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Psychopharmacology (Berl). Author manuscript; available in PMC 2010 August 1.

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Published in final edited form as:
Psychopharmacology (Berl). 2009 August ; 205(2): 327–335. doi:10.1007/s00213-009-1548-x.

Selective reduction of alcohol drinking in Sardinian alcohol-preferring rats by a sigma-1 receptor antagonist

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Abstract

Rationale and objectives—Sigma receptors have been implicated in appetitive effects of psychostimulants and in high levels of ethanol intake. This study tested the hypothesis that the sigma-1 receptor subtype (Sig-1R) may modulate ethanol intake.

Material and methods—The effects of acute and repeated treatment with the potent, selective Sig-1R antagonist NE-100 on ethanol intake (10%) were studied in adult, male Sardinian alcohol-preferring (sP) rats, a model of genetic predisposition to high ethanol drinking. To assess the specificity of action, the acute effects of NE-100 on intake of an equally preferred sucrose solution and of a higher concentration of ethanol that sP rats did not prefer over water (28%), were determined. Finally, the ability of NE-100 administration to prevent the increased ethanol intake that occurs after deprivation was evaluated.

Results—Acute treatment with NE-100 dose-dependently (10–30 mg/kg) reduced 1- and 3-h intake of 10% ethanol solution in sP rats, while increasing concurrent water intake and not affecting food intake. NE-100 (17.8–30 mg/kg) comparably reduced intake of the 28% ethanol solution, while not suppressing 1.25% sucrose solution intake, suggesting selectivity of action against ethanol intake. Acute NE-100 (30 mg/kg) also prevented an increase in ethanol intake after a 7-day deprivation period. Repeated, daily NE-100 (30 mg/kg) treatment continued to reduce 24-h ethanol intake across 7 days of administration, with some, but incomplete, tolerance, evident by day 6.

Conclusions—The results implicate the Sig-1R system in alcohol drinking, identifying a potential therapeutic target for the treatment of alcohol use disorders.

Keywords

Ethanol; Addiction; Rat; Alcoholism; Treatment

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Disclosure/conflicts of interest The authors declare no conflicts of interest.

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Introduction

Alcoholism, a global health problem, is a chronic relapsing disorder of compulsive alcohol use (McLellan et al. 2000). Despite progress in understanding the etiology of alcohol dependence, effective, well-tolerated treatments remain elusive. Sigma receptor (SigR) antagonists have been hypothesized to be putative pharmacotherapies for addiction to ethanol and psychostimulants (Brammer et al. 2006; Martin-Fardon et al. 2007; Maurice and Romieu 2004; Menkel et al. 1991; Sabino et al. 2009). SigRs were originally categorized as members of the opiate receptor family (Martin et al. 1976) and a high-affinity phencyclidine (PCP) binding-site (Quirion et al. 1981), but more recent data suggest that SigRs are unique binding sites that differ from other known mammalian proteins (Gundlach et al. 1985; Walker et al. 1990). SigRs are widely expressed in rat brain, throughout the limbic system and brainstem motor structures. The highest levels of immunoreactivity are observed in the olfactory bulb, hypothalamus, and hippocampus; the ventral and dorsal striatum and amygdala also show moderately concentrated, intense labeling (Alonso et al. 2000; Bouchard and Quirion 1997). SigRs are immunohistochemically localized to synaptic contacts, and the anatomical distribution of SigRs (Alonso et al. 2000; Maurice et al. (2002) suggests that SigRs may modulate motivationally relevant synaptic transmission, including that of substances of abuse. Two SigR subtypes are known, Sig-1R and Sig-2R, differing in their binding profile and molecular weight (Hanner et al. 1996; Moebius et al. 1993).

Relevant to ethanol use disorders, nonselective SigR antagonists previously were shown to attenuate ethanol-induced locomotion, block ethanol-induced place and taste conditioning in mice (BD-1047; Maurice et al. 2003), and reduce ethanol intake in rat models of excessive drinking (BD-1063; Sabino et al. 2009). However, the receptor subtype mediating these actions remains unclear. Data implicate the Sig-1R subtype in the actions of cocaine and methamphetamine (Matsumoto et al. 2002; Romieu et al. 2004). Also, Sig-1R gene functional polymorphisms that correlate with altered receptor transcription were over-represented in Japanese alcoholics (Miyatake et al. 2004). In contrast, some pharmacological evidence suggests that some actions which previously have been attributed exclusively to Sig-1Rs may also (or alternatively) involve Sig-2Rs (Matsumoto and Mack 2001; Nuwayhid and Werling 2006). For example, (±)-SM 21, a compound with high and preferential affinity for Sig-2R, relative to the Sig1R subtype has been shown to attenuate some behavioral and toxic effects of cocaine (Matsumoto and Mack 2001; Matsumoto et al. 2007). In addition, while 1,3-di(2-tolyl) guanidine (DTG), a specific, but subtype-nonselective Sig-1R/Sig-2R agonist, markedly worsens cocaine-induced toxicity, (+)pentazocine—a selective Sig-1R agonist—did not significantly alter the responsiveness of the animals to the convulsive effects of cocaine (Matsumoto and Mack 2001; Matsumoto et al. 2007).

The purpose of the present study was to test the hypothesis that the Sig-1R subtype modulates alcohol drinking. We examined the effects of systemic administration of the selective Sig-1R antagonist NE-100 on alcohol drinking in Sardinian alcohol-preferring (sP) rats. The selectively bred sP rat (Colombo 1997) provides a model for identifying potential pharmacotherapies for alcoholism (Colombo et al. 2006; McBride and Li 1998). This line voluntarily drinks ethanol (10% *v*/*v*) in high quantities and preference over water, exhibits a heritable component similar to human ethanol dependence (Cloninger et al. 1981; Prescott and Kendler 1999; Sigvardsson et al. 1996), and possesses predictive validity to identify pharmacotherapies for alcohol dependence.

To assess the specificity of action against consumption of ethanol, as opposed to an action on preferred solutions per se, the effects of NE-100 were also evaluated on the intake of an equally consumed, preferred sucrose solution and on the intake of a more concentrated alcohol solution (28% *v*/*v*) which sP rats do not prefer over water. The ability of NE-100 to suppress the increase

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in ethanol intake that occurs after deprivation (the "alcohol deprivation effect"; Koob 2000; Li 2000), a model of relapse drinking in human alcoholics (Boening et al. 2001; Colombo et al. 2006; McBride et al. 2002), was also assessed. Finally, the effects of repeated, daily treatment with NE-100 on ethanol intake were studied to investigate changes in effectiveness that may occur with repeated administration.

Thus, the present study investigated the effects and the selectivity of action of a selective Sig-1R antagonist, NE-100, on alcohol intake in alcohol-preferring sP rats. The overall hypothesis under test was that the Sig-1R system promotes alcohol intake in sP rats such that a subtype-selective antagonist, NE-100, would specifically reduce ethanol intake.

Materials and methods

Animals

Male, genetically selected TSRI Sardinian alcohol-preferring rats (*N*=60 Scr:sP; 300 g at study onset) were individually housed in a humidity- and temperature-controlled vivarium on a 12-h light–dark cycle (lights off, 10:00A.M.). Rats were generated from the 22nd to the 24th generations of intra-line breeding at The Scripps Research Institute from sP rats obtained after 32 generations of selection from Prof. G.L. Gessa (University of Cagliari, Italy). Different sets of rats were used for each experiment. Procedures adhered to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute.

Drugs

Ethanol solutions (10% and 28% *v*/*v*) and sucrose solution (1.25% *w*/*v*) were prepared using 95% ethyl alcohol or sucrose (Sigma Aldrich) in tap water. NE-100, a gift from Taisho Pharmaceutical, Tokyo, Japan, was dissolved in bacteriostatic saline and injected subcutaneously (s.c., 1 ml/kg), 15 min before drinking sessions (dark cycle onset). NE-100 is a selective Sig-1R antagonist (K_i=1.09 vs. 212 nM at Sig-1R and Sig-2R, respectively; Berardi et al. 2001) that does not show significant affinity for systems other than sigma, including histaminergic, dopaminergic, adrenergic, serotonergic, cholinergic, or glutaminergic receptors (Tanaka et al. 1995).

Procedure for ethanol drinking experiments

Before the experiments, rats received continuous (24 h/day) two-bottle choice access to ethanol (10% *v*/*v*) vs. tap water for 8 to 10 consecutive weeks, unless otherwise specified, and were habituated to handling and s.c. injections. Test days in within-subject design experiments were spaced by 2 to 4 intervening treatment-free days. To determine the effect of NE-100 on ethanol drinking, rats (*N*=10) were pretreated with NE-100 (0, 10, 17.8, and 30 mg/kg, s.c.) in a within-subjects Latin square design. We used the doses of 10 and 30 mg/kg per convention, as approximate half-log intervals. The mid-dose, 17.8 mg/kg, was chosen because it is a quarter-log interval greater than 10 and thus approximately midway between the 10 and 30 doses in log-scale. Ethanol (10% *v*/*v*), water, and food intake were determined by weighing bottles and food before session onset using a scale with 0.1 g precision and again 60 and 180 min later.

To determine the effects of NE-100 on intake of a more concentrated ethanol solution that sP rats do not prefer relative to water (28% *v*/*v*), sP rats (*N*=8) received continuous (24 h/day) two-bottle choice access to ethanol (10% *v*/*v* for 6 weeks, then 28% *v*/*v* for 3 weeks) vs. water and then were pretreated with NE-100 (0 and 30 mg/kg, s.c.) in a counterbalanced design.

In the alcohol deprivation effect experiment, sP rats (*N*=14) received continuous (24 h/day) two-bottle choice access to ethanol (10% *v*/*v*) vs. tap water for 8 weeks. Rats were then

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pretreated with vehicle. Ethanol, water, and food intake were measured after 60 and 180 min, constituting *non-deprived intake*. After 2 further days of access to ethanol, rats received a 7-day period of deprivation from ethanol, during which water was the only fluid available. Rats were then pretreated with NE-100 (30 mg/kg) or vehicle in a between-subjects design (n=7/group), and ethanol, water, and food intake were measured after 60 and 180 min, constituting *deprived intake*.

To determine the effect of repeated treatment with NE-100 on voluntary ethanol drinking, rats (N=9–10/group) received NE-100 (0 or 30 mg/kg, s.c.) daily 15 min before the onset of the dark cycle for 7 consecutive days in a between-subjects design. Ethanol, water, and food were weighed each day, 3 and 24 h after dark cycle onset.

Procedure for sucrose drinking experiment

To determine the effect of acute NE-100 treatment on consumption of a preferred, ethanol-free solution, rats first received continuous (24 h/day) two-bottle choice access to a sucrose solution (1.25% *w*/*v*) vs. tap water for 3 consecutive weeks. Rats (N=8) then were pretreated with NE-100 (s.c., 0, 17.8 and 30 mg/kg) in a within-subjects Latin square design. Sucrose solution, water, and food were postweighed 60 and 180 min after the dark cycle onset.

Procedure for blood alcohol level measurements

To determine whether NE-100 altered ethanol pharmacokinetics, rats (n=8/group) were pretreated with NE-100 (30 mg/kg, s.c.) or vehicle in a between-subjects design before receiving ethanol by gavage (1 g/kg, 16% *w*/*v*, p.o.) at dark cycle onset. Blood samples were collected from the rats' tails 15, 30, 60, and 120 min after ethanol administration. Plasma was assayed for alcohol content (Analox Instruments, Lunenburg, MA, USA).

Statistical analysis

Incremental intake in each time bin was normalized for body weight (g/kg, ml/kg, and g/kg, for ethanol, water–sucrose solution, and food, respectively). Intake data from the ethanol and sucrose solution experiments were analyzed by a two-way, repeated-measures analysis of variance (ANOVA), with Antagonist Treatment and Time as within-subject factors. Data on first-hour intake from the alcohol deprivation effect experiment were analyzed by Student's t-test. Data from the repeated treatment experiment were analyzed by separate two-way, mixed-design ANOVAs (for 3- and 24-h intake), with Antagonist as a between-subjects factor and Day as a within-subjects factor. Significant interactions were interpreted by simple main effects analysis. For pairwise comparisons, Dunnett's tests were used to determine whether treatment altered performance compared with vehicle conditions. Statistical significance was set at $P<0.05$.

Results

Effect of acute NE-100 administration on ethanol drinking

As shown in Fig. 1, treatment with the selective Sig-1R antagonist NE-100 dose-dependently reduced intake of a 10% *v*/*v* ethanol solution [$F(3,27)=10.84$, $P<0.0001$], while showing a strong trend toward increased concurrent water intake [$F(3,27)=2.92$, $P=0.052$]. Pairwise comparisons revealed that the doses of 17.8 and 30 mg/kg both significantly reduced ethanol intake (Fig. 1a, 27% and 58% decrease at the 3-h timepoint, for the 17.8 and 30 mg/kg doses, respectively). Thus, NE-100 decreased 3-h preference for an otherwise preferred ethanol solution (89.8% preference over water in vehicle-treated condition, vs. 72.3% for 17.8 mg/kg and 45.0% for 30 mg/kg). NE-100 treatment did not reliably alter total fluid [$F(3,27)=2.78$, n.s.] (data not shown) or food intake [$F(3,27)=0.85$, n.s.] (Fig. 1c).

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NE-100 treatment (30 mg/kg) also reduced 3-h consumption of a more concentrated solution of ethanol (28% *v*/*v*) that was not preferred over water (45.8% baseline preference) [$F(1,8)=12.26$, $P<0.01$] (vehicle, 1.68±0.20 vs. NE-100: 0.92± 0.12 g/kg). In this study, NE-100 treatment did not reliably alter concurrent water, food, or total fluid intake (data not shown). Thus, the ability of NE-100 to reduce ethanol intake did not simply reflect reduced intake of a preferred solution.

Effect of acute NE-100 administration on sucrose drinking

As shown in Fig. 2, treatment with the selective Sig-1R antagonist NE-100 increased sucrose solution intake in a time-dependent manner [Treatment×Time, $F(2,16)=6.09$, $P<0.05$]. *Post hoc* comparisons revealed that the 30 mg/kg dose transiently increased sucrose solution intake by 84% within the first hour compared with vehicle conditions, but not thereafter. NE-100 treatment did not alter intakes of water, food, or total fluid [Treatment, $F(2,16)=1.14$, 1.92, and 0.84, respectively; Treatment×Time, $F(2,16)=0.84$, 2.15, and 1.68, respectively, all n.s.].

Effect of NE-100 on the alcohol deprivation effect

As expected, an imposed 7-day deprivation from ethanol increased ethanol intake during the first hour of renewed access in vehicle-treated subjects [deprived vs. non-deprived intake, $t(6)=4.77$, $P<0.01$]. In contrast, as shown in Fig. 3, rats pretreated with NE-100 (30 mg/kg) before renewed access did not show increased ethanol intake during the first hour compared with their nondeprived intake [$t(6)=0.07$, n.s.]. Consequently, vehicle-treated rats drank more than NE-100-treated rats under deprived conditions [$t(12)=2.48$, $P<0.05$].

Effect of NE-100 on blood alcohol levels

Blood alcohol levels changed across time following oral ethanol gavage (1 g/kg) [$F(3,33)=29.16$, $p<0.0001$]. Table 1 shows that pretreatment with 30 mg/kg NE-100 did not significantly alter blood alcohol levels 15–120 min following ethanol administration [Treatment, $F(1,11)=0.46$, n.s., Treatment×Time, $F(3,33)=0.05$, n.s.].

Effect of repeated NE-100 administration on ethanol drinking

Daily NE-100 (30 mg/kg) administration reduced both 3-and 24-h ethanol intake during the 7 days of treatment [Treatment, 3 h, $F(1,17)=11.40$, $p<0.01$; 24 h, $F(1,17)=5.14$, $p<0.05$], with no reliable change in this effect observed across the treatment period [Day×Treatment, 3 h, $F(6,102)=2.11$, n.s.; 24 h, $F(6,102)=1.42$, n.s.]. As shown in Fig. 4a, b, pairwise analyses showed that the NE-100-treated group drank significantly less ethanol through the fifth day of treatment.

As shown in Fig. 4c, d, repeated NE-100 treatment increased water intake [Treatment, 3 h, $F(1,17)=16.31$, $p<0.001$; 24 h, $F(1,17)=14.07$, $p<0.01$], leading to a nonsignificant tendency toward increased total fluid intake [Treatment, 3 h, $F(1,17)=2.51$, n.s.; 24 h, $F(1,17)=4.35$, n.s.] (data not shown). Figure 4e shows that NE-100 transiently reduced food intake for up to 3 h but only during the first 3 days of treatment [Treatment, $F(1,17)=7.95$, $p<0.05$]. However, as Fig. 4f shows, NE-100 treatment did not alter 24 h food intake at any time across the 7-day treatment period [Treatment, $F(1,17)=1.12$, n.s.].

Discussion

The present study showed that the selective Sig-1R antagonist NE-100 dose-dependently reduced ethanol intake in alcohol-preferring sP rats, a model of genetic predisposition to high ethanol drinking. NE-100 also prevented the increase in ethanol intake that otherwise occurs after a 7-day deprivation period. Suppressive actions of NE-100 were ethanol-selective and

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not due to changes in ethanol pharmacokinetics, general reductions in preferred fluid intake, or general decreases in fluid intake. NE-100 treatment reduced ethanol intake across 5 days of repeated, daily administration, with little tolerance evident until day 6. The results support the hypothesis that Sig-1R systems modulate alcohol intake.

The highest dose of NE-100 administered (30 mg/kg) decreased 3 h ethanol intake by 58% under continuous access conditions in sP rats, conditions with predictive validity for alcoholism pharmacotherapies (Colombo et al. 2006). The selective reduction of ethanol intake, and not of sucrose solution intake or concurrent food or water intake, suggests that NE-100 did not produce malaise-like, sedative, or other nonspecific behavior-impairing effects. NE-100 treatment also reduced intake of a solution of ethanol which subjects did not prefer over water, discounting the alternative explanation that NE-100 reduced intake of preferred solutions in general. The specific preference shift away from ethanol supports the potential relevance of NE-100, and possibly other Sig-1R antagonists, as therapeutics for ethanol use disorders.

The present results are consistent with findings that subtype nonselective SigR antagonists attenuated behavioral and motivational effects of acute passive ethanol administration in mice (BD-1047; Maurice et al. 2003) and reduced ethanol self-administration in rat models of excessive ethanol intake (BD-1063; Sabino et al. 2009). The findings obtained here with NE-100 more specifically suggest that the Sig-1R subtype may contribute to oral ethanol's reinforcing effects. This hypothesis is consistent with the overrepresentation of Sig-1R gene functional polymorphisms in alcoholics (Miyatake et al. 2004). Differences in nucleus accumbens Sig-1R transcription also have been observed in relation to genetic preference for ethanol and to ethanol exposure-induced dependence (Sabino et al. 2009). Finally, the present results are also consistent with the apparent role of Sig-1R receptors in the psychomotor stimulant (Menkel et al. 1991; Ujike et al. 1992) and rewarding (Romieu et al. 2000) effects of other substances of abuse.

NE-100 suppressed the alcohol deprivation effect, defined as the transient increase in voluntary alcohol intake seen after alcohol abstinence (Agabio et al. 2000; Romieu et al. 2000). The increase in alcohol intake observed in vehicle-treated rats after a 7-day abstinence period was comparable in magnitude (73% increase) and duration (~1 h) to that reported previously in sP rats (Agabio et al. 2000; Serra et al. 2003). The alcohol deprivation effect models the uncontrolled alcohol consummatory behavior that characterizes alcohol relapse in human alcoholics (Boening et al. 2001) and is attenuated by drugs which reduce relapse frequency in alcoholics (Heilig and Egli 2006), providing some additional predictive validity.

Repeated, daily s.c. NE-100 treatment (30 mg/kg) significantly reduced alcohol intake by 44% on the first day of treatment, with a peak reduction of 61% by the third treatment day. Beginning from the sixth treatment day, some tolerance to NE-100's actions was evident. This time course is similar to that of clinically utilized opioid receptor antagonists (e.g., naloxone and naltrexone), for which tolerance develops after 5–14 days of treatment (Cowen et al. 1999; Overstreet et al. 1999; Parkes and Sinclair 2000).

Ethanol is not thought to interact directly with SigRs, but SigR ligands may alter reinforcing actions of ethanol by indirectly modulating activity of other ethanol-sensitive transmitter systems. For example, SigR ligands affect the synthesis, release, and uptake of dopamine (Bastianetto et al. 1995; Iyengar et al. 1990; Weatherspoon and Werling 1999; Weiser et al. 1995), and they also modulate the firing activity of both the A9 and A10 dopaminergic pathways, with an activation of SigRs generally increasing firing rate (Minabe et al. 1999; Sanchez-Arroyos and Guitart 1999; Steinfels and Tam 1989). Therefore, Sig-1R antagonists might attenuate ethanol reinforcement by reducing mesolimbic dopaminergic transmission. The hypothesis that NE-100 might alter the reinforcing properties of ethanol by interfering

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with the mesolimbic dopaminergic pathway is, however, challenged by the fact that the effect of the drug does not correspond with preference/palatability, either on ethanol solutions or on sucrose solution. Rather than altering mesolimbic responsiveness in general, NE-100 might perhaps alter the pharmacological interaction of ethanol with mesolimbic neurons. Alternatively, NE-100's action may be mediated via a different neural substrate.

For example, ethanol actions are thought to be mediated by neurosteroids, which are endogenous SigR ligands (for a review, see Maurice 2004). Therefore, an alternative (or complementary) hypothesis is that Sig-1R antagonists might prevent the ethanol-induced release, synthesis, or action of neurosteroids (Maurice 2004; Ueda et al. 2001). While the brain regions subserving Sig-1R antagonist-induced decreases in ethanol intake are unknown, the distribution of Sig-1Rs (Alonso et al. 2000) suggests that the nucleus accumbens, which integrates reward-relevant information with the generation of goal-directed behaviors (Carelli and Wightman 2004), may be involved.

In summary, acute or repeated administration of NE-100, a selective Sig-1R antagonist, selectively reduced ethanol intake in a model of genetic predisposition to high ethanol drinking. The effects were specific to ethanol and did not involve general reductions in total fluid intake or preferred solution intake. The results implicate the Sig-1R subtype in ethanol drinking and suggest the therapeutic potential of NE-100, or other selective Sig-1R antagonists, for the detoxification and abstinence phases of alcohol use disorders.

Acknowledgments

Research was supported by grants from the National Institute on Alcohol Abuse and Alcoholism (5K99AA016731-02, 2P60AA006420-25, and 5R01AA012602-09) and by the Pearson Center for Alcoholism and Addiction Research. This is manuscript number 19878 from The Scripps Research Institute. The authors thank Taisho Pharmaceuticals for the gift of NE-100, the excellent technical assistance of Maury Cole, Molly Brennan, Jeanette Helfers and Robert Lintz, and the editorial assistance of Mike Arends.

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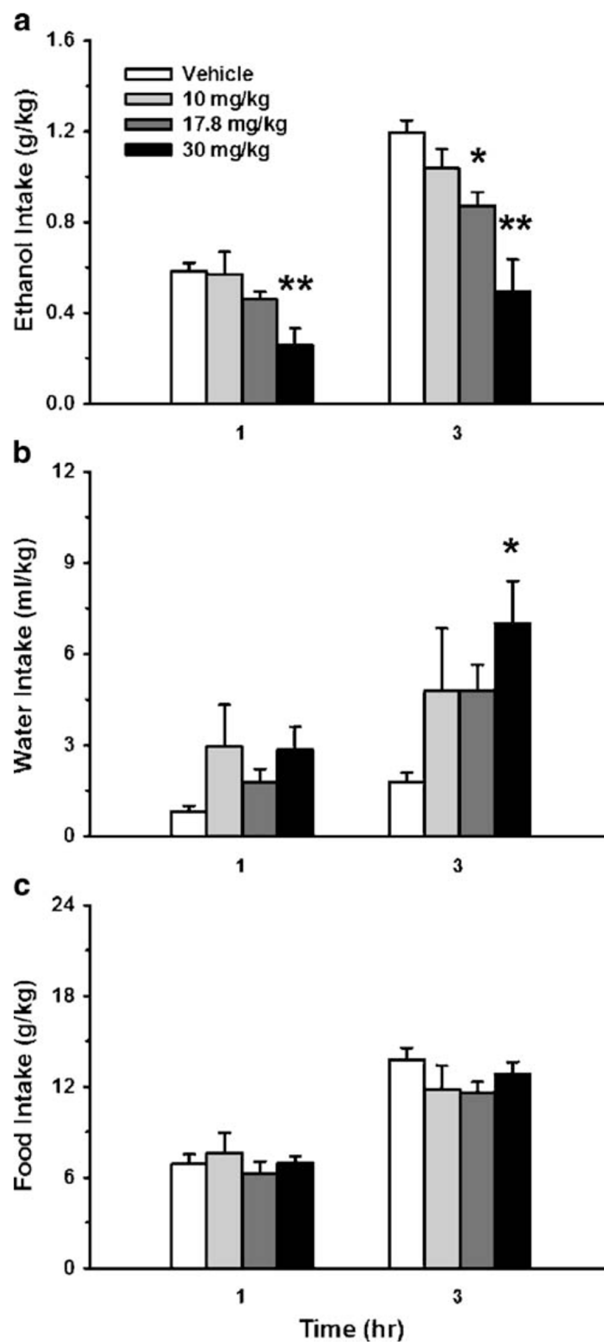

Fig. 1.

Effect of acute s.c. pretreatment (−15 min) with the selective sigma-1 receptor (Sig-1R) antagonist NE-100 on 1- and 3-h alcohol (**a**), water (**b**), and food intake (**c**), relative to dark cycle onset. Subjects were Sardinian alcohol-preferring (sP) rats (*N*=10), tested under continuous access (24 h) conditions. Data represent mean+SEM intake, normalized for body weight. **P* <0.05, ***P*<0.01 vs. vehicle-treated group (Dunnett's test)

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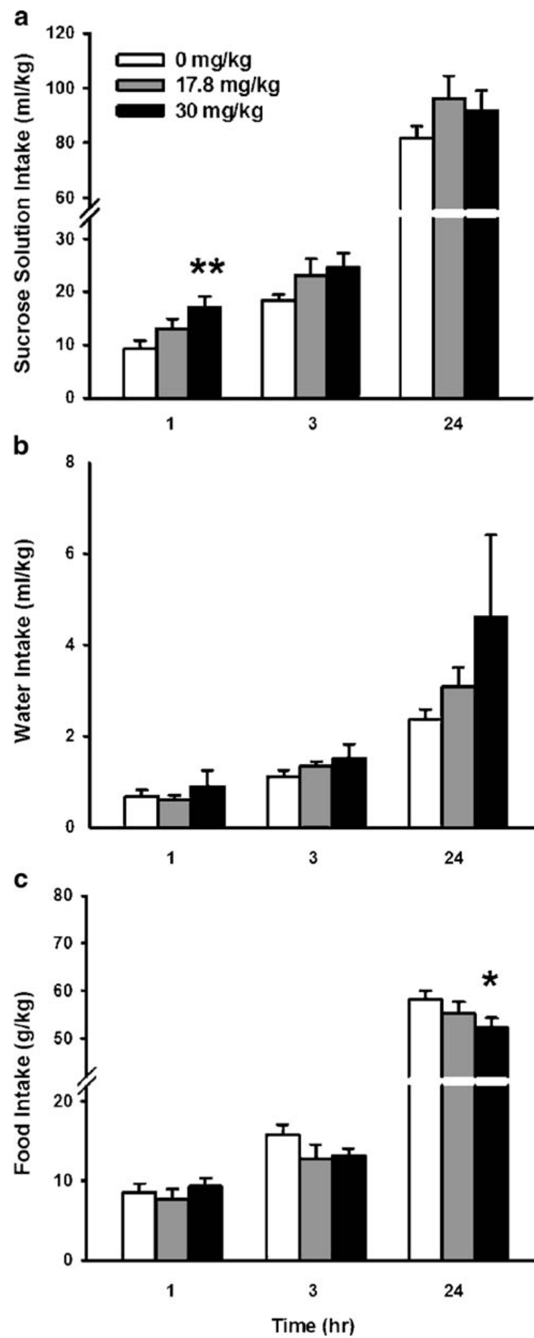

Fig. 2.

Effect of acute s.c. pretreatment (−15 min) with the selective sigma-1 receptor (Sig-1R) antagonist NE-100 on 1-, 3-, and 24-h intake of 1.25% *w*/*v* sucrose solution (**a**), water (**b**), and food (**c**), relative to dark cycle onset in sP rats under continuous access (24 h) conditions ($n=8$). Data represent mean+SEM intake, normalized for body weight. *$P< 0.05$, **$P<0.01$ vs. vehicle-treated group (Dunnett's test)

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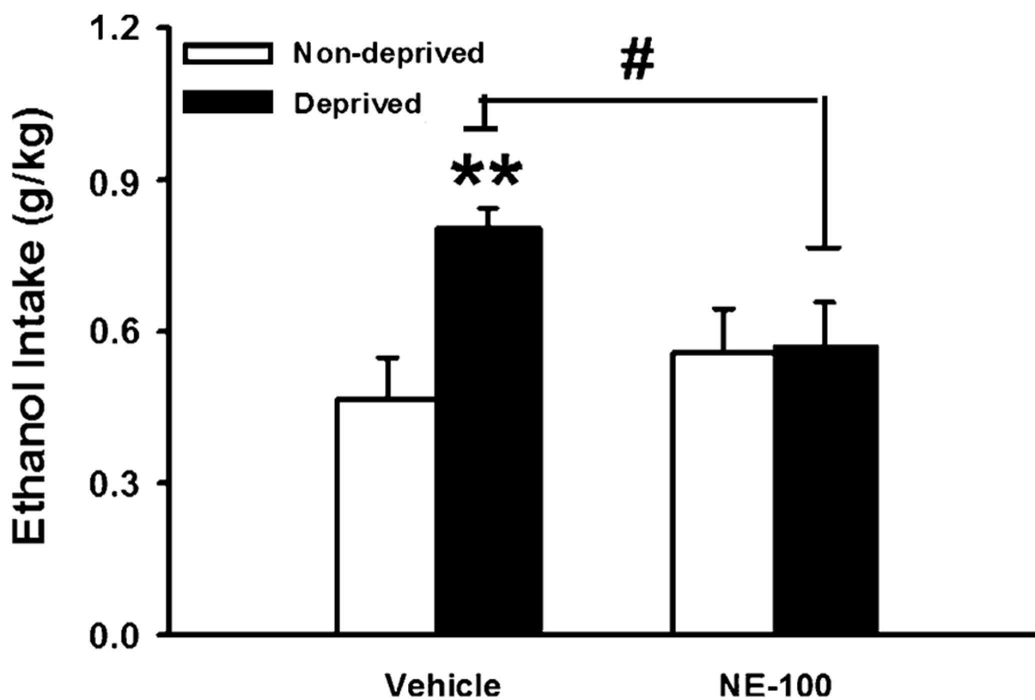

Fig. 3.
Effect of acute s.c. pretreatment (−15 min) with the selective sigma-1 receptor (Sig-1R) antagonist NE-100 on 1-h alcohol intake in sP rats (n=7/group) after an imposed 7-day deprivation from ethanol. Data represent mean+SEM intake normalized for body weight. $**P< 0.01$ vs. non-deprived group, $^{\#}P<0.05$ vs. vehicle-treated deprived group (Student's t tests)

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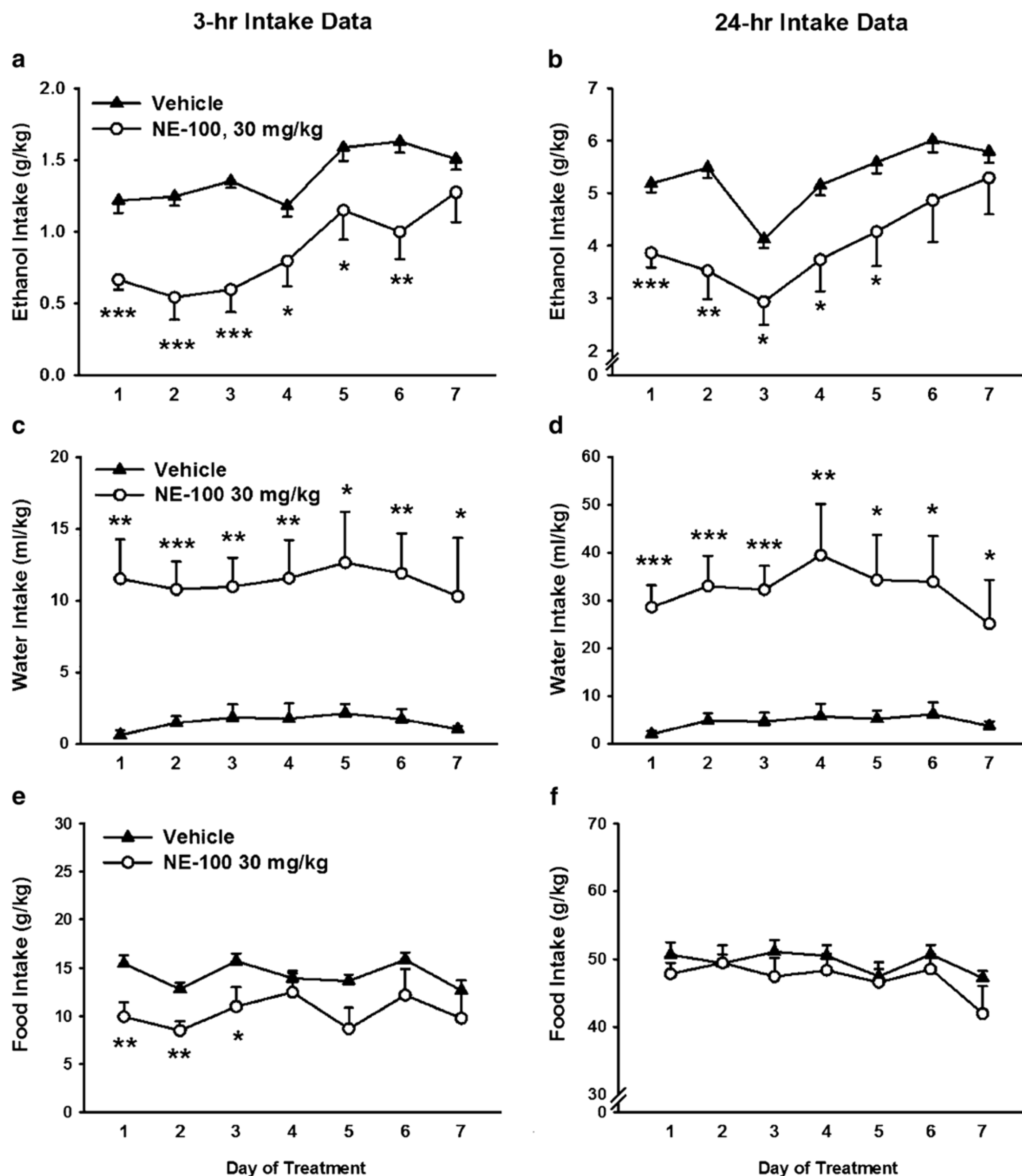

Fig. 4.

Effect of repeated, 7-day s.c. pretreatment (−15 min) with the selective sigma-1 receptor (Sig-1R) antagonist NE-100 on 3- (**a**, **c**, **e**) and 24-h (**b**, **d**, **f**) intake of alcohol (**a** and **b**), water (**c** and **d**), and food (**e** and **f**), relative to dark cycle onset in sP rats (*N*=9–10/group), tested under continuous access (24-h) conditions. Data represent mean±SEM intake, normalized for body weight. *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs. vehicle-treated group (Student's *t* tests)

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Table 1

Effect of acute s.c. pretreatment (−15 min) with the selective sigma-1 receptor (Sig-1R) antagonist NE-100 on blood alcohol levels 15, 30, 60, and 120 min following gavage administration of ethanol (1 g/kg, p.o.) to sP rats (n=8/group)

	15 min	30 min	60 min	120 min
Vehicle	67.5±14.2	104.2±4.4	93.2±5.4	66.4±7.4
NE-100, 30 mg/kg	59.4±12.3	100.6±8.6	85.5±9.2	58.5±6.5

Data represent the mean±SEM of mg of alcohol per deciliter of plasma.