$, but not on supersac drinking ($P > 0.05$). Water intake was not affected in either of the two groups (Table 2).

Discussion

The present series of experiments was designed to explore a rat model of binge-like alcohol drinking and the neuropharmacological mechanisms involved in this type of alcohol drinking. Animals voluntarily and orally self-administered amounts of alcohol sufficient to reliably produce BALs greater than 0.08 g% following 30-min self-administration sessions (two-bottle choice and operant situations) in the absence of food or water deprivation. The satisfaction of these criteria qualifies this animal model as one with face validity for human binge drinking, as defined by NIAAA. In the present investigation, rats consumed substantial amounts of alcohol and exhibited pharmacologically relevant post-session BALs after a modest period of training, a pragmatic advantage of this model.

Other animal models of binge-like alcohol exposure are limited by a reliance on either nonoral routes of alcohol administration, exposure via forced alcohol administration, or selective breeding for high alcohol preference. Binge alcohol exposure procedures that utilize forced alcohol administration that is either passive (e.g. repeated experimenter-administered intragastric alcohol infusions; Crews *et al.*, 2000; Crews and Braun, 2003) or active (e.g., bout drinking via consumption of alcohol liquid diet as the sole source of nutrition; Fidler *et al.*, 2006; N.W. Gilpin and G.F. Koob, unpublished findings), produce significant neurobiological perturbations that are likely associated with dependence-induced drinking. Other models of binge-like alcohol self-administration have employed water deprivation (e.g., Hubbell *et al.*, 1986; Reid *et al.*, 1996; Gardell *et al.*, 1997) or food deprivation (e.g., MacDonall and Marcucella, 1979; Falk and Tang, 1988) to promote ethanol self-administration during daily limited-access periods. These designs are problematic, however, because animals are primarily motivated by thirst during self-administration sessions and body weight gain is slowed or completely blocked with such procedures. Collectively, these models have weak construct validity for the human condition (i.e., humans do not consume ethanol because they are hungry or thirsty). Other studies have produced voluntary ethanol consumption by rats during limited access sessions without the use of any of these manipulations but those studies either did not produce BALs (0.08 g%) determined by NIAAA to be the defining factor in binge alcohol drinking (e.g., Stewart and Grupp, 1984; Gill *et al.*, 1986; Linseman, 1987) or did not measure BALs (e.g., Macdonall and Marcucella, 1979). Finally, genetic manipulations have been used to produce rats selectively bred for high alcohol preference based on either continuous access ethanol intakes (e.g., alcohol-preferring P rats; Murphy *et al.*, 1986) or limited access ethanol intakes (i.e., high alcohol-consuming HARF rats; Lê *et al.*, 2001). P rats exhibit binge-like patterns of voluntary alcohol drinking behavior and achieve BALs that exceed 0.08 g% under a variety of alcohol access conditions (Murphy *et al.*, 1986) and have been used extensively for the study of genetic and neurobiological mechanisms of alcoholism. HARF rats also appear to achieve ethanol intake levels sufficient to qualify as binge-like drinking (Lê *et al.*, 2001).

In this experiment, intake of both sweetened alcohol and supersac tended to be higher in the two-bottle choice situation than in the operant situation, an effect that might be attributable to the increased work (lever pressing versus drinking from spout) required of rats to attain those solutions during operant sessions. In both experiments, there was some oscillation in alcohol intake over time; however, supersac intake by control rats exhibited the same pattern of change across time. In both experiments, there was also a strong correlation between alcohol intake and BALs but it is curious that this function exhibits a substantial rightward shift in two-bottle choice alcohol binge rats versus operant alcohol binge rats. This discrepancy could be due to different contributions of food intake to alcohol absorption rates in the two experiments. From a circadian perspective, rats typically consume the most food during the first 2–3 h of the dark cycle and then again just before the end of the dark cycle (Whishaw and Kolb, 2005). In Experiments 1 and 2 of the present investigation, self-administration sessions occurred at different time points during the dark cycle. More specifically, self-administration sessions

occurred during the middle of the dark cycle in Experiment 1, but at the start of the dark cycle in Experiment 2. Presumably, rats in Experiment 1 (two-bottle choice drinkers) consumed more food during the hours preceding self-administration sessions relative to rats in Experiment 2 (operant responders). Therefore, the rightward shift in the BAL versus intake function of two-bottle choice alcohol drinkers versus operant alcohol responders might be due to the delay in alcohol absorption that occurs in animals with full stomachs (Goldberg, 1943).

This model combines saccharin and low glucose concentrations in a solution shown to have high palatability in rats (Valenstein *et al.*, 1967). Adding sweeteners to ethanol solutions to produce higher ethanol intake is not a new experimental strategy. Some of the disadvantages of earlier procedures are, however, circumvented in the present model, such as the need for food deprivation (e.g., Macdonall and Marcucella, 1979) or water deprivation (e.g., Hubbell *et al.*, 1986; Reid *et al.*, 1996; Gardell *et al.*, 1997), or the lack of a defined BAL criterion (e.g., Stewart and Grupp, 1984; Gill *et al.*, 1986; Linseman, 1987; Sinclair *et al.*, 1992). Although genetic manipulations have been used to produce animals that reliably and voluntarily consume large quantities of ethanol (e.g., HARF animals, Lê *et al.*, 2001; P rats, Murphy *et al.*, 1986), selective breeding is not a practical solution for most laboratories. In addition, existing rat lines are in high demand and can be difficult to acquire. The model presented here combines a previously used strategy for the induction of pharmacologically meaningful ethanol drinking in rats with a sweetening procedure (Valenstein *et al.*, 1967) that appears to optimize ethanol consumption by nonalcohol-dependent rats.

Using a flavored vehicle (i.e., supersaccharin) with positive reinforcing properties as a control procedure is an advantage of the present procedure. The majority of ethanol self-administration studies allows rats a choice between an unsweetened ethanol solution and water, where water is the only alternate reinforcer available for intake comparisons. Such an experimental design has some limitations for testing the behavioral specificity of subsequent pharmacological manipulations because water has little reinforcing value in water-sated rats. That is, low water responding can produce a floor effect and make it difficult to discuss the behavioral specificity of drug effects. Furthermore, analysis of limited-access water response data is complicated by the fact that rats have continuous access to water during the nonexperimental periods, presumably resulting in lower reinforcement value for water during experimental sessions; indeed, water availability has complex effects on operant responding for a variety of reinforcers (Freed and Mendelson, 1977; Johnson *et al.*, 1991). The model used here is more conducive to pharmacological manipulations that suppress ethanol intake because the effects on ethanol intake can be compared with effects on the intake of a highly reinforcing alternate solution (i.e., ethanol vehicle). Thus, effects of pharmacological manipulations on the intake of sweetened ethanol solution in the absence of effects on supersaccharin can be more aptly called ‘behaviorally specific.’ Treatments that produce generalized suppressive effects on consumption lack specificity for ethanol, although such behavioral changes could result from drug effects on a reinforcement pathway common to both ethanol and natural reinforcers.

Another important point to address is the difference in intake levels between sweetened ethanol and supersaccharin in this investigation. Rats consume substantially more supersaccharin alone than they do supersaccharin plus ethanol. This aspect of the procedure, however, also exhibits face validity with the human condition. Rats and humans, given the choice between sweet solutions that do and do not contain ethanol, generally prefer solutions that do not contain ethanol as ethanol has aversive taste properties in both species (Myers and Ewing, 1980; Shoaib and Almeida, 1996). This, however, does not diminish the significance of ethanol consumption by those same humans and rats, especially when ethanol is being consumed in a pathological behavioral pattern (e.g., binge drinking). That is, excessive or detrimental consumption patterns of sweetened ethanol are made no less relevant by the fact that rats and humans consume less total volume of those solutions than they do of sweetened solutions that do not contain ethanol.

Related to this point, P rats exhibit an increased preference for saccharin solutions relative to their nonpreferring counterparts and also relative to outbred Wistar rats, lending support for a genetic correlation between ethanol preference and saccharin preference (Sinclair *et al.*, 1992). Similarly, rats selectively bred for high saccharin consumption consume more unsweetened ethanol than their low-saccharin-consuming counterparts (Dess *et al.*, 1998). It should, however, be noted that outbred rats that show an initial low preference for ethanol (versus both water and saccharin) eventually, given prolonged access, self-administer amounts of ethanol identical to Wistar rats that show an initial high preference for ethanol (Kampov-Polevoy *et al.*, 1990).

Binge alcohol drinking can be considered either as a phase in the development of alcoholism that precedes physical and psychological dependence on alcohol (NIAAA, 2004) or as a separate entity. As a result, binge alcohol drinkers often do not exhibit somatic or motivational signs of alcohol dependence. One approach for differentiating the behaviors and neural mechanisms associated with binge-like alcohol drinking and dependence-induced drinking is to examine a profile of treatment with different pharmacological agents. Following stabilization of drinking behavior by animals in the alcohol binge groups and supersac controls, the groups were tested for the effects of various drugs on drinking behavior.

Duloxetine is a SSNRI used in humans to treat major depression, pain from diabetic peripheral neuropathy, and stress urinary incontinence (Westanmo *et al.*, 2005). Selective serotonin reuptake inhibitors suppress nondependent alcohol drinking in rats (Gill and Amit, 1989) and early stage problem drinking in humans (Naranjo and Sellers, 1989), but not drinking by later-stage human alcoholics (Kabel and Petty, 1996). Furthermore, low serotonin functioning in humans has long been associated with impulsivity and a predisposition to alcohol dependence (Linnoila *et al.*, 1994). In the current investigation, duloxetine exhibited differential effects based on the drinking model: in the two-bottle choice situation, duloxetine dose-dependently suppressed alcohol binge-like drinking, but did not affect supersac drinking, whereas in an operant situation, duloxetine suppressed responding for both alcohol and supersac. As some selective serotonin reuptake inhibitors have been reported to have anorexic actions in rats (Gill and Amit, 1989), it could be argued that duloxetine suppressed intake of alcohol owing to its caloric value. Duloxetine, however, did not affect supersac intake by two-bottle choice drinkers, indicating that its effects are likely not attributable to any potential anorexic actions. It is not clear why duloxetine suppressed supersac drinking in an operant situation, and not in a two-bottle choice situation, but one hypothesis would be the work requirement for operant self-administration. This effect of work requirement might manifest owing to locomotor or motivational properties of the drug, although the locomotor explanation is unlikely as duloxetine does not appear to affect activity in rats (Brocco *et al.*, 2002).

Naltrexone is a nonselective opioid antagonist used clinically in the treatment of alcoholism. Naltrexone has long been known to suppress alcohol drinking by rats (e.g., Altshuler *et al.*, 1980; Reid and Hunter, 1984; Walker and Koob, 2007), and this effect is exaggerated in selectively bred Sardinian alcohol-preferring (sP Sabino *et al.*, 2006 rats. In the current investigation, a very low dose (50 μg/kg) of naltrexone suppressed alcohol binge-like drinking, whereas a three-fold higher dose was required to reduce supersac consumption. These results are consistent with past findings that high doses of naltrexone (5–10 mg/kg; Reid *et al.*, 1996; Gardell *et al.*, 1997) suppress consumption of a sweetened alcohol solution [5% (w/v) sucrose + 6% ethanol (w/v)]. The heightened sensitivity of alcohol binge-like drinking to the suppressive effects of naltrexone is comparable to the ability of a very low naltrexone dose (50 μg/kg) to suppress alcohol drinking by sP rats (Sabino *et al.*, 2006). A low naltrexone dose (100 μg/kg) also suppresses operant responding for unsweetened alcohol by nondependent Wistar rats, but naltrexone is considerably less efficacious in alcohol-dependent rats as a considerably higher dose (500 μg/kg) is required to suppress operant alcohol responding in

those rats (Walker and Koob, 2007). Together, these findings indicate that naltrexone is more effective in suppressing excessive binge-like alcohol drinking than excessive drinking related to alcohol dependence.

The ability of naltrexone to suppress supersac consumption in the current investigation is in agreement with other reports in which naltrexone blocked the development of preference for a sucrose diet (Levine *et al.*, 2002) and consumption of saccharin solution (Goodwin *et al.*, 2001). These results are consistent with the hypothesis that blockade of opioid receptors by naltrexone at higher doses blocks the positive reinforcing effects of both natural and drug reinforcers.

Extra-hypothalamic CRF systems are thought to be perturbed during the transition to alcohol dependence, and to be an important factor in subsequent relapse alcohol drinking (Koob, 2003). MPZP is a CRF antagonist that effectively blocks CRF_1 receptors (George *et al.*, 2007; Specio *et al.*, 2007). Previous findings from this laboratory indicate that this analog suppresses operant alcohol responding in dependent, but not nondependent, Wistar rats (Richardson *et al.*, 2007). In the current investigation, MPZP did not affect alcohol binge-like drinking or supersac drinking, but there was a significant upward linear trend of dose on alcohol drinking. This result is consistent with the hypothesis that the activation of extra-hypothalamic CRF systems is more involved in alcohol drinking motivated by the negative reinforcing properties of the drug (i.e., dependence-induced drinking), but not drinking motivated by the positive reinforcing effects of the drug (i.e., binge-like alcohol drinking and nondependent alcohol drinking; Koob, 2003). Consistent with this notion, nondependent CRF_1-receptor knockout mice have been observed to drink more alcohol than wild-type controls (Sillaber *et al.*, 2002), but CRF_1-receptor knockout mice do not exhibit the dependence-induced increases in alcohol drinking observed in wild-type controls (Chu *et al.*, 2007). It remains to be determined if long-term binge-like drinking in a model such as the one used here is capable of eventually producing the motivational symptoms associated with alcohol dependence.

It is unlikely that the drug effects observed in the present investigation are because of nonspecific drug effects (e.g. activity, taste sensitivity, thirst, hunger). Naltrexone suppresses water intake induced by angiotensin II injection at doses similar to those used in the present investigation (Ruegg *et al.*, 1994). The absence of effects of naltrexone on water intake by any group in the present investigation, however, indicates that the observed suppression of experimental solutions was not because of nonspecific thirst effects. Systemically administered naltrexone also suppresses food intake (Hobbs *et al.*, 1994), and opioid antagonists typically suppress locomotor activity (Leventhal *et al.*, 1996), but these effects occur at doses significantly higher than those used in the present investigation. Duloxetine suppresses locomotor activity (Bymaster *et al.*, 2005) and food intake (Jackson *et al.*, 1997) in rodents, and can also affect salivation (Katoh *et al.*, 1995), but these effects occur at doses much higher (30–200 mg/kg) than those used in the present investigation. Relative to naltrexone and duloxetine, less is known about nonspecific behavioral effects of MPZP. MPZP suppresses anxiety-like behavior and dependence-induced alcohol drinking (Richardson *et al.*, 2007). Brain CRF systems are involved in feeding behavior (Zorrilla *et al.*, 2003), but those effects are likely mediated by CRF_2 receptors (Ohata *et al.*, 2002; Cottone *et al.*, 2007). CRF_1-receptor antagonists may be capable of suppressing locomotor activity (Ohata *et al.*, 2002), but this effect likely did not alter behavior in this study because MPZP produced either increases or no effect on drinking in the various binge groups.

In summary, in the absence of food or water deprivation, rats consumed alcohol voluntarily and orally in amounts sufficient to produce BALs that define alcohol binge drinking in humans. This binge-like model is highly sensitive to compounds that suppress drinking via opioidergic (naltrexone) and serotonergic (duloxetine) mechanisms, but not sensitive to compounds that

suppress drinking via decreases in CRF activity. Animal models of binge-like alcohol drinking will be valuable in assessing the motivational aspects and neural consequences of predependence heavy alcohol drinking behavior. The different profiles of compounds that affect alcohol binge-like drinking versus dependence-induced drinking should advance the effort to develop potential pharmacotherapeutics for subpopulations of alcohol abusers and alcoholics (Egli, 2005).

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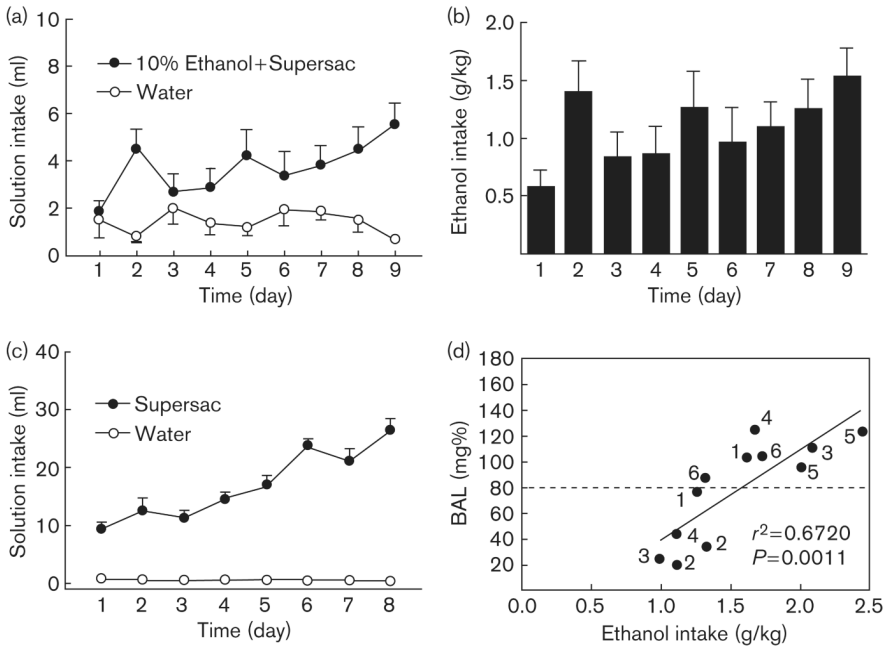

Fig. 1.

(a and b) Mean ± SEM intake (ml and g/kg) of sweetened (3% glucose + 0.125% saccharin) 10% (w/v) alcohol solution during the initial 9-day baseline binge period; (c) mean ± SEM intake (ml) of supersac (3% glucose + 0.125% saccharin) by controls during the initial 8-day baseline binge period; and (d) scatter plot of blood–alcohol levels produced by alcohol intake (g/kg) by animals in the alcohol binge group during two representative drinking sessions. Points are individually labeled with rat identification numbers to show that five of the six animals in the alcohol binge group achieved BALs ≥ 0.08 g% during at least one of the two drinking sessions.

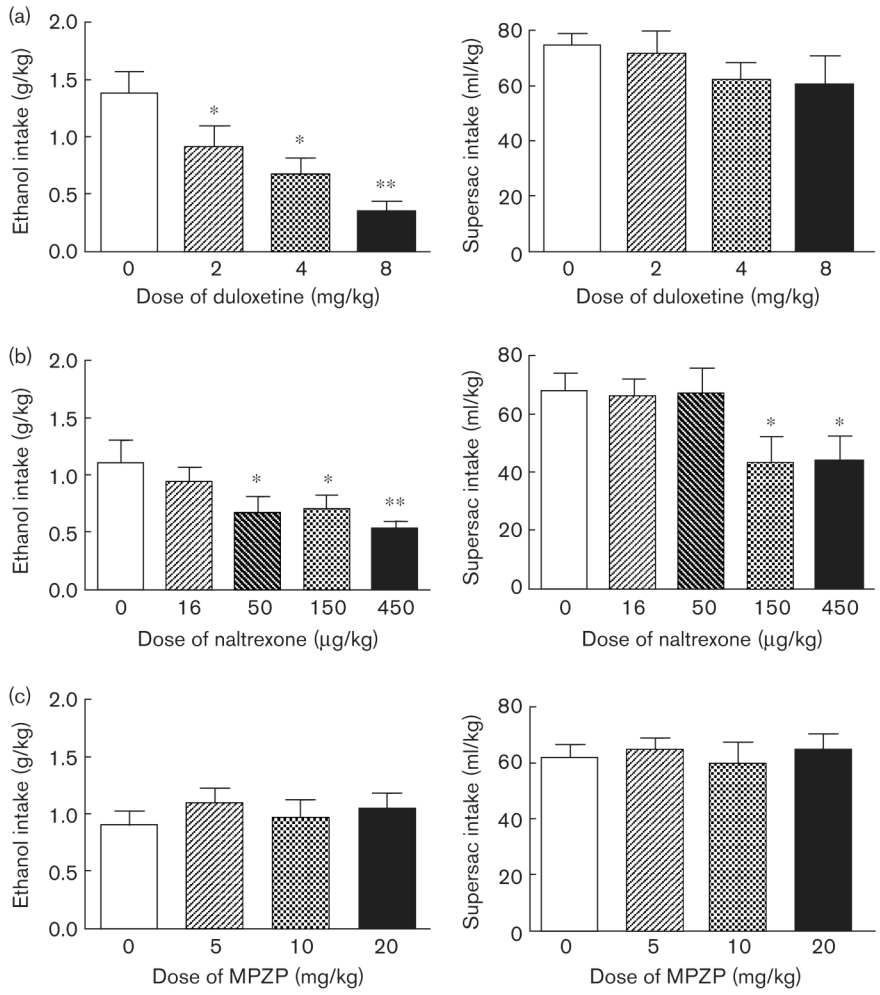

Fig. 2.

Mean ± SEM alcohol intake (g/kg) by animals in the alcohol binge group (n = 6; left panel) and supersac intake (ml/kg) by supersac controls (n = 6; right panel) following pretreatment with (a) one of four doses (0, 2, 4, 8 mg/kg) of duloxetine (intraperitoneal injection 40 min prior to drinking session), (b) one of five doses (0, 16, 50, 150, 450 μg/kg) of naltrexone (subcutaneous injection 30 min prior to drinking session), or (c) one of four doses (0, 5, 10, 20 mg/kg) of MPZP (subcutaneous injection 60 min prior to drinking session). *P<0.05, **P<0.001 significant difference from vehicle condition.

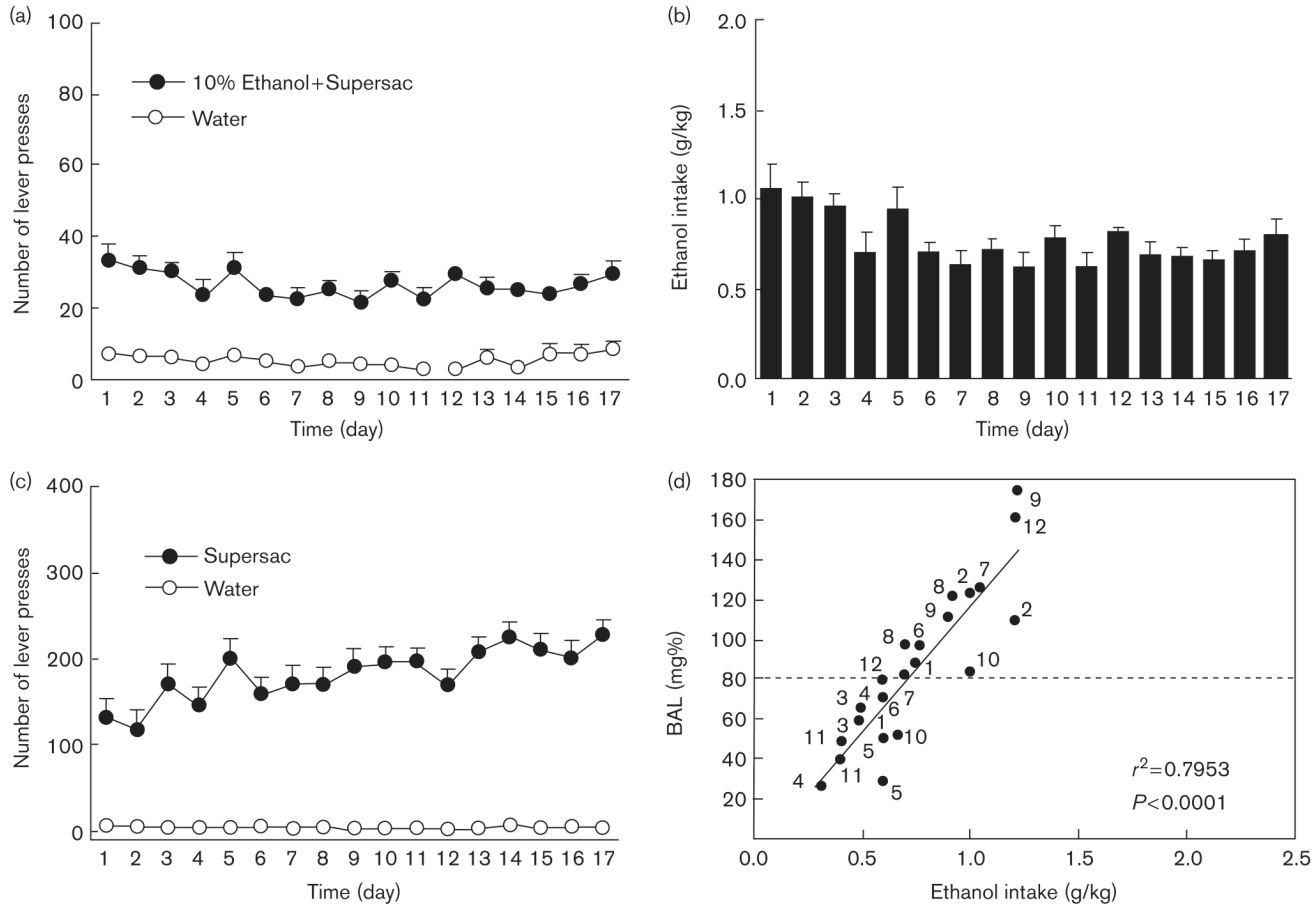

Fig. 3.

(a) Mean ± SEM number of lever presses for sweetened (3% glucose + 0.125% saccharin) 10% (w/v) alcohol solution, and (b) mean ± SEM intake (g/kg) of sweetened alcohol solution during the initial 17-day baseline binge period; (c) mean ± SEM number of lever presses for supersac (3% glucose + 0.125% saccharin) by controls during the initial 17-day baseline binge period; and (d) scatter plot of blood–alcohol levels produced by alcohol intake (g/kg) by animals in the alcohol binge group during two representative drinking sessions. Points are individually labeled with rat identification numbers to show that 8 of the 12 animals in the alcohol binge group achieved BALs ≥ 0.08 g% during at least one of the two drinking sessions.

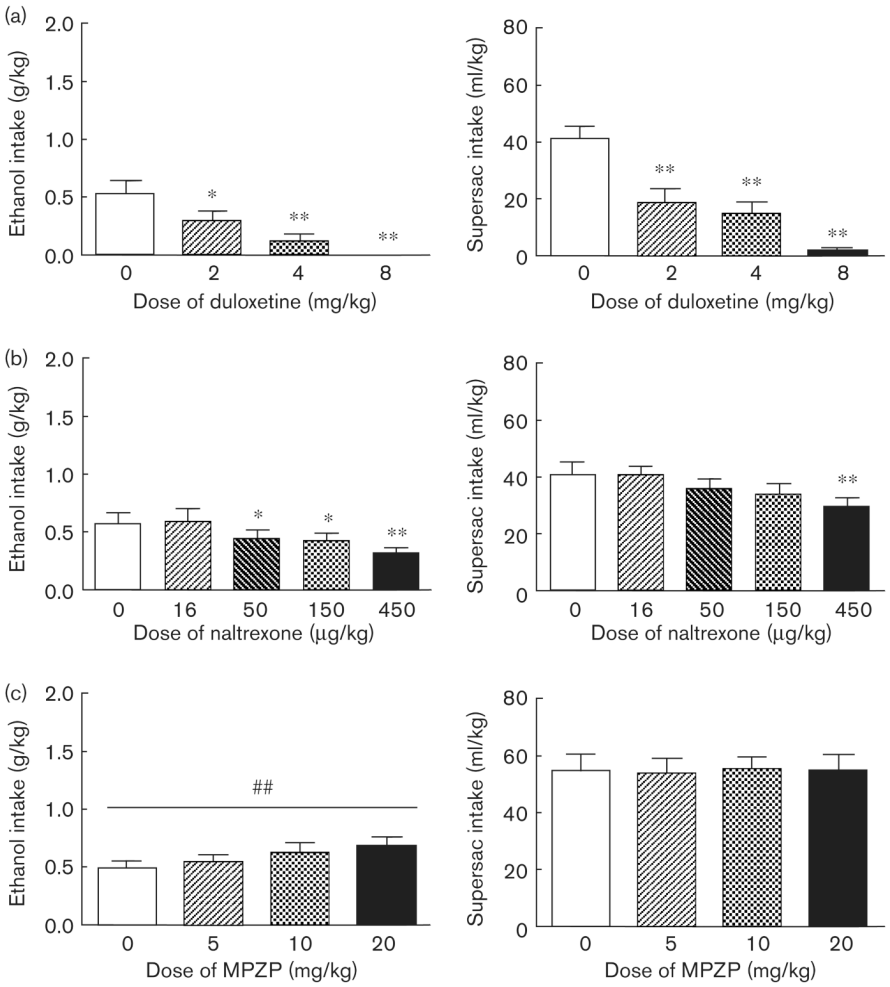

Fig. 4.

Mean ± SEM alcohol intake (g/kg) by animals in the alcohol binge group (n = 11; left panel) and supersac intake (ml/kg) by supersac controls (n = 12; right panel) following pretreatment with (a) one of four doses (0, 2, 4, 8 mg/kg) of duloxetine (intraperitoneal injection 40 min prior to drinking session), (b) one of five doses (0, 16, 50, 150, 450 µg/kg) of naltrexone (s.c. injection 30 min prior to drinking session), or (c) one of four doses (0, 5, 10, 20 mg/kg) of MPZP (s.c. injection 60 min prior to drinking session). *P<0.05, **P<0.01 significant difference from vehicle condition.

Table 1

Effects of duloxetine, naltrexone, and MPZP on water intake, total fluid intake, and preference [ethanol/supersac:total (E/S:T)] for sweetened alcohol solution by animals in the two-bottle choice (experiment 1) alcohol binge group, and of supersac solution by supersac controls

	Water intake (ml)	Total fluid (ml)	Preference (E/S:T)
Duloxetine			
Alcohol binge drinkers			
0 mg/kg	0.88 ± 0.25	6.12 ± 0.98	0.85 ± 0.04
2 mg/kg	0.72 ± 0.11	4.30 ± 0.81	0.79 ± 0.05
4 mg/kg	0.57 ± 0.05	3.22 ± 0.61*	0.79 ± 0.06
8 mg/kg	0.58 ± 0.11	2.00 ± 0.38**	0.67 ± 0.08*
Supersac controls			
0 mg/kg	0.32 ± 0.06	26.77 ± 1.55	0.99 ± 0.00
2 mg/kg	0.28 ± 0.06	26.17 ± 3.92	0.99 ± 0.00
4 mg/kg	0.22 ± 0.07	22.02 ± 2.75	0.99 ± 0.00
8 mg/kg	0.20 ± 0.04	21.02 ± 4.02	0.99 ± 0.00
Naltrexone			
Alcohol binge drinkers			
0 μg/kg	0.70 ± 0.15	5.88 ± 0.92	0.86 ± 0.04
16 μg/kg	0.87 ± 0.25	5.17 ± 0.50	0.83 ± 0.05
50 μg/kg	1.07 ± 0.55	4.13 ± 1.01	0.73 ± 0.08
150 μg/kg	0.80 ± 0.37	4.02 ± 0.60	0.80 ± 0.07
450 μg/kg	0.87 ± 0.29	3.27 ± 0.38*	0.74 ± 0.09
Supersac controls			
0 μg/kg	0.28 ± 0.08	32.75 ± 3.56	0.99 ± 0.00
16 μg/kg	0.33 ± 0.09	31.63 ± 3.37	0.99 ± 0.00
50 μg/kg	0.47 ± 0.21	32.10 ± 3.90	0.98 ± 0.01
150 μg/kg	0.45 ± 0.23	21.17 ± 4.71**	0.95 ± 0.04
450 μg/kg	0.23 ± 0.04	21.38 ± 4.40**	0.99 ± 0.00
MPZP			
Alcohol binge drinkers			
0 mg/kg	0.45 ± 0.13	5.22 ± 0.61	0.90 ± 0.04
5 mg/kg	0.63 ± 0.24	6.30 ± 0.49	0.90 ± 0.04
10 mg/kg	0.52 ± 0.13	5.48 ± 0.84	0.89 ± 0.04
20 mg/kg	0.58 ± 0.14	5.98 ± 0.56	0.89 ± 0.03
Supersac controls			
0 mg/kg	0.37 ± 0.06	35.23 ± 2.39	0.99 ± 0.00
5 mg/kg	0.33 ± 0.08	37.20 ± 3.85	0.99 ± 0.00
10 mg/kg	0.37 ± 0.07	33.68 ± 4.26	0.99 ± 0.00
20 mg/kg	0.32 ± 0.09	36.22 ± 3.20	0.99 ± 0.00

* $P<0.05$,

** $P<0.01$ relative to vehicle condition.

Table 2

Effects of duloxetine, naltrexone, and MPZP and naltrexone on water intake, total fluid intake, and preference [ethanol/supersac:total (E/S:T)] for sweetened alcohol solution by animals in the operant (experiment 2) alcohol binge group and supersac solution by supersac controls

	Water intake (ml)	Total fluid (ml)	Preference (E/S:T)
Duloxetine			
Alcohol binge drinkers			
0 mg/kg	0.88 ± 0.30	3.68 ± 0.47	0.70 ± 0.10
2 mg/kg	0.43 ± 0.18	1.93 ± 0.42**	0.72 ± 0.08
4 mg/kg	0.33 ± 0.16	0.92 ± 0.34**	0.57 ± 0.14
8 mg/kg	0.01 ± 0.01 **	0.03 ± 0.03**	N/A
Supersac controls			
0 mg/kg	0.40 ± 0.10	21.30 ± 2.44	0.98 ± 0.00
2 mg/kg	0.08 ± 0.04**	9.84 ± 2.55**	0.99 ± 0.00
4 mg/kg	0.07 ± 0.03**	7.43 ± 2.23**	0.98 ± 0.01
8 mg/kg	0.03 ± 0.02**	0.74 ± 0.39**	0.91 ± 0.06
Naltrexone			
Alcohol binge drinkers			
0 μg/kg	1.30 ± 0.34	3.91 ± 0.36	0.68 ± 0.08
16 μg/kg	1.29 ± 0.27	4.17 ± 0.54	0.67 ± 0.06
50 μg/kg	1.33 ± 0.38	3.45 ± 0.42	0.63 ± 0.08
150 μg/kg	1.12 ± 0.33	3.15 ± 0.41	0.66 ± 0.08
450 μg/kg	0.93 ± 0.25	2.42 ± 0.34**	0.62 ± 0.07
Supersac controls			
0 μg/kg	0.22 ± 0.10	19.23 ± 2.30	0.99 ± 0.01
16 μg/kg	0.23 ± 0.13	19.13 ± 1.43	0.98 ± 0.01
50 μg/kg	0.09 ± 0.04	16.87 ± 1.70	1.00 ± 0.00
150 μg/kg	0.24 ± 0.12	16.05 ± 1.93	0.98 ± 0.01
450 μg/kg	0.14 ± 0.10	13.70 ± 1.79**	0.99 ± 0.01
MPZP			
Alcohol binge drinkers			
0 mg/kg	1.04 ± 0.16	3.00 ± 0.15	0.65 ± 0.05
5 mg/kg	1.00 ± 0.43	3.18 ± 0.39	0.75 ± 0.08
10 mg/kg	1.06 ± 0.29	3.58 ± 0.42	0.71 ± 0.06
20 mg/kg	1.09 ± 0.26	3.80 ± 0.39	0.72 ± 0.07
Supersac controls			
0 mg/kg	0.23 ± 0.08	22.18 ± 2.26	0.99 ± 0.00
5 mg/kg	0.19 ± 0.08	21.62 ± 2.07	0.99 ± 0.01
10 mg/kg	0.28 ± 0.06	22.29 ± 1.74	0.99 ± 0.00
20 mg/kg	0.23 ± 0.07	21.96 ± 2.25	0.98 ± 0.01

** $P<0.01$ relative to vehicle condition;

N/A indicates denominator (total fluid intake) was zero for many of the individual rats.