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Conditioned Place Aversion Mediated by Self-Administered Ethanol in the Rat: A Consideration of Blood Ethanol Levels

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STEWART, R. B. AND L. A. GRUPP. *Conditioned place aversion mediated by self-administered ethanol in the rat: A consideration of blood ethanol levels.* PHARMACOL BIOCHEM BEHAV 32(2) 431–437, 1989.—A previous experiment has shown that rats will avoid environmental cues that have been associated with a history of ethanol self-administration. One possible explanation for this conditioned place aversion may be related to the temporal parameters of that experiment. During the initial segment of each 90-min conditioning/drinking trial (when most of the drinking occurred) the blood ethanol levels (BELs) were low and may well have produced positive effects at that time. However, as the drug continued to be absorbed and BELs increased during the remainder of the 90-min trial, the final (and conditioned) drug effects may have been aversive. In the present experiment the trial length was shortened to a 15-min period so that only low BELs would be temporally paired with the conditioning environment. A conditioned preference for that environment was predicted. Twelve rats were trained to self-administer ethanol in one environment and had water available in a different environment. Eight control animals had only water in both environments. BELs were measured and found to be low (16.8 to 57.6 mg%) and rising during the conditioning trials. However, when given a choice between the two environments, the rats avoided the environment in which they formerly consumed ethanol. No change in preference was noted for the control animals. This result was in accordance with previous findings but did not support the hypothesis that low, excitatory BELs would mediate a conditioned place preference in the rat.

Ethanol self-administration Place conditioning Aversion Reinforcement Blood ethanol levels

PERHAPS the most important and obvious criterion for establishing a valid animal model of human alcohol use and abuse is that the animals should self-administer ethanol (9,14). However, the measurement of drug self-administration is not the only way the motivational properties of a drug can be assessed. The place conditioning procedure, for example, involves exposing a rat to the effects of a drug while in a novel and distinctive environment. Subsequent approach or avoidance behavior directed toward or away from that environment in the absence of the drug indicates that the rat has come to associate either positive or aversive aspects of the drug effect with the location in which the drug was experienced. Using this procedure, preferences have been demonstrated for environments that have been previously associated with a number of other drugs of abuse such as morphine (2), heroin (5), amphetamine (18) and cocaine (24). Thus, there appears to be a good correspondence between the ability of drugs to function as reinforcers, i.e., to be self-administered, and the ability of the same drugs to mediate conditioned place preferences. However, this correspondence apparently breaks down in the case of ethanol. Although an initial report (4) indicated that rats would develop a preference for an environment paired with intraperitoneal (IP) injections of ethanol, other investigators (10, 11, 26) have failed to demonstrate such a preference using nearly identical procedures including the same route of administration and dose. Subsequently, extensive studies have examined the dose-response relationship between ethanol and place conditioning using IP (1, 26, 28), intragastric (21,31) and intravenous (31) routes of ethanol administration. The general finding has been that low doses of ethanol (usually below 1.0 g/kg) fail to produce conditioned place preferences or aversions while high ethanol doses result in the avoidance of the ethanol-paired environments.

Stewart and Grupp (29) had previously hypothesized that the general failure to produce an ethanol-mediated place preference may have been due to the nonoral routes of administration that were used. In order to test this hypothesis they trained rats to self-administer ethanol orally in one distinctive environment with water available in a different environment. A control group had only water available in both environments. Animals in the experimental group drank more ethanol solution than water and consumed ethanol at a rate which exceeded their capacity to metabolize the drug as

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confirmed by the measurement of blood ethanol levels. However, when later offered a choice between the two environments in the absence of the drug, these rats avoided the environment in which they previously consumed ethanol. The control animals showed no change in preference for the two environments which were both associated with water. The conditioned place aversion observed with the oral self-administration of ethanol was in accordance with previous studies in which rats were dosed passively using nonoral routes of administration (10, 11, 21, 26, 28, 31), but this result was unexpected and paradoxical. The ethanol self-administration behavior indicated that the drug was functioning as a positive reinforcer, yet the avoidance of the drug-paired environment indicated that the ethanol was functioning as an aversive stimulus or a punisher.

One way to resolve this apparent discrepancy is to postulate that ethanol may have both reinforcing and aversive properties. This is not a new concept and it has been incorporated into a theory of the aversive control of drug-taking behavior (6). According to this hypothesis, the reinforcing properties of a drug motivate the initiation and maintenance of drug intake while the aversive effects act to modulate or stop the drug self-administration. Whether the reinforcing or aversive effects of ethanol predominate in a given situation may depend, at least in part, on pharmacokinetic factors such as the absolute levels of ethanol in the blood and/or whether the blood ethanol levels (BELs) are rising (in the absorption phase of the BEL time curve) or falling (in the elimination phase of the BEL time curve).

In the place conditioning study by Stewart and Grupp (29) described above, each ethanol conditioning trial consisted of placing the rats in conditioning chambers with an ethanol solution continuously available for 90 min. It was observed that most of the ethanol drinking occurred during the first 10 or 15 min of the conditioning trial. It is likely that during this initial segment of each 90-min trial the BELs were low and rising. These low BELs may have produced reinforcing effects during the initial segment of each conditioning trial. However, as the drug continued to be absorbed and BELs increased during the remainder of the trial the drug effect may have become aversive, thus producing conditioned aversion to the environment associated with ethanol consumption. If this was the case, then shortening the trial length so that only the initial, putatively reinforcing, part of the drinking episode would take place in the conditioning environment might result in the development of a preference for that environment.

This prediction was somewhat corroborated in a recent study by Reid *et al.* (19) who demonstrated a conditioned preference for an environment paired with IP injections of ethanol when very short (4 min) conditioning trials were used. Reid *et al.* (19) hypothesized that only the low, excitatory BELs that presumably followed shortly after the ethanol injection could produce positive effects that would ultimately result in the formation of a conditioned place preference. However, the short trial length was not a sufficient condition to produce a preference since only rats that had previous experience with ethanol prior to conditioning trials developed a place preference with the injected drug. This previous ethanol experience consisted of 26 daily periods of access to a 6% ethanol solution under conditions of 20-hr fluid deprivation. Groups of rats that did not undergo this pretreatment did not show a place preference with the injected drug even with the short conditioning trial length. The mechanism by which this pretreatment contributed to the development of the place preference is unclear. Perhaps tolerance had developed to the aversive effects of the drug so that the reinforcing ethanol effects were enhanced during conditioning. Reid *et al.* did not test other trial lengths in that experiment so it is not known whether a preference would also have been seen with longer trial lengths, provided that the rats had the previous experience with ethanol. Blood ethanol levels during or immediately following conditioning trials were not measured or reported. Nevertheless, the idea that ethanol pharmacokinetics may be an important variable in place conditioning studies would appear to be reasonable.

The strategy used in the present study was to repeat the procedures that were used in the previous self-administration place conditioning experiment of Stewart and Grupp (29) with the important exception that the length of each conditioning trial was shortened to 15 min. Previous work (27) has indicated that with a trial of this length the environmental cues of the conditioning apparatus will be exclusively paired with both the actual consumption of the ethanol and with low BELs in the rising phase of the BEL time curve. It was expected that shortening the conditioning trials would result in a conditioned preference for the environment in which ethanol was consumed, as was found by Reid *et al.* (19) with nonoral ethanol administration.

METHOD

Subjects

Twenty male Long Evans hooded rats (Charles River, Quebec), weighing 334–368 g at the beginning of the experiment, were housed individually and kept on a 12/12-hr light/dark cycle with lights on at 7:00 p.m. All animals were weight-reduced prior to the experiment and kept at 80% of their initial free-feeding weights for the duration of the study. The rats were fed sufficient amounts of Purina No. 5001 Rodent Chow in the home cages to maintain their reduced body weights and this feeding took place at least two hr after conditioning trials and choice tests. Water was available at all times in the home cages. Conditioning and choice test trials were carried out at approximately the same time every day (during the dark portion of the light/dark cycle).

Drug Preparation

Solutions of 2, 4 and 8% ethanol (weight/volume) were prepared with absolute ethanol in tap water at least 12 hr before use and kept in sealed containers at room temperature.

Apparatus

The apparatus consisted of three conditioning/test boxes, 86×38×38 cm. Each box was divided into three sections or compartments. The middle section (10×30×38) had a sheet metal floor and was painted grey. This middle section could be separated from the two side compartments by removable partitions. One side compartment, 38×38×38 cm, was painted white with the floor area covered by 1 cm grid wire screening. The other side compartment, also 38×38×38 cm, was black and had a smooth plywood floor. Both side compartments could be equipped with single 100 ml graduated drinking tubes and were covered by wire screen lids.

Preconditioning Choice Test

The partitions were removed from the conditioning/test box and for three consecutive days all the animals were

placed individually in the grey middle section of the box and then allowed to move freely among the black, white and grey sections for 15 min. The first two days served to familiarize the rats with the apparatus and on the third day the amount of time spent in each compartment was measured. Choice tests were monitored remotely using a video camera.

Experimental Groups

The animals were assigned to either the Ethanol self-administration group (Ethanol-SA group) n=12, or the Water-only group (H_2O group) n=8. Half of the animals in the Ethanol-SA group were given access to ethanol in the black compartment and the remaining rats were given access to ethanol in the white compartment. In order to control for the time between the last ethanol conditioning trial and the postconditioning choice test, half of each of these subgroups were designated to receive ethanol on even numbered days while the remainder received ethanol on odd numbered days. The H_2O group was subdivided in a similar manner. For each rat in the H_2O group a compartment type (black or white) was designated as the "control" compartment in the same way that for each rat in the Ethanol-SA group a compartment type was designated as the ethanol compartment. Thus, half of the H_2O group had the black compartment designated as the "control" compartment and the other half had the white compartment designated as the "control." The time spent in the "control" compartment by the H_2O group was used in the statistical analysis for comparison with the time spent in the ethanol compartment by the Ethanol-SA group. This assignment of the rats to groups resulted in a mean time spent in the ethanol compartment during the pretest by the Ethanol-SA group (432.46 sec) which was similar to the mean time spent in the "control" compartment by the H_2O group (427.51 sec).

Place Conditioning Trials

On the day following the preconditioning choice test, daily place conditioning trials were begun. The partitions were replaced in the conditioning/test box so that the black and the white compartments were physically separated. Each conditioning trial consisted of removing the animals from their home cages and placing them individually into one of the compartments for 30 min. During the final 15 min of the trial a graduated drinking tube filled with the appropriate fluid (ethanol or water) was attached to the compartment which provided the animals with only 15-min access to the fluid before being removed from the conditioning environment and returned to the home cages. In order to induce high levels of ethanol consumption, a training procedure was used that was based on the prandial drinking technique developed by Meisch (16,17). Thus, for the first 46 trials the rats in both experimental groups were given 6 g of lab chow in the black or white compartments at the beginning of each conditioning trial. This acted to facilitate fluid consumption during the 15-min fluid access phase of each conditioning trial. In order to produce stable drinking in the compartments before the introduction of ethanol trials, during the first ten trials the rats in both groups had access to water only in both the black and white compartments. Assignment to compartment was alternated daily so that each rat was given five water trials in both the black and white compartments. Beginning on the 11th day the rats in the Ethanol-SA group were given access to ethanol in one compartment every other day and access to water in the other compartment on intervening days. Two percent ethanol was available during the first two ethanol trials, then 4% ethanol for four trials and finally 8% ethanol for 12 trials. Ethanol and water trials were alternated daily and ethanol was always paired with one environment and water with a different environment. After 46 trials the training procedure was complete and food was no longer placed in the compartments at the beginning of the conditioning trials. Forty-six additional trials were then given with 8% ethanol and water continuing to be alternated daily, each fluid paired with its distinctive conditioning environment.

The eight animals in the H_2O group were treated identically to the rats in the Ethanol-SA group except that water was the only available fluid during all the place conditioning trials. For each rat in the H_2O group one of the compartments (black or white) was designated as the "control" compartment for the purpose of statistical analysis (see the Experimental Groups section above). However, the rats in the H_2O group had access to water in both compartments and were otherwise treated identically for all trials. The amount of fluid consumed during each trial was measured.

Postconditioning Choice Test

On the day following the completion of 92 place conditioning trials, the partitions were removed from the conditioning/test box and a second 15-min test trial was given in which each rat was allowed to move freely among the black, white and grey sections. The amount of time spent in each compartment was measured in order to determine the amount of time spent by the Ethanol-SA group in the compartment which was associated with ethanol consumption and the amount of time spent by the H_2O group in the compartment designated as the "control" compartment. No ethanol or water was available during the postconditioning choice test.

Blood Sampling and Analysis

The rats continued to be maintained at reduced body weights for seven days after the postconditioning choice test. At that time the animals of the Ethanol-SA group were placed in the conditioning compartments in the usual manner and were allowed to drink 8% ethanol solution for 15 min. They were then removed from the compartment and had 50 μl samples of blood drawn from the cut tip of the tail. Blood samples from the same animals were then collected at 15-min intervals for the first two hr and at 60-min intervals during hr 2–4 following cessation of drinking. The amount of ethanol consumed during that drinking session was noted.

Analysis of blood samples was done using a Hewlett-Packard gas chromatograph (HP5798) fitted with a flame ionization detector and an HP3390A Integrator-Recorder. A four-foot pyrex column, 4 mm inside diameter, packed with 5% Carbowax 20M on Holoport 30/60 mesh was used in an on-column injection system. Temperatures were maintained as follows: column 100°C, injector 150°C, detector 180°C. Nitrogen was used as the carrier gas with a rate of 30 ml/min. 1-Butanol, 0.1% w/v, was used as the internal standard.

RESULTS

Ethanol and Water Consumption

Figure 1a shows the mean amount of fluid consumed during each place conditioning trial by the animals in the Ethanol-SA group. Half of this group had access to ethanol

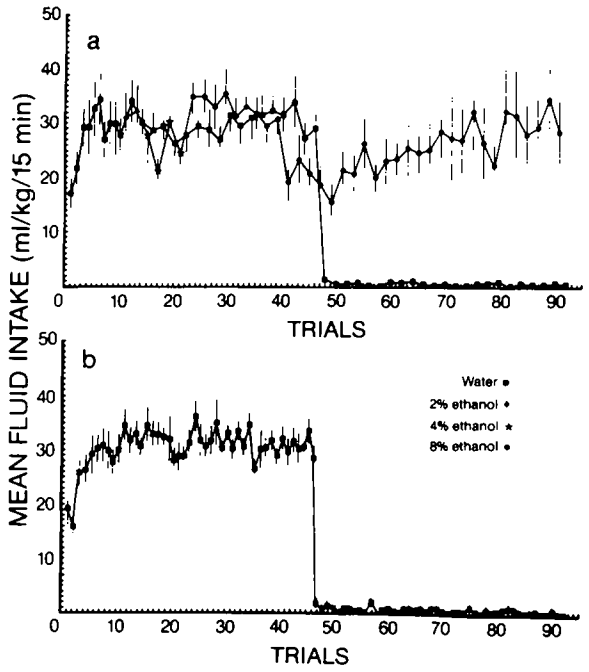

FIG. 1. Mean fluid intake (ml/kg) during 92 daily 15-min place conditioning trials for (a) the 12 rats in the Ethanol-SA group and (b) for the eight rats in the H_2O group. For each of the first 46 trials, 6 g of food was available and for the last 46 trials no food was given to the animals. The vertical lines indicate the standard error of the mean.

during odd numbered trials and the remainder had ethanol during even numbered trials. However, in order to avoid unnecessary complexity in this figure, the ethanol consumption for all the animals is shown above odd numbers and the water intake is shown above even numbers. For the first 46 trials 6 g of food was always placed in the conditioning compartments during both ethanol and water trials and the consumption of these two fluids did not differ. However, during the last 46 trials (trials 47–92) food was no longer available in the conditioning compartments and 8% ethanol consumption remained elevated while water drinking on intervening days was reduced to very low levels since the drinking was no longer prandially induced. For the purpose of statistical analysis the mean fluid consumption for each rat for the last 23 ethanol trials and the last 23 water trials was calculated. Mean ethanol consumption for individual animals ranged from 12.97 to 47.07 ml/kg/trial (1.04–3.77 g/kg/trial) with a group mean of 25.89 ml/kg/trial (2.07 g/kg/trial). Mean water consumption for individual animals ranged from 0.15 to 1.57 mg/kg/trial with a group mean of 0.57 ml/kg/trial. Ethanol drinking statistically exceeded water intake during the last 46 trials, $t(11)=5.49$, $p<0.01$.

Figure 1b shows the mean amount of water consumed during each place conditioning trial by the eight animals in the H_2O group. Water intake for this group was similar to the water consumption observed for the Ethanol-SA group's alternate day water trials, i.e., water drinking was elevated during the first 46 trials in which 6 g of food was available and then dropped off sharply during the last 46 trials when food was no longer present.

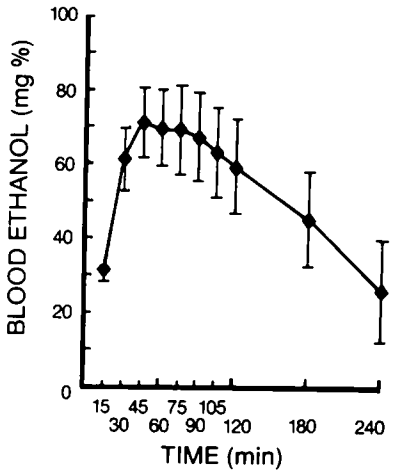

FIG. 2. Mean blood ethanol levels (mg%) for the 12 animals in the Ethanol-SA group following a 15-min trial during which they could drink an 8% w/v solution. The first (15 min) blood sampling time coincided with the cessation of drinking and additional samples were taken at the times indicated.

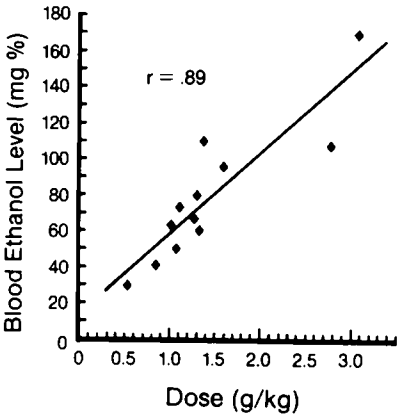

FIG. 3. Peak blood ethanol levels (mg%) for samples obtained after a 15-min drinking trial plotted as a function of dose (ml 8% w/v ethanol consumed converted to g/kg). The points represent individual samples obtained from each of the 12 rats in the Ethanol-SA group. Dose and blood ethanol levels were positively correlated (r=.89).

Blood Ethanol Analysis

Figure 2 shows mean blood ethanol levels (BELs) for the 12 animals in the Ethanol-SA group following a 15-min trial during which they could consume 8% ethanol. The blood samples were taken at intervals for four hr after the cessation of drinking. Of particular interest is the initial 15-min sampling time which shows the BELs for the rats immediately after the drinking trial. The mean BEL at this time was 31.5 mg% with a range of 16.8 to 57.6 mg%. Thereafter, the BELs increased for a period of about 30 min after the drinking took place, reached peak levels at approximately 45–90 min, and then declined steadily for the remainder of the sampling times. The data for each individual animal showed a similar pattern to that illustrated by the group means in Fig. 2. The BELs for all the rats were on the rise at 15 min.

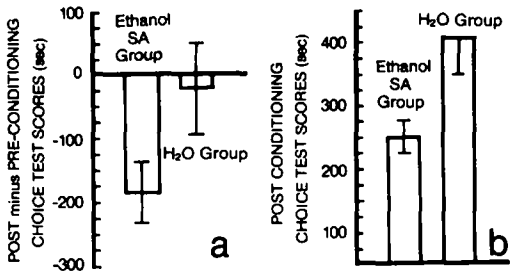

FIG. 4. (a) Mean change in time (sec) spent in the ethanol-paired compartment of the choice test apparatus by animals in the Ethanol-SA group and in the "control" water-paired compartment by the animals in the H_2O group. The data were obtained by subtracting each rat's preconditioning choice test time from its postconditioning choice test time. The brackets indicate the standard error of the mean. (b) Mean times (sec) that rats in the Ethanol-SA and H_2O groups spent in the ethanol-paired and "control" compartments respectively during the 15-min postconditioning choice test. The brackets indicate the standard error of the mean.

Figure 3 shows the peak BELs for individual rats plotted as a function of dose (ml 8% ethanol consumed converted to g/kg). Dose and BEL were positively correlated, $r(10)=0.89$, $p<0.01$, two tailed. This suggests that the measurement of the volume of drug consumed was a reliable indicator of the BEL achieved. The mean peak BEL was 78.6 mg% with a range of from 29.3 to 168.4 mg%. The mean dose was 1.44 g/kg. This was less than the mean (2.07 g/kg) dose consumed during actual conditioning trials and therefore the BELs reported here may underestimate the values achieved during the place conditioning trials.

Place Conditioning Results

Figure 4a shows the mean change in the time spent in the ethanol-paired compartment of the test apparatus for animals in the Ethanol-SA group, calculated by subtracting each animal's single preconditioning choice test time from its single postconditioning choice test time. The Ethanol-SA group showed a significant, $t(11)=3.79$, $p<0.01$, reduction in the time spent in the compartment associated with the drug. Figure 4a also shows no difference, $t(7)=0.28$, n.s., in the time that the rats of the H_2O group spent in the compartment that was designated as the "control" compartment when pre- and postconditioning choice test trials were compared. The mean change in time for the Ethanol-SA group was significantly different from the mean change in time for the H_2O group only in a one-tailed test, $t(18)=1.97$, $p<0.05$.

Figure 4b shows the mean times that animals in the Ethanol-SA and H_2O groups spent in the ethanol-paired and "control" compartments respectively during the postconditioning choice test. The Ethanol-SA group spent significantly less time in the ethanol-paired compartment than the H_2O group spent in the "control" compartment, $t(18)=2.95$, $p<0.01$, again indicating that the animals were avoiding the location in which they self-administered the drug.

DISCUSSION

The hypothesis tested in this investigation was that low, presumably excitatory, BELs would produce positive effects that would mediate a conditioned preference for an environment associated with oral ethanol self-administration. In a previous experiment, Stewart and Grupp (29) found that rats avoided an environment that had been paired with ethanol consumption. However, in that study the conditioning trial length was sufficiently long (90 min) that both low and then high BELs were probably experienced in the conditioning environment and the high BELs may have produced the place aversion. In the present experiment, the conditioning trial length was shortened to 15 min so that only low BELs, in the rising phase of the BEL time curve, would be paired with the environment in which the ethanol self-administration occurred.

The results of this experiment were nearly identical to those obtained previously by Stewart and Grupp (29). Similar ethanol intake during the conditioning trials was achieved (mean dose for the present study=2.07 g/kg/trial; mean dose for the previous study=2.04 g/kg/trial) in spite of the fact that the trial length in the present experiment was much shorter. This confirmed the observations made by Stewart and Grupp (27) and others (17) that when similar methods are used to induce ethanol self-administration most of the drinking occurs at the beginning of a session. Therefore, the reduction in trial length from 90 to 15 min had no effect on the total amount of ethanol consumed. The drinking resulted in measurable blood ethanol levels and ethanol intake was significantly higher than water intake. The drug was functioning as a positive reinforcer yet, the rats avoided the environment in which they consumed the drug even when BELs were low and rising during the time of conditioning. Thus, this experiment replicated the previous finding that rats would show a conditioned place aversion for the same environment in which they had self-administered ethanol. This result is also in agreement with the preponderance of other place conditioning experiments in which ethanol was tested (10, 11, 21, 26, 28, 31).

One exception to the general finding of ethanol-mediated conditioned place aversions was a study by Reid *et al.* (19) which showed a conditioned preference for an environment associated with ethanol, administered by IP injection. Two aspects of their methods seemed to be important in determining whether or not a place preference developed. First, the rats had a history of experience with ethanol. In their study, this consisted of the water-deprivation-induced oral consumption of a 6% ethanol solution in the home cages (approximately 1.6–2.2 g/kg/day) for 26 daily one-hr periods, followed by a 35-day ethanol-free period before conditioning trials with injected drug commenced. Second, Reid *et al.* used a short conditioning trial in order to ensure that only low, excitatory, BELs were paired with the conditioning environment. The rats were placed in the conditioning compartment for four min, beginning four min after each 1.0 g/kg IP injection.

These two methodological aspects were approximated in the present experiment, albeit the ethanol was self-administered rather than passively administered for conditioning trials. Thus, the rats in the present study also had a prolonged period of ethanol exposure consisting of 41 conditioning/drinking trials with comparable daily doses (a mean of 2.07 g/kg) to the preexposure period in the study by Reid *et al.* In addition, the conditioning trials were sufficiently short in the present study to encompass only low BELs. However, the rats showed a behavioral response that was opposite in direction to that found by Reid *et al.*, i.e., the rats in the present study avoided the environment in which the drug was experienced. This result does not necessarily represent a failure to replicate Reid *et al.* since many aspects of the methods differed, comparing the two experiments. However, it is suggested that the achievement of a conditioned

place preference using ethanol is not simply a matter of arranging the temporal parameters to "capture" putatively reinforcing low BEL-induced effects of the drug.

The method that was used to establish ethanol self-administration in this study and in the previous study by Stewart and Grupp (29) involves maintaining the animals at 80% of their free-feeding body weights. Weight reduction does account for most of the ethanol intake that is achieved when this method is used (17, 27, 30), although it is not clear that the animals are therefore consuming the drug exclusively for its caloric content (8). Nevertheless, it is possible that in the place conditioning experiments the rats may be consuming the ethanol as a food and that the postingestional effects are undesired aversive "side-effects" of this consumption that led to the observed place aversion. With this interpretation of the results, one might ask why the rats continue to self-administer the drug if its consequences are aversive. The literature concerned with the conditioned taste aversion phenomemon suggests that rats are more predisposed or "prepared" to associate aversive consequences with the taste of a consumed substance than with environmental cues (20). Indeed, this predisposition is apparent in studies using other drugs, for example, rats will develop a conditioned aversion to the taste of a fluid that is associated with IV drug self-administration (32). Along similar lines, morphine and amphetamine have been used to produce conditioned taste aversions, yet conditioned place preferences have been demonstrated with the same drugs, even when the same rats are simultaneously tested with both taste and place conditioning procedures (18,22). These findings illustrate that "paradoxical" effects are not confined to studies using ethanol, but also show the expected pattern that the taste of a consumed substance paired with drug administration most often acquires conditioned aversive properties. The ethanol self-administration in the present study showed no sign of a decrease as would be the case if an aversion to the taste of the fluid was developing. Instead, an aversion emerged for the environment associated with the drug. This suggests that under certain conditions when ethanol is tested, the predisposition may be stronger to associate aversive consequences with environmental cues than with taste. In this regard, Sherman *et al.* (21) reported a finding which parallels the present results. They tested weight-reduced rats and found that the same animals would simultaneously develop both a conditioned preference for a flavour associated with ethanol (given by gastric intubation) and a conditioned place aversion for the environment in which the drug was administered. These effects were dose-dependent since they occurred at 0.5 g/kg but not at 1.0 or 2.0 g/kg doses of ethanol. For the highest dose, Sherman *et al.* (21) found that the drug produced both taste and place aversions.

In order to account for the avoidance of the drug-paired environment in the present study, the simplest explanation is that the postingestional effects of the drug were aversive and that the rats associated these aversive effects with the ethanol-paired environment. Thus, the conditioned place aversion seen with ethanol may have been similar in mechanism to that produced by emetics such as apomorphine (3), i.e., the drug made the animals ill and therefore they avoided the place where they were previously ill. However, a different interpretation may be that the direct pharmacological effects of ethanol are not aversive in themselves but rather that, in the absence of the drug, the environment which had previously been associated with ethanol consumption may elicit a conditioned physiological response that is aversive. Stimuli associated with drug-administration have been shown to elicit conditioned compensatory responses which resemble withdrawal-like symptoms (23). The avoidance of the drinking environment might then be explained if the following assumptions are considered: 1) the association between ethanol self-administration and the drinking environment had resulted in the conditioning of compensatory physiological responses which are revealed when the animal is exposed to the environment in the absence of the drug, 2) these conditioned physiological responses were aversive and 3) during the choice test, when no ethanol was available, the animals stayed away from the cues that elicited an aversive conditioned compensatory response. Such an explanation remains speculative in the absence of the direct measurement of any conditioned physiological responses within the parameters of the place conditioning experiments. In addition, like ethanol, morphine has been used to condition compensatory responses (23) but, unlike ethanol, morphine can be used to condition a preference in rats for an environment previously associated with its administration (2). However, this explanation has the advantage of accounting for the paradox revealed in the present study, i.e., that rats would both self-administer ethanol and avoid the place where this drinking took place during a test trial without drug.

One difference between this study and all of the other place conditioning studies which tested ethanol was the physical presence of the filled drinking tubes during conditioning trials. It could be argued that the absence of the drinking tubes during the choice test may have produced a change in the stimulus complex which in turn may have provoked a "frustration" effect or some other artifact of extinction that may account for the aversion seen. However, when place conditioning mediated by food has been examined (25), choice tests were done in the absence of food stimuli (e.g., the sight and smell of the food). The rats may well have been frustrated, yet a conditioned preference for the environment associated with food was observed.

Aversive properties of ethanol have been demonstrated in rats using several different experimental procedures (7, 12, 13, 15). The conclusion usually drawn is that the manifestation of aversive effects is a function of the dose administered, i.e, if the dose or BEL is sufficiently high then negative effects occur. It has also been suggested that this may be a factor that limits ethanol intake in the sense that animals may learn to avoid aversive high-dose effects by adjusting their drug consumption (6). The results of the present experiment seem to indicate that such a conclusion may be overly simplistic since aversive effects were shown during the period when the most positive aspects of ethanol might be expected—when BELs are low and rising and the rat is actively consuming the drug.

On the other hand, it is possible that the environmental cues acquired conditioned aversive properties not because they were temporally paired with the ethanol self-administration itself, but rather because they reliably *predicted* that aversive effects would occur later on. For example, after the rats had been returned to their home cages they may have experienced aversive high BELs or, later still, even hangover. It is, of course, possible to question why the rats don't learn to avoid the drug's aversive consequences by adjusting their intake. In a sense, this question lies at the center of the addiction problem. Why do those who abuse alcohol persist in drinking intoxicating amounts in spite of aversive consequences that surely will follow?

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