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Pharmacology, Biochemistry and Behavior 77 (2004) 731–743
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
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Conditioned place aversion induced by intragastric administration of ethanol in rats☆

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Received 4 September 2003; received in revised form 16 January 2004; accepted 23 January 2004

Abstract

Most experiments investigating ethanol-induced place conditioning in rats have produced conditioned place aversion (CPA). In one of the few reports of ethanol-induced conditioned place preference (CPP) in rats, selectively bred alcohol-preferring (msP) rats showed CPP in a biased procedure when ethanol was administered via intragastric (IG) catheter but not when ethanol was administered via intraperitoneal injection or by gavage. This finding suggests the importance of both route of administration and genetic variables to the outcome of place conditioning studies. We conducted three experiments examining place conditioning induced by IG ethanol in genetically heterogeneous rats to test the generality of the earlier finding. We employed an unbiased procedure that is more sensitive to detecting preference changes in either direction (preference or aversion). Ethanol-naive (Experiment 1) and ethanol-experienced Sprague–Dawley rats (Experiment 2) showed robust CPA. In Experiment 3, infusion rate was varied to see if the CPA observed in Experiments 1 and 2 was a result of the rapidity of the transition from the sober to the intoxicated states. Both groups showed strong CPA. Overall, the present findings are consistent with previous findings of CPA in heterogeneous rats, suggesting that the aversive postabsorptive effects of ethanol produce CPA.

Keywords: Place conditioning; Preference; Aversion; Ethanol; Activity; Rats

1. Introduction

Although determinants of ethanol-induced conditioned place preference (CPP) are now relatively well established in mice, the conditions under which ethanol will reliably produce a CPP in rats remain elusive (Cunningham et al., 2000; Tzschentke, 1998). Attention has focused on a number of important variables including route of administration, strain or line of rats employed, ethanol experience before conditioning, and the apparatus (biased or unbiased). However, examination of Table 1 reveals that holding any one of these variables constant still reveals a range of conditioning outcomes. Of particular interest is a series of studies by Ciccocioppo et al. (1999) in which conditioning parameters were held constant and route of administration was varied. Ciccocioppo et al. found CPP when ethanol was administered via a surgically implanted intragastric (IG) catheter, but saw no conditioning when ethanol was administered by gavage or by intraperitoneal injection (ip) to selectively bred Marchigian Sardinian alcohol-preferring (msP) rats. Ethanol-naive msP rats showed CPP when the dose given via IG catheter was 0.7 g/kg, but not when the dose was lower (0.35 g/kg) or higher (1.5 g/kg). However, after 25 days exposure to 10% ethanol in the home cage (15 days of continuous access followed by 10 days of limited access), CPP was obtained at both 0.7 and 1.5 g/kg but not at 0.35 or 2.8 g/kg. In contrast to these findings, van der Kooy et al. (1983) showed conditioned place aversion (CPA) in ethanol-naive Wistar rats when ethanol was administered via IG or intravenous (iv) catheter at doses above 1.0 g/kg and no conditioning at lower doses.

The reasons behind this discrepant pattern of findings are unclear. The studies of Ciccocioppo et al. (1999) strongly suggested differences due to route of administration, with CPP developing only when ethanol was infused via an implanted catheter and not when ethanol was given ip or

☆ Experiment 1 was presented at the Midwestern Psychological Association Annual Meeting in Chicago, IL, May 2001. Experiment 2 was presented at the Research Society on Alcoholism (RSA) Annual Scientific Meeting in Montreal, Quebec, Canada, June 2001. Experiment 3 was presented at the RSA meeting in San Francisco, CA, July 2002.

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doi:10.1016/j.pbb.2004.01.010

Table 1
Results of ethanol-induced place conditioning studies sorted by route of administration

Citation	Experiment no.	Result	Preexposure	Dose, g/kg	Strain/Line	Biased apparatus	Biased procedure
Administration via IG catheter							
Ciccocioppo et al. (1999)	2	CPP	No	0.7	msP	Yes	Yes
Ciccocioppo et al. (1999)	2	CPP	Yes	0.7 and 1.5	msP	Yes	Yes
Ciccocioppo et al. (1999)	2	No conditioning	No	0.35 and 1.5	msP	Yes	Yes
Ciccocioppo et al. (1999)	2	No conditioning	Yes	0.35 and 2.8	msP	Yes	Yes
van der Kooy et al. (1983)	2	CPA	No	1.0–5.0	Wistar	Unspecified	No
van der Kooy et al. (1983)	2	No conditioning	No	0.3–0.6	Wistar	Unspecified	No
Administration via gavage							
Bagrov et al. (1999)	na	CPP	No	1.2	Wistar	No	No
Bedingfield et al. (1999)	na	CPA	No	2.25	Sprague–Dawley	No	Yes
Bedingfield et al. (1999)	na	No conditioning	No	1.5	Sprague–Dawley	No	Yes
Ciccocioppo et al. (1999)	3	No conditioning	Yes	0.35–1.5	msP	Yes	Yes
Gauvin and Holloway (1992)	na	CPA	No	2.0	Sprague–Dawley	Yes	Yes
Gauvin and Holloway (1992)	na	No conditioning	Yes	2.0	Sprague–Dawley	Yes	Yes
Gauvin et al. (1994)	na	CPA	No	4.0	Sprague–Dawley	Yes	Yes
Gauvin et al. (2000)	na	CPP	Yes	2.0	AA	Yes	Yes
Gauvin et al. (2000)	na	No conditioning	Yes	2.0	AA	Yes	Yes
Gauvin et al. (2000)	na	CPP	Yes	2.0	ANA	Yes	Yes
Gauvin et al. (2000)	na	No conditioning	Yes	2.0	ANA	Yes	Yes
Patkina and Zvartau (1998)	1	CPP	No	1.2	Wistar	No	No
Sherman et al. (1983)	1	CPA	No	0.5–2.0	Sprague–Dawley	Unspecified	No
Oral consumption of ethanol							
Gauvin and Holloway (1992)	na	CPP	Yes	Not specified	Sprague–Dawley	Yes	Yes
Stewart and Grupp (1986)	na	CPA	No	~2	Long Evans	Yes	No
Stewart and Grupp (1989)	na	CPA	No	~2	Long Evans	Yes	No
Intraperitoneal administration of ethanol							
Biala and Kotlinska (1999)	na	CPP	Yes	0.5	Wistar	Yes	Yes
Bienkowski et al. (1995)	na	CPP	Yes	0.5	Wistar	Yes	Yes
Bienkowski et al. (1995)	na	No conditioning	Yes	1.0	Wistar	Yes	Yes
Bienkowski et al. (1996)	2 and 4	No conditioning	No	0.5	Wistar	Yes	Yes
Bienkowski et al. (1996)	3	No conditioning	No	1.0	Wistar	Yes	Yes
Bienkowski et al. (1996)	4	No conditioning	Yes	1.0	Wistar	Yes	Yes
Bienkowski et al. (1996)	4	CPP	Yes	0.5	Wistar	Yes	Yes
Bienkowski et al. (1996)	5	CPP	No	0.5	Wistar	Yes	Yes
Black et al. (1973)	1 and 2	CPP	No	1.0	Sprague–Dawley	Yes	Yes
Bormann and Cunningham (1997)	1	CPA	No	1.8	Holtzman	No	No
Bormann and Cunningham (1997)	2	No conditioning	No	1.2	Holtzman	No	No
Bormann and Cunningham (1998)	na	CPA	No	1.0 and 1.5	Holtzman	No	No
Ciccocioppo et al. (1999)	4	No conditioning	Yes	0.35–1.5	msP	Yes	Yes
Cunningham (1979)	na	CPA	No	1.5	Holtzman	No	No
Cunningham (1981)	na	CPA	No	1.0 and 2.0	Holtzman	No	No
Cunningham and Niehus (1993)	na	CPA	No	1.2	Holtzman	No	No
Cunningham et al. (1993)	na	CPA	No	1.5	Holtzman	No	No
Cunningham and Niehus (1993)	na	CPA	No	1.8	Holtzman	No	No
Holloway et al. (1992)	2	CPP	Yes	1.5	Sprague–Dawley	Yes	Yes
Holloway et al. (1992)	2	CPA	No	1.5	Sprague–Dawley	Yes	Yes
Reid et al. (1985)	na	CPP	Yes	1.0	Sprague–Dawley	Ambiguous	No
Reid et al. (1985)	na	No conditioning	No	1.0	Sprague–Dawley	Ambiguous	No
Administration via IV catheter							
van der Kooy et al. (1983)	1	CPA	No	1.1	Wistar	Unspecified	No
van der Kooy et al. (1983)	1	No conditioning	No	0.1–0.6	Wistar	Unspecified	No

by gavage. Moreover, their study implied that use of an ethanol preferring genotype was important. However, both of these observations are challenged by findings of ethanol CPP after gavage in ethanol nonpreferring ANA rats (Gauvin et al., 2000) and in outbred Wistar rats (Patkina and Zvartau, 1998). Furthermore, although both selected line studies indicated that ethanol preexposure facilitated development of CPP induced by IG ethanol, the studies con-

ducted in Wistar rats suggest that preexposure is not necessary. Both of the selected line studies used a biased apparatus and biased stimulus assignment procedure. In both cases, IG ethanol was paired with each rat's initially nonpreferred compartment, raising the possibility that ethanol produced CPP because of "antiaversive" effects rather than rewarding effects (Cunningham et al., 2003a). Another potential problem with biased procedures is that they maximize the opportunity to observe change in a single direction due to floor effects. In contrast, the studies conducted in Wistar rats used an unbiased procedure (Patkina and Zvartau, 1998), which provides stronger support for a reward interpretation.

In light of these disparate findings, the present experiments were designed to shed additional light on the possibility that CPP might be induced in genetically heterogeneous (nonselected) rats by infusing ethanol directly into the stomach via a surgically implanted catheter. We focused on this technique of IG administration rather than gavage because it eliminates the pretrial restraint stress produced by manual insertion of a feeding tube on each conditioning trial. The primary goal of Experiments 1 and 2 was to see whether ethanol CPP could be induced in outbred rats in an unbiased procedure using conditioning parameters similar to those of Ciccocioppo et al. (1999). Experiment 3 was designed to address the role of infusion duration (i.e., rate of ethanol infusion).

2. Experiments 1 and 2

The two previous place conditioning studies in which rats were given ethanol via implanted gastric catheters differed in several ways that might have produced the opposite outcomes reported in those studies. For example, the study that yielded only CPA involved genetically heterogeneous rats (Wistar) tested in an unbiased place conditioning procedure (van der Kooy et al., 1983), whereas the study that yielded CPP involved selectively bred rats (msP) tested in a biased procedure (Ciccocioppo et al., 1999). Another potentially important procedural difference between these studies was the temporal relationship between infusion of ethanol and exposure to the compartment that serves as the conditioned stimulus (CS). In the study that produced CPP, ethanol infusion was completed outside the apparatus just before rats were placed into the CS compartment (Ciccocioppo et al., 1999). However, in the study that produced CPA, ethanol infusion did not begin for several minutes after placement in the CS compartment (van der Kooy et al., 1983). Based on recent place conditioning studies in mice, exposure to ethanol's effects after onset of the CS has been shown to be more likely to produce CPA, whereas exposure to ethanol before CS onset is more likely to produce CPP (Cunningham et al., 2003b). Thus, development of CPA in the van der Kooy et al. (1983) study could have been caused by the temporal delay between CS onset and ethanol infusion.

Experiments 1 and 2 were designed to determine whether IG infusion of ethanol would produce CPP or CPA in unselected Sprague–Dawley rats using the same conditioning parameters shown to produce CPP in selectively bred msP rats. Ethanol was infused before exposure to the CS compartment to maximize the likelihood of inducing CPP. However, in contrast to the studies of Ciccocioppo et al. (1999), these studies used an unbiased procedure to avoid the interpretive complications that arise with biased procedures (Carr et al., 1989; Cunningham et al., 2003a; Swerdlow et al., 1989). Experiments 1 and 2 differed only in the treatment given between implantation of the catheter and the start of place conditioning. In Experiment 1, all rats were ethanol naive and received only infusions of water before the first pretest. Given the findings of Ciccocioppo et al., we expected that the 0.7 g/kg group would show CPP or a weaker aversion than the 1.5 g/kg group. Experiment 2 was designed to examine the possibility that repeated exposure to ethanol before conditioning would enhance the likelihood of producing CPP. Thus, half of the rats were given a single ethanol infusion per day for 15 consecutive days prior to the first pretest. Assuming the results of Ciccocioppo et al. would generalize to nonselected rats, we expected that ethanol preexposed groups would show CPP or a weaker aversion than ethanol-naive groups.

2.1. Method

2.1.1. Subjects

Forty-one adult male Sprague–Dawley rats (Harlan–Holtzman) were used in the first experiment and 76 in the second experiment. Rats arrived in the laboratory at 2 months of age and were individually housed under a 12-h light–dark schedule with lights on at 7:00 a.m. All experimental manipulations occurred during the light portion of the cycle. Water was always available in the home cage and food was available ad lib except during the 24 h immediately before surgery. Rats were handled and weighed daily except on the day after surgery. The experimental protocols were approved by the OHSU IACUC and procedures complied with the NIH *Guide for Care and Use of Laboratory Animals*.

2.1.2. Surgery

Rats were allowed 7 to 11 days to adapt to the colony prior to surgery. Each animal was anesthetized with isoflurane gas (5% loading dose; 2–3% maintenance) for implantation of an IG catheter. This catheter was constructed using a 17-cm length of Dow Corning Silastic tubing (ID 0.04 × OD 0.085 in). A "knob" was created at the intragastric end of the catheter by slipping a short piece (2–3 mm) of larger Silastic tubing (ID 0.078 × OD 0.125 in.) over one end of the catheter and fixing it in place with

Dow Corning Medical Adhesive A. A piece of polypropylene mesh (Davol) was also attached to the catheter approximately 1 cm from the knob end. The surgical procedure was similar to those described previously (Koopmans, 1987; Lukas and Moreton, 1979). Briefly, the stomach was externalized through an incision in the animal's left side caudal to the rib cage. The knob end of the catheter was inserted into the stomach through a puncture and secured with a purse-string suture and polypropylene mesh. The stomach was returned to the cavity and the incision through the muscle and peritoneum was sutured. The catheter was threaded subcutaneously to a small incision on the back just posterior to the scapulae, trimmed and attached to the back mount, which consisted of an L-shaped piece of 20-gauge hypodermic tubing embedded in a male luer tip and cemented to a piece of polypropylene mesh with cranioplastic cement (Plastics One). The back mount was secured subcutaneously with a stitch into muscle tissue and skin incisions were sutured. Body temperature was maintained during surgery using an isothermal heating pad (Braintree Scientific). At the end of surgery, rats were injected subcutaneously with 0.3 ml amoxicillin (250 mg/ml) to protect against postsurgical infection and approx. 12 ml of sterile saline to help maintain hydration. After recovering from anesthesia, rats were returned to their home cages with food and water available. Catheters were flushed with sterile water at the end of surgery and with 3.0 ml sterile water 48 h post-surgery in order to maintain catheter patency. The surgeries for each experiment were performed over a period of 4–5 days; each rat was allowed at least 5 days recovery before the experiment began.

2.1.3. Apparatus

Behavioral testing was conducted in eight identical place conditioning boxes (47.5 × 15.5 × 18 cm) that have been described previously (Bormann and Cunningham, 1998). Each box consisted of a single chamber (47.5 × 15.5 × 18 cm) with acrylic side panels, aluminum end panels, and was enclosed in a separate light- and sound-attenuating chamber (Kalt, Portland, OR). Five sets of infrared light sources and detectors were mounted along the sides of the boxes 5 cm above the floor. One set of photodetectors was placed in the center of the walls and two additional sets were 7 cm apart on either side of center. Tactile features of the floors on each side of the box were manipulated to provide CSs for place conditioning. The grid floors were made from 2.3-mm stainless steel rods mounted 13 mm apart in an acrylic frame. The hole floors were made from perforated stainless steel (16-gauge) with 13-mm round holes on 19-mm staggered centers. During conditioning trials the tactile cues on both sides of the box were identical. During choice tests, one side of the chamber had a grid floor and the other side a hole floor (counterbalanced). After each session, the place conditioning chambers and floors were wiped with a damp sponge.

2.2. Procedure

2.2.1. Overview

The general procedure for Experiments 1 and 2 included the following: (a) a preexposure phase (3 days in Experiment 1; 15 days in Experiment 2), (b) two pretests, (c) a series of 10 conditioning trials (5 CS+ and 5 CS−) followed by a drug-free choice test, and (d) four additional conditioning trials (2 CS+ and 2 CS−) followed by a second choice test. Within each experiment, rats were assigned to one of three dose groups: 0 (water), 0.7 or 1.5 g/kg (see Table 2).

2.2.2. Preexposure

In Experiment 1, all rats were weighed, infused with sterile water (18.75 ml/kg), and then returned to their home cages on each of the 3 days before the first pretest. No ethanol infusions were given during this phase. In Experiment 2, however, half of the rats in each dose group were randomly assigned to the ethanol preexposure group and the rest were assigned to the water-preexposure group (Table 2). Ethanol-preexposed rats received a single IG infusion of ethanol (1.5 g/kg, 10% vol/vol in sterile water, 18.75 ml/kg) every day for 15 days. Water-preexposed rats received a single IG infusion of sterile water (18.75 ml/kg) every day. On each preexposure day, rats were weighed, infused and then returned to their home cages. Infusions were given by hand over approximately 30 s. The last preexposure infusion occurred 4 days before the first pretest to minimize carry-over drug effects. Preexposure was the only phase in the two experiments in which rats were treated differently.

2.2.3. Pretests

Two pretests were conducted 24 h apart to assess unconditioned floor preferences so that conditioning subgroups could be matched on this measure. For each pretest, rats were weighed, infused with sterile water and placed into the conditioning chambers for 15 min. Rats had access to the entire chamber, which was configured with a grid floor on one side and a hole floor on the other side (position counterbalanced). Infusion volumes were 18.75 ml/kg for the 0 and 1.5 g/kg ethanol dose groups and 8.75 ml/kg for the 0.7 g/kg ethanol dose group. Rats were returned to their home cages immediately after each pretest session.

Table 2
Number of subjects per preexposure, dose and conditioning subgroup in each experiment

	Ethanol dose (g/kg)				
	0	0.7		1.5	
	Grid−/Hole−	Grid+	Hole+	Grid+	Hole+
Experiment 1	8	7	6	8	7
Experiment 2					
Water preexposure	4	8	8	7	8
Ethanol preexposure	5	8	7	8	8

2.2.4. Conditioning trials

An unbiased stimulus assignment procedure was used for place conditioning. Rats within each ethanol dose group (0.7 or 1.5 g/kg) were assigned to one of two conditioning subgroups (GRID+ or GRID−) matched for time on the grid floor during the second pretest (Table 2) ensuring groups had equivalent preferences for the floor stimuli prior to stimulus–ethanol pairings. These subgroups were then exposed to a series of differential Pavlovian conditioning trials in which one floor stimulus was paired with infusion of the assigned ethanol dose and the other floor stimulus was paired with infusion of water. Rats in the GRID+ subgroups were immediately placed on the grid floor after ethanol infusions (CS+ trials) and on the hole floor after water infusions (CS− trials). These contingencies were reversed for rats in the GRID− subgroups. Rats assigned to the 0 g/kg (water) group were treated similarly except that water was infused before placement on either floor. A total of seven CS+ and seven CS− trials were given on alternating days (counterbalanced order within each subgroup). Floor texture was identical on both sides of the apparatus and rats had access to the entire chamber during each 60-min trial.

The primary reason for including the groups given water only during conditioning trials was to determine whether the apparatus was biased or unbiased (see Cunningham et al., 2003a), not to provide a "control" for assessing the presence of place conditioning. As noted elsewhere, a vehicle-only group is not an adequate control in drug conditioning studies (Cunningham, 1993). An unpaired-drug design or discrimination design such as that used here offers much better control for possible nonassociative effects of drug exposure on cue preference.

2.2.5. Preference tests

Tests 1 and 2 were choice tests to assess conditioned preference or aversion for the grid and hole floors. The tests were identical to the pretests except that they were 60 min in duration. Before being placed on the floor (half grid and half hole in the same configuration as in the pretests), rats were infused with sterile water in the same volume used on conditioning trials. Test 1 was conducted on the day after completion of 10 conditioning trials (5 CS+ and 5 CS−) and Test 2 was conducted on the day after completion of four additional conditioning trials (2 CS+ and 2 CS−).

2.2.6. Data analysis

The primary dependent measure was the amount of time (s/min) spent on the grid floor during choice tests. [A comparison of the time spent on the hole floor would produce reciprocal results since in our apparatus the animals are defined (by the pattern of photobeam breaks) as being on either the grid floor or the hole floor.] In this counterbalanced discrimination design, the difference between the GRID+ and GRID− subgroups within each ethanol dose group defines the presence of place conditioning (Cunningham, 1993). Conditioned place preference is indicated when GRID+ groups spend significantly more time on the grid floor than GRID− groups. Conversely, CPA is obtained when GRID+ groups spend significantly less time on the grid floor than GRID− groups. Activity, expressed as counts (number of photobeam breaks) per minute, was also recorded during conditioning trials and tests. Activity data from conditioning trials were analyzed to provide an independent assessment of the ethanol dose and preexposure manipulations. Test session activity data were analyzed to determine whether there were group differences in activity that might complicate interpretation of preference data. In all cases, data were analyzed using analysis of variance (ANOVA) with the alpha level set at .05. Ethanol dose and conditioning subgroup (GRID+ vs. GRID−) were treated as between-groups factors, whereas trial type (CS+ vs. CS−), conditioning trial and test session were treated as within-group factors. Preexposure treatment (water vs. ethanol) was an additional between-subjects factor in Experiment 2. Significant interactions were further examined by performing simple effects analyses.

2.3. Results

Four rats were dropped from Experiment 1 and 5 rats from Experiment 2 due to postsurgical complications or problems with the catheters. All data for these animals were removed. An additional rat from Experiment 1 became ill after the first preference test and was removed from the study. Its data were also excluded from all analyses.

2.3.1. Pretests

Rats showed a slight preference for the grid floor prior to conditioning in both experiments. In Experiment 1, average time spent on the grid floor was 34.9 ± 0.9 and 35.0 ± 1.1 s/min (± S.E.M.) on the first and second pretests, respectively. In Experiment 2, these means were 34.6 ± 0.7 and 33.4 ± 0.8, respectively. The matching procedure was successful in creating conditioning subgroups that did not differ in average time on the grid floor during the second pretest (Fs <1 for the main effect of groups in both experiments). Mean grid times ranged from 32.5 to 36.8 s/min across subgroups in Experiment 1 and from 30.5 to 37.4 s/min in Experiment 2.

2.3.2. Conditioning trial activity

Preliminary analyses indicated a general decrease in activity across conditioning trials in both experiments (data not shown). Because overall conclusions about effects of other independent variables did not vary importantly as a function of trials, the trials factor was eliminated from analyses reported below to simplify presentation.

2.3.2.1. Experiment 1. Mean activity rates (counts/min ± S.E.M.) averaged across the seven CS+ (ethanol) and seven CS− (water) conditioning trials are shown in Fig. 1

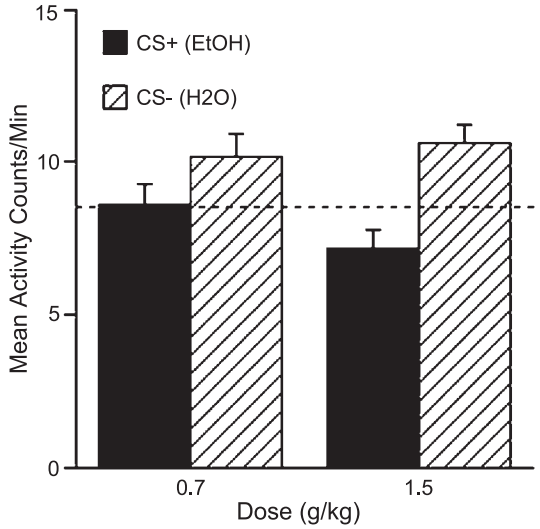

Fig. 1. Mean activity counts per minute (+S.E.M.) averaged across all CS+ (dark bars) and CS− (hatched bars) conditioning trials for both ethanol-conditioned groups in Experiment 1 ($n=13-15$/group). The dashed line depicts mean activity rate for the group that received only water infusions on all trials ($n=8$). Rats in the ethanol-conditioned groups received IG infusions of ethanol (0.7 or 1.5 g/kg) before CS+ trials and IG infusions of water before CS− trials. Data are collapsed over GRID+ and GRID− conditioning subgroups.

for both of the ethanol-treated groups (collapsed across conditioning subgroup). The dashed line depicts mean activity rate of the water-only group, which was treated identically on both types of trials. In general, activity was lower after ethanol infusion (CS+ trials) than after water infusion (CS− trials). Two-way (Dose × Trial Type) ANOVA applied to data from ethanol-treated groups supported this observation, yielding a significant main effect of trial type [$F(1,26)=55.7$, $P<.0001$]. The main effect of ethanol dose was not significant ($F<1$). However, the difference between activity on ethanol and saline trials was greater in the 1.5 g/kg dose group than in the 0.7 g/kg group, suggesting that the depressant effect of ethanol was dose dependent [Dose × Trial Type interaction: $F(1,26)=7.9$, $P<.01$]. Follow-up analyses showed that within-group differences between CS+ and CS− trial activity rates were significant in both dose groups (both Ps $<.0005$), confirming that both ethanol doses produced a decrease in activity. As expected, a simple main effect (between-group) comparison showed no dose group difference in activity on CS− trials ($P>.6$). However, although there was a trend toward lower activity at the higher dose on CS+ trials, the dose group comparison was not significant [$F(1,26)=2.7$, $.05<P<.12$].

A planned comparison (one-way ANOVA) between the water-only group (dashed line in Fig. 1) and ethanol-treated groups on their CS− (water) trials indicated no significant difference among groups [$F(2,33)=2.0$, $P>.15$].

2.3.2.2. Experiment 2. Fig. 2 depicts average activity rates on CS+ and CS− trials for the water-preexposed groups (left panel) and ethanol-preexposed groups (right panel). Dashed lines show activity in the water-only groups. As in Experiment 1, activity on CS+ (ethanol) trials was lower than activity on CS− (water) trials, again indicating a general depressant effect of ethanol [main effect of trial type: $F(1,55)=114.0$, $P<.0001$]. A three-way (Preexposure × Dose × Trial Type) ANOVA also revealed a significant main effect of ethanol dose [$F(1,55]=4.4$, $P<.05$], reflecting a generally lower overall level of activity in the 1.5 g/kg groups. However, the effect of ethanol dose was due primarily to group differences on CS+ trials as indicated by a significant Dose × Trial Type interaction [$F(1,55)=21.8$, $P<.0001$]. This interpretation was confirmed by simple effect follow-up analyses (collapsed across preexpo-

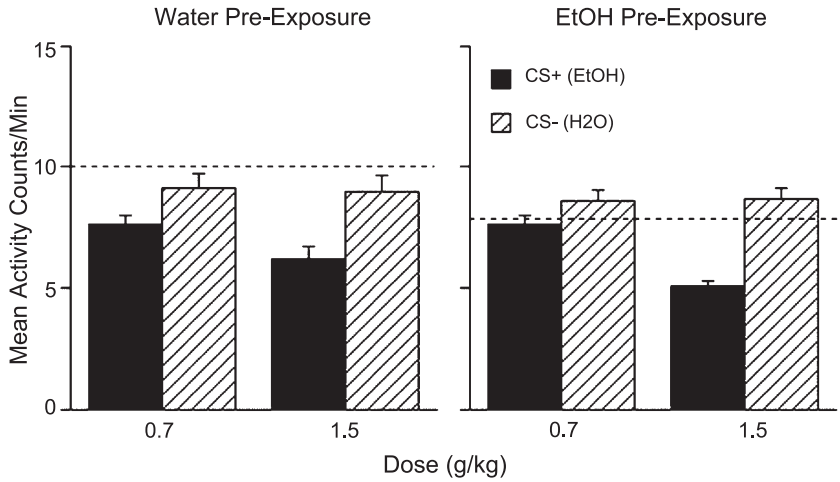

Fig. 2. Mean activity counts per minute (+S.E.M.) averaged across all CS+ (dark bars) and CS− (hatched bars) conditioning trials for ethanol-conditioned groups in Experiment 2 ($n=14-16$/group). Before conditioning, rats received a series of 15 home-cage infusions of water (left panel) or 1.5 g/kg ethanol (right panel). The dashed lines depict activity rate averaged across all trials for the water-conditioned group preexposed to water ($n=4$) or ethanol ($n=5$) in the home cage. Rats in the ethanol-conditioned groups received IG infusions of ethanol (0.7 or 1.5 g/kg) before CS+ trials and IG infusions of water prior to CS− trials. Data are collapsed over GRID+ and GRID− conditioning subgroups.

sure treatment) that showed no dose group difference on CS− trials ($F<1$), but a significant dose effect on CS+ trials [$F(1,57)=19.2$, $P<.0001$]. Additional simple effects analyses (also collapsed across preexposure treatment) showed that the difference between CS+ and CS− trials was significant in both dose groups (both Ps $<.0001$). The three-way ANOVA yielded no significant effects involving preexposure treatment. Thus, IG infusion of both ethanol doses produced a depressant effect on activity that was unaffected by prior exposure to ethanol infusions in the home cage.

Comparison between the two water-only groups (dashed lines in Fig. 2) showed no significant effect of ethanol preexposure, although there was a trend toward lower activity in ethanol-preexposed rats [$F(1,7)=3.7$, $.05<P<.10$]. A planned comparison between the water-only groups and ethanol-treated groups on their CS− (water) trials (collapsed across preexposure treatment) indicated no significant differences among groups ($F<1$).

2.3.3. Preference tests

Preliminary analyses of test session data based on shorter periods of time within the 60-min session (e.g., first 15 or first 30 min) yielded conclusions that were identical to those based on analyses of data averaged over the entire session. Thus, to simplify presentation, only analyses based on the full 60 min are reported here.

2.3.3.1. Experiment 1. Fig. 3 shows mean grid times for the 0.7 and 1.5 g/kg GRID+ and GRID− subgroups averaged across both preference tests in Experiment 1. In both dose groups, GRID+ subgroups spent less time on the grid floor than GRID− subgroups, indicating development of CPA. This observation was confirmed by a three-way ANOVA (Dose × Conditioning Subgroup × Test) that yielded a significant main effect of conditioning subgroup [$F(1, 24)=35.5$, $P<.0001$], but no other main effects or interactions. The absence of dose and test session effects suggests that our conditioning parameters had rapidly produced an asymptotic level of conditioning in both groups.

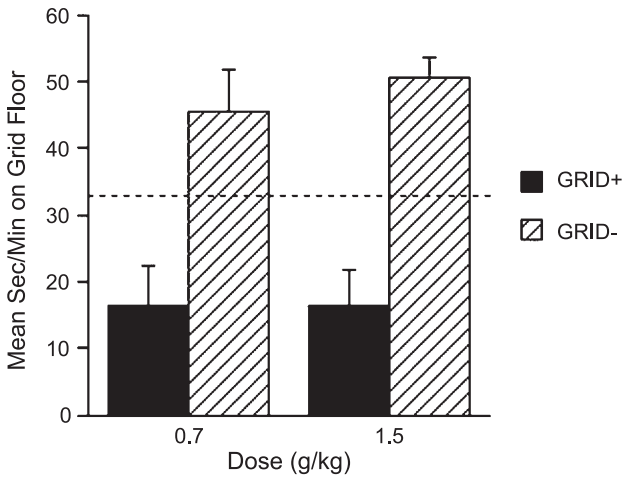

Fig. 3. Mean seconds per minute (+S.E.M.) spent on the grid floor averaged across both 60-min tests in Experiment 1. The floor of the apparatus was half grid and half hole (position counterbalanced). All rats were infused with water before testing. During the conditioning phase, rats in the GRID+ subgroups ($n=7-8$/subgroup) had been placed on the grid floor after ethanol infusions and on the hole floor after water infusions. These contingencies were reversed for rats in the GRID− subgroups ($n=6-7$/subgroup). The dashed line depicts mean preference of rats that had been infused with water before placement on either floor during the conditioning phase ($n=8$).

The water infusion group spent an average of 33.0 ± 6.5 s/min on the grid floor across both tests. Post hoc comparisons (Fisher's PLSD) indicated a significant difference between the water group and all of the ethanol-treated groups (Ps $<.05$) except the 0.7 g/kg GRID− subgroup. Although a vehicle-only group is generally not considered an adequate control in drug conditioning studies (Cunningham, 1993), the difference from only one of the two 0.7 g/kg subgroups could be viewed as evidence for weaker CPA at the lower ethanol dose.

Test session activity rates were also analyzed to determine whether there were any group differences that might complicate interpretation of preference test results. In general, activity decreased over time during both 60-min tests (data not shown), but there were no differences among groups during either test. Activity rates averaged across both 60-min tests were 8.9 ± 1.7, 9.4 ± 0.9 and 9.3 ± 0.5 counts/min for the 0, 0.7 and 1.5 g/kg groups, respectively ($F<1$).

2.3.3.2. Experiment 2. Fig. 4 shows mean time spent on the grid floor averaged over both choice tests for animals that received water (left panel) or ethanol (right panel) preexposure in Experiment 2. As in Experiment 1, rats that received ethanol–grid pairings (GRID+ subgroups) spent less time on the grid floor than rats that received ethanol–hole pairings (GRID− subgroups), indicating development of CPA. However, neither ethanol dose nor ethanol preexposure had an appreciable effect on magnitude of place aversion. These observations were supported by a four-way ANOVA (Preexposure × Dose × Conditioning Subgroup × Test) that yielded only a significant main effect of conditioning subgroup [$F(1, 54)=55.1$, $P<.0001$]. No other main effects or interactions were significant.

To address the possibility that ethanol dose or preexposure might have affected the rapidity with which CPA was initially expressed, a separate post hoc analysis (Preexposure × Dose × Conditioning Subgroup ANOVA) was applied to data from the first 15 min of the first test (Fig. 5). Consistent with the analysis based on the entire 60 min, this ANOVA yielded a significant main effect of conditioning subgroup [$F(1,54)=54.3$, $P<.0001$]. No other main effects or interactions were significant at the .05 criterion level, but both the main effect of preexposure [$F(1,54)=3.4$, $.05<P<.07$] and the Dose × Conditioning Subgroup interaction [$F(1,54)=3.6$, $.05<P<.07$] were close to that criterion. The marginal preexposure effect is due to each of the

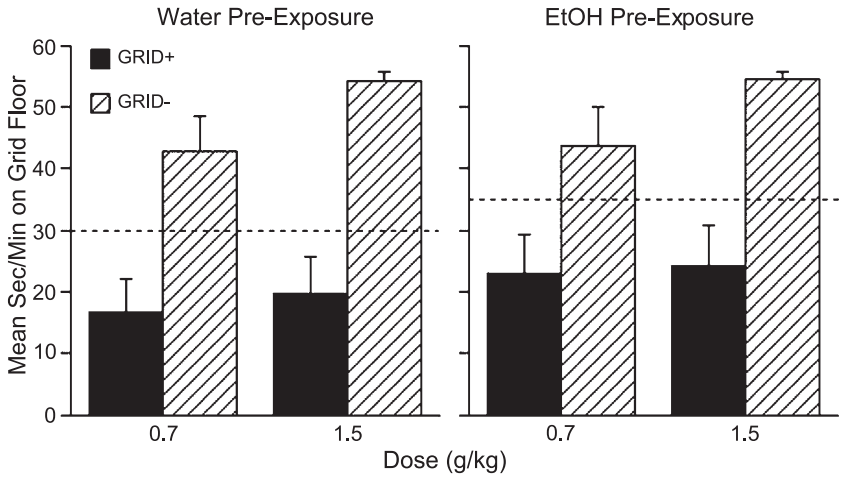

Fig. 4. Mean seconds per minute (+S.E.M.) spent on the grid floor averaged across both 60-min tests in Experiment 2. The floor of the apparatus was half grid and half hole (position counterbalanced). All rats were infused with water before testing. Before conditioning, rats had received a series of 15 home-cage infusions of water (left panel) or 1.5 g/kg ethanol (right panel). During the conditioning phase, rats in the GRID+ subgroups ($n=7-8$/subgroup) had been placed on the grid floor after ethanol infusions and on the hole floor after water infusions. These contingencies were reversed for rats in the GRID− subgroups ($n=7-8$/subgroup). The dashed lines depict mean preference of rats that had been infused with water before placement on either floor during the conditioning phase ($n=4-5$/preinfusion group).

water-preexposed groups spending less time on the grid floor than their ethanol-preexposed counterparts. The marginal interaction between dose and conditioning subgroup was due to a smaller difference between the GRID+ and GRID− subgroups conditioned with the lower ethanol dose (collapsed across preexposure groups). At the 0.7 g/kg dose, mean times (s/min ± S.E.M.) spent on the grid floor were 24.4 ± 3.2 and 39.6 ± 3.1 for the GRID+ and GRID− subgroups, respectively. At the 1.5 g/kg dose, the means were 21.9 ± 3.2 and 48.1 ± 1.7, respectively. Although these data suggest that CPA induced by the lower dose may have emerged a little more slowly during testing, there was no evidence that ethanol preexposure affected the rate at which aversion was expressed.

The groups that received water only during conditioning trials did not differ in time spent on the grid floor as a function of whether they had received water infusions (30.1 ± 7.3 s/min) or ethanol infusions (35.3 ± 10.7 s/min) during the preexposure phase [$F(1,7) < 1$] (averaged over all 60 min of both tests). Post hoc comparisons (Fisher's PLSD) indicated that the combined water-conditioned groups differed significantly from the ethanol-preexposed 1.5 g/kg GRID− group ($P < .01$), the water-preexposed 1.5 g/kg GRID− group ($P < .01$) and the water-preexposed 0.7 g/

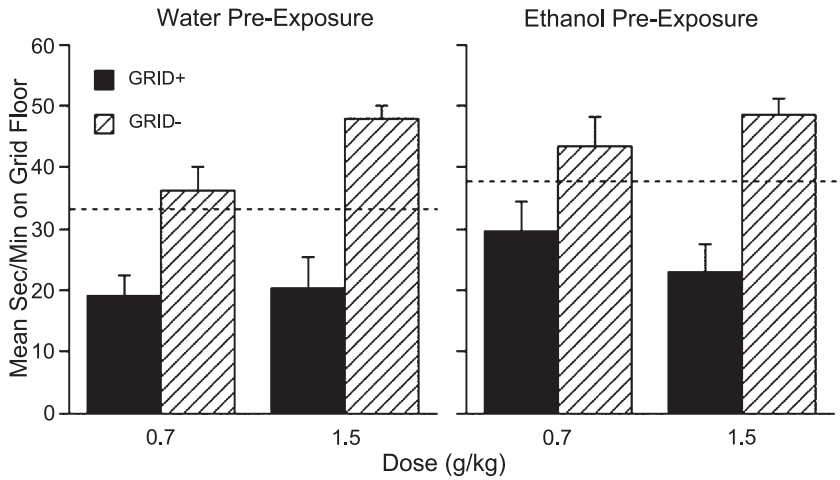

Fig. 5. Mean seconds per minute (+S.E.M.) spent on the grid floor during the first 15 min of the first preference test in Experiment 2. The floor of the apparatus was half grid and half hole (position counterbalanced). All rats were infused with water before testing. Before conditioning, rats had received a series of 15 home-cage infusions of water (left panel) or 1.5 g/kg ethanol (right panel). During the conditioning phase, rats in the GRID+ subgroups ($n=7-8$/subgroup) had been placed on the grid floor after ethanol infusions and on the hole floor after water infusions. These contingencies were reversed for rats in the GRID− subgroups ($n=7-8$/subgroup). The dashed lines depict mean preference of rats that had been infused with water before placement on either floor during the conditioning phase ($n=4-5$/preinfusion group).

kg GRID+ group. Differences between the combined water-conditioned groups and other ethanol treated groups were not significant ($.09 < P < .25$).

A two-way (Preexposure × Dose) ANOVA of test session activity (averaged over both 60-min tests) yielded no significant main effects or interactions. Thus, interpretation of preference data is not compromised by group differences in test session activity. Mean ± S.E.M. activity rates (collapsed across preexposure group) were 7.8 ± 0.8, 7.9 ± 0.4 and 7.6 ± 0.5 counts/min for the 0, 0.7 and 1.5 g/kg dose groups, respectively.

2.4. Discussion

Despite use of conditioning parameters and an administration route previously shown to produce CPP in selectively bred msP rats (Ciccocioppo et al., 1999), Experiments 1 and 2 yielded a robust ethanol-induced CPA in outbred rats. Thus, these findings are in general agreement with the previous study in outbred rats reported by van der Kooy et al. (1983). Because ethanol was infused prior to CS onset in the present studies, our data suggest that development of CPA in the van der Kooy et al. study was probably not due to the time delay inserted between CS onset and ethanol infusion. However, this conclusion must be tempered by the possibility that onset of ethanol's pharmacological effects may nevertheless have been delayed for some time after CS onset due to the slowness of gastric absorption, even though the IG infusion was given immediately before the CS. This possibility is strengthened by recent data showing that IG infusion of ethanol immediately before the CS produces CPA in mice, whereas IG infusion of ethanol 5 min before CS exposure produces CPP (Cunningham et al., 2002). Thus, slower gastric absorption due to genotype or to uncontrolled variables such as the amount of food or water in the stomach might explain, in part, why our studies yielded CPA, while those of Ciccocioppo et al. (1999) yielded CPP.

Activity data recorded during conditioning trials generally showed an activity-suppressing effect of ethanol that was dose dependent in the range tested here. The absence of a dose effect on CPA despite this effect on activity suggests dissociation between ethanol's locomotor and aversive motivational effects in rats. This dissociation is generally consistent with ethanol place conditioning studies in mice, which have also failed to show any relationship between ethanol's acute activating effects and development of ethanol-induced CPP (Cunningham, 1995; Risinger et al., 1994).

3. Experiment 3

Experiment 3 was designed to test the possibility that insertion of a longer time interval between onset of ethanol infusion and onset of CS exposure might increase the likelihood of producing CPP in outbred rats. Thus, for half of the rats, ethanol infusion began 10 min before rather than immediately before exposure to the CS. Because a previous study had shown that CPP was not produced when a 10-min delay was used in a study that involved rapid ip injections of ethanol (Bormann and Cunningham, 1998), we also used a very slow rate of infusion in an attempt to minimize any aversion that might be related to the otherwise rapid transition from the sober to the intoxicated state (Cunningham et al., 1997). We hypothesized that a relatively slow IG infusion initiated 10 min before CS exposure would be more likely to condition CPP than a rapid IG infusion given immediately before CS exposure (Cunningham et al., 2002).

The number of conditioning trials in this experiment was reduced to four of each CS type before the first preference test (from five in Experiments 1 and 2) to bring it more in line with previous work from our laboratory (e.g., Bormann and Cunningham, 1998). The trial duration was reduced from 60 to 15 min since previous data from our laboratory indicated that shorter trial durations were sufficient to produce significant place conditioning. A water-only control group was not included in this experiment because we felt that the inclusion of such a group in the previous two experiments was sufficient to show that our apparatus was relatively unbiased.

3.1. Subjects, surgery, apparatus

Adult male rats ($n = 26$) were obtained at about 3 months of age from the same vendor and housed as in previous studies. After 7–10 days adaptation to the colony, each rat was surgically implanted with an IG catheter and allowed 5–8 days recovery before the experiment began. The place conditioning apparatus was that used in Experiments 1 and 2. Timed IG infusions were administered using a programmable syringe pump (Cole-Parmer Model 74900-10). During these infusions, rats were placed in an acrylic cylinder (24 cm diameter × 30.5 cm high) on cob bedding. The experimental protocol was approved by the OHSU IACUC and procedures complied with the NIH *Guide for Care and Use of Laboratory Animals*.

3.2. Procedure

3.2.1. Overview

The general procedure for Experiment 3 included the following: (a) an infusion habituation phase (two water sessions followed by two ethanol sessions), (b) a series of eight conditioning trials (4 CS+ and 4 CS−) followed by a drug-free choice test and (c) four additional conditioning trials (2 CS+ and 2 CS−) followed by a second choice test. All rats were conditioned with the same dose of ethanol (1.0 g/kg), but were randomly assigned to groups that differed in the infusion duration (30 vs. 600 s). The long-duration infusion began 10 min before placement in the chamber, whereas the short-duration infusion began 30 s before

placement in the chamber. The ethanol dose was selected because it is in the range of doses that other laboratories have reported to produce ethanol CPP in outbred rats (Bozarth, 1990; Reid et al., 1985).

3.2.2. Habituation

The purpose of this phase was to habituate animals to the potentially stressful effects of exposure to the infusion apparatus and procedure. On each of four consecutive days, each rat was weighed, attached to the syringe pump and placed into the infusion chamber for 10 min. The syringe pump was activated immediately for rats assigned to the long (600-s) infusion group, but activation was delayed 9.5 min for rats assigned to the short (30-s) infusion group. Sterile water (12.5 ml/kg) was infused during the first two sessions whereas ethanol (1.0 g/kg, 10% vol/vol in sterile water, 12.5 ml/kg) was infused during the last two sessions. Based on results of Experiment 2, preexposure to ethanol during the habituation phase was not expected to affect subsequent development of place conditioning.

3.2.3. Conditioning trials

Beginning 48 h after the last habituation session, rats were exposed to a series of discriminative place conditioning trials using an unbiased stimulus assignment procedure similar to that described for Experiments 1 and 2. Within each infusion duration group, rats were randomly assigned to GRID+ and GRID− conditioning subgroups. Six CS+ and six CS− trials were given on alternating days (counterbalanced order within each subgroup). Each conditioning trial began with a 10-min placement in the infusion chamber where rats received either ethanol (CS+ trials) or water (CS− trials) infusions at the assigned duration (30 vs. 600 s). Immediately after infusion, rats were placed into the conditioning chamber on the appropriate floor (grid or hole depending on subgroup assignment and trial type) for a 15-min conditioning trial. Although this trial duration was shorter than that used in Experiments 1 and 2, previous studies in this laboratory have shown that relatively short (5–15 min) trial durations are still quite effective for producing ethanol place conditioning in rats (Bormann and Cunningham, 1998; Cunningham, 1981; Cunningham et al., 1993). Moreover, it has been suggested that a relatively short CS exposure on the rising limb of the blood ethanol curve may be best for demonstrating ethanol CPP in rats (Reid et al., 1985).

3.2.4. Preference tests

All rats received two 60-min preference tests like those described for Experiments 1 and 2. Test 1 was conducted on the day after completion of the fourth trial of each type and Test 2 was conducted on the day after the last trial of each type. Each test was preceded by 10-min placement in the infusion chamber accompanied by sterile water infusion at the assigned duration.

3.3. Results

The analyses reported below exclude data from two rats that were removed from the study due to problems with the catheter. The remaining number of rats in each conditioning subgroup ranged from 5 to 7.

3.3.1. Conditioning trial activity

As in the previous studies, preliminary analyses showed a general decrease in activity across conditioning trials (data not shown). Because conclusions about effects of infusion duration did not vary as a function of trials, the trials factor was eliminated from analyses reported below to simplify presentation.

Fig. 6 depicts mean activity rates averaged over all six CS+ (ethanol) trials and all six CS− (water) trials for both infusion duration groups (collapsed across conditioning subgroup). Activity was generally higher in this experiment than in Experiments 1 and 2, reflecting use of a shorter conditioning trial duration. As in the previous experiments, activity was significantly lower on ethanol (CS+) trials than on water (CS−) trials [main effect of trial type: $F(1,22) = 23.7$, $P < .0001$]. However, there was no main effect or interaction involving infusion duration [Infusion Duration × Trial Type ANOVA: both Fs < 1]. Thus, despite a relatively large difference in infusion rate, the activity-suppressing effect produced by 1 g/kg ethanol was similar.

3.3.2. Preference tests

Mean times spent on the grid floor averaged across both preference tests are shown for all groups in Fig. 7. At both infusion durations, GRID+ subgroups spent less time on the

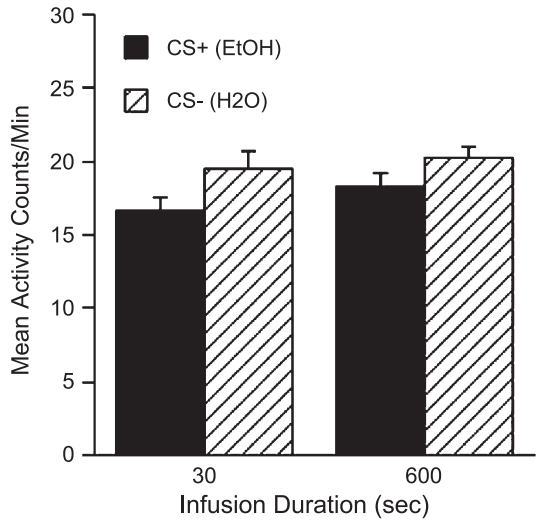

Fig. 6. Mean activity counts per minute (+S.E.M.) averaged over all CS+ (dark bars) and CS− (hatched bars) conditioning trials for all groups in Experiment 3 ($n = 12$/group). Rats received short (30-s) or long (600-s) duration IG infusions of ethanol (1.0 g/kg) or water before CS+ and CS− trials, respectively. Data are collapsed over GRID+ and GRID− conditioning subgroups.

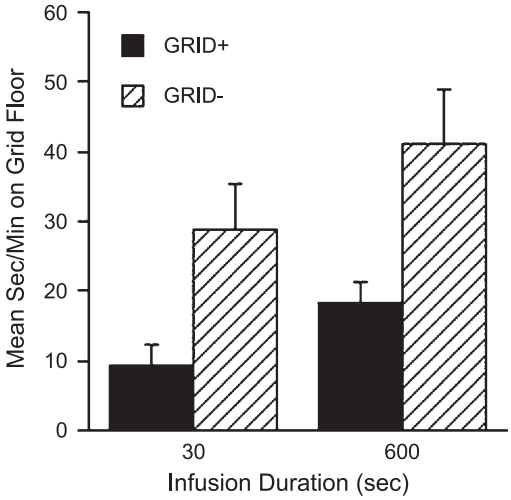

Fig. 7. Mean seconds per minute (+S.E.M.) spent on the grid floor averaged across both 60-min tests in Experiment 3. The floor of the apparatus was half grid and half hole (position counterbalanced). All rats were infused with water at the assigned duration before testing. During the conditioning phase, rats in the GRID+ subgroups ($n=6-7$/subgroup) had been placed on the grid floor after ethanol infusions and on the hole floor after water infusions. These contingencies were reversed for rats in the GRID− subgroups ($n=5-6$/subgroup).

grid floor than GRID− subgroups, indicating development of CPA [main effect of conditioning subgroup: $F(1,20)=16.5$, $P<.001$]. Three-way ANOVA (Infusion Duration × Conditioning Subgroup × Test) did not yield any other significant main effects or interactions. Thus, infusion duration did not affect strength of ethanol-induced CPA. Moreover, as in Experiments 1 and 2, the absence of significant test session differences suggests that place conditioning had reached asymptote after the initial series of conditioning trials.

Inspection of Fig. 7 suggests that rats given 30-s infusions generally spent less time on the grid floor, regardless of floor assignment subgroup (GRID+ or GRID−). This observation is supported by a marginally nonsignificant main effect of infusion duration [$F(1,20)=4.2$, $.05<P<.06$]. Given the similarity between strength of CPA in the 30-s duration group (as defined by the difference between GRID+ and GRID− subgroups) and the place aversions shown by groups in Experiments 1 and 2 (which received similarly short duration infusions), it does not appear that this general bias against grid interfered with ability to detect place aversion. Because this bias was not seen in any of the groups in the previous experiments, this marginal main effect of infusion duration may simply be the result of sampling error.

Analysis of test session activity rates showed no difference between infusion duration groups [$F(1,22)=1.2$, $P>.25$], indicating that interpretation of test session preference data was not complicated by differences in test activity. Mean activity rates were 9.4 ± 1.1 and 10.7 ± 0.6 counts/min for the 30-s and 600-s groups, respectively.

3.4. Discussion

Experiment 3 showed that a relatively slow IG infusion of ethanol initiated 10 min before CS exposure produced a CPA similar in magnitude to that produced by rapid infusion of the same ethanol dose immediately before CS exposure. Thus, in contrast to recent findings in mice (Cunningham et al. 2002), it does not appear that the direction of place conditioning induced by intragastrically administered ethanol can be reversed in rats by inserting a delay between the onset of ethanol infusion and onset of the CS. Furthermore, the fact that ethanol-induced CPA was insensitive to a 20-fold difference in infusion rate suggests that the CPA induced by ethanol in rats may not be related to the rapidity of the transition from the sober to the intoxicated state. Rather, these findings suggest that aversive postabsorptive effects of ethanol produce CPA in rats.

4. General discussion

Overall, these experiments showed development of a robust CPA that was insensitive to ethanol dose in the 0.7 to 1.5 g/kg range (Experiments 1 and 2), 15 days of ethanol preexposure (Experiment 2) and a 20-fold difference in ethanol infusion rate (Experiment 3). When compared to the two previous place conditioning studies in which rats have received ethanol via a surgically implanted gastric catheter, the present findings are clearly more consistent with those of van der Kooy et al. (1983) than with those of Ciccocioppo et al. (1999). Our use of an outbred rat strain rather than a line selectively bred for alcohol preference may be responsible for the discrepancy between our findings and those of Ciccocioppo et al. However, it is also possible that differences in apparatus or procedure contributed to the different outcomes. One significant difference is the use of a biased procedure by Ciccocioppo et al., which may have promoted development of CPP through a negative reinforcement mechanism. That is, the CPP observed in msP rats may not have been due to a conditioned rewarding effect of ethanol, but to ethanol's alleviation of an unconditioned aversive state elicited by the nonpreferred compartment (Carr et al., 1989; Cunningham et al., 2003a; Swerdlow et al., 1989). This argument is supported by data showing enhanced sensitivity to ethanol's anxiolytic effects in selectively bred alcohol preferring rats (Colombo et al., 1995; Swerdlow et al., 1989). Moreover, because Ciccocioppo et al. paired ethanol with the initially nonpreferred CS compartment, their ability to detect CPA may have been reduced due to a floor effect (Cunningham et al., 2003a).

The finding that IG ethanol produced CPA in outbred rats is also consistent with the results of many other studies showing that similar or higher doses of ethanol consumed orally (Stewart and Grupp, 1986, 1989) or injected ip (Bormann and Cunningham, 1997, 1998; Cunningham, 1979, 1981; Cunningham and Niehus, 1993; Cunningham

et al., 1993) produce CPA in drug-naive outbred rats (Table 1). These similarities in outcome despite differences in route of administration suggest that CPA produced by ip injection is not simply an artifact of pain or discomfort produced by peritoneal irritation. Moreover, the similar outcomes across different routes offer additional support for the suggestion that ethanol-induced CPA reflects postabsorptive aversive effects.

The absence of a drug preexposure effect on CPA (Experiment 2) is not consistent with previous rat studies suggesting that preexposure reduces CPA (Gauvin and Holloway, 1992) or facilitates induction of CPP (Biala and Kotlinska, 1999; Bienkowski et al., 1995, 1996; Ciccocioppo et al., 1999; Gauvin et al., 2000; Gauvin and Holloway, 1992; Holloway et al., 1992; Reid et al., 1985). However, interpretation of these earlier preexposure studies is potentially clouded by use of a biased apparatus and procedure. Thus, effects of ethanol preexposure in these studies could reflect a change in ethanol's antiaversive or anxiolytic effects rather than a change in its rewarding effect (Cunningham et al., 2003a). Moreover, several of these preexposure studies either did not include concurrent vehicle treated controls (Biala and Kotlinska, 1999; Bienkowski et al., 1995; Gauvin and Holloway, 1992) or did not provide evidence of a preexposure effect based on direct comparison between ethanol- and vehicle-pretreated groups (Ciccocioppo et al., 1999). Indeed, one study showed that both ethanol and saline preexposures produced the same enhancing effect on CPP, suggesting it was due to nonspecific (i.e., nonpharmacological) aspects of the preexposure treatment (Bienkowski et al., 1996). In light of these complications in the interpretation of previous preexposure studies in rats, it is difficult to know whether the absence of a preexposure effect in Experiment 2 should be considered anomalous.

Of course, a preexposure effect might have been obtained with more extensive exposure to ethanol (e.g., higher frequency and/or higher dose). Ethanol preexposure takes a variety of forms across experiments and it is possible that these procedural differences account for differences in outcome. In some cases, ethanol preexposure took the form of either limited or continuous access home cage drinking (Ciccocioppo et al., 1999; Gauvin and Holloway, 1992; Reid et al., 1985) for up to 120 days (Gauvin and Holloway, 1992). Ciccocioppo et al. (1999) reported that their ethanol-experienced msP rats consumed approximately 1.5 g/kg ethanol per day when ethanol was available during a 2-h limited access period. We selected the 1.5 g/kg dose for ethanol preexposure so that our rats would have comparable experience with ethanol in the days leading up to the start of the place conditioning procedure. Differences between the effects of self-administered and experimenter-administered ethanol may be responsible for the lack of a preexposure effect in our experiments. Nevertheless, some experiments suggest that experimenter-administered ethanol could have an impact on subsequent conditioning (Bienkowski et al., 1995, 1996). Perhaps our ethanol preexposure dose should have been lower and/or more prolonged in order to reduce CPA.

The observed findings of ethanol-induced CPA or CPP appear to result from a complex interaction among a number of variables including genetics, route of administration, design (biased vs. unbiased) and ethanol experience. Examination of ethanol-induced place conditioning with selectively bred alcohol preferring (and nonpreferring) rats using an unbiased design and IG administration might shed some light on the relative importance of the different variables. If the alcohol-preferring rats still showed CPP (as in Ciccocioppo et al., 1999) then this might suggest that genetics are a more important determinant of preference and that the postabsorptive drug effects are positive in alcohol-preferring rats. If, however, the selectively bred rats showed CPA (as did the heterogeneous rats in the three experiments presented here) then the assertion that biased designs show CPP because of ''antiaversive'' effects rather than rewarding effects would be supported.

Acknowledgements

This research was supported by NIH/NIAAA grant AA07702 and the Oregon Health Sciences Foundation N. L. Tartar Research Fund.

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