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Injection timing determines whether intragastric ethanol produces conditioned place preference or aversion in mice

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Abstract

Previous studies have shown that mice develop conditioned place preference (CPP) when ethanol is administered by intraperitoneal (ip) or intravenous (iv) injection. The present studies examined CPP in mice using the intragastric (ig) route of administration. Inbred mice were surgically implanted with chronic intragastric cannulae and exposed to an unbiased place conditioning procedure in which infusion of ethanol (2 or 4 g/kg) was paired with a conditioned stimulus (CS+). A different CS was paired with water. In Experiments 1–2, ethanol was infused just before exposure to CS+. Contrary to previous studies involving intraperitoneal injection, infusion of 4 g/kg ig ethanol produced a significant conditioned place aversion (CPA). However, when a 5-min delay was inserted between infusion and CS exposure (Experiments 3–4), the same dose produced CPP. These outcomes are not consistent with expectations derived from a recent study in selectively bred rats, suggesting that sensitivity to ethanol reward is enhanced by intragastric administration. However, the finding that intragastric ethanol can produce either CPP or CPA depending on dose and injection timing is consistent with previous intraperitoneal ethanol studies in mice. Although the parameters differ for each route of administration, it appears that the same underlying processes can be invoked to explain how manipulation of injection timing affects the direction of ethanol-induced place conditioning. More specifically, in both cases, CPA can be attributed to an initial, short-lived aversive effect, whereas CPP can be attributed to a delayed rewarding effect of ethanol.

Keywords: Place conditioning; Preference; Aversion; Locomotor activity; Sensitization; DBA/2 inbred mice

1. Introduction

The place conditioning procedure is a widely accepted technique for assessing the rewarding and aversive effects of most abused drugs including ethanol (see review by Tzschentke, 1998). However, sensitivity of this procedure to ethanol's rewarding effect, as demonstrated by conditioned place preference (CPP), appears to differ markedly between rats and mice (e.g., Cunningham et al., 1993). Whereas CPP has been readily obtained in drug-naive mice at moderate to high doses across a wide variety of strains and lines (e.g., Chester et al., 1998; Cunningham, 1995; Cunningham et al., 1991, 1992, 2000; Grahame et al., 2001; Itzhak and Martin, 2000; Nocjar et al., 1999; Risinger and Oakes, 1996; Risinger et al., 1994), there are relatively few such demonstrations in rats (Black et al., 1973; Bozarth, 1990). Typically, ethanol produces conditioned place aversion (CPA) in drug-naive rats (e.g., Bormann and Cunningham, 1997, 1998; Cunningham, 1979, 1981; Cunningham and Niehus, 1993; Gauvin and Holloway, 1992; Gauvin et al., 1994; Holloway et al., 1992; Sherman et al., 1983; Stewart and Grupp, 1986, 1989; van der Kooy et al., 1983), including rats selectively bred for ethanol preference (Stewart et al., 1996). To produce CPP in rats, one must give extensive ethanol preexposure (e.g., Gauvin and Holloway, 1992; Holloway et al., 1992; Reid et al., 1985), coadminister food or other drugs (Marglin et al., 1988; Stewart and Grupp, 1981, 1985) or combine place conditioning with a fear-conditioning procedure (e.g., Matsuzawa et al., 1998, 1999, 2000).

Although early studies appeared to eliminate a role for route of administration in determining whether ethanol would produce CPP or CPA in rats (van der Kooy et al., 1983), a recent report suggests that sensitivity to ethanol's

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rewarding effect may be enhanced in genetically selected alcohol-preferring rats (msP) when ethanol is administered via a chronic intragastric (ig) cannula (Ciccocioppo et al., 1999). These experiments showed a significant CPP when ethanol was given via intragastric cannula at doses that did not produce any place conditioning when ethanol was given by gavage (po) or by intraperitoneal (ip) injection. The authors suggested that ethanol's rewarding effect might have been reduced by the latter routes of administration due to stress. They also suggested that intraperitoneal administration might interfere with CPP due to gastrointestinal disturbance or because the intraperitoneal route allowed the more rapid attainment of a higher brain ethanol level than the intragastric route. The implication of the latter suggestion is that more rapid changes in brain ethanol level and/or higher brain ethanol levels are less rewarding, more aversive, or both.

Nearly all of the published studies of ethanol place conditioning in mice have involved intraperitoneal injection. The one exception is a study in which intravenous (iv) infusion of ethanol was found to produce CPP in C57BL/6 mice (Kelley et al., 1997). To date, however, there have been no studies of ethanol place conditioning in mice involving the intragastric route of administration. Given the possibility that this route enhances sensitivity to ethanol reward (Ciccocioppo et al., 1999), the present experiments were designed to examine ethanol-induced place conditioning in mice using intragastric administration.

2. Experiments 1–2

In Experiment 1, two ethanol doses (2 or 4 g/kg) were tested in a counterbalanced, unbiased place conditioning procedure using an inbred mouse strain (DBA/2J). To assess the reliability and generality of the effect observed in Experiment 1, the higher dose was tested again in Experiment 2. However, due to the vendor's inability to supply DBA/2J mice, Experiment 2 used a different DBA/2 substrain (DBA/2N Tac). Although ethanol-induced place conditioning has not previously been studied in this substrain, there was no reason to expect that it would differ from the DBA/2J substrain. Based on a preliminary study showing relatively rapid onset of ethanol's activating effects after intragastric infusion, temporal parameters in Experiments 1 and 2 were identical to those used in previous ethanol place conditioning studies involving the intraperitoneal route of administration. That is, ethanol (or water) was infused immediately before a 5-min exposure to the conditioning chamber.

2.1. Method

2.1.1. Subjects

Naive adult male inbred mice were purchased at 6–8 weeks of age and allowed to adapt to the colony for at least 2 weeks before surgery. Mice (DBA/2J) used in Experiment 1 ($n = 41$) were shipped from the Jackson Laboratory (Bar Harbor, ME), whereas mice (DBA/2N Tac) used in Experiment 2 ($n = 42$) were shipped from Taconic Farms (Germantown, NY). Animals were housed in polycarbonate cages with cob bedding in a Thoren rack. Before surgery, animals were housed in groups of three or four. After surgery, all mice were housed individually to minimize damage to the cannula implants. The colony room was maintained at 21 ± 1 °C with a normal 12-h light–dark cycle (lights on at 7 AM). Testing occurred during the light cycle. Food was available at all times in the home cage, except for 16–23 h prior to surgery. Water was available at all times except for 3 h after each ethanol infusion to minimize the possibility that mice would drown in the water dish. The experimental protocol was approved by the OHSU IACUC and procedures complied with the NIH "Guide for Care and Use of Laboratory Animals."

2.1.2. Apparatus

The place conditioning apparatus was identical to that used in previous studies in which ethanol was administered by intraperitoneal injection (e.g., Cunningham, 1995). Twelve aluminum and acrylic chambers ($30 \times 15 \times 15$ cm) were contained in separate ventilated sound- and light-attenuating enclosures. The animal's position and locomotor activity were detected by infrared photodetectors and light sources interfaced to a computer recording system. The conditioned stimuli (CSs) were tactile cues presented by interchangeable floor halves placed beneath the conditioning chamber. More specifically, the grid floor was made from 2.3-mm stainless-steel rods mounted 6.4 mm apart in acrylic rails; the hole floor was constructed from perforated stainless-steel sheet metal (16 gauge) containing 6.4-mm round holes on 9.5-mm staggered centers. This combination of floor stimuli was chosen on the basis of many previous studies showing that drug-naive mice spend about 50% of the time on each floor type during choice tests (e.g., Cunningham, 1995; Cunningham et al., 1997). The inside of the chamber and floors were wiped with a damp sponge, and the litter paper beneath the floors was changed after each animal.

2.1.3. Procedure

2.1.3.1. Surgery

All mice were fully anesthetized with isoflurane gas (5% loading dose; 1.5–2% maintenance) for surgical implantation of a gastric cannula constructed from silastic tubing (0.020 inches i.d. × 0.037 inches o.d.). The procedure was similar to that described by Koopmans (1987). Briefly, a silastic knob attached to the internal end of the cannula was inserted through a puncture in the stomach wall and secured to the stomach with a purse-string suture and polypropylene mesh. The external end was then tunneled subcutaneously to an incision on the back where it connected to hypodermic

tubing within a plastic mount (Plastics One single guide cannula, C313G-5UPSPC) encased in cranioplastic cement attached to polypropylene mesh. All incisions were closed with suture and the animal received a 1-ml subcutaneous injection of saline to reduce dehydration. Mice were kept warm and monitored until completely recovered from anesthesia before being returned to the home cage. Moistened food was available during the first few days of recovery. Mice were allowed 3–7 days recovery before habituation in Experiment 1 and 6–14 days recovery in Experiment 2. Cannulae were flushed daily with sterile distilled water to prevent blockage by stomach contents.

2.1.3.2. Place conditioning

In Experiment 1, nine mice were removed from the study due to complications from surgery or problems with the cannula, leaving a total of 32 mice. In Experiment 2, five mice were removed for similar reasons, leaving a total of 37 mice.

Each experiment included three phases: Habituation (1 day), Conditioning (12 days) and Preference Testing (2 days). On the habituation day, all mice were infused with distilled water and placed in the apparatus on a smooth paper floor for 5 min. For conditioning, mice in Experiment 1 were randomly assigned to one of two ethanol dose groups: 2 or 4 g/kg ($n = 16$/group); in Experiment 2, all mice received 4 g/kg. Within each dose group, mice were also assigned randomly to one of two conditioning subgroups, GRID+ or GRID −. On alternate days, mice in the GRID+ subgroups received an intragastric infusion of ethanol immediately before exposure to the grid floor (CS+) and an intragastric infusion of distilled water just before exposure to the hole floor (CS −). These relationships were reversed for the GRID − subgroups. All infusions were done manually and lasted about 30–45 s. Ethanol dose was manipulated by varying the volume of a 20% v/v solution of ethanol in distilled water. Each conditioning trial was 5 min long and mice had access to the entire chamber, which contained one floor type throughout. The order of exposure to CS+ and CS − was counterbalanced within each subgroup.

Two 60-min floor preference tests were given. The first test occurred 24 h after the eighth conditioning trial (i.e., after four CS+ and four CS − trials), whereas the second test occurred 24 h after the twelfth conditioning trial (i.e., after six CS+ and six CS − trials). Two-day breaks were inserted after the first four conditioning trials and after the first preference test; otherwise, the experiment was conducted on consecutive days.

2.1.4. Data analysis

Data from each experiment were analyzed separately using analysis of variance (ANOVA) with the alpha level set at .05. Ethanol Dose and Conditioning Subgroup were treated as between-groups factors, whereas CS Type (CS+ vs. CS −) and Trial were treated as within-groups factors. In the unbiased, counterbalanced experimental design used here, place conditioning is defined by the difference between the GRID+ and GRID − conditioning subgroups within each of the main dose groups (Cunningham, 1993).

2.2. Results

2.2.1. Conditioning activity

Intragastric administration of ethanol on CS+ trials produced a dose-dependent increase in locomotor activity during the first 5 min after infusion (see Fig. 1). Moreover, repeated infusion produced sensitization to ethanol's locomotor stimulant effect, which was strongest at the 4-g/kg dose in Experiment 1 (left panel, Fig. 1). In contrast, activity after water infusions on CS − trials tended to remain relatively stable over trials. Factorial ANOVAs of data from

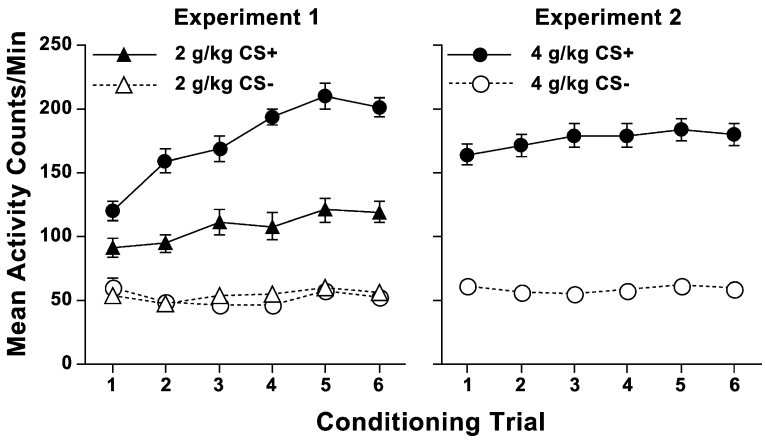

Fig. 1. Mean activity counts per minute (± S.E.M.) during each CS+ (solid symbols) and CS − (open symbols) conditioning trial in Experiments 1 (left panel) and 2 (right panel). DBA/2J mice were used in Experiment 1, whereas DBA/2N Tac mice were used in Experiment 2. All mice received an intragastric ethanol (2 or 4 g/kg) infusion before each CS+ trial and a water infusion before each CS − trial. Data are collapsed over GRID+ and GRID − conditioning subgroups (Experiment 1: $n = 16$ per dose group; Experiment 2: $n = 37$).

Table 1
Mean activity counts (±S.E.M.) during each minute of the first CS+ (E = ethanol) and CS − (W = water) conditioning trials

	Minute				
Dose group	1	2	3	4	5
Exp 1, 2 g/kg (n = 16)	E: 64.8 ± 4.5	E: 86.1 ± 8.4	E:109.4 ± 10.1	E:110.4 ± 10.3	E:106.9 ± 8.9
	W: 59.7 ± 3.0	W: 49.2 ± 3.2	W: 46.0 ± 3.3	W: 49.2 ± 2.4	W: 47.9 ± 3.5
Exp 1, 4 g/kg (n = 16)	E: 84.7 ± 5.2	E: 153.8 ± 7.0	E: 151.2 ± 9.6	E: 111.8 ± 13.7	E: 101.1 ± 12.0
	W: 67.9 ± 5.9	W: 63.1 ± 9.7	W: 57.1 ± 10.1	W: 59.2 ± 9.6	W: 54.2 ± 7.9
Exp 2, 4 g/kg (n = 37)	E: 94.8 ± 4.6	E: 152.3 ± 6.8	E: 191.9 ± 6.3	E: 197.3 ± 6.0	E: 187.8 ± 7.2
	W: 80.6 ± 0.1	W: 62.4 ± 3.6	W: 57.6 ± 2.7	W: 55.6 ± 3.3	W: 53.3 ± 2.6
Exp 3, 2 g/kg (n = 19)	E: 146.5 ± 8.6	E: 111.8 ± 6.6	E: 102.9 ± 6.0	E: 90.1 ± 5.9	E: 93.9 ± 4.1
	W: 89.9 ± 4.2	W: 68.3 ± 3.5	W: 58.3 ± 3.9	W: 49.7 ± 3.2	W: 48.9 ± 2.6
Exp 3, 4 g/kg (n = 19)	E: 201.1 ± 5.6	E: 180.8 ± 5.2	E: 175.9 ± 7.6	E: 159.7 ± 7.6	E: 149.7 ± 8.5
	W: 88.7 ± 4.8	W: 72.1 ± 3.4	W: 67.5 ± 2.6	W: 57.4 ± 3.3	W: 50.7 ± 2.6
Exp 4, 4 g/kg (n = 26)	E: 182.3 ± 8.0	E: 167.2 ± 6.4	E: 155.2 ± 7.5	E: 145.1 ± 7.5	E: 139.6 ± 8.2
	W: 87.8 ± 6.2	W: 68.9 ± 4.8	W: 61.5 ± 4.1	W: 58.2 ± 4.9	W: 53.6 ± 4.9

Trials began immediately after infusion in Experiments 1 and 2, but were delayed until 5 min after infusion in Experiments 3 and 4.

Experiment 1 (Dose × CS Type × Trials) and Experiment 2 (CS Type × Trials) yielded significant outcomes for all main effects and interactions (smallest $P < .002$). To assess development of sensitization, we conducted separate planned comparisons of activity on the first and last trials of each type within each group. Analyses of CS+ data confirmed a significant increase in activity over trials at both doses [2 g/kg: $F(1,15) = 9.9$, $P < .01$; 4 g/kg (Experiment 1): $F(1,15) = 109.3$, $P < .0001$; 4 g/kg (Experiment 2): $F(1,36) = 6.1$, $P < .02$]. Similar analyses showed no significant change in activity over CS − trials in any dose group (all F's < 1.4).

Due to interest in the rapidity of the onset of ethanol's effects after intragastric infusion, we also examined activity on a minute-by-minute basis during the first trial of each type (see Table 1). In general, activity on the first CS+ (ethanol) trial was positively related to dose and increased over the first few minutes before reaching a plateau or decreasing. In contrast, activity on the first CS − (water) trial generally decreased over time. Within-groups planned comparisons between CS+ and CS − activity scores during the initial minutes were used to determine how soon the ethanol effect could be detected. At the 2-g/kg dose (Experiment 1), there was no difference between mean activity counts after ethanol versus water in the first minute ($F < 1$). However, a significant difference had emerged by the second minute [$F(1,15) = 31.2$, $P < .0001$], and this difference increased as time passed, peaking during Minutes 3–4. At the 4-g/kg dose, the difference between ethanol and water was significant in the first minute [Experiment 1: $F(1,15) = 4.7$, $P < .05$; Experiment 2: $F(1,36) = 13.3$, $P < .001$]. Activation produced by 4 g/kg peaked during Minutes 2–3 in Experiment 1, and during Minutes 3–4 in Experiment 2.

2.2.2. Preference tests

Outcomes of the two preference tests are depicted in Figs. 2 (Experiment 1) and 3 (Experiment 2). In general, there was little evidence of place conditioning during the

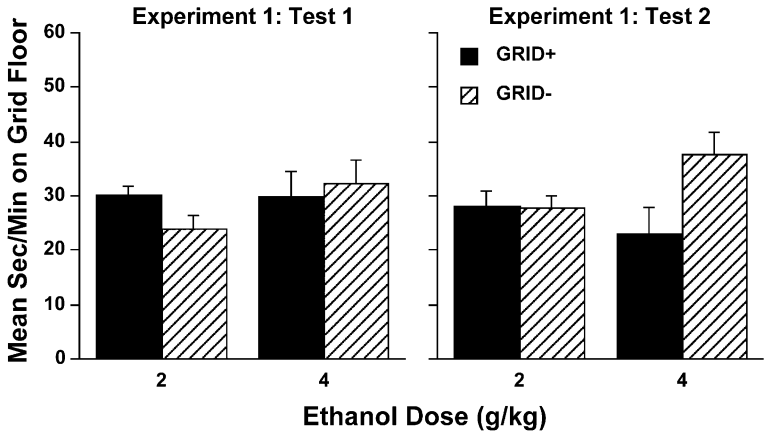

Fig. 2. Mean seconds per minute (+S.E.M.) spent on the grid floor during two 60-min test sessions in Experiment 1. Test 1 (left panel) was given after the first eight conditioning trials (four CS+ and four CS − trials), whereas Test 2 (right panel) was given after four additional conditioning trials (i.e., a total of six CS+ and six CS − trials). During the conditioning phase, mice in the GRID+ conditioning subgroups received an intragastric ethanol (2 or 4 g/kg) infusion immediately before 5-min exposure to the grid floor on CS+ trials; water was infused before exposure to the hole floor on CS − trials. These contingencies were reversed for mice in the GRID − conditioning subgroups. Each conditioning subgroup contained seven to nine mice.

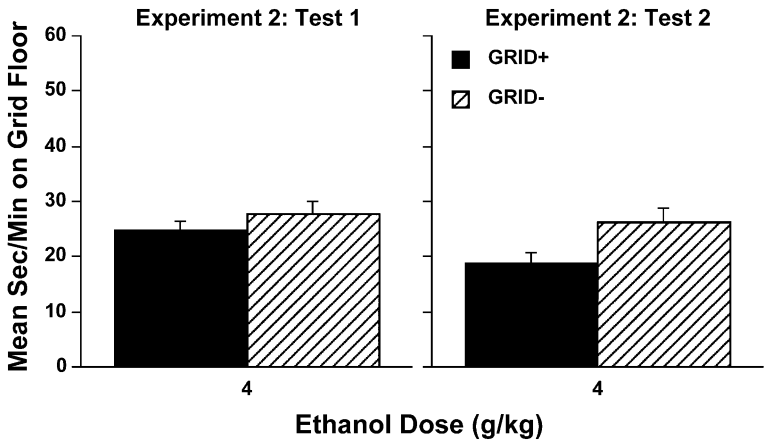

Fig. 3. Mean seconds per minute (+S.E.M.) spent on the grid floor during two 60-min test sessions in Experiment 2. Test 1 (left panel) was given after the first eight conditioning trials (four CS+ and four CS − trials), whereas Test 2 (right panel) was given after four additional conditioning trials (i.e., a total of six CS+ and six CS − trials). During the conditioning phase, mice in the GRID+ conditioning subgroups received an intragastric ethanol (4 g/kg) infusion immediately before 5-min exposure to the grid floor on CS+ trials; water was infused before exposure to the hole floor on CS − trials. These contingencies were reversed for mice in the GRID − conditioning subgroups. Each conditioning subgroup contained 18–19 mice.

first test after four conditioning trials of each type, although there was a trend toward place preference in the 2-g/kg group (left panel, Fig. 2). Between-groups factorial (Experiment 1: Dose × Conditioning Subgroup) and one-way (Experiment 2: Conditioning Subgroup) ANOVAs confirmed that there was no significant effect of Conditioning Subgroup during Test 1 in either experiment (all P's > .05). After two more conditioning trials, the 4-g/kg dose produced a significant CPA during Test 2 in both experiments. This conclusion was supported by the finding of a significant Dose × Conditioning Subgroup interaction in Experiment 1 [$F(1,28) = 4.3$, $P < .05$] and simple effect follow-up analyses showing a Conditioning Subgroup difference at 4 g/kg [$F(1,14) = 5.2$, $P < .04$], but not at 2 g/kg ($F < 1$). The main effect of Conditioning Subgroup was also significant for the 4-g/kg group in Experiment 2 [$F(1,35) = 5.9$, $P = .02$]. Mean activity rates for each dose group during the preference tests ranged between 35.1 and 40.4 cpm. There were no significant differences in test activity between the two dose groups in Experiment 1 and no difference between the two 4-g/kg groups across experiments.

2.2.3. Blood-ethanol concentrations

In order to characterize blood-ethanol levels produced by intragastric infusion, a subset of mice from Experiment 2 received an additional infusion (2 or 4 g/kg) about 10–14 days after the second preference test. Tail-blood samples (20 μl) were taken at 5, 15, 30 and 60 min after injection and were analyzed by gas chromatograph using previously published procedures (Crabbe et al., 1982). Mean blood-ethanol concentrations at each time point are listed in Table 2. As can be seen, blood-ethanol levels were positively related to infused dose and peaked at about 30 min. Blood-ethanol concentration at the time point corresponding to the end of the conditioning trial (5 min) was appreciable, but still only 40–45% of peak level (30 min). Two-way ANOVA (Dose × Time) yielded significant main effects of Dose [$F(1,10) = 20.6$, $P < .002$] and Time [$F(3,30) = 13.6$, $P < .0001$]; the interaction was not significant [$F(3,30) = 2.4$, $P = .09$].

Table 2
Blood ethanol concentrations after intragastric infusion of ethanol (mg/ml ± S.E.M.)

Dose	5 min	15 min	30 min	60 min
2 g/kg ($n = 6$)	0.48 ± 0.09	0.95 ± 0.10	1.08 ± 0.13	0.87 ± 0.12
4 g/kg ($n = 6$)	0.84 ± 0.13	1.52 ± 0.14	2.12 ± 0.30	1.87 ± 0.26

2.3. Discussion

In contrast to recent findings in genetically selected rats (Ciccocioppo et al., 1999), Experiments 1–2 offered no evidence that the intragastric route of administration enhanced sensitivity to ethanol's rewarding effect in mice. In fact, under conditions in which intraperitoneal ethanol injection normally produces a robust CPP in DBA/2 mice, intragastric administration produced either no place conditioning (2 g/kg) or place aversion (4 g/kg). The latter finding was quite unexpected because there are no previous reports of CPA in mice given intraperitoneal ethanol injections under identical conditions. In fact, this procedure has consistently been found to produce CPP in mice given intraperitoneal ethanol (e.g., Chester and Cunningham, 1998, 1999a,b; Chester et al., 1998; Cunningham, 1995; Cunningham and Prather, 1992; Cunningham et al., 1991, 1992, 1993, 1995, 1997, 1998, 2000; Dickinson and Cunningham, 1998; Risinger and Oakes, 1996; Risinger et al., 1992, 1994; Nocjar et al., 1999; Itzhak and Martin, 2000; Grahame et al., 2001; Thrasher et al., 1999), even at doses of 4 g/kg (Cunningham et al., 1992, 1996).

At first glance, this outcome suggests the puzzling conclusion that intragastric ethanol has aversive effects in mice whereas intraperitoneal and intravenous ethanol have rewarding effects. Such differences might be explained, at least in part, by arguing that ethanol reward actually depends on the relatively rapid increase in brain ethanol level produced by intraperitoneal or intravenous administration. In the absence of this rapid increase, intragastric ethanol fails to produce a rewarding effect and is actually aversive, perhaps due to nausea. Note, however, that this explanation is exactly opposite the one offered to explain why the intragastric route increases sensitivity to ethanol reward in genetically selected rats (Ciccocioppo et al., 1999).

3. Experiments 3–4

One might be able to resolve the apparent discrepancy between effects of intragastric and intraperitoneal ethanol in mice by considering the results of recent studies showing that intraperitoneal injection of ethanol produces CPA in mice when the injection is given immediately *after* exposure to the CS+ (Cunningham and Henderson, 2000; Cunningham et al., 1997, 1998, in press). Although the mechanisms underlying this reversal of intraperitoneal ethanol's effect in the place conditioning procedure are not yet known, it has been suggested that CPP and CPA reflect independently mediated rewarding and aversive effects of ethanol, respectively (Cunningham and Henderson, 2000; Cunningham et al., in press). More specifically, intraperitoneal injection of ethanol has been hypothesized to produce an initial short-duration aversive effect that is followed by a longer-lasting rewarding effect (Cunningham and Henderson, 2000; Cunningham et al., 1997). Presumably, the initial aversive effect has substantially dissipated and been replaced by the delayed rewarding effect by the time of CS+ exposure in the standard place conditioning procedure, resulting in a preference for CS+. However, when mice are injected after CS+ exposure, the forward temporal relationship facilitates association of CS+ with ethanol's initial aversive effect.

To extend the foregoing analysis to the present situation, one can assume that intragastric ethanol also produces bivalent motivational effects, but that these effects are slightly delayed due to slower absorption via the intragastric route (Nurmi et al., 1994). Analysis of conditioning trial activity data generally supports this suggestion by showing peak activation around the third minute of the trial, which is about a minute later than the time of peak effect when DBA/2 mice receive ethanol by intraperitoneal injection (Cunningham and Prather, 1992). Because the onset of ethanol's initial aversive effect is presumably delayed until shortly after the onset of the CS+, one can argue that pre-CS intragastric administration is functionally similar to post-CS intraperitoneal administration in promoting the development of a learned aversion to the CS+. A further implication of this interpretation is that one should be able to reduce aversive conditioning and promote the development of CPP after intragastric infusion by allowing absorption for several minutes outside the chamber before CS exposure. This prediction was tested in Experiments 3–4.

3.1. Method

DBA/2J mice were shipped from the Jackson Laboratory at 6–8 weeks of age about 2 weeks before surgery. Of the mice that were successfully implanted with gastric cannulae ($n = 43–45$ in each experiment), four (Experiment 4) to seven (Experiment 3) mice were later removed due to cannula problems, poor health or procedural errors. The remaining mice were exposed to place conditioning procedures that were identical to those used in our earlier experiments with the following exceptions: (a) Ethanol (CS+ trial) or water (CS − trial) infusions were given 5 min *before* the mouse was placed into the conditioning chamber on the assigned floor. Mice were returned to their home cage between the end of the infusion and placement in the chamber. (b) Mice received only four conditioning trials of each type followed by a single 60-min preference test. The design of Experiment 3 was similar to that of Experiment 1, with half the mice randomly assigned to either a 2- or 4-g/kg dose group ($n = 19$/group). The 4-g/kg dose was tested again in Experiment 4 using identical procedures ($n = 26$). Experiment 4 also included a Water-Only group ($n = 13$), which received the same exposure to both floors, but was infused with water 5 min before each trial. Test data for one ethanol-treated mouse in Experiment 4 were lost due to an equipment problem.

3.2. Results

3.2.1. Conditioning activity

The overall pattern of activity on CS+ trials during the second 5 min after intragastric ethanol infusion in Experiments 3 and 4 (Fig. 4) was generally similar to that observed during the first 5 min after infusion in Experiments 1 and 2 (Fig. 1). That is, ethanol produced an acute dose-dependent increase in activity and repeated exposure produced sensitization at the 4-g/kg dose. Data from ethanol-treated mice in Experiments 3 (Dose × CS Type × Trials) and 4 (CS Type × Trials) were analyzed by factorial ANOVA. Both analyses yielded significant main effects of CS Type and Trials and a significant CS Type × Trials interaction (all P's $\leq .001$). The analysis of Experiment 3 also showed a significant effect of Dose, a significant Dose × CS Type interaction, and a significant three-way interaction (all P's $< .02$). Separate planned comparisons of activity on the first and last trials of each type within each group indicated a significant increase in ethanol-stimulated activity (i.e., sensitization) in the 4-g/kg groups in both experiments [Experiment 3: $F(1,18) = 8.5$, $P < .01$; Experiment 4: $F(1,25) = 46.7$, $P < .0001$], but not in the 2-g/kg group

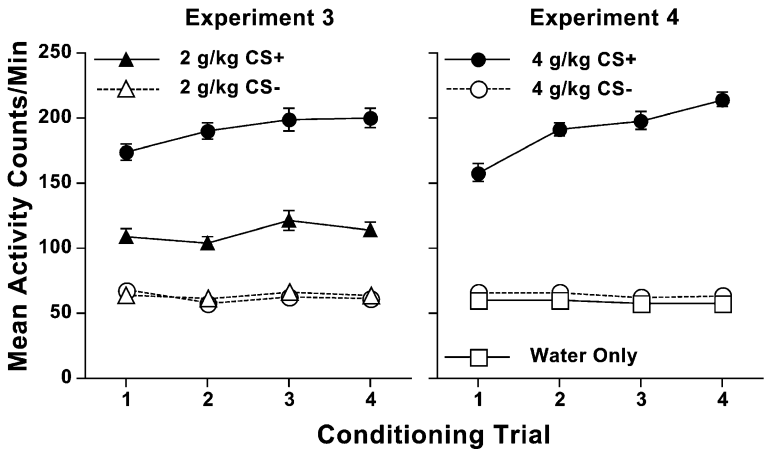

Fig. 4. Mean activity counts per minute (±S.E.M.) during each CS+ (solid symbols) and CS− (open symbols) conditioning trial in Experiments 3 (left panel) and 4 (right panel). DBA/2J mice were used in both experiments. All mice received an intragastric ethanol (2 or 4 g/kg) infusion 5 min before each CS+ trial and a water infusion 5 min before each CS− trial. The Water-Only group (Experiment 4) received water infusions on all trials. Data for ethanol-treated mice are collapsed over GRID+ and GRID− conditioning subgroups (Experiment 3: $n=19$ per dose group; Experiment 4: $n=26$ in the 4-g/kg group; $n=13$ in the Water-Only group).

($F<1$). The 4-g/kg group showed a significant decrease across CS− trials in Experiment 3 [$F(1,18)=4.7$, $P<.05$], but not in Experiment 4 [$F(1,25)=1.3$]. The 2-g/kg group showed no change across CS− trials ($F<1$). Finally, a Group × Trials ANOVA showed no differences between the Water-Only group's activity (averaged across consecutive pairs of conditioning trials) and the CS− trial activity of the 4-g/kg group in Experiment 4 (all F's < 1.9).

Activity scores during each minute of the first trial of each type are listed in Table 1. As can be seen, the temporal pattern of activity on the first CS+ (ethanol) trial in Experiments 3–4 was different from that observed in Experiments 1–2. That is, ethanol-induced activation had already reached its peak by the first minute and declined steadily over the 5-min trial. Planned comparisons showed a highly significant difference between CS+ and CS− during the first minute at both doses in both experiments (all F's>65, P's < .0001). That difference remained relatively constant over time. Activity on the first CS− (water) trial decreased over time, paralleling the decline seen on the CS+ trial.

3.2.2. Preference test

Fig. 5 shows the outcome of the preference test for both experiments. As in Experiment 1, administration of 2 g/kg

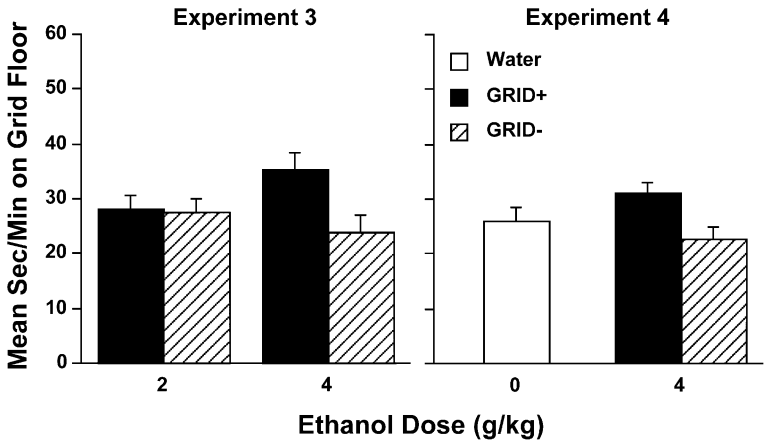

Fig. 5. Mean seconds per minute (+S.E.M.) spent on the grid floor during the 60-min test session in Experiments 3 (left panel) and 4 (right panel). The test was given after eight conditioning trials (four CS+ and four CS− trials). During the conditioning phase, mice in the GRID+ conditioning subgroups received an intragastric ethanol (2 or 4 g/kg) infusion 5 min before a 5-min exposure to the grid floor on CS+ trials; water was infused 5 min before exposure to the hole floor on CS− trials. These contingencies were reversed for mice in the GRID− conditioning subgroups. Mice in the Water-Only group received water infusions on both types of trials. Each conditioning subgroup contained 8–14 mice.

ig ethanol did not produce place conditioning. However, in contrast to Experiments 1–2, 4 g/kg ethanol produced CPP. Thus, insertion of a 5-min delay between infusion and exposure to CS+ caused a reversal in the direction of place conditioning induced by the higher ethanol dose. A two-way (Dose × Conditioning Subgroup) ANOVA of data from Experiment 3 yielded a significant main effect of Conditioning Subgroup [$F(1,34) = 4.8$, $P < .04$]. A one-way (Conditioning Subgroup: GRID+ vs. GRID − vs. Water) ANOVA of data from Experiment 4 also revealed a significant main effect [$F(2,35) = 4.1$, $P < .03$]. The Dose × Conditioning Subgroup interaction (Experiment 3) fell short of the criterion for significance [$F(1,34) = 3.5$, $P = .07$]. Planned comparisons between the GRID+ and GRID − subgroups within each dose group confirmed the development of CPP in both 4-g/kg groups (Bonferroni-corrected P's < .03), but not in the 2-g/kg group (Bonferroni-corrected $P > .9$). The Water-Only group, whose test performance fell between that of the GRID+ and GRID − subgroups in Experiment 4, did not differ significantly from either of those subgroups. A two-way (Dose × Conditioning Subgroup) ANOVA showed no differences in Experiment 3 test session activity rates, which ranged between 38.2 and 41.2 cpm. In Experiment 4, one-way (Conditioning Subgroup) ANOVA yielded a significant group difference in test activity [$F(2,35) = 4.2$, $P < .03$], reflecting higher activity rates in the GRID+ subgroup (48.2 ± 5.1) than in the GRID − (36.5 ± 1.3) and Water-Only (38.6 ± 2.1) subgroups.

3.3. Discussion

Experiments 3–4 showed development of CPP when intragastric infusion of a high (4 g/kg) ethanol dose was separated from CS+ exposure by a 5-min delay. This finding supports the suggestion that the delayed effects of intragastric ethanol, like those of intraperitoneal ethanol, are rewarding. By allowing a few minutes for intragastric ethanol to be absorbed before CS exposure, we presumably avoided pairing the CS with the short-lived aversive effect that has been hypothesized to accompany the onset of ethanol intoxication in drug-naive mice (Cunningham et al., 1997, in press). This delay period is apparently required in intragastric studies, but not in intraperitoneal studies, due to slower onset of ethanol's effects via the intragastric route. Minute-by-minute analysis of ethanol's locomotor activating effect confirmed that a 5-min delay was sufficient to allow substantial expression of at least one prominent behavioral effect of intragastric ethanol prior to the onset of CS exposure.

Although the finding of CPP after intragastric infusion of ethanol in mice is generally consistent with the finding of CPP after intragastric infusion in genetically selected rats (Ciccocioppo et al., 1999), Experiments 3–4 do not support the suggestion that the intragastric route of administration enhanced sensitivity to ethanol's rewarding effect in mice. Given previous reports of robust CPP induced by intraperitoneal injection of the lower (2 g/kg) ethanol dose using a delay as long as 30 min (Cunningham et al., 1997), it appears that the intragastric route caused a reduction in sensitivity to ethanol's rewarding effect (i.e., a rightward shift in the CPP dose–effect curve). Because peak brain ethanol levels after intragastric infusion are generally lower than those produced by intraperitoneal injection of the same ethanol dose (Nurmi et al., 1994), this outcome suggests that ethanol's rewarding effect is positively related to peak ethanol concentration in mice. At the same time, these studies clearly show that other factors, such as the temporal relationship between ethanol and CS exposure, play a critical role in determining whether CPP is observed.

Even at the high ethanol dose, the magnitude of CPP observed with the intragastric procedure was relatively modest compared to that typically produced by intraperitoneal injection of the lower dose. For example, we recently reported that DBA/2J mice given four conditioning trials with 2 g/kg ip ethanol spent about 75% of the test on the ethanol-paired floor (Cunningham et al., in press) compared to the approximately 58% time spent by the 4-g/kg ig ethanol groups in Experiments 3–4. Although statistically significant differences between our GRID+ and GRID − subgroups provide sufficient evidence of place conditioning (Cunningham, 1993), one might interpret the lack of difference between either of those subgroups and the Water-Only group (Experiment 4) as further evidenced that the CPP produced by intragastric ethanol was relatively weak. One possible explanation for the modest CPP is that a 5-min delay does not provide optimal overlap between the CS and intragastric ethanol's rewarding effect. This suggestion is consistent with the general observation of a longer delay to peak brain ethanol after intragastric infusion than after intraperitoneal injection (Nurmi et al., 1994) and with our finding that blood-ethanol concentration did not peak until 30 min after infusion (Table 2). Future CPP studies will need to examine other delay intervals to address this issue.

4. General discussion

Overall, these experiments showed that effects of intragastric ethanol on place conditioning in mice are completely reversed when a short temporal delay (5 min) separates the infusion from exposure to the CS+. Whereas immediate exposure to the CS after infusion produced CPA (Experiments 1–2), delayed exposure produced CPP (Experiments 3–4). This effect was observed only at the higher (4 g/kg) ethanol dose. The lower (2 g/kg) dose failed to produce place conditioning under either condition.

The present findings do not support the suggestion from the rat place conditioning literature that ethanol's rewarding effect is enhanced when the drug is given via the intragastric route (Ciccocioppo et al., 1999). More generally, these studies do not suggest a way to reconcile the disparate findings from ethanol place conditioning studies conducted

in rats and mice. However, the overall conclusions from our intragastric place conditioning studies in mice are generally consistent with those from our previous intraperitoneal studies. That is, administration of ethanol by either route is capable of producing either CPP or CPA depending on ethanol dose and the temporal relationship between exposure to ethanol and exposure to CS+. Although the parameters differ for each route of administration, it appears that the same underlying processes can be invoked to explain how manipulation of injection timing affects the direction of ethanol-induced place conditioning. More specifically, in both cases, CPA can be attributed to an initial, short-lived aversive effect, whereas CPP can be attributed to a delayed rewarding effect of ethanol (Cunningham et al., 1997).

The exact nature of the hypothesized short-duration aversive effect is unclear. The observation that CPA can be produced by either intraperitoneal or intragastric ethanol argues against the suggestion that CPA produced by intraperitoneal injection of ethanol is caused simply by peritoneal irritation (Cunningham et al., 1997). We have previously proposed that the aversive effect may be related to novelty of the rapid transition from the sober to the intoxicated state produced by bolus drug injections (Cunningham and Henderson, 2000; Cunningham et al., 1997, in press). This suggestion is supported by studies showing that CPA induced by post-CS injection is reduced in mice that are already intoxicated at the time of the conditioning trial (Cunningham et al., in press) and in mice given repeated home-cage exposure to ethanol before conditioning (Cunningham and Henderson, 2000; Cunningham et al., in press). Moreover, this interpretation is consistent with studies showing that post-CS injection of other abused drugs such as nicotine and amphetamine also produce CPA (Cunningham et al., 2001; Fudala and Iwamoto, 1987, 1990). The fact that a higher dose and a larger number of conditioning trials (4 g/kg $\times$ 6 trials) were required to produce CPA with intragastric ethanol than with intraperitoneal ethanol (2 g/kg $\times$ 2 trials: Cunningham et al., in press) might indicate that the aversive nature of the drug state transition is reduced because of the slower onset or lower peak level produced by intragastric infusion. However, it is also possible that the weaker CPA reflects CS overlap with ethanol's delayed rewarding effect.

Locomotor activity data recorded during conditioning trials in the present studies are of interest for several reasons. First, these data appear to provide the first demonstration of ethanol-induced activation and sensitization in mice given ethanol via the intragastric route. Based on comparison to recent studies in our laboratory involving intraperitoneal administration, it appears that intragastric administration shifted the dose–effect curve to the right. For example, the activation and sensitization produced by intragastric infusion of 4 g/kg (Figs. 1 and 4) are quite similar to that produced by intraperitoneal injection of 2 g/kg in DBA/2J mice (e.g., Cunningham et al., in press). The locomotor activity data are also of interest because they support conclusions from many previous studies involving intraperitoneal injection in showing dissociation between ethanol-induced locomotor activation/sensitization and ethanol's rewarding effects as indexed by CPP (e.g., Cunningham, 1995; Risinger et al., 1994). That is, despite producing a robust activation and sensitization during CS+ conditioning trials, the high ethanol dose did not always produce CPP.

In summary, these studies are the first to examine place conditioning and locomotor activation induced by intragastric ethanol administration in mice. They demonstrate that intragastric ethanol can produce either CPP or CPA, outcomes that are qualitatively similar to those produced by intraperitoneal ethanol in mice. Our interpretation of these findings is that ethanol has time-dependent, bivalent motivational (i.e., aversive and rewarding) effects that must be considered whenever ethanol is given to mice via the intragastric or intraperitoneal route of administration.

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References

Black RW, Albiniak T, Davis M, Schumpert J. A preference in rats for cues associated with intoxication. Bull Psychonom Soc 1973;2:423–4.

Bormann NM, Cunningham CL. The effects of naloxone on expression and acquisition of ethanol place conditioning in rats. Pharmacol, Biochem Behav 1997;58:975–82.

Bormann NM, Cunningham CL. Ethanol-induced conditioned place aversion in rats: effect of interstimulus interval. Pharmacol, Biochem Behav 1998;59:427–32.

Bozarth MA. Evidence for the rewarding effects of ethanol using the conditioned place preference method. Pharmacol, Biochem Behav 1990; 35:485–7.

Chester JA, Cunningham CL. Modulation of corticosterone does not affect the acquistion or expression of ethanol-induced conditioned place preference in DBA/2J mice. Pharmacol, Biochem Behav 1998;59:67–75.

Chester JA, Cunningham CL. Baclofen alters ethanol-stimulated activity but not conditioned place preference or taste aversion in mice. Pharmacol, Biochem Behav 1999a;63:325–31.

Chester JA, Cunningham CL. $GABA_A$ receptors modulate ethanol-induced conditioned place preference and taste aversion in mice. Psychopharmacology 1999b;144:363–72.

Chester JA, Risinger FO, Cunningham CL. Ethanol reward and aversion in mice bred for sensitivity to ethanol withdrawal. Alcohol: Clin Exp Res 1998;22:468–73.

Ciccocioppo R, Panocka I, Froldi R, Quitadamo E, Massi M. Ethanol induces conditioned place preference in genetically selected alcohol-preferring rats. Psychopharmacology 1999;141:235–41.

Crabbe JC, Janowsky JS, Young ER, Kosobud A, Stack J, Rigter H. Tolerance to ethanol hypothermia in inbred mice: genotypic correlations with behavioral responses. Alcohol: Clin Exp Res 1982;6:446–58.

Cunningham CL. Flavor and location aversions produced by ethanol. Behav Neural Biol 1979;27:362–7.
Cunningham CL. Spatial aversion conditioning with ethanol. Pharmacol, Biochem Behav 1981;14(2):1–2.
Cunningham CL. Pavlovian drug conditioning. In: van Haaren F, editor. Methods in behavioral pharmacology. Amsterdam: Elsevier, 1993. pp. 349–81.
Cunningham CL. Localization of genes influencing ethanol-induced conditioned place preference and locomotor activity in BXD recombinant inbred mice. Psychopharmacology 1995;120:28–41.
Cunningham CL, Henderson CM. Ethanol-induced conditioned place aversion in mice. Behav Pharmacol 2000;11:591–602.
Cunningham CL, Niehus JS. Drug-induced hypothermia and conditioned place aversion. Behav Neurosci 1993;107:468–79.
Cunningham CL, Prather LK. Conditioning trial duration affects ethanol-induced conditioned place preference in mice. Anim Learn Behav 1992;20:187–94.
Cunningham CL, Hallett CL, Niehus DR, Hunter JS, Nouth L, Risinger FO. Assessment of ethanol's hedonic effects in mice selectively bred for sensitivity to ethanol-induced hypothermia. Psychopharmacology 1991; 105:84–92.
Cunningham CL, Niehus DR, Malott DH, Prather LK. Genetic differences in the rewarding and activating effects of morphine and ethanol. Psychopharmacology 1992;107:385–93.
Cunningham CL, Niehus JS, Noble D. Species difference in sensitivity to ethanol's hedonic effects. Alcohol 1993;10:97–102.
Cunningham CL, Dickinson SD, Okorn DM. Naloxone facilitates extinction but does not affect acquisition or expression of ethanol-induced conditioned place preference. Exp Clin Psychopharmacol 1995;3:330–43.
Cunningham CL, Okorn DM, Howard CE. Ethanol-induced conditioned place preference and activation in 15 inbred mouse strains. Alcohol: Clin Exp Res 1996;20:59A.
Cunningham CL, Okorn DM, Howard CE. Interstimulus interval determines whether ethanol produces conditioned place preference or aversion in mice. Anim Learn Behav 1997;25:31–42.
Cunningham CL, Henderson CM, Bormann NM. Extinction of ethanol-induced conditioned place preference and conditioned place aversion: effects of naloxone. Psychopharmacology 1998;139:62–70.
Cunningham CL, Howard MA, Gill SJ, Rubinstein M, Low MJ, Grandy DK. Ethanol-conditioned place preference is reduced in dopamine D2 receptor-deficient mice. Pharmacol, Biochem Behav 2000;67:693–9.
Cunningham CL, Tull LE, Lee LE, Clemans JM. Post-CS injection of abused drugs produces conditioned place aversion. Soc Neurosci Abstr 2001;27 (Program No. 647.19).
Cunningham CL, Tull LE, Rindal KE, Meyer PJ (in press). Distal and proximal pre-exposure to ethanol in the place conditioning task: tolerance to aversive effect, sensitization to activating effect, but no change in rewarding effect. Psychopharmacology.
Dickinson SD, Cunningham CL. Altered ambient temperature and ethanol-induced conditioned place preference in mice. Alcohol 1998;16:13–8.
Fudala PJ, Iwamoto ET. Conditioned aversion after delay place conditioning with nicotine. Psychopharmacology 1987;92:376–81.
Fudala PJ, Iwamoto ET. Conditioned aversion after delay place conditioning with amphetamine. Pharmacol, Biochem Behav 1990;35:89–92.
Gauvin DV, Holloway FA. Historical factors in the development of EtOH-conditioned place preference. Alcohol 1992;9:1–7.
Gauvin DV, Briscoe RJ, Goulden KL, Holloway FA. Aversive attributes of ethanol can be attenuated by dyadic social interaction in the rat. Alcohol 1994;11:247–51.
Grahame NJ, Chester JA, Rodd-Henricks K, Li T-K, Lumeng L. Ethanol place preference conditioning in high- and low-alcohol preferring selected lines of mice. Pharmacol, Biochem Behav 2001;68:805–14.
Holloway FA, King DA, Bedingfield JB, Gauvin DV. Role of context in ethanol tolerance and subsequent hedonic effects. Alcohol 1992;9:109–16.
Itzhak Y, Martin JL. Blockade of alcohol-induced locomotor sensitization and conditioned place preference in DBA mice by 7-nitroindazole. Brain Res 2000;858:402–7.
Kelley BM, Bandy AL, Middaugh LD. A study examining intravenous ethanol-conditioned place preference in C57BL/6J mice. Alcohol: Clin Exp Res 1997;21:1661–6.
Koopmans HS. Surgical methods in the study of ingestive behavior. In: Toates FM, Rowland NE, editors. Feeding and drinking. Amsterdam: Elsevier, 1987. pp. 317–65.
Marglin SH, MacKechnie DK, Mattie ME, Hui Y, Reid LD. Ethanol with small doses of morphine establishes a conditioned place preference. Alcohol 1988;5:309–13.
Matsuzawa S, Suzuki T, Misawa M. Conditioned fear stress induces ethanol-associated place preference in rats. Eur J Pharmacol 1998;341:127–30.
Matsuzawa S, Suzuki T, Misawa M, Nagase H. Different roles of μ-, δ- and κ-opioid receptors in ethanol-associated place preference in rats exposed to conditioned fear stress. Eur J Pharmacol 1999;368:9–16.
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;65:281–8.
Nocjar C, Middaugh LD, Tavernetti M. Ethanol consumption and place-preference conditioning in the alcohol-preferring C57BL/6 mouse: relationship with motor activity patterns. Alcohol: Clin Exp Res 1999;23: 683–92.
Nurmi M, Kiianmaa K, Sinclair JD. Brain ethanol in AA, ANA, and Wistar rats monitored with one-minute microdialysis. Alcohol 1994;11:315–21.
Reid LD, Hunter GA, Beaman CM, Hubbell CL. Toward understanding ethanol's capacity to be reinforcing: a conditioned place preference following injections of ethanol. Pharmacol, Biochem Behav 1985;22: 483–7.
Risinger FO, Oakes RA. Dose- and conditioning-trial dependent ethanol-induced conditioned place preference in Swiss–Webster mice. Pharmacol, Biochem Behav 1996;55:117–23.
Risinger FO, Dickinson SD, Cunningham CL. Haloperidol reduces ethanol-induced motor activity stimulation but not conditioned place preference. Psychopharmacology 1992;107:453–6.
Risinger FO, Malott DH, Prather LK, Niehus DR, Cunningham CL. Motivational properties of ethanol in mice selectively bred for ethanol-induced locomotor differences. Psychopharmacology 1994;116:207–16.
Sherman JE, Hickis CF, Rice AG, Rusiniak KW, Garcia J. Preferences and aversions for stimuli paired with ethanol in hungry rats. Anim Learn Behav 1983;11:101–6.
Stewart RB, Grupp LA. An investigation of the interaction between the reinforcing properties of food and ethanol using the place preference paradigm. Prog Neuropsychopharmacol 1981;5:609–13.
Stewart RB, Grupp LA. Some determinants of the motivational properties of ethanol in the rat: concurrent administration of food or social stimuli. Psychopharmacology 1985;87:43–50.
Stewart RB, Grupp LA. Conditioned place aversion mediated by orally self-administered ethanol in the rat. Pharmacol, Biochem Behav 1986;24: 1369–75.
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;32:431–7.
Stewart RB, Murphy JM, McBride WJ, Lumeng L, Li T-K. Place conditioning with alcohol in alcohol-preferring and -nonpreferring rats. Pharmacol, Biochem Behav 1996;53:487–91.
Thrasher MJ, Freeman PA, Risinger FO. Clozapine's effects on ethanol's motivational properties. Alcohol: Clin Exp Res 1999;23:1377–85.
Tzschentke TM. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. Prog Neurobiol 1998;56:613–72.
van der Kooy D, O'Shaughnessy M, Mucha RF, Kalant H. Motivational properties of ethanol in naive rats as studied by place conditioning. Pharmacol, Biochem Behav 1983;19:441–5.