Published in final edited form as:
Alcohol. 2012 November ; 46(7): 643–648. doi:10.1016/j.alcohol.2012.06.001.

EVIDENCE FOR CONDITIONED PLACE PREFERENCE TO A MODERATE DOSE OF ETHANOL IN ADULT MALE SPRAGUE-DAWLEY RATS

Melissa Morales, M.S., **Elena I. Varlinskaya, Ph.D.**, and **Linda Patia Spear, Ph.D.**
Center for Development and Behavioral Neuroscience, Department of Psychology, Binghamton University, Binghamton, New York 13902-6000

Abstract

The present series of experiments examined affective properties of a moderate dose of ethanol using the conditioned place preference (CPP) paradigm in ethanol-naïve, adult male Sprague-Dawley rats. The apparatus and the procedure used were both unbiased. In Experiment 1, rats were given four 30 min conditioning sessions with 1.5 g/kg ethanol (i.p.) or an equivalent volume of saline on the paired side. Animals were found to demonstrate CPP to the ethanol-paired side, an unexpected finding at this relatively high dose in rats. To replicate this finding, and to examine the possibility of non-associative conditioning, an unpaired control group was included in Experiment 2. Once again, rats showed a CPP to the side paired with ethanol relative to either control group. Given that testing in an unfamiliar environment typically results in elevated levels of anxiety and that animals in Experiments 1 and 2 were not exposed to the apparatus prior to conditioning, Experiment 3 was conducted to examine the potential role of context unfamiliarity for induction of ethanol CPP in this test situation by varying whether animals were exposed to the apparatus prior to conditioning. In this study, pre-exposure to the CPP apparatus was found to eliminate the CPP to ethanol observed in rats who were not familiarized with the apparatus. Collectively, these studies demonstrate that ethanol-naïve rats can find ethanol reinforcing as indexed by the CPP test, and provide some evidence for the conditions under which this uncommon finding is observed.

Keywords

Ethanol place conditioning; Unbiased; Rat; Novelty; Anxiety; Unpaired control

Introduction

In order to study the neurobiological basis for dependence and drug addiction, animal models have been developed to explore contributors to the rewarding effects of alcohol and other drugs of abuse (Shippenberg and Koob, 2002). Among the most commonly used behavioral tests is place conditioning, a classical conditioning paradigm used frequently to index the reinforcing as well as aversive properties of a variety of drugs of abuse (see Bardo and Bevins, 2000; Tzschentke, 1998,for review), including morphine, cocaine (Campbell et al., 2000), and amphetamine (Bardo et al., 1999). In the place preference procedure, two

Address correspondence to: Melissa Morales, Department of Psychology, Binghamton University PO Box 6000, Binghamton, New York 13902-6000, mmorale2@binghamton.edu Phone: (607) 777-6418 Fax: (607) 777-6418.

distinct environmental contexts are paired with drug versus vehicle exposure across several conditioning sessions. After a number of pairings with each drug and their distinct environment, a drug-free test is given in which the animal is no longer restricted to one compartment but now has the opportunity to explore the apparatus. During the test, time spent in each compartment is recorded and a conditioned place preference (CPP) is present if the animal spends more time on the drug-paired side on test day, whereas an avoidance of the drug-paired side is used to index a conditioned place aversion (CPA).

While CPP is readily induced by most drugs of abuse (see Bardo and Bevins, 2000, for review), ethanol has produced inconsistent findings. Although mice often display CPP across a variety of ethanol doses (see Tzschentke, 1998, for review), in rats findings are more mixed. In rats, low doses of ethanol often induce neither a CPP nor CPA (Busse et al., 2004), with aversions most typically appearing at moderate to high (>1.0 g/kg) doses (Cunningham, 1981; Bedingfield et al., 1999; Funk et al., 2004). The few studies that have reported a place preference in adult rats have generally conditioned animals following many pairings with ethanol (Bozarth, 1990), used rats with a history of ethanol consumption (Reid et al., 1985) or utilized genetically selected alcohol-preferring rats (Ciccocioppo et al., 1999; although see Stewart et al., 1996). As place aversions are commonly seen in ethanol-naïve adult rats given limited pairings with moderate doses of ethanol, the goal of the first experiment was to simply establish a place aversion to 1.5 g/kg ethanol; however, the results unexpectedly revealed appetitive motivational properties. Factors contributing to these unexpected results were then explored.

Experiment 1: Place conditioning following 1.5 g/kg ethanol

Materials and Methods

Subjects—Male Sprague-Dawley rats (P69-70 at onset of testing) derived from our breeding facility were used, with a total of 8 animals placed in each condition. On postnatal day (P) 1, all litters were culled to 8–10 pups with a sex ratio of six males and four females kept whenever possible, with females used in other studies. Pups were weaned and pair housed with a same-sex littermate on P21. Rats were given ad libitum access to food (Purina Lab chow, Lowell, MA) and water, and were maintained in a temperature-controlled vivarium with a 14:10 hr light-dark cycle (lights on at 0700 hr). At all times animals were treated in accordance with guidelines for animal care established by the National Institutes of Health under protocols approved by the Binghamton University Institutional Animal Care and Use Committee.

Apparatus—The conditioned place preference apparatus consisted of three visually distinct compartments. One of the two outer compartments (30.5 × 25 × 32 cm) had solid black walls while the other had vertical black and white striped walls. The central compartment (11 × 25 × 32 cm) had white walls and all three compartments had a metal bar floor. Photobeam detectors were located along the bottom walls of each box and measured the rat's location while in the apparatus. Following each conditioning session, the apparatus was wiped clean with 3% peroxide and allowed to dry before the next rat was conditioned in the chamber. All conditioning and test session were conducted between 1100 – 1200 hrs.

The place conditioning apparatus used in the present experiments was configured to produce no initial bias in animals, with preliminary data showing that adult rats spend roughly equivalent amounts of time in the black versus stripe compartments (i.e., 39% and 42%, respectively, with time spent in the central compartment being 18%).

Drugs—Ethanol was administered intraperitoneally (i.p.) as a 12.6% v/v solution in 0.9% saline (Sal) at a dose of 1.5 g/kg. Saline was administered at an equivalent volume (1.5%

body weight). All drugs were administered at room temperature and immediately before conditioning trials.

Methods—Adult male rats in Experiment 1 (N=16) received a total of four conditioning sessions. Rats were assigned to one of two groups that were injected with either EtOH (EtOH-treated) or Sal (Sal-treated controls) on the paired side (CS+), with both of these groups receiving Sal on the unpaired (CS−) trials. One session (30 min) was conducted daily with order of drug (EtOH, Sal) counterbalanced for each group. Each drug group was further subdivided into compartment subgroups (i.e., black, stripe) that were counterbalanced as well. The day after the final conditioning session (i.e., test day: day 5), animals were placed into the central compartment of the CPP apparatus for 15 min, and time spent in each compartment was recorded.

Statistical analysis—Preference scores were analyzed a variety of ways (e.g., time in sec on paired side, difference scores at test between CS+ (i.e., paired) and CS− (i.e., unpaired), and as percentage (%) time on EtOH side, all of which yielded the same results. Thus, data are presented as % time on EtOH side for Exp. 1 and 2. For saline-treated rats, % time on EtOH side reflected time spent on the matched compartment (i.e., CS+). Data were analyzed via two-way ANOVAs for drug group (EtOH, Sal) and compartment (black, stripe), to determine if place preference differed as a function of pairing EtOH with a distinct environment (see Cunningham et al., 2003). Any significant effects were explored further using Tukey's post hoc test.

Results

The two-way ANOVA of % time on EtOH-paired side revealed a main effect of drug group [$F(1,12) = 7.80$, $p<0.05$], which surprisingly reflected a place preference for the compartment paired with EtOH among the EtOH-exposed rats. The lack of an interaction between drug group and compartment indicates that, in this case, the visual cue (i.e., black vs stripe) did not impact the preference that was observed. Average time spent (in sec ± SEM) in the central compartment for Sal- and EtOH-treated rats was 135.91±27.12 and 128.59±75.75, respectively.

Experiment 2: Ethanol place conditioning following 1.5 g/kg ethanol using unpaired controls

The purpose of Experiment 2 was to replicate the results of Experiment 1, as well as to confirm that the results obtained from Experiment 1 were, in fact, due to associative conditioning.

Materials and Methods

Adult male rats (N=24) in Experiment 2 were given four conditioning sessions. Rats were assigned to one of three groups, EtOH-paired, EtOH-unpaired and Sal controls. Conditioning and test procedure for the Sal control and EtOH-paired animals were the same as for the Sal and EtOH groups in Experiment 1. Rats in the EtOH-unpaired control group received Sal prior to exposure to both the paired (CS+) and unpaired (CS-) compartments. Animals in this group received the same number and dose of EtOH administrations as for the EtOH-paired group, but with these injections given in the home cage 2 hr post-conditioning.

Results

The factorial ANOVA of % time on EtOH side revealed only a main effect of group [$F(1,18) = 4.41$, $p<0.05$]. Tukey's post hoc test showed that rats in the EtOH-paired group developed a place preference to the compartment paired with EtOH relative to both the Sal-paired and unpaired control groups, with no difference observed between the two control groups (Figure 2). As was seen in Experiment 1, regardless of which compartment was paired with EtOH (CS+), rats in the EtOH-paired group showed a place preference for that compartment (i.e., lack of drug group X compartment interaction). The average time (in sec ±SEM) in the central compartment for Sal-treated, EtOH-paired, and unpaired groups was 104.95±23.70, 79.87±16.65, and 80.95±21.42, respectively.

Experiment 3: Role of pre-exposure to the apparatus on conditioned place preference to ethanol

Testing in an unfamiliar environment is anxiety-provoking for animals and EtOH has been shown to reverse anxiety induced by unfamiliarity of the test situation (Varlinskaya and Spear, 2002). Therefore, it is possible that the EtOH-induced CPP observed in Experiments 1 and 2 was related to the anxiolytic effects of EtOH. In order to examine the role of familiarity/unfamiliarity of the environment paired with EtOH, rats in Experiment 3 were either exposed to the CPP apparatus prior to conditioning or not.

Materials and Methods

Rats in Experiment 3 (N=32) received a total of four conditioning sessions and were assigned to either EtOH (EtOH-treated) or Sal (Sal-treated controls) drug groups, using the same conditioning and test procedure as used in Experiment 1. To test the anxiolytic effects of conditioning in an unfamiliar environment, rats were either habituated (15 min) to the apparatus on the day before conditioning, or not.

Results

A factorial ANOVA of % time on EtOH-paired side revealed a drug group X pre-exposure interaction [$F(1,24) = 6.78$, $p<0.05$]. Tukey's post hoc test revealed that rats conditioned with ethanol who were not habituated to the CPP apparatus developed a place preference to the ethanol-paired side relative to Sal controls of the same pre-exposure group (i.e., no habituation)—replicating the preference observed in Experiments 1 and 2. On the other hand, rats that were pre-exposed to the apparatus developed neither a preference nor aversion to the ethanol-paired side (Figure 3). As was the case in Experiments 1 and 2, there was no effect or interaction with compartment, suggesting that regardless of the CS+ with which EtOH was paired, animals treated with EtOH developed a preference for that compartment. The average time spent (in sec ± SEM) in the central compartment for Sal- and EtOH-treated rats was 241.61±19.13 and 204.95±14.79, respectively.

Discussion

The present experiments demonstrate consistent evidence for ethanol place preference in adult male rats following a moderate (1.5 g/kg) dose of ethanol, with rats in all experiments spending significantly more time on the ethanol-paired side. This was unexpected given that adult rats often (Cunningham, 1981; Bedingfield et al., 1999) although not always (Der-Avakian et al., 2007; also see habituation group in Exp. 3 of present study) have been found to display an aversion to doses of 1 g/kg ethanol or above, especially with such few pairings. Typically, for a place preference to ethanol to emerge, rats need to be conditioned over the course of many pairings (10 or more) or have a history of ethanol consumption (Bozarth, 1990; Reid et al., 1985).

The second experiment was conducted to replicate the unexpected results from Experiment 1, as well as to include an EtOH-unpaired control group to determine whether effects observed in Experiment 1 were a result of associative conditioning. Given that these unpaired controls received the same number of ethanol injections (in their home cage) as EtOH-paired animals, as well as the same number of compartment exposures, any residual effects of ethanol that might affect test performance should be equivalent between these two EtOH-exposed groups. Hence, any difference in time spent on the paired side (CS+) between the unpaired and the EtOH-paired group should be attributable to an association between the compartment and ethanol (i.e, a place preference). Indeed, we demonstrated that only rats in the EtOH-paired group showed a place preference, relative to both EtOH unpaired and saline animals. EtOH unpaired animals did not differ from Sal controls, hence there was no evidence that an association between the compartment and delayed ethanol injection was formed. These data provide evidence that the place preference observed in the EtOH-paired group was due to associative conditioning, While this effect has been seen with ethanol in mice (Cunningham and Noble, 1992) and cocaine in rats (Miller and Marshall, 2004), to our knowledge, this is the first report demonstrating a place preference with ethanol in rats using both Sal-paired and unpaired control groups.

The third experiment was conducted to determine the role of pre-exposure (i.e., habituation) on ethanol-induced place preference. Rats are often habituated to the place conditioning apparatus for at least one day prior to subsequent conditioning sessions, whereas rats in the first experiment were not exposed to the CPP environment prior to the onset of conditioning. In a meta-analysis of studies examining cocaine-, morphine-, and amphetamine-induced CPP, Bardo and colleagues (1995) found place preferences were weaker when animals were pre-exposed to the apparatus. Therefore, it is possible that by eliminating pre-conditioning exposure to the apparatus in the first experiment, we may have facilitated expression of ethanol CPP. Indeed, when habituation was tested as a contributing factor (Experiment 3), a significant interaction revealed that only rats that were not habituated to the apparatus expressed an ethanol-induced CPP, with no effect observed in those rats habituated to the apparatus. This lack of significant place conditioning in rats pre-exposed (habituated) to the apparatus is reminiscent of another finding of a lack of aversive conditioning to moderate doses of EtOH (e.g., 1 or 2 g/kg) after only a few drug pairings in rats (Der-Avakian et al., 2007). The circumstances that produce place aversions, no conditioning or place preferences still remain unclear, although a number of procedural variables (e.g., 2 vs. 3 compartment, pre-test vs no pre-test) have been shown to impact results using the place preference paradigm (e.g., see Bardo et al., 1995). These results suggest that eliminating pre-exposure to the place conditioning apparatus may be an important factor for observing CPP to ethanol in rats under these test circumstances. There are two possible explanations for this effect. First, it is possible that rats in the current experiments displayed a place preference to a moderate dose of ethanol because anxiogenic effects associated with the novel environment may have been eliminated by EtOH's anxiolytic properties, thereby enhancing the appetitive effects of ethanol. Indeed, there is evidence demonstrating that moderate doses of EtOH can be anxiolytic in adult rats (Ferreira and Morato, 1997). For example, EtOH-treated rats (1.2 g/kg) entered and spent significantly more time (%) in the open arms of an elevated plus maze than saline-treated rats—an indication of EtOH's anxiolytic effects at doses that closely resemble that which was used in our experiments (Ferreira and Morato, 1997). In previous work from our laboratory, levels of anxiety seen in adult rats in the elevated plus maze were found to be negatively correlated with amount of pretest perturbation (Doremus et al., 2004). Thus the minimal handling prior to conditioning plus the absence of a habituation day may have enhanced the anxiolytic properties of ethanol, thereby facilitating expression of ethanol-induced place preference. Our laboratory has previously shown that the suppression of social activity induced by testing in an unfamiliar (anxiogenic) environment was reversed by ethanol (Varlinskaya and Spear, 2002).

A second possible explanation as to why the absence of pre-conditioning facilitated ethanol CPP may be because this procedure avoids latent inhibition. Although there has been little specific study of the role of latent inhibition in CPP, it is possible that familiarizing animals to the conditioning context prior to CPP training may weaken subsequent conditioning to that stimulus, making induction of CPP less likely. This would be analogous to other incidences of latent inhibition – i.e., attenuated conditioning seen when animals receive pre-training exposure to the to-be-conditioned stimulus (see Domjan, 2006).

While place preferences to ethanol were observed in all three experiments, paired side of the compartment (black vs. stripe) was counterbalanced rather than included as a specific factor in these experiments. Hence, as designed these studies were insufficiently powered to detect statistically significant interactions of drug group with compartment. Indeed, GPOWER software (Erdfelder et al., 1996) revealed that sample sizes of >30 would be necessary to yield sufficient power to reveal significant interactions at the recommended 0.80 level (Cohen, 1992) (in contrast to the sample sizes used in the present experiments that yielded power levels of only ~.10 in each study for detection of this interaction). Thus, although in all experiments the conditioning effect was sufficiently robust to emerge regardless of paired compartment assignment, from inspect of Figure 3 (see data for “no habituation” group on the right) it appears that the main effect of conditioning in Experiment 3 was driven largely by animals receiving EtOH in the striped compartment. A somewhat similar trend was also evident in Experiment 2 (see Figure 2) but not Experiment 1 (see Figure 1). On average, animals in the saline control groups spent ~5-10% less time in the striped compartment than the black one in all experiments; although these differences were not statistically significant (perhaps due to power issues), they could perhaps represent a modest compartment bias. Thus, it is possible that in Experiment 3, ethanol exposure may have served to counteract the “aversiveness” of the striped compartment, hence producing CPP via negative reinforcement. Evidence that ethanol can serve to negate an aversion to a distinctive environment has been reported in a study of the role of compartment bias in ethanol CPP in mice (Cunningham et al., 2003).

Unless facilitated by other factors (i.e., history of alcohol, stress), rats generally do not display a conditioned place preference to ethanol doses of 1 g/kg or greater. Due to the many failed attempts to produce a place preference, it has been suggested that perhaps rats do not find the effects of ethanol at these doses rewarding. However, the present findings add additional evidence that under certain circumstances, formerly ethanol-naïve rats can find ethanol reinforcing at moderate doses. These results also point to the important relationship between stress (due to anxiety) and ethanol, a factor that has been implicated in alcohol use (see Pohorecky, 1981 for review). Other stressors (e.g. foot shock) have been shown to result in a place preference to ethanol in rats (Matsuzawa et al., 2000). However, no studies to date have directly examined the potential stress of ethanol conditioning in a familiar versus unfamiliar place preference apparatus—a methodological manipulation which clearly has the potential to alter the effects of ethanol. Thus, although the relationship between the motivational and aversive properties of ethanol need to be studied further, the present series of experiments demonstrate the conditions under which a moderate dose of ethanol can elicit a place preference in ethanol-naïve adult rats.

Acknowledgments

This work was supported by the National Institute on Alcohol Abuse and Alcoholism [R01 AA018026 to LPS].

References

Bardo MT, Bevins RA. Conditioned place preference: what does it add to our preclinical understanding of drug reward? Psychopharmacology (Berl). 2000; 153:31–43. [PubMed: 11255927]

Bardo MT, Rowlett JK, Harris MJ. Conditioned place preference using opiate and stimulant drugs: a meta-analysis. Neurosci. Biobehav. Rev. 1995; 19:39–51. [PubMed: 7770196]

Bardo MT, Valone JM, Bevins RA. Locomotion and conditioned place preference produced by acute intravenous amphetamine: role of dopamine receptors and individual differences in amphetamine self-administration. Psychopharmacology (Berl). 1999; 143:39–46. [PubMed: 10227078]

Bedingfield JB, King DA, Holloway FA. Peripheral opioid receptors may mediate a portion of the aversive and depressant effect of EtOH: CPP and locomotor activity. Alcohol. 1999; 18:93–101. [PubMed: 10456559]

Bozarth MA. Evidence for the rewarding effects of ethanol using the conditioned place preference method. Pharmacol. Biochem. Behav. 1990; 35:485–487. [PubMed: 2320661]

Busse GD, Lawrence ET, Riley AL. The modulation of cocaine-induced conditioned place preferences by alcohol: effects of cocaine dose. Prog. Neuropsychopharmacol. Biol. Psychiatry. 2004; 28:149–155. [PubMed: 14687869]

Campbell JO, Wood RD, Spear LP. Cocaine and morphine-induced place conditioning in adolescent and adult rats. Physiol. Behav. 2000; 68:487–493. [PubMed: 10713288]

Ciccocioppo R, Panocka I, Froldi R, Quitadamo E, Massi M. Ethanol induces conditioned place preference in genetically selected alcohol-preferring rats. Psychopharmacology(Berl). 1999b; 141:235–241. [PubMed: 10027504]

Cohen J. Statistical power analysis. Current directions in psychological science. 1992; 1:98–101.

Cunningham CL. Spatial aversion conditioning with ethanol. Pharmacol. Biochem. Behav. 1981; 14:263–264. [PubMed: 7208566]

Cunningham CL, Ferree NK, Howard MA. Apparatus bias and place conditioning with ethanol in mice. Psychopharmacology (Berl). 2003; 170:409–422. [PubMed: 12955296]

Cunningham CL, Noble D. Conditioned activation induced by ethanol: role in sensitization and conditioned place preference. Pharmacol. Biochem. Behav. 1992; 43:307–313. [PubMed: 1409816]

Der-Avakian A, Bland ST, Rozeske RR, Tamblyn JP, Hutchinson MR, Watkins LR, Maier SF. The effects of a single exposure to uncontrollable stress on the subsequent conditioned place preference responses to oxycodone, cocaine, and ethanol in rats. Psychopharmacology (Berl). 2007; 191:909–917. [PubMed: 17211647]

Domjan, M. The Principles of Learning and Behavior. 5th edn. Belmont, CA: Wadsworth/Thomson Learning; 2003.

Doremus TL, Varlinskaya EI, Spear LP. Age-related differences in elevated plus maze behavior between adolescent and adult rats. Ann. N.Y. Acad. Sci. 2004; 1021:427–430. [PubMed: 15251922]

Erdfelder E, Faul F, Buchner A. GPOWER: A general power analysis program. Behavior research methods, instruments, and computers. 1996; 28:1–11.

File SE, Seth P. A review of 25 years of the social interaction test. Eur. J. Pharmacol. 2003; 463:35–53. [PubMed: 12600701]

Funk D, Vohra S, Le AD. Influence of stressors on the rewarding effects of alcohol in Wistar rats: studies with alcohol deprivation and place conditioning. Psychopharmacology (Berl). 2004; 176:82–87. [PubMed: 15064919]

Matsuzawa S, Suzuki T, Misawa M. Ethanol, but not the anxiolytic drugs Buspirone and Diazepam, produces a conditioned place preference in rats exposed to conditioned fear stress. Pharmacol. Biochem. Behav. 2000; 65:281–288. [PubMed: 10672981]

Miller CA, Marshall JF. Altered prelimbic cortex output during cue-elicited drug seeking. J. Neurosci. 2004; 24:6889–6897. [PubMed: 15295023]

Pohorecky LA. The interaction of alcohol and stress A review. Neurosci. Biobehav. Rev. 1981; 5:209–229. [PubMed: 6115346]

Reid LD, Hunter GA, Beaman CM, Hubbel CL. Toward understanding ethanol's capacity to be reinforcing: a conditioned place preference following injections of ethanol. Pharmacol. Biochem. Behav. 1985; 22:483–487. [PubMed: 3991762]

Shippenberg, TS.; Koob, GF. Recent advances in animal models of drug addiction and alcoholism. In: Davis, KL.; Charney, D.; Coyle, JT.; Nemeroff, C., editors. Neuropsychopharmacology: The fifth generation of progress. Philadelphia: Lippincott Williams and Wilkins; 2002. p. 1381-1397.

Stewart RB, Grupp LA. Conditioned place aversion mediated by self-administered ethanol in the rat: a consideration of blood ethanol levels. Pharmacol. Biochem. Behav. 1989; 32:431–437. [PubMed: 2727002]

Stewart RB, Murphy JM, McBride WJ, Lumeng L, Li TK. Place conditioning with alcohol in alcohol-preferring and -nonpreferring rats. Pharmacol. Biochem. Behav. 1996; 53:487–491. [PubMed: 8866945]

Substance Abuse and Mental Health Services Administration. Results from the 2009 National Survey on Drug Use and Health. Volume I. Summary of National Findings (Office of Applied Studies, NSDUH Series H-38A, HHS Publication No. SMA 10-4586). Rockville, MD: 2010.

Tzschentke TM. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. Prog. Neurobiol. 2008; 56:613–672. [PubMed: 9871940]

Varlinskaya EI, Spear LP. Acute effects of ethanol on social behavior of adolescent and adult rats: role of familiarity of the test situation. Alcohol. Clin. Exp. Res. 2002; 26:1502–1511. [PubMed: 12394283]

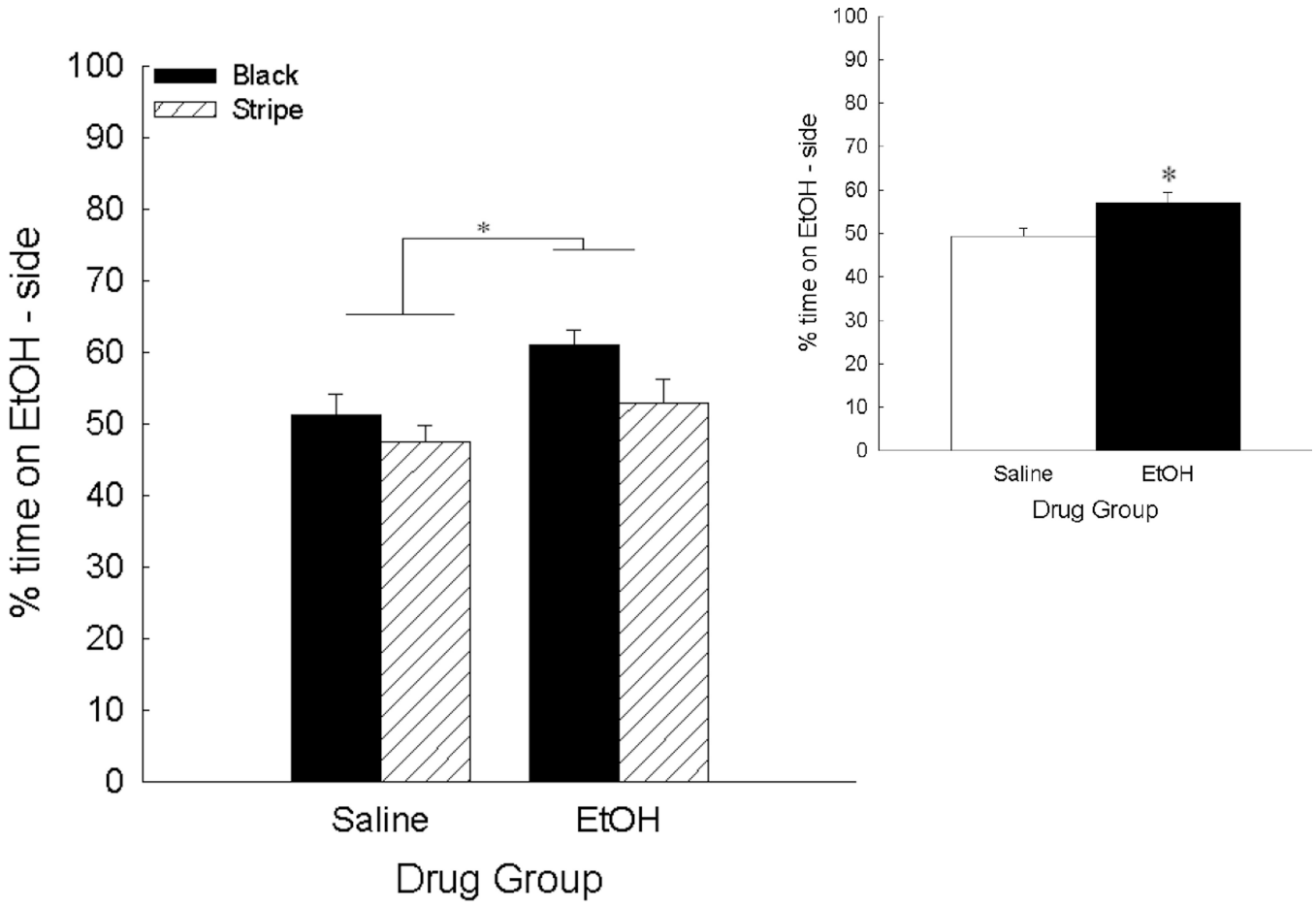

Figure 1.

Percentage (%) time (±SEM) spent on the EtOH-paired side by each subgroup (i.e., black vs. stripe environment). The factorial ANOVA revealed a significant place preference in EtOH-treated as compared to Sal-treated rats, regardless of compartment (main effect of drug group, * $p<0.05$). Insert depicts this significant main effect.

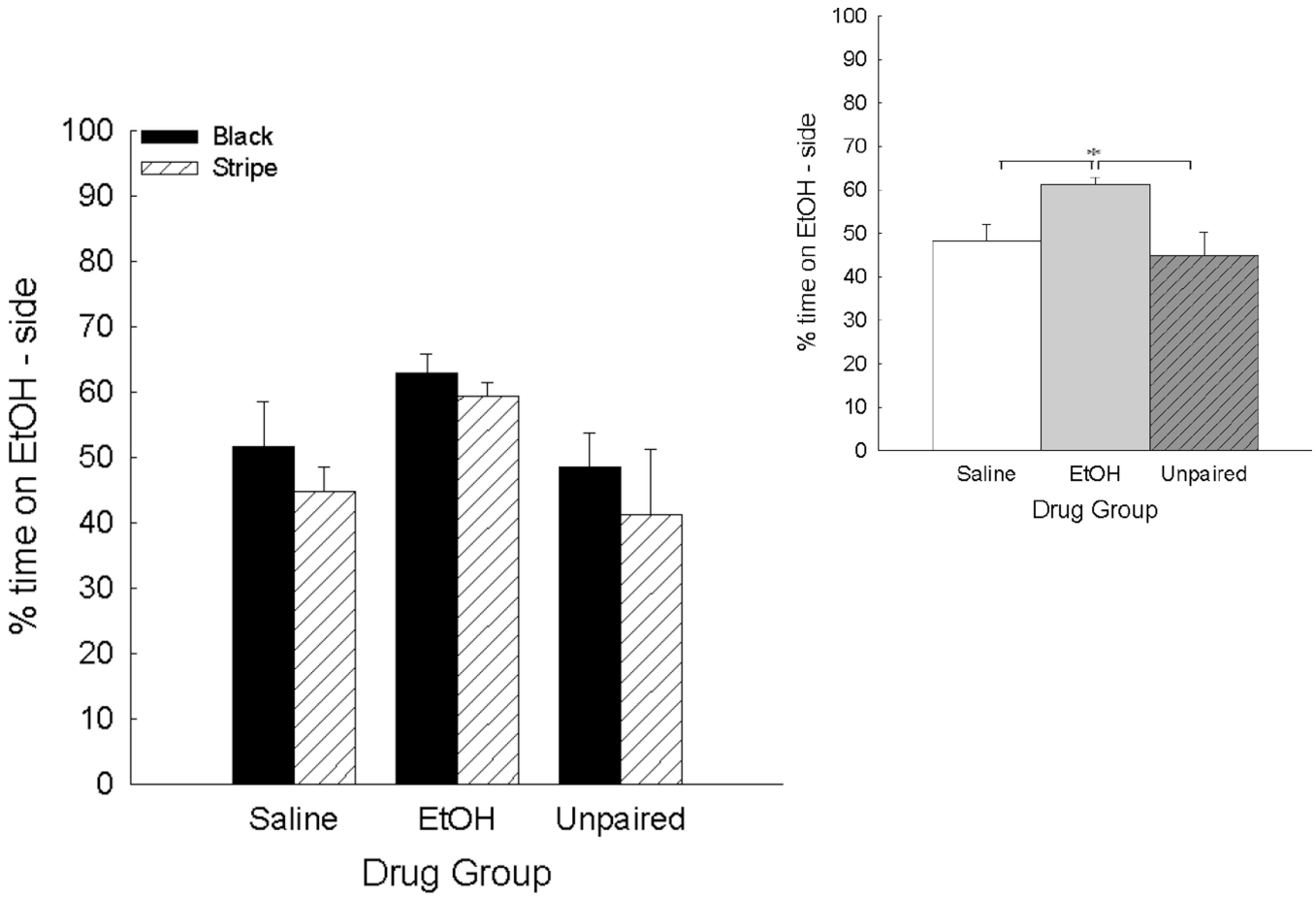

Figure 2.

Percentage (%) time (±SEM) spent on the EtOH-paired side. The factorial ANOVA revealed a main effect of drug group ($p<0.05$), revealing that EtOH-treated rats spent more time on the EtOH-paired compartment relative to both Sal-treated and unpaired controls (see insert). There were no differences between the Sal-treated and unpaired control groups.

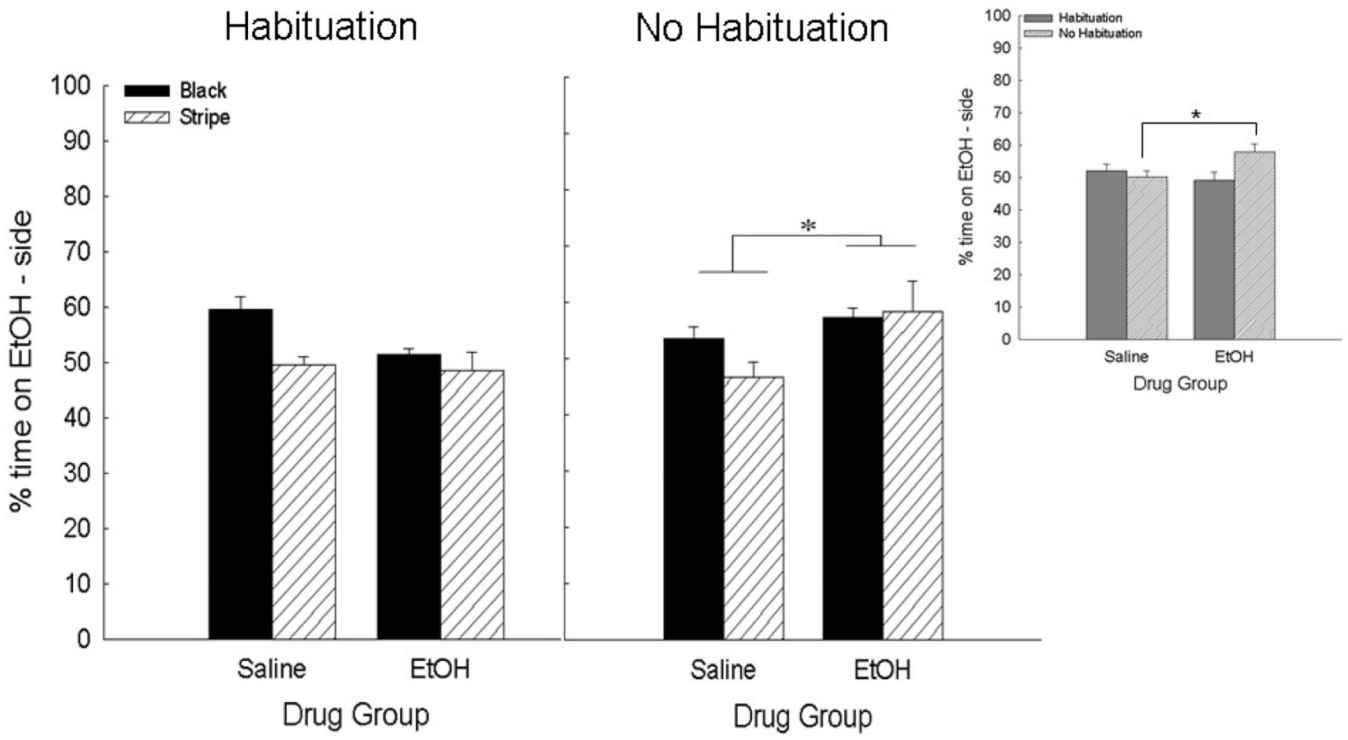

Figure 3.

Percentage (%) time (±SEM) spent on the EtOH-paired side. A significant pre-exposure X drug group interaction ($p<0.05$) revealed that EtOH-treated animals in the no habituation group showed a preference for the ethanol-paired side relative to Sal controls of the same pre-exposure group (denoted by * -this significant interaction is depicted in the insert). Rats in the no habituation group showed no difference.