Conditioning trial duration affects ethanol-induced conditioned place preference in mice

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The present experiments were designed to determine the effect of conditioning trial duration on strength of ethanol-induced conditioned place preference in mice. In a counterbalanced, differential conditioning procedure, DBA/2J mice received four pairings of a distinctive tactile (floor) stimulus with injection of ethanol (2 g/kg); a different floor stimulus was paired with saline. Different groups were exposed to the floor stimuli for 5, 15, or 30 min after injection. Conditioned place preference was inversely related to trial duration, with mice in the 5-, 15-, and 30-min groups, spending 83%, 74%, and 66% of their time, respectively, on the ethanol-paired floor during a choice test. This outcome was replicated in a second experiment, which also showed that context familiarity can influence conditioned place preference. In general, these findings suggest that ethanol's rewarding effect is greatest shortly after injection.

Study of the rewarding pharmacological effect of ethanol has been limited primarily to examination of ethanol drinking and preference (Myers & Veale, 1972) or operant self-administration by various routes (Meisch, 1977; Samson, 1987; Samson, Pfeffer, & Tolliver, 1988; Winger, Young, & Woods, 1983). Although these studies offer convincing evidence of ethanol's positive reinforcing effect, they also suggest that ethanol is a relatively weak reinforcer and that special techniques must be used to initiate ethanol-maintained behavior (Samson et al., 1988; Winger et al., 1983). Attempts to use alternative paradigms for studying ethanol reward have had only limited success. For example, although the place conditioning task has been widely used as an alternative to self-administration for studying the rewarding properties of many abused substances, including heroin, morphine, amphetamine, and cocaine (Carr, Fibiger, & Phillips, 1989; Swerdlow, Gilbert, & Koob, 1989), it has generally proved rather disappointing as an alternative for studying ethanol's rewarding effects (Sherman, Jorenby, & Baker, 1988). Most ethanol place conditioning experiments with rats have yielded conditioned place aversion (e.g., Cunningham, 1979, 1981; Sherman, Hickis, Rice, Rusiniak, & Garcia, 1983; Stewart & Grupp, 1981, 1985, 1986,

1989; van der Kooy, O'Shaughnessy, Mucha, & Kalant, 1983). In those few instances where ethanol has produced conditioned place preference, magnitude of preference is rather small and it appears to depend on extensive pre-exposure to ethanol (Reid, Hunter, Beaman, & Hubbell, 1985), a large number of conditioning trials (Bozarth, 1990), or concurrent exposure to another reinforcer (Marglin, MacKechnie, Mattie, Hui, & Reid, 1988; Stewart & Grupp, 1981, 1985).

Recent studies in this laboratory suggest that examination of ethanol's rewarding properties might be more favorably pursued using mice as experimental subjects. This suggestion is based on a number of experiments showing that several inbred or selectively bred lines of mice consistently develop a conditioned preference for ethanol-paired tactile stimuli using a relatively standard place conditioning procedure (Crabbe, Phillips, Cunningham, & Belknap, in press; Cunningham & Noble, in press-a; Cunningham et al., 1991; Cunningham, Niehus, Malott, & Prather, in press; Risinger, Dickinson, & Cunningham, in press; Risinger, Malott, Riley, & Cunningham, in press). The dose-response study reported by Cunningham, Niehus, et al. (in press) is especially interesting because of its potential implications for understanding the variables that affect strength of ethanol-induced conditioned preference. In that study, inbred mice (DBA/2J) exposed to a Pavlovian differential conditioning procedure displayed a dose-dependent preference for a tactile (floor) stimulus presented for 30 min immediately after injection. Specifically, reliable conditioned preference was obtained at doses of 3 and 4 g/kg, and a nonsignificant trend toward preference was observed at 2 g/kg, but no conditioning occurred at 1 g/kg. The finding of conditioned preference at 4 g/kg is especially intriguing, because it is quite likely that most mice in-

jected with that dose were unconscious within 2–3 min after injection (Cunningham, Niehus, et al., in press). Although the occurrence of reliable conditioned place preference at 3 g/kg (which did not result in loss of righting reflex) indicated that the effect did not depend on loss of consciousness, these findings raise the possibility that most, if not all, of the learning about the relationship between tactile cues and ethanol's effects occurred within the first few minutes after injection. Interestingly, even the large dose of ethanol exerted an excitatory effect on locomotor activity during that period of time (Cunningham, Niehus, et al., in press), a finding that appears consistent with theories suggesting a common biological basis for the rewarding and activating effects of addictive drugs (e.g., Wise & Bozarth, 1987).

EXPERIMENT 1

Experiment 1 was designed to examine the role of conditioning trial duration in the development of conditioned preference for ethanol-paired tactile stimuli in DBA/2J mice. Specifically, the 30-min trial duration used by Cunningham, Niehus, et al. (in press) was compared with durations of 5 and 15 min. If ethanol's rewarding effects, like its locomotor activating effects, are greatest during the first few minutes after injection, one would predict that strength of conditioned place preference produced by a short trial duration should be as strong or stronger than that observed at a relatively long duration.

Method

Subjects

Ninety-six adult male inbred mice (DBA/2J) were obtained from the Jackson Laboratory (Bar Harbor, ME) at 6 weeks of age and were allowed to acclimate to the animal colony for 2 weeks before training. They were housed in groups of 4 in polycarbonate cages (27.9×9.5×12.7 cm) with cob bedding at an ambient temperature of 21°±1°C. Water and lab chow were available at all times in the home cage. Experimental procedures were conducted between 0900 and 1600 h during the light phase of a 12:12-h light:dark cycle (lights on at 0700 h).

Apparatus

The apparatus consisted of 12 identical acrylic and aluminum boxes (30×15×15 cm) enclosed in separate, ventilated, light- and sound-attenuating enclosures (Coulbourn Instruments Model E10-20). Infrared light sources and photodetectors (a total of six sets) were mounted opposite each other at 5-cm intervals on the long walls of each box, 2.2 cm above the floor. Occlusion of the infrared light beams was used both as a measure of general activity and to detect the animal's position (left vs. right side) within the box. Three photodetectors monitored activity on one side of the apparatus, while the other three detected activity on the opposite side. A change in side was recorded when all photobeams on one side were inactivated and at least one beam on the opposite side was occluded. Total activity counts and amount of time spent on each side of the chamber were recorded every minute by an Apple II microcomputer (10-msec resolution).

The floor of each box consisted of interchangeable halves made of one of two textures. The grid floor was composed of 2.3-mm stainless steel rods mounted 6.4 mm apart in acrylic rails. The hole floor was made from perforated stainless steel (16 ga) with 6.4-mm round holes on 9.5-mm staggered centers. This combination of floor textures was selected on the basis of previous studies showing that drug-naive control groups spend about half their time on each floor type during preference tests (Cunningham, Niehus, et al., in press; Cunningham & Noble, in press-b). The floors and inside of the box were wiped with a damp sponge, and the litter paper beneath the floors was changed after each animal.

Procedure

The experiment involved three phases: habituation (one session), conditioning (eight sessions), and testing (one session). Sessions were conducted 5 days a week with a 2-day break between the first four and second four conditioning sessions. Each mouse was weighed and injected (i.p.) immediately before being placed in the center of the apparatus for each session.

Habituation. The habituation session was intended to reduce the novelty and stress associated with handling, injection, and exposure to the apparatus. All mice were injected with saline and placed in the conditioning box for 30 min on a smooth floor covered with paper. In order to avoid latent inhibition, the subjects were not exposed to the distinctive floor textures.

Conditioning. The mice were randomly assigned to one of three trial duration groups during the conditioning phase: 5, 15, or 30 min. At each trial duration, the mice were also randomly assigned to one of two conditioning subgroups ($n = 14$–16/group) and exposed to a Pavlovian discriminative conditioning procedure. On all conditioning trials, the subjects had access to both sides of the apparatus and floor texture was homogeneous (cf. Vezina & Stewart, 1987a). On alternate days, mice in the GRID+ subgroups received ethanol prior to placement on the grid floor (CS+ trial), and they received saline prior to placement on the hole floor (CS– trial). In contrast, mice in the GRID– subgroups received saline before placement on the grid floor (CS– trial), and they received ethanol before placement on the hole floor (CS+ trial). Ethanol dose was 2 g/kg (20% v/v in saline vehicle). This dose was selected because it does not induce loss of righting reflex in DBA/2J mice and because other studies conducted in this laboratory suggested it would produce conditioned place preference in a range that might be sensitive to variations in trial duration. Four conditioning trials of each type were given over an 8-day period. Order of exposure to CS+ and CS– was counterbalanced within each subgroup. Because the two conditioning subgroups at each trial duration were matched for overall exposure to each floor type, drug, and saline, and differed only in the floor-drug contingency, any differences between subgroups during preference testing must be attributed to development of a Pavlovian association between the CS+ floor and drug (cf. Cunningham, in press).

Place preference test. The floor preference test was given 24 h after the last conditioning trial. All subjects received a saline injection just before placement in the apparatus with half grid floor and half hole floor. Relative position of the floors (i.e., left vs. right) was counterbalanced within each subgroup. The primary dependent variable was the amount of time spent on the grid floor during the 30-min test session.

Results

Due to procedural errors during the conditioning phase, 3 subjects were eliminated from Experiment 1. Activity and preference test data were analyzed by an unweighted means analysis of variance (ANOVA) using an alpha level of .05. Probability levels for individual statistical tests are indicated only in the instances where $.01 < p < .05$; for all other significant outcomes, $p < .01$.

Conditioning Trials

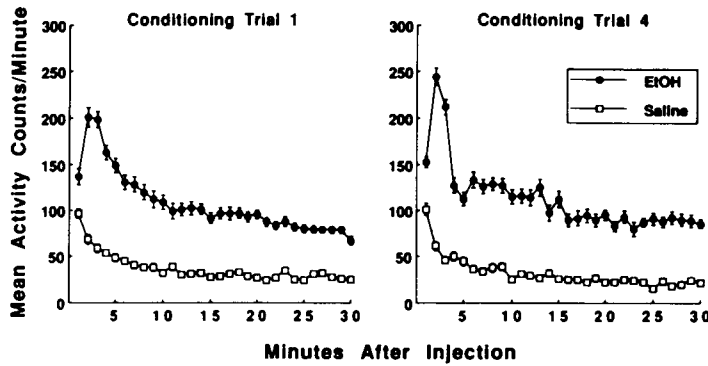In general, ethanol produced an elevation in locomotor activity that increased over trials. The temporal pat-

Figure 1. Mean (±*SEM*) activity counts per minute on the first (left panel) and last (right panel) ethanol and saline conditioning trials for the 30-min group in Experiment 1. Data are collapsed over conditioning subgroups.

tern of activity within trials is illustrated in Figure 1, which shows mean (±*SEM*) activity counts per minute recorded during successive minutes on the first (left panel) and last (right panel) conditioning trials for the 30-min groups. As can be seen in Figure 1, ethanol's stimulant effect was especially pronounced during the first few minutes after injection and persisted for at least 30 min after injection.

In order to compare activity levels across the different duration groups, mean activity rates during the first 5 min of each trial were computed for each mouse. Table 1 lists group mean (±*SEM*) activity rates on the first and last ethanol and saline conditioning trials (averaged across conditioning subgroups). In general, ethanol-induced activity increased over trials, whereas activity recorded after saline injection decreased over trials. The ethanol and saline data shown in Table 1 were examined separately by a two-way (trial duration × conditioning trial) ANOVA. Floor type (i.e., grid vs. hole) was not included as a factor because preliminary analyses indicated no effect of this variable on activity after either ethanol or saline. The analysis of ethanol-stimulated activity yielded significant effects of conditioning trial [$F(1,90) = 26.6$] and trial duration × conditioning trial [$F(2,90) = 8.0$]. Follow-up analyses indicated several reasons for the interaction. First, the 5- and 15-min groups showed reliable increases in activity across trials [for the 5-min group, $F(1,28) = 28.9$; for the 15-min group, $F(1,31) = 8.9$], whereas the 30-min group did not. Moreover, trial duration differences were significant on Trial 4 [$F(2,90) = 4.4, p < .02$], but

not on Trial 1. Activity levels on Trial 4 were inversely related to conditioning trial duration.

Analysis of activity on saline trials indicated significant effects of trial duration [$F(2,90) = 6.1$] and conditioning trial [$F(1,90) = 18.6$]. In general, activity declined across saline trials, with the 30-min group displaying slightly higher activity levels than those of either of the other trial duration groups.

Place Preference Test

Figure 2 depicts mean percentage of total time (+*SEM*) spent on the grid floor by all groups during the 30-min preference test. Magnitude of place conditioning is represented by the difference between conditioning subgroups at each trial duration. As can be seen, groups that had previously received grid-ethanol pairings (GRID+) spent more time on grid than did groups that had received grid-saline pairings (GRID−), indicating a conditioned preference for the ethanol-paired floor at all three trial durations. However, strength of conditioned place preference was inversely related to trial duration. An ANOVA (trial duration × conditioning group) supported these observations, yielding significant effects of conditioning group [$F(1,87) = 100.6$] and trial duration × conditioning group [$F(2,87) = 4.1, p < .02$]. Follow-up analyses indicated that the interaction obtained in the overall analysis was due primarily to differences between the 5- and 30-min conditioning groups [trial duration × conditioning group, $F(1,57) = 8.3$]; differences between either of those duration groups and the 15-min groups were not

Table 1
Mean (±*SEM*) Activity Counts per Minute During the First 5 Minutes of the First and Last Conditioning Trials in Experiment 1

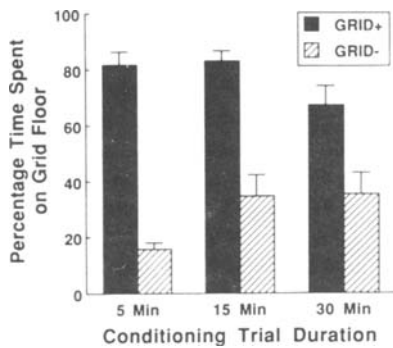| Duration Group	n	Ethanol		Saline	
		Trial 1	Trial 4	Trial 1	Trial 4
5 min	29	152.7±6.8	196.9±6.6	57.6±2.5	48.6±2.5
15 min	32	154.6±5.5	179.7±8.0	60.4±2.7	47.8±3.0
30 min	32	169.4±6.1	169.7±4.6	65.3±2.9	60.9±3.1

Figure 2. Mean (±*SEM*) percentage of total time spent on the grid floor by each conditioning subgroup during the 30-min floor preference test in Experiment 1.

significant. Separate tests of the conditioning-group difference at each trial duration indicated significant conditioned preference at all durations [for the 5-min group, $F(1,27) = 158.6$; for the 15-min group, $F(1,30) = 31.7$; for the 30-min group, $F(1,30) = 10.0$].

Activity levels during the preference test did not differ across duration groups [$F(2,90) = 1.3$]. Mean (±*SEM*) activity counts per minute were 30.2 ± 1.5, 32.6 ± 1.3, and 30.1 ± 0.9 for the 5-, 15-, and 30-min groups, respectively.

Discussion

Magnitude of ethanol-induced conditioned place preference was greatest at the shortest conditioning trial duration (5 min) and diminished in strength as trial duration was increased (to 30 min). In general, this outcome is consistent with the view that ethanol's rewarding effect is greatest shortly after injection (e.g., Reid, Hunter, Beaman, & Hubbell, 1985). Moreover, it suggests that the previous finding of conditioned preference at an ethanol dose that produces loss of righting reflex (Cunningham, Niehus, et al., in press) was probably due to learning about positive ethanol effects occurring within the first few minutes after injection. There may be several reasons why longer conditioning trial durations produced weaker conditioned place preference, even though brain ethanol concentrations were presumably quite high for most of a 30-min trial (Goldstein, 1983, p. 7). One possibility is that ethanol's positive motivational effects are greatest or occur only during the period of time when brain ethanol levels are rising. A conditioned stimulus (CS) whose duration closely overlapped the peak effect of the unconditioned stimulus (US) would be expected to produce stronger conditioning than would a CS whose duration extended well beyond the US's effect (Barnes, 1956; Schneiderman, 1966), either because the CS-US contingency is degraded by a long duration CS or because the extended period of CS exposure produces extinction. Another possibility is that the hedonic effect of an ethanol US actually changes from positive to negative as a function of time after injection (Solomon, 1977). In this case, the weaker preference in the 30-min group might be attributed

to the partial conditioning of a delayed aversive effect of ethanol (see Risinger & Cunningham, in press).

As reported previously, ethanol produced an increase in the activity of DBA/2J mice (e.g., Crabbe, Kosobud, Young, & Janowsky, 1983; Frye & Breese, 1981), and repeated exposure to ethanol on conditioning trials enhanced this stimulant effect (e.g., Crabbe, Johnson, Gray, Kosobud, & Young, 1982; Cunningham, Niehus, et al., in press; Cunningham & Noble, in press-a; Risinger, Dickinson, & Cunningham, in press). Of greater interest, however, is the apparent relationship between ethanol-induced activation and strength of conditioned place preference. Two aspects of the data encourage further consideration of this relationship. First, the trial duration that was most effective in producing conditioned place preference overlapped and was restricted to the time period during which ethanol's activating effect was greatest. Second, the group that showed the strongest place conditioning also showed the greatest sensitization to ethanol's activating effect. In light of data showing that sensitization to ethanol's activating effect is mediated in large part by Pavlovian conditioning (Cunningham & Noble, in press-a), the present findings offer further support for the suggestion that development of behavioral sensitization and conditioned preference are both mediated by a common learning process (Cunningham & Noble, in press-a).

EXPERIMENT 2

Interpretation of the results of Experiment 1 may be complicated by the fact that manipulation of trial duration varied not only the duration of overlap between floor cues and drug but also the total duration of exposure to the test apparatus. One might argue, for example, that strength of the conditioned motivational effect due to CS-drug overlap was identical in the 5- and 30-min groups, but that the greater familiarity of 30-min groups with the test apparatus interfered somehow with learning or expression of the conditioned preference. Experiment 2 was designed to determine whether the weaker conditioned place preference obtained with 30-min conditioning trials was due to greater familiarity with the place conditioning apparatus. As in Experiment 1, one pair of conditioning groups was exposed to the tactile CSs for 5 min after injection (5-min groups), whereas a second pair was exposed to the CSs for 30 min after injection (30-min groups). A third pair of conditioning groups (25–5 groups) was also exposed to the CSs for 5 min after injection. However, these groups received an additional 25-min exposure to the apparatus in the absence of the distinctive floor cues (i.e., on a smooth surface covered with paper) just before each 5-min conditioning trial. Thus, although mice in the latter groups were exposed to the same relationship between tactile cues and ethanol as were mice in the 5-min groups, their overall exposure to the apparatus was identical to that of mice in the 30-min groups. If differences among groups in Experiment 1 depended solely on the duration of overlap between tactile cues and

Table 2
Mean (±SEM) Activity Counts per Minute During the First 5 Minutes
of the First and Last Conditioning Trials in Experiment 2

Duration Group	n	Ethanol		Saline	
		Trial 1	Trial 4	Trial 1	Trial 4
5	26	157.4±5.3	204.6±7.3	59.1±1.8	49.9±2.6
25-5	26	117.7±5.2	176.2±6.1	47.5±2.0	46.8±2.9
30	27	155.8±7.3	174.2±6.7	65.3±3.3	52.6±2.9

ethanol's effects, the 25-5 groups should show a conditioned preference similar to that seen in the 5-min groups. However, if overall familiarity with the apparatus interferes with learning or expression of conditioned preference, the 25-5 groups ought to behave more like the 30-min groups.

Method

Subjects and Apparatus

Eighty-four naive inbred mice (DBA/2J) were obtained from the same vendor and maintained under the same conditions described earlier. The apparatus was that used for Experiment 1.

Procedure

The general procedure was similar to that described for Experiment 1, consisting of one habituation session, eight conditioning sessions, and one preference test session. The subjects were randomly assigned to one of two conditioning subgroups (i.e., GRID+ vs. GRID−) within each of three trial-duration treatment conditions (n = 13-14/conditioning subgroup). Mice in the 25-5 groups were first placed in the apparatus on a paper floor for 25 min. They were then removed briefly from the apparatus, injected with saline (CS− trial) or ethanol (CS+ trial), and returned to the apparatus for an additional 5 min in the presence of the appropriate floor. Mice in the 5- and 30-min groups received conditioning treatments nearly identical to those received by the 5- and 30-min groups in Experiment 1. However, in order to equate those groups for the extra handling involved in the 25-5 procedure, mice in the 5-min groups were weighed and returned to the home cage 25 min before their injection, whereas mice in the 30-min groups were picked up and replaced in the apparatus 25 min after their injection. The experiment concluded with a preference test identical to that used in Experiment 1.

Results

Due to procedural errors during the conditioning phase, 4 subjects were eliminated from Experiment 2. In addition, a computer error resulted in the loss of preference test data from 1 mouse. Data were analyzed as in Experiment 1.

Conditioning Trials

Table 2 presents average activity rates during the first 5 min of the first and last ethanol and saline conditioning trials in Experiment 2. As in Experiment 1, activity after ethanol generally increased over trials, whereas activity after saline decreased over trials. Moreover, ethanol-stimulated activity on Trial 4 was once again greatest in the 5-min group. On Trial 1, 25-min exposure to the apparatus on a paper floor suppressed the activity response to both ethanol and saline in Group 25-5. However, by Trial 4, Group 25-5's activity levels were nearly identical to those seen in the 30-min group. A two-way (trial

duration × conditioning trial) ANOVA applied to the ethanol data revealed significant main effects of trial duration [$F(2,77) = 9.6$] and conditioning trial [$F(1,77) = 129.0$], as well as a significant interaction [$F(2,77) = 10.7$]. Follow-up analyses indicated that the increase across trials was reliable in each group [lowest, $F(1,26) = 6.5$, $p < .02$] and that the group effect was reliable on each trial [both $Fs(2,77) > 6.5$].

Activity declined across saline trials, with the 25-5 group displaying slightly lower levels than the other groups. An ANOVA (trial duration × conditioning trial) confirmed these observations, yielding significant main effects of trial duration [$F(2,77) = 9.5$] and conditioning trial [$F(1,77) = 12.8$]; the interaction was not significant.

Place Preference Test

The outcome of the preference test for Experiment 2 is shown in Figure 3. As in Experiment 1, conditioned preference for the ethanol-paired floor was greater in the 5-min groups than it was in the 30-min groups. The conditioned preference shown by the 25-5 groups was intermediate to that shown by the other trial duration groups. An ANOVA (trial duration × conditioning group) yielded significant effects of conditioning group [$F(1,73) = 61.1$] and trial duration × conditioning group [$F(2,73) = 3.7$, $p < .03$]. Follow-up analyses revealed that the interaction obtained in the overall analysis was due primarily to differences between the 5- and 30-min conditioning groups [trial duration × conditioning group, $F(1,49) = 7.9$]. Similar comparisons between each of those duration groups and the 25-5 groups were not significant. Separate tests of the conditioning-group difference at each du-
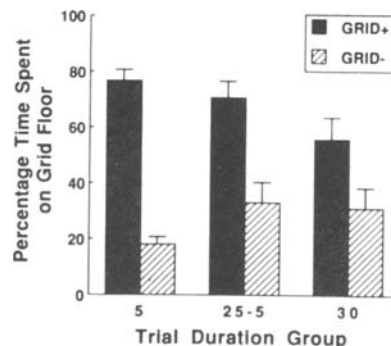

Figure 3. Mean (±SEM) percentage of total time spent on the grid floor by each conditioning subgroup during the 30-min floor preference test in Experiment 2.

ration indicated significant conditioned preference in all cases [5-min group, $F(1,24) = 137.2$; 25-5 group, $F(1,24) = 15.2$; 30-min group, $F(1,25) = 5.1$, $p = .03$].

Mean ($\pm SEM$) activity counts per minute during the preference test were 32.0 ± 1.2, 27.8 ± 1.4, and 37.1 ± 1.6 for the 5-min, 25-5, and 30-min groups, respectively. An ANOVA indicated the group difference was significant [$F(2,76) = 10.7$].

Discussion

As in Experiment 1, mice exposed to 5-min conditioning trials showed stronger conditioned place preference than did mice exposed to 30-min trials. Group 25-5, which received a 5-min overlap of CS and drug but 30-min exposure to the apparatus on each trial, displayed a conditioned preference of intermediate strength. The intermediate preference shown by Group 25-5 might mean that the difference between the 5- and 30-min groups is due both to an effect of CS-US overlap duration and to an effect of general familiarity with the experimental apparatus. There are several ways in which familiarity with the apparatus could have influenced conditioned place preference. One possibility is that context familiarity interfered with expression of conditioned preference at the time of testing. In other words, the difference in the conditioned hedonic values of the two floor cues might have been less effective in controlling locomotor choice behavior in a more familiar context. This interpretation, however, is not consistent with studies suggesting that context familiarity *enhances* the expression of conditioned place preference in rats (Vezina & Stewart, 1987b). An alternative possibility is that context familiarity interfered somehow with the increment in associative strength that occurred on each conditioning trial. This suggestion is clearly not consistent with predictions that might be made on the basis of the presumed relative saliences of the context and tactile cues. That is, one would ordinarily expect a reduction in the novelty/salience of context cues to enhance acquisition of associative strength by a novel CS paired with the US in the presence of those context cues (cf. Rescorla & Wagner, 1972). However, context familiarity might have more directly influenced the increment in associative strength by decreasing the magnitude of the unconditioned response evoked by ethanol. The greatly reduced level of ethanol-stimulated activity in Group 25-5 on the first conditioning trial supports this suggestion (see Table 2) and may indicate that ethanol's positive affective properties were also reduced by context familiarity. Finally, it is also possible that expression of the preference conditioned in Group 25-5 was reduced because apparatus exposure without drug produced extinction of a context-ethanol association that normally interacted with (e.g., was modulated by) the floor-ethanol association (see Cunningham & Noble, in press-a).

The reduced level of ethanol-stimulated activity shown by Group 25-5 on the first conditioning trial was not expected and suggests that the attempt to match groups for overall context familiarity was confounded by the addition of another variable. The smaller stimulant effect was probably due to giving the additional 25-min apparatus exposure *immediately* before ethanol injection. In other words, there might have been a rather localized effect of context familiarity on the ethanol response, which might not have occurred if the additional apparatus exposure had been separated from ethanol injection by several hours. Thus, although the outcome of Experiment 2 suggests a role of context familiarity in the conditioning of place preference, the 25-5 and 30-min groups might have differed from the 5-min group for different reasons. As suggested earlier, conditioned preference in the 30-min groups might have been reduced by association of the CS+ floor with a delayed aversive effect of ethanol, whereas conditioned preference in the 25-5 groups might have been reduced by a localized inhibitory effect of apparatus exposure on the unconditioned rewarding effect of ethanol.

GENERAL DISCUSSION

In two experiments, strength of ethanol-induced conditioned place preference was found to be inversely related to trial duration. Specifically, a stronger preference was observed in mice that had previously received 5-min exposure to the CS after ethanol injection than in mice that had received 30-min exposure to the CS. These findings are generally consistent with other drug conditioning studies showing that CS duration affects strength of conditioning (Paletta & Wagner, 1986; Schwarz-Stevens & Cunningham, 1992). However, these results contrast with two previous studies reporting no effect of conditioning trial duration (range: 10-100 min) on strength of conditioned place preference induced by opiate drugs in rats (Bozarth, 1987; Mucha, van der Kooy, O'Shaughnessy, & Bucinieks, 1982). Although differences in species, apparatus, and procedure make reconciliation of these findings difficult, the different outcomes may reflect a difference between the temporal dynamics of the hedonic effects of ethanol and opiate drugs. This conclusion is supported, in part, by an unpublished study conducted in this laboratory that yielded no effect of trial duration (5 vs. 30 min) on conditioned place preference induced by morphine (8 mg/kg) in DBA/2J mice using procedures identical to those described here. This conclusion is also consistent with the observation that the activating effects of ethanol and morphine, which are often linked to their hedonic effects (e.g., Wise & Bozarth, 1987), have quite different time courses (Cunningham, Niehus, et al., in press).

Consideration of locomotor activity levels during conditioning trials may lend some support to theories postulating a relationship between the rewarding and activating effects of addictive drugs (e.g., Wise & Bozarth, 1987). Of particular interest is the finding in both experiments of a positive relationship between strength of conditioned place preference and degree of sensitization to ethanol's activating effect. As one means of evaluating the significance of this apparent relationship, group means from both experiments for (1) time spent on ethanol-paired floor during preference testing and (2) activity counts during the

final ethanol conditioning trial were used as scores to compute a Pearson correlation coefficient. Analysis indicated a significant positive correlation between the group means of these two dependent variables [$r(4) = +0.88, 0.01 < p < .05$].[1] As suggested earlier, this relationship supports the hypothesis that behavioral sensitization and conditioned preference are mediated by a common learning process. However, this finding does not necessarily mean that the brain systems mediating the activating and rewarding effects of ethanol are identical. For example, it has recently been shown that haloperidol, a dopamine receptor blocker, can greatly reduce the activating effect of ethanol without influencing either the learning or the expression of conditioned place preference (Cunningham, Malott, Dickinson, & Risinger, in press; Risinger, Dickinson, & Cunningham, in press).

These experiments add to a growing list of studies that encourage further use of the mouse in place conditioning studies of ethanol's rewarding effect (e.g., Crabbe et al., in press; Cunningham et al., 1991; Cunningham, Niehus, et al., in press). Moreover, the consistency with which a robust conditioned preference is obtained using relatively standard training procedures has already led to studies aimed at delineating the neuropharmacological bases of ethanol reward (e.g., Cunningham, Malott, et al., in press; Risinger, Dickinson, & Cunningham, in press; Risinger, Malott, et al., in press). The present experiments also illustrate one advantage of a Pavlovian conditioning technique over oral preference or operant self-administration in the study of drug effects. Specifically, because the experimenter can control duration of CS exposure and its temporal relation to a drug's effect, the place conditioning task offers a means of examining time-dependent affective changes produced by a drug. In contrast, because the subject controls its exposure to drug during self-administration, it is difficult to draw meaningful conclusions about temporal variations in drug effect.

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NOTE

1. Correlations based on data from individual subjects were also computed. These correlations, however, were all nonsignificant [Experiment 1, $r(91) = +0.03$; Experiment 2, $r(77) = -0.08$; both experiments combined, $r(170) = -0.02$]. The reason for the discrepancy between the correlation derived from group mean scores and that derived from individual subject scores may be related to use of an inbred strain in these experiments. This precluded any contribution of genotype to individual differences in either of the two dependent variables, leaving environmental factors as the sole determinants of any correlation. Because the two dependent variables were measured on 2 different days, it may be reasonable to assume there was greater variability in the "uncontrolled" environmental variables influencing individual subjects on each occasion than there was in the "controlled" environmental variable (i.e., trial duration on previous conditioning trials) governing group means. By this analysis, individual subject differences in uncontrolled environmental influences on each day might have produced sufficient "noise" to obscure the environmental correlation between Trial 4 activity and test session preference. However, when that noise was eliminated by using group means, the environmental correlation was revealed. Use of a genetically heterogeneous subject population would not necessarily have eliminated the possibility of a discrepancy between a group means correlation and an individual scores correlation. However, the addition of genetic variation to the factors influencing individual subjects can significantly alter a phenotypic correlation if there is a genetic correlation between the two dependent variables. Given a true genetic correlation, congruence between a phenotypic correlation based on group means and one based on individual scores may be more likely in an experiment involving genetically heterogeneous subjects than in an experiment involving genetically homogeneous subjects because of the general tendency toward correlation between genetic correlations and environmental correlations (see DeFries, Kuse, & Vandenberg, 1979).