Pharmacology Biochemistry & Behavior, Vol. 19, pp. 441–445, 1983. © Ankho International Inc. Printed in the U.S.A.

Motivational Properties of Ethanol in Naive Rats as Studied by Place Conditioning

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Received 2 March 1983

VAN DER KOOY, D., M. O'SHAUGHNESSY, R. F. MUCHA AND H. KALANT. *Motivational properties of ethanol in naive rats as studied by place conditioning.* PHARMACOL BIOCHEM BEHAV 19(3) 441–445, 1983.—The reinforcing properties of ethanol were examined in naive adult male rats by means of a place conditioning paradigm that has previously demonstrated the positive reinforcing properties of food, water and some drugs, and the aversive properties of punishers such as electric shock and lithium chloride. Only doses of 0.8–1.0 g/kg and higher produced clear place conditioning, and this was only conditioned place aversion; rats spent significantly more time on the side of the place conditioning box in which they received the vehicle than on the side in which they received ethanol. Doses between 0.1 g/kg and 0.8 g/kg produced increases in general activity, but did not produce any place conditioning. Control experiments indicated that the pattern of effects was not specific to the route of ethanol administration (intravenous or intragastric), rate of infusion, concentration, or vehicle. It was concluded that ethanol, in the doses used here, has only punishing or neutral motivational effects in naive rats and does not serve as a primary positive reinforcer in this model. The conclusions are discussed in relation to the relative difficulty encountered in attempts to produce ethanol self-administration, and the findings are viewed as consistent with a proposal that prolonged training and experience with ethanol are important for ethanol self-administration by the rat.

Ethanol Reinforcing properties Rat Place conditioning Aversion Alcohol experience

THE WIDESPREAD consumption of ethanol by humans is obvious. However, it is more difficult to observe reinforcement by ethanol in naive laboratory animals than it is with other substances commonly consumed by humans. There are important species and strain differences in spontaneous consumption of ethanol. However, naive rats, which readily ingest food and water, do not rapidly acquire oral consumption of ethanol in amounts producing appreciable pharmacological effects. Similarly, naive rats rapidly acquire intravenous self-administration of cocaine, opiates, and amphetamine, but do so with considerable difficulty in the case of ethanol. When learned self-administration does occur, it is typically after considerable training and experience with the drug [1, 6, 7, 8, 11, 17, 20, 24, 27, 28, 33, 37]. Experiments using oral preference for ethanol indicate that pre-exposure to ethanol reduces its ability to produce conditioned taste-aversion and promotes intake of flavors associated with ethanol [2, 9, 10, 22]. These experiments suggest that prolonged training and experience with ethanol play a critical role in ethanol intake. This fact does not preclude the existence of a primary positive reinforcing effect of ethanol, but it increases the difficulty of distinguishing clearly between primary and secondary reinforcement mechanisms.

The difficulty of interpretation might be reduced if reinforcing properties were assessed by a method that involved minimal previous exposure to ethanol. The principal aim of the present paper is to examine the reinforcing properties of ethanol in rats by the use of place conditioning. In this classical conditioning procedure, controlled training and testing of naive rats is completed in a short period and with few drug exposures [26,35]. Distinctive locations in a test apparatus constitute the conditioned stimuli and administration of a drug is the unconditioned stimulus (UCS). The conditioned response is seen in trained rats as a preference for a location paired with an appetitive reinforcer and an avoidance of a location paired with a punisher. With only 1 to 4 administrations of drug we were able to demonstrate conditioned place preference with morphine and cocaine and conditioned place aversions with naloxone and lithium chloride [26,35]. This procedure also has an important advantage over taste conditioning procedures which cannot distinguish between drugs that are well-known punishers such as lithium chloride and those that are well-known positive reinforcers such as morphine. Indeed, taste classical conditioning paradigms have failed to reveal a positive reinforcing effect of ethanol in animals previously naive to the drug [10, 13, 22].

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Brief reports of classically conditioned place preference studies of ethanol in the rat have indicated contradictory motivational effects [3,5]. The present report describes a more detailed and comprehensive study of the phenomenon. Since ethanol has a biphasic dose-effect relation on many behaviors [30,36], place conditioning was studied with a wide range of doses of ethanol. In addition, parameters of ethanol infusion rates, ethanol concentrations and routes of administration were manipulated.

METHOD

Adult male Wistar rats (Canadian Breeding Laboratories, St. Constant, Quebec), weighing 250–350 g on arrival in the laboratory, underwent implantation of intravenous (IV) cannulae according to procedures described by Mucha *et al.* [26] and of intragastric (IG) cannulae according to a modification of the procedures of Deutsch and Koopmans [10]. Our IG cannulae were constructed of polyethylene (0.965 mm outer diameter, Clay Adams) and silicone (0.064 mm outer diameter, Silastic) tubing that were thinner than those used previously. Also, the silicone tubing was expanded with solvent and then allowed to shrink tightly over the polyethylene tubing rather than being attached with glue. Rats were housed individually at an ambient temperature of 22–23°, in rooms with lights on from 0700 to 1900 hr. Purina rat chow and tap water were available ad lib.

The experimental design and place conditioning procedure were identical to those used by Mucha *et al.* [26]. Briefly, the training was carried out in two square boxes differing in color, texture, and smell: black walls, smooth Plexiglas floor, and smell of vinegar, versus white walls, wood chip floor, and smell of wood. Each rat usually received 4 infusions of ethanol in the presence of one set of environmental cues and the same number of vehicle infusions in the other. Prior to each infusion, the rat was connected to the infusion system by a length of polyethylene tubing and allowed to walk freely about the appropriate box over the entire 30 min trial. Two min after the rat was placed in the box the infusion started. Treatment was on consecutive days and on each day rats received one infusion of ethanol and one of vehicle. One infusion was in the morning, one in the late afternoon or evening. The order of ethanol and vehicle presentation, and the choice of environment paired with ethanol, were counterbalanced for the rats in each group. Different groups of rats were used for each combination of dose, concentration and route of administration.

Testing was carried out in a large rectangular box with the two training environments on opposite sides, separated by a grey area with a grid floor. One day after the last infusion, rats were simply placed in the grey area and the amount of time (in seconds) spent in the two treatment environments over the next 15 min was scored.

Ethanol solutions (5 to 50% v/v) were prepared from 95% ethanol and saline. Tap water was used instead of saline in one set of experiments. Constant infusions were delivered at rates of about 0.25 to 2.4 ml/min for different durations of time. A wide range of ethanol doses was used: 0.055 to 1.1 g/kg IV and 0.5 to 5 g/kg IG.

The data are presented as mean±SEM. Student's *t*-tests were used and effects were considered significant when $p<0.05$. The tests were two-tailed except for those used in experiments involving IG administration of ethanol. Since these were designed to confirm the effects found with ethanol administered IV, the direction of the effects was known, justifying the use of one-tailed tests. To minimize the possibility of type II errors, statistical tests were carried out only when necessary.

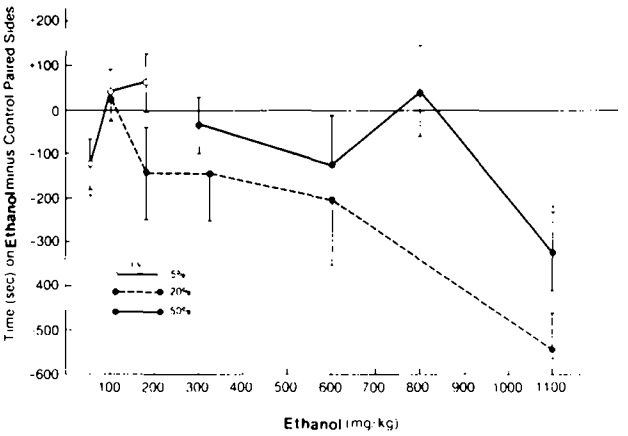

FIG. 1. Place conditioning produced by various doses and concentrations of ethanol administered intravenously. Data represent means±S.E.M. for 6–14 rats per group, except for the group receiving 0.1 g/kg at 5%, which was made up of 19 rats. *$p<0.05$.

RESULTS

IV Infusions

The first experiment involved IV infusions of doses of 0.1, 0.18, 0.32, 0.6, and 1.1 g/kg ethanol by varying the duration of infusion of a 20% ethanol solution (0.25 ml/min). Behaviors seen after the various doses during training were bidirectionally different from those after infusion of saline. After saline, the rats generally explored and groomed during the first half of the 30-min training session and slept or rested in the last half. After 1.1 g/kg ethanol they were typically inactive or ataxic, and after lower doses they explored the apparatus or groomed throughout the session. Behaviors produced by ethanol doses as low as 0.1 g/kg are well-known and therefore were not quantitated here (see [23]).

Appreciable place conditioning was seen, but only in rats receiving 1.1 g/kg as a 20% solution (see Fig. 1, solid circles with broken lines). These rats showed a clear aversion to the side of the test box paired with the ethanol. The mean times on the ethanol and vehicle sides were 66±26 sec and 613±70 sec, respectively. The difference between the times spent on the two sides was significant ($t=6.23$, $n=6$, $p<0.005$). Although some of the groups at lower doses appeared to show place aversions, these effects were not appreciable; at 0.6 g/kg, for example, the difference between the times on the two sides of the test box was not significant ($t=1.39$, $n=6$, $p>0.20$), despite the use of a powerful statistical test.

Since ethanol is not a potent drug, high concentrations and volumes were used as described above to study place conditioning. To control for the possible involvement of local irritant effects of high concentrations of ethanol, we contrasted effects seen after infusion of ethanol as a 20% solution at 0.25 ml/min, as described above, to those seen when other parameters of administration were used. In one set of experiments ethanol was infused as a 50% solution at a

rate of 1.2 ml/min. Acute behavioral effects of ethanol were similar to those described above with one exception: occasionally, animals receiving doses lower than 1.1 g/kg showed a transient depressant effect just after the ethanol administration, followed by the stimulant effect. The initial depressant effect was not seen when ethanol was given as a 20% solution and at a lower rate of infusion. Variations in effect associated with different parameters of administration of the same IV dose of ethanol have been reported previously [29]. With regard to place conditioning (see Fig. 1), the dose/response patterns found with the two sets of infusion parameters were generally similar. Indeed, only with the 1100 mg/kg dose of 50% ethanol was the amount of time spent on the ethanol side of the test box significantly less than that on the vehicle side ($t=3.65$, n=10, $p<0.01$). There was also a suggestion that place aversions were less marked with 50% ethanol than with 20%. The higher volumes required for administration of the 20% ethanol solution may have caused the greater aversiveness, but since this apparent effect was small and the general pattern was similar, this question was not pursued.

The effects of ethanol infused as a 5% solution at a rate of 0.25 ml/min were also studied. To avoid the possible aversive effects of high volumes of administration, only doses of 0.055, 0.1 and 0.18 g/kg were studied. Behavioral effects were seen only after doses of 0.1 and 0.18 g/kg ethanol; they comprised locomotor stimulation and increased grooming. The results of the place conditioning tests at these doses are shown in Fig. 1. Although there appears to be a difference at 0.18 g/kg between rats receiving 5% ethanol and those receiving 20%, there was no significant conditioned place preference in the 5% group at this dose ($t=0.97$, n=11, $p>0.30$). It should be noted that the data for 0.1 g/kg actually represent the combined results of several replications. During an initial experiment, it appeared that some rats were showing a preference for the ethanol side of the test box, but attempts to replicate this failed. A significant but small aversive effect of the lowest dose (0.055 g/kg) of the 5% solution was observed; no explanation of this is readily apparent.

Black *et al.* [3] reported that rats showed a preference for a place paired on 8 or 10 occasions with ethanol (1 g/kg). Therefore, we also tested the effects of 8 pairings. The group tested after receiving 4 pairings with 0.8 g/kg ethanol given as a 50% solution (1.2 ml/min) were given an additional 4 pairings with ethanol and tested a second time. On the first test day (Fig. 1) there was no apparent place conditioning, but on the second test day the rats showed an aversion to the side paired with ethanol that approached statistical significance (mean difference on the ethanol minus vehicle side = −206 sec, $t=2.01$, n=6, $p<0.1$). Thus, we failed to replicate the findings of Black *et al.* [3]. Their effects were probably not related to their use of the intraperitoneal route, as Cunningham [5] used this route and saw a conditioned place aversion. In addition, the following experiments with the IG route confirmed our findings with the IV route.

IG Infusions

Doses of ethanol were administered as 10, 20, or 50% solutions, at infusion rates of 1.2 or 2.4 ml/min. The rates of infusion were not systematically manipulated, because it was noted that normal ingestion can deliver fluid to the stomach at rates higher than those used to administer the ethanol here [15].

The patterns of behavior and place conditioning with IG ethanol were similar to those seen with IV ethanol. Following doses of 3.5 and 5.0 g/kg, clear depressant effects were seen, whereas after lower doses stimulant effects predominated. There was, however, a noticeable latency to onset of the behaviors with the IG route that was not seen after IV injection.

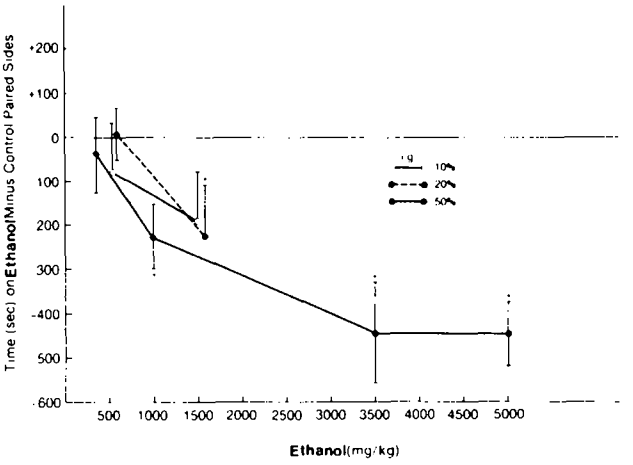

FIG. 2. Place conditioning produced by various doses and concentrations of ethanol administered intragastrically. Data represent means ± S.E.M. for at least 6 rats per group, except for the 3.5 and 5.0 g/kg groups which comprised 4 and 3 rats, respectively. *$p<0.05$.

As with IV ethanol, there was no obvious preference for the ethanol or saline side of the test box at IG doses lower than 1 g/kg, but at higher doses rats avoided the ethanol side. Statistical tests on the two smallest mean differences in the high-dose range revealed that at 1.5 g/kg given as a 20% solution the aversive effect was significant ($t=2.12$, n=9, $p<0.05$) and at 1.5 g/kg given as a 10% solution it approached significance ($p<0.1$). It is important to point out that the experiments with 1.5 g/kg given as a 20% solution actually comprised two groups differing in the nature of the vehicle. In most experiments saline was used, but since humans occasionally find intubations of saline aversive, this particular dose condition was repeated with tap water as a vehicle. The place aversion appeared more substantial in the group with the water vehicle. However, an additional experiment comparing the times spent on the side of the test box paired with IG saline and that paired with IG water failed to confirm this (mean time spent on the saline minus water side of the test box was −6 ± 127 sec, n=9).

DISCUSSION

The most important finding in the present report is the lack of demonstrable positive reinforcing effects of ethanol in the place conditioning paradigm. In previous studies opiates, cocaine, and amphetamine produced conditioned place preferences, the same effect seen with known positive reinforcers such as food and water (cf., [26,31]). In contrast, use of a similar experimental protocol in the present study showed that ethanol produced only conditioned place aversion. This effect was the same as those of generally acknowledged punishers such as footshock [19], lithium chloride, and naloxone [26]. It is concluded that in the place conditioning

paradigm ethanol is a punishing stimulus. Results with different parameters of administration suggest that this stimulus arises from the systemic pharmacological effects of ethanol, rather than from local irritant effects.

Ethanol readily produced place aversions only at doses of 1 g/kg or more, but its behavioral effects were evident at doses as low as one-tenth of this (present observations; [23,30]). Other investigators have found that ethanol served as an effective discriminative stimulus at doses that failed to produce place conditioning in the present study [18,38]. Therefore, low doses of ethanol were pharmacologically active, but as a stimulus for place conditioning they were neutral. Since a stimulus that does not normally produce a CR by itself generally can do so after pairing with effective UCSs such as food or shock [21], low doses of ethanol were considered to be neutral UCSs.

It is unlikely that the present conclusions are limited to the use of the place conditioning paradigm. It is well-known that ethanol is punishing in taste aversion experiments and the present dose/response curves are almost identical to those in the taste conditioning studies of Cappell *et al.* [4]. Also, many self-administration studies have indicated that in relatively naive animals ethanol does not readily produce an increase in responding (see Introduction). This is consistent with ethanol being punishing or neutral during the initial phases of training, although conclusive evidence for this is usually lacking. Numan [28] showed that untrained rats lever-pressed less when pressing was paired with ethanol than when paired with saline. However, two recent operant experiments [32,33] showed that ethanol is actually self-administered at certain low doses by naive rats. It should be noted that these studies [32,33], employed only continuous reinforcement schedules which do not allow the discrimination of the locomotor excitatory effects of ethanol from its reinforcing effects.

The present findings are consistent with the relatively weak reinforcing properties of ethanol revealed by numerous self-administration studies in experimental animals [12, 14, 25]. The long training and experience with ethanol that are usually necessary for the acquisition of self-administration create some difficulty in assessing the primary reinforcing properties, since they increase the possibility that other mechanisms may be involved. First, Deutsch *et al.* [9,10] and Numan [28] suggested that prolonged ethanol treatment, resulting in physical dependence, might provide a basis for reinforcement through reduction or postponement of withdrawal symptoms by ethanol. However, animals will voluntarily stop self-administering ethanol and subject themselves to severe withdrawal reactions [7], suggesting that this mechanism may play only a partial role.

Second, tolerance develops at different rates to different effects of ethanol [16]. If tolerance to the aversive effects develops more rapidly than to the initially weak positive reinforcing effects, the latter might be unmasked. This explanation is plausible, but conclusive evidence is not yet available.

Third, conditioned or secondary reinforcement [21] might be added to the weak primary reinforcing properties, and thus strengthen self-administration behavior. Through appropriate scheduling of ethanol administration together with effective primary reinforcers such as food, water, sex, social reinforcers, or other drugs, the reinforcing properties of ethanol might be significantly enhanced. Indeed, preliminary results in this laboratory suggest that small doses of ethanol paired with food may be effective in the place conditioning model [34]. Pairing with social reinforcement might prove to be a fruitful line of research.

ACKNOWLEDGMENTS

Supported in part by a grant from the Natural Sciences and Engineering Research Council of Canada to D. van der Kooy. R. F. Mucha is a Fellow of the MRC of Canada and gratefully acknowledges the use of facilities in the Experimental Psychology Department, Cambridge University, England, during preparation of the manuscript. We are indebted to Mrs. V. Cabral for typing the final manuscript.

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