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Dose- and Conditioning Trial-Dependent Ethanol-Induced Conditioned Place Preference in Swiss–Webster Mice

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RISINGER, F. O. AND R. A. OAKES. *Dose- and conditioning trial-dependent ethanol-induced conditioned place preference in Swiss–Webster mice.* PHARMACOL BIOCHEM BEHAV **55**(1) 117–123, 1996.—The motivational effects of ethanol were examined in Swiss–Webster mice using an unbiased place conditioning design. Adult male Swiss–Webster mice received six 5-min pairings of a tactile stimulus with different doses of ethanol (1, 2, 3, or 4 g/kg, IP). A different tactile stimulus was paired with saline injections. A 60-min preference test was given after the first four conditioning trials and an additional 30-min preference test after the sixth conditioning trial. During conditioning, ethanol initially produced locomotor stimulation at the 2 g/kg dose and locomotor depression at the 4 g/kg dose. However, after repeated ethanol exposure, all doses produced overall increases in activity relative to saline, suggesting sensitization to ethanol's stimulant effect. After four conditioning trials ethanol-induced conditioned place preference was noted in mice receiving 3 and 4 g/kg ethanol. After two additional conditioning trials all ethanol doses produced conditioned place preference. These results indicate that ethanol has dose-dependent rewarding effects measured in an unbiased place-conditioning paradigm using a standard outbred mouse strain. Further, additional place-conditioning trials enhance the development of preference at lower (1 or 2 g/kg) ethanol doses.

Ethanol Mice Reward Locomotor activity Place conditioning

PLACE-CONDITIONING procedures are based on the establishment of a Pavlovian relationship between distinctive environmental cues and some effects of a drug (5). Subsequent approach or withdrawal behaviors to drug-paired cues are thought to index drug motivational properties. A number of place-conditioning designs have established that many abused or self-administered (i.e., reinforcing) drugs will produce conditioned place preference (5,23,48). One frequent exception, however, has been ethanol (43). Although a number of studies have shown ethanol conditioned place preference (2,4,28,34,44, 45), the majority of reports indicate that, in rats, ethanol produces either no conditioning or place aversion (1,9,10,15,22, 42,44–47,51).

Although rats have been a species used frequently in drug place-conditioning studies (5,23,48,50), mice also appear suited to the study of motivational drug effects using these designs [e.g. (13,17,25,37)]. In particular, mice appear to be sensitive to place conditioning with ethanol. Beginning in 1991, Cunningham and colleagues (12) began reporting reliable ethanol-induced conditioned place preference in inbred and selectively bred mice using a procedure based on pairing distinctive tactile cues with IP ethanol exposure (7,12,13,40). Using four 30-min conditioning trials, a range of ethanol doses have been shown to produce reliable place preference. In a study with inbred DBA/2J (D2) and C57BL/6J (B6) mice using 1–4 g/kg ethanol doses, Cunningham et al. (13) found conditioned preference in D2 mice with a 3 g/kg or 4 g/kg ethanol dose. B6 mice did not display conditioned place preference. In a study with selectively bred FAST and SLOW mice [cf. (6,33)] using 0.8–2.0 g/kg ethanol, Risinger et al. found conditioned preference in both strains at 1.0, 1.2, and 2.0 g/kg ethanol doses (40). These reports generally have stood in contrast to results with rats that have often shown that ethanol produces conditioned place aversion at doses of 1 g/kg or above (9,10,22, 42,45–47,51). Further, using comparable procedures and the same dose of ethanol (1.5 g/kg) in both species, Cunningham et al. reported that the finding of conditioned place preference in mice and conditioned place aversion in rats reflects a species

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difference in sensitivity to ethanol's motivational effects (15). The basis for this species difference in ethanol conditioned place preference remains undefined. For example, rats and mice may differ in pharmacokinetic or neurochemical processes [see also discussion in (15)]. However, both species are sensitive to ethanol's aversive effect as measured by taste conditioning (9,12,35,36,40,42).

Although 30-min conditioning trials are effective, ethanol place preference is enhanced when shorter conditioning trial durations are used. Specifically, four 5-min trials produce robust conditioned preference in D2 mice using a 2 g/kg dose (19). Longer trials (i.e., 15 min and 30 min) are associated with the development of less preference. The reliability at which inbred D2 mice acquire ethanol conditioned place preference using these parameters encouraged other studies by this same group investigating various neurochemical mechanisms thought to be related to ethanol's rewarding effects (14,16,38,39). Throughout these studies, ethanol-induced conditioned place preference was reliably seen in D2 mice using a 5-min trial duration and a 2 g/kg ethanol dose [see also (8)].

The purpose of the present experiment was to characterize ethanol-induced conditioned place preference in outbred Swiss–Webster mice. As previously reviewed, a 2 g/kg ethanol dose using four 5-min conditioning trials is optimal for obtaining robust ethanol conditioned place preference in D2 mice. However, a number of preliminary studies in this lab indicated the magnitude of conditioned place preference obtained using these parameters with Swiss–Webster mice was not nearly as great or reliable as that seen in D2 mice, hindering examination of potential neurobiological mechanisms and drug-ethanol interactions [e.g., (14,16,38,39)]. Therefore, in the present study, a range of ethanol doses was used to establish the ethanol doses necessary for robust and reliable place conditioning in this strain. Generally, ethanol was expected to produce conditioned place preference at higher ethanol doses after four conditioning trials [cf. (13)]. Also, additional conditioning trials were given to determine whether enhanced place preference would be seen at lower ethanol doses.

METHOD

Subjects

Male Swiss–Webster mice were obtained from Simonson (Gilroy, CA) at 7 weeks of age and allowed to acclimate to the colony for 6 days prior to the beginning of the experiment. They were housed in polypropylene cages (33 × 16 × 13 cm) with cob type bedding replaced twice weekly. A 12 L:12 D cycle was in effect with the onset of the light portion of the cycle beginning at 0700 h. Experimental procedures were conducted during the light portion of the cycle starting at approximately 0900 h. Food and water were continuously available in the home cage and the colony room temperature was maintained at 22 ± 2°C.

Apparatus

The place-conditioning apparatus was eight identical acrylic and aluminum chambers (30 × 15 × 15 cm), each enclosed in a ventilated, light- and sound-attenuating box (Med Associates ENV-015M; St. Albans, VT). Infrared light sources and detectors were positioned opposite each other at 5-cm intervals on the long walls of each place conditioning chamber, 2.2 cm above the floor surface. Occlusion of the infrared light beams was used both as a measure of general activity and to determine the animal's position (left or right side) in the chamber. Data were recorded each minute by computer.

The floor of each box consisted of interchangeable halves with one of two distinctive textures: hole floors were made from perforated stainless steel with 6.4-mm round holes on 9.5-mm staggered centers; grid floors were composed of 2.3-mm stainless steel rods mounted 6.4 mm apart in Plexiglas rails. This combination of floor types results in equal unconditioned preference in saline-treated Swiss–Webster mice (37).

Procedure

The experimental sequence was as follows: habituation (one 5-min session), conditioning (eight 5-min sessions), testing (one 60-min session), conditioning (four 5-min sessions), testing (one 30-min session). Sessions were conducted daily (5 day/week) with the habituation session occurring on a Monday and both test sessions occurring on a Friday.

Habituation

During habituation, all subjects received saline (10 ml/kg) and were immediately placed in the conditioning apparatus for 5 min on a smooth floor covered with paper. Subjects were not exposed to the distinctive floor textures to avoid the development of latent inhibition (27). The habituation session was intended to reduce the novelty and stress associated with handling, injection and exposure to the apparatus.

Conditioning

During the conditioning phase, mice were randomly assigned to one of four ethanol dose groups: 1.0, 2.0, 3.0, or 4.0 g/kg ethanol. Dose was manipulated by varying the volume of injection of a 20% v/v ethanol/saline mixture [cf. (26)]. Conditioning was conducted using a between-group discrimination design (11). Within each ethanol dose group mice were randomly assigned to one of two conditioning subgroups (n = 14–16/group) and exposed to an unbiased differential conditioning procedure. On alternate days mice received ethanol (CS+ days) prior to placement on the grid floor (Grid+ subgroup) or the hole floor (Grid− subgroup). Mice received saline (CS− days) prior to placement on the other floor type. Therefore, one complete conditioning trial consisted of a pairing of a distinctive floor after ethanol exposure and a pairing of a different floor with saline. Presentation of CS+ and CS− days was counterbalanced for order of presentation. Thus, the conditioning subgroups within each ethanol dose group were matched for exposure to ethanol and floor type, and differed only in the specific floor–ethanol relationship [cf. (11)].

Testing

For the preference tests, all subjects received saline injections before placement in the apparatus for a 60-min session (test 1) or 30-min session (test 2) with half grid floor and half hole floor (left/right position counterbalanced within groups).

Data Analysis

Conditioning activity data were analyzed by unweighted means analysis of variance (ANOVA) using an alpha level of 0.05. For the preference tests, initial analyses consisted of between-group ANOVA comparisons of time on the grid floor with ethanol dose and conditioning subgroup as factors. Planned between-group comparisons of conditioning subgroup at each ethanol dose were conducted using a Bonferroni correction

TABLE 1

MEAN (± SEM) ACTIVITY COUNTS PER MINUTE DURING CONDITIONING

Ethanol (g/kg)	Trial 1	Trial 2	Trial 3	Trial 4*	Trial 5	Trial 6
1 CS+	52.1 (3.7)	34.7 (2.6)	36.5 (2.5)	33.4 (2.8)	52.3 (3.6)	43.7 (2.6)
CS−	49.0 (3.4)	31.9 (2.4)	31.3 (2.8)	28.4 (3.0)	43.6 (3.1)	32.4 (2.5)
2 CS+	75.7 (5.5)	65.7 (5.0)	62.4 (3.5)	58.7 (4.3)	61.5 (4.1)	59.9 (4.5)
CS−	46.7 (2.7)	34.4 (2.1)	29.9 (1.8)	29.3 (2.7)	42.0 (3.4)	32.1 (2.6)
3 CS+	46.6 (4.2)	46.7 (4.3)	55.0 (5.3)	53.7 (5.1)	57.3 (5.0)	59.5 (5.5)
CS−	48.5 (1.7)	31.3 (2.4)	28.7 (2.5)	20.9 (2.5)	33.9 (3.1)	25.4 (2.7)
4 CS+	18.1 (1.9)	20.4 (3.0)	28.1 (3.3)	27.9 (3.0)	33.5 (4.0)	29.4 (3.1)
CS−	45.4 (2.1)	30.4 (2.0)	24.1 (2.2)	19.8 (2.4)	30.1 (3.2)	22.2 (2.3)

*Preference test 1 occurred between conditioning trials 4 and 5.

(24) for family-wise error (alpha of 0.05/four comparisons = corrected alpha of 0.0125 for each followup analysis). Statistically reliable results are $p < 0.01$ unless otherwise noted.

RESULTS

Conditioning

Mean (± SEM) activity counts for each conditioning trial are given in Table 1. During the first conditioning trial ethanol produced locomotor stimulation at the 2 g/kg dose and locomotor depression at the 4 g/kg dose. However, after four ethanol exposures, locomotor stimulation was noted in mice receiving 2, 3, and 4 g/kg ethanol. After six ethanol exposures, all ethanol doses produced locomotor stimulation compared to saline trials. Saline activity levels declined over the course of conditioning.

Overall analysis of activity levels on conditioning trial 1 yielded reliable effects of ethanol dose, $F(3, 121) = 19.0$, and ethanol dose × trial type, $F(1, 121) = 33.8$. Reliable effects of ethanol exposure were seen in the 2 g/kg group, $F(1, 31) = 39.1$, and the 4 g/kg group, $F(1, 29) = 105.0$, but not in the 1 g/kg group, $F(1, 31) = 0.7$, or the 3 g/kg group, $F(1, 30) = 0.2$. On each subsequent trial, reliable effects of ethanol dose and ethanol dose × trial type were seen (all Fs > 4.6). Reliable effects of trial type were seen in the 1 g/kg group on trial 3, $F(1, 31) = 5.5$, $p < 0.03$, and trials 5 and 6 (both Fs > 9.0). In the 2 g/kg group, reliable effects of trial type were seen on each trial (all Fs > 22.9). In the 3 g/kg group, reliable effects of trial type were seen on trials 2–6 (all Fs > 10.5). In the 4 g/kg group, reliable effects of trial type were seen on trial 2, $F(1, 29) = 9.6$, trial 4, $F(1, 29) = 4.3$, $p < 0.04$, and trial 6, $F(1, 29) = 4.0$, $p < 0.05$.

Analysis of activity changes over CS+ conditioning yielded reliable effects of ethanol dose, $F(3, 121) = 26.4$, conditioning trial, $F(5, 605) = 6.2$, and ethanol dose × conditioning trial, $F(15, 605) = 5.7$. Analysis of conditioning trial within each dose group showed reliable trial effects at all doses (all Fs > 2.9, ps < 0.02. Analysis of CS− activity over trials yielded reliable effects of ethanol dose, $F(3, 121) = 3.2$, $p < 0.03$, conditioning trial, $F(5, 605) = 77.6$, and ethanol dose × conditioning trial, $F(15, 605) = 1.7$, $p < 0.05$. Reliable effects of conditioning trial were seen in each ethanol dose group (all Fs > 13.1).

Figures 1 and 2 depict minute by minute activity levels for trials 1 and 6, respectively. During trial 1, 2 g/kg ethanol produced locomotor stimulation after the first minute. In contrast, 4 g/kg ethanol caused locomotor depression after the first minute. During trial 6, the 1 and 2 g/kg ethanol doses produced locomotor activation that was highest after the first 2 min of CS+ trial 6. The 3 g/kg ethanol dose produced locomotor stimulation after the first minute; however, the level of activation decreased over the course of the session. The 4 g/kg ethanol dose produced locomotor stimulation during the first 2 min of the trial, no net locomotor effect during minutes 3 and 4, and locomotor depression during minute 5.

Overall ethanol dose × trial type × minute effects were seen on all trials (all Fs(12, 484) > 9.7. During trial 1, all ethanol dose groups showed a trial type × minute interaction (all Fs > 2.7, ps < 0.03). Follow-up analysis of activity during each minute for the 1 g/kg group yielded a reliable trial type effect only during minute 4, $F(1, 31) = 6.4$, $p < 0.02$. The 2 g/kg group showed reliable trial type effects on minutes 2–5 [all Fs(1, 31) > 17.3]. The 3 g/kg group showed reliable trial type effects only during minute 1, $F(1, 30) = 7.7$. The 4 g/kg group showed reliable trial type effects during minutes 2–5 [all Fs(1, 29) > 5.8, ps < 0.03]. During trial 6, all ethanol dose groups also showed a reliable trial type × minute interaction [all Fs > 7.2]. Follow-up analysis of activity changes in the 1 g/kg group showed reliable trial type effects during minutes 3, 4, and 5 [all Fs(1, 31) > 11.0]. Analysis of the 2 g/kg group showed reliable trial type effects during minutes 2–5 [all Fs(1, 31) > 34.1]. Analysis of the 3 g/kg groups showed reliable trial type effects during minutes 1–4 [all Fs(1, 30) > 6.7, $p <$ 0.02]. Analysis of the 4 g/kg group showed reliable trial type effects during minutes 1, 2, and 5 [all Fs(1, 29) > 5.1, $p < 0.03$].

Preference Tests

Figure 3 depicts the mean (± SEM) seconds per minute on the grid floor during preference testing for both subgroups within each drug treatment condition. The results of the first test (after four trials) are shown on the left and the results of the second test (after two additional trials) are shown on the right. As indicated by the between-group difference between the Grid+ and Grid− subgroups, mice receiving 3 or 4 g/kg ethanol displayed conditioned preference for the ethanol-paired floor during the first test, while the 1 and 2 g/kg groups showed a trend towards preference. However, after two additional trials (i.e., six trials total) all ethanol doses produced conditioned place preference.

Ethanol dose × conditioning group analysis of the first preference test data yielded reliable effects of conditioning group, $F(1, 117) = 57.1$, and ethanol dose × conditioning group, $F(3, 117) = 4.1$. Planned between-group comparisons of conditioning group at each ethanol dose showed conditioned

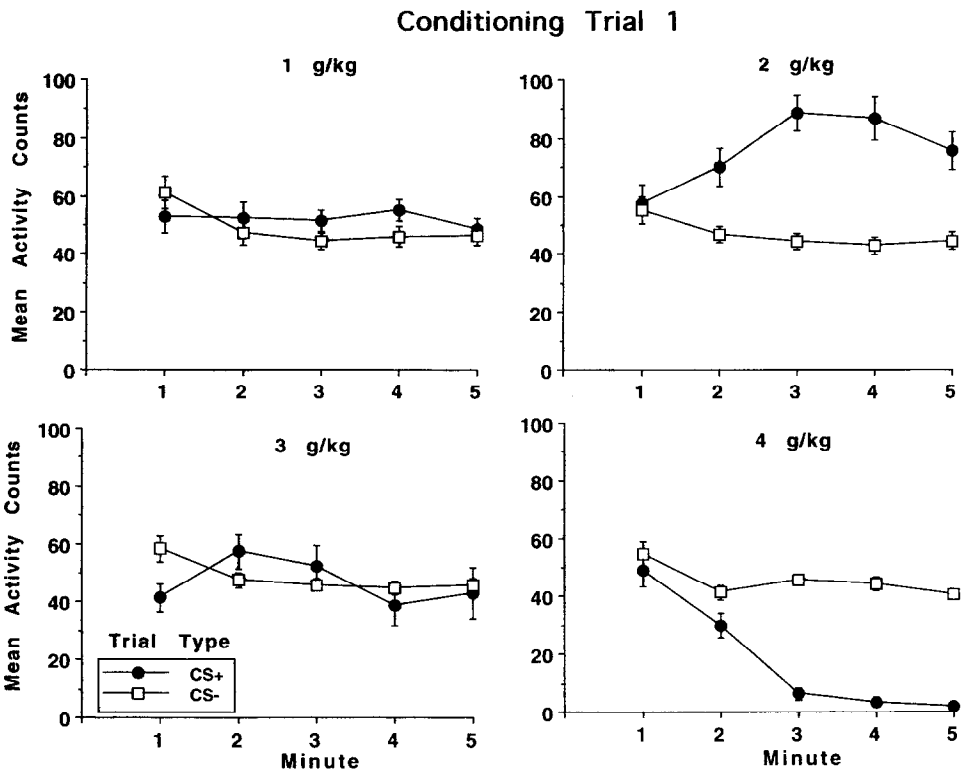

FIG. 1. Mean (± SEM) activity counts during each minute of conditioning trial 1.

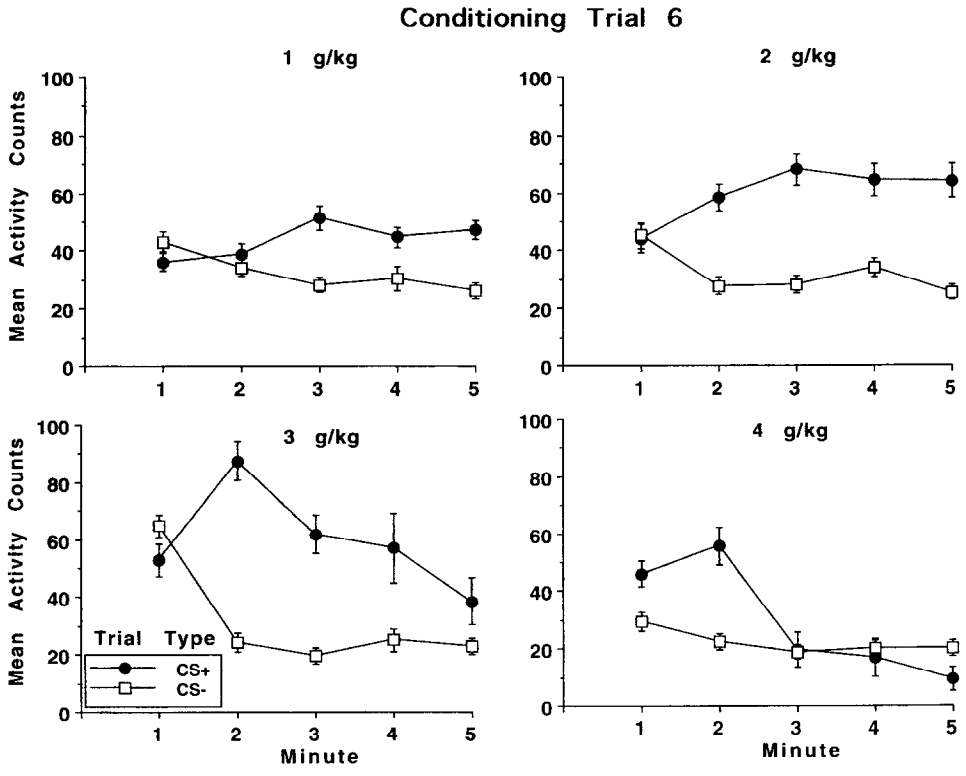

FIG. 2. Mean (± SEM) activity counts during each minute of conditioning trial 6.

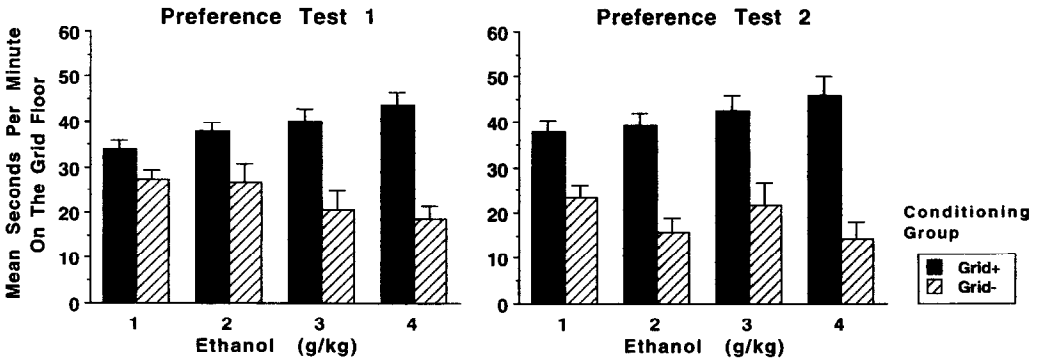

FIG. 3. Mean seconds per minute (± SEM) spent on the grid floor during floor choice testing. Test 1 (after four conditioning trials) is shown in the left panel. Test 2 (after two additional conditioning trials) is shown in the right panel. Grid+ groups had previously received pairings of the grid floor with ethanol (and hole floor with saline), whereas grid− groups had previously received pairings of the grid floor with saline (and hole floor with ethanol). Conditioned place preference is shown when time spent on grid floor by the grid+ group exceeds time spent on the grid floor by the grid− group. Preference for these floor types in the absence of drug is equal in Swiss–Webster mice (37).

preference at the 3 g/kg dose, $F(1, 29) = 14.9$, and the 4 g/kg dose, $F(1, 28) = 45.7$. A trend towards preference was noted in the 1 g/kg group, $F(1, 30) = 5.2$, $p < 0.03$, and the 2 g/kg group, $F(1, 30) = 6.0$, $p < 0.02$. Analysis of the second test showed a reliable effect of conditioning group, $F(3, 117) = 8.3$, but no ethanol dose × conditioning group interaction, $F(3, 117) = 1.9$. Between-group comparisons of conditioning group in each ethanol dose showed reliable preference at all doses (all Fs > 11.8).

An additional analysis compared preference on the first and second test. Overall analysis showed reliable effects of conditioning group, $F(1, 117) = 97.1$, ethanol dose × conditioning group, $F(3, 117) = 3.6$, $p < 0.02$, and conditioning group × test, $F(1, 117) = 8.0$.

Activity levels during each preference test were highest at the start of the sessions and declined over time. All groups showed similar levels of activity during test 1; however, the 3 and 4 g/kg ethanol groups showed a trend towards lower activity levels, as suggested by an ANOVA comparison of ethanol dose, $F(3, 121) = 2.4$, $p < 0.06$. Mean (± SEM) activity counts during test 1 were as follows: 1 g/kg, 35.3 ± 1.7; 2 g/kg, 34.7 ± 2.0; 3 g/kg, 31.5 ± 2.8; 4 g/kg, 28.2 ± 1.9. During test 2, the 4 g/kg ethanol dose group showed lower activity. Mean (± SEM) activity counts during test 2 were as follows: 1 g/kg, 32.7 ± 1.4; 2 g/kg, 33.8 ± 1.9; 3 g/kg, 29.7 ± 3.0; 4 g/kg, 21.8 ± 2.0. Analysis showed reliable effects of ethanol dose, $F(3, 121) = 6.3$.

DISCUSSION

This study provides a within-experiment analysis of dose and trial frequency effects in the acquisition of ethanol-induced conditioned place preference. Ethanol produced reliable conditioned place preference in Swiss–Webster mice, indicating that a readily available standard mouse strain is sensitive to ethanol reward in this design. More importantly, the magnitude of preference depended on both ethanol dose and number of conditioning trials, indicating that consideration of these conditioning parameters is important in determining ethanol's rewarding efficacy in this design. Unlike most other ethanol place-conditioning studies in mice, the present experiment did not use selectively bred (7,12,40) or inbred mice (8,13,14,16,18,19,38,39). Indeed, only one other report of conditioning place preference in mice has used a genetically heterogenous mouse strain (15). In that study, using HS-derived mice, ethanol produced a small conditioned place preference at a 1.5 g/kg dose using four 5-min conditioning trials.

Place conditioning procedures have been used quite frequently in the determination of drug motivational effects (5,23,48). The advantages of this procedure include drug-free testing, sensitivity to both rewarding and aversive drug effects [e.g. (37)], and strict control over the conditions of drug exposure (5). One disadvantage of place-conditioning procedures has been difficulty in characterizing dose–response differences [e.g. (31,41), though see (3)]. The results of the present experiment indicate clear dose-dependent differences in the magnitude of ethanol conditioned preference after four conditioning trials. However, after additional conditioning trials, all ethanol doses produced comparable levels of preference suggesting that dose-dependent differences in this procedure can be obscured or eliminated by development of enhanced preference. Also, the relatively rapid development of robust preference in the present design attests to the sensitivity of the place conditioning procedure to drug motivational effects [cf. (5)].

In mice, ethanol reliably produces locomotor stimulation at moderate dose ranges (20,21,29,30). This response has been subjected to considerable attention, including genetic selection and gene mapping studies (6,32). Further, a homologous relationship between ethanol-stimulated activity and ethanol reinforcement has been proposed (52), although these responses can be dissociated (38). In accord with previous observations [e.g., (20,29,30), see also (15)] ethanol produced locomotor stimulation. However, the notion of a positive relationship between ethanol-stimulated activity and ethanol reward was not generally supported, as evidenced by the finding of similar levels of conditioned preference at doses shown to differ in the profile of ethanol activation. For example, both 3 and 4 g/kg ethanol produced robust conditioned place preference, yet on initial exposure these doses produced either no locomotor stimulation (3 g/kg) or locomotor depression (4 g/kg). Also, after six conditioning trials all ethanol doses produced comparable levels of conditioned preference, while consis-

tently producing different levels of locomotor stimulation throughout conditioning.

Sensitization to ethanol's locomotor activating effect has also been suggested as related to ethanol conditioned preference (18). In the present study, enhanced locomotor stimulation after ethanol was noted with each ethanol dose. Specifically, compared to CS− trials, ethanol produced locomotor stimulation, except at the 4 g/kg dose where the initial response (i.e., on conditioning trials 1 and 2) was locomotor depression. As saline activity levels decreased, ethanol activation was noted at all ethanol doses, suggesting that high ambient activity levels may obscure detection of ethanol's stimulant effects. Alternatively, enhancement of ethanol-stimulated activity is thought to occur after repeated exposure [e.g., (30)]. Sensitization to ethanol-stimulated activity is mediated, in part, by conditioning processes; however, the role of sensitization in development of conditioned preference is unclear (18). The change in ethanol-induced locomotor effects may also be interpreted as due to the development of resistance (i.e., tolerance) to ethanol's locomotor depressant effect (49). In particular, the replacement of locomotor depression with locomotor stimulation in the 4 g/kg ethanol groups is consistent with the development of ethanol tolerance [cf. (29)].

A number of studies have used a design similar to that used in the present experiment in determination of the influence of neurotransmitter selective drugs on the acquisition or expression of ethanol conditioned place preference (14,16,38,39). These studies used D2 mice, a 2 g/kg ethanol dose and four 5-min conditioning trials. However, as indicated by the present results, these conditioning parameters do not yield robust place preference with ethanol in Swiss–Webster mice. Therefore, in neurobiological investigations using this strain, consideration should be given to the choice of ethanol dose and number of conditioning trials that differ from parameters effective with D2 mice. For example, choosing either a higher ethanol dose (e.g., 3 g/kg) or six conditioning trials would allow for analysis of pharmacological reduction in ethanol reward [cf. (38,39)]. Alternatively, if one expects certain pharmacological treatments to enhance ethanol reward, choosing lower ethanol doses and four conditioning trials would be appropriate.

In conclusion, the present experiment provides parametric information on the acquisition of ethanol-induced conditioned place preference, and demonstrates the interaction between ethanol dose and number of conditioning trials in determining the magnitude of ethanol conditioned preference. Similar designs have been used successfully in characterizing ethanol's rewarding effects (8,12–16,18,19,38,39,40), as well as the motivational effects of morphine, methamphetamine, and nicotine (13,17,37). As previously reviewed, place conditioning procedures offer a number of advantages in studies of the neural basis of drug reward (5). Further, recent investigations with inbred mouse strains have suggested partial overlap in the genetic mechanisms controlling ethanol drinking and ethanol-induced conditioned place preference (8). The present study extends these findings by showing ethanol dose and conditioning trial influences on the development of ethanol conditioned place preference. Also, these results enhance the generality of this procedure by establishing the parameters necessary for robust ethanol conditioned place preference in Swiss–Webster mice. Thus, future investigations using this design need not rely on the use of relatively special mouse strains [e.g. (7,8,13,44)].

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