LOW DOSE OF ETHANOL INDUCES CONDITIONED PLACE PREFERENCE IN RATS AFTER REPEATED EXPOSURES TO ETHANOL OR SALINE INJECTIONS

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(Received 5 February 1996; in revised form 22 July 1996; accepted 30 July 1996)

Abstract — Using the place conditioning paradigm (biased design), we have shown that five conditioning sessions with ethanol (0.5 or 1.0 g/kg, i.p.) did not result in place conditioning. Similarly, 15 conditioning sessions with ethanol (1.0 g/kg) did not produce significant place conditioning response. In contrast, rats that received 20 injections of ethanol (0.5 g/kg) or saline, before the conditioning procedure, showed significant place preference to the compartment paired with 0.5 g/kg ethanol (but not 1.0 g/kg).

INTRODUCTION

Conditioned place preference (CPP) has been used widely to study the rewarding effects of various drugs of abuse (Barr et al., 1985; Shoaib et al., 1994; Shippenberg and Heidbreder, 1995). This procedure measures drug reward by assessing the association developed between certain environmental stimuli and the drug effects. After several conditioning sessions, animals increase the amount of time spent in the compartment associated with the effects of rewarding drugs such as cocaine (Shippenberg and Heidbreder, 1995) or morphine (Mucha and Iversen, 1984; Barr et al., 1985).

Studies of the effect of ethanol (EtOH) on the conditioned place preference in rats have generated conflicting results. The most commonly observed outcome is a failure to establish a CPP after lower ethanol doses (Asin et al., 1985) or a conditioned place aversion (CPA) after higher (1.0 g/kg) doses of the drug (Cunningham, 1979; van der Kooy et al., 1983). These results taken together with some self-administration studies (Numan, 1981; Numan et al., 1984) suggest that EtOH possesses rather weak primary reinforcing properties in rats. Some authors, however, were able to show EtOH-induced CPP or reduction in EtOH-induced CPA if the rats had previously been exposed to ethanol (Reid et al., 1985; Gauvin and Holloway, 1991; Holloway et al., 1992). Bozarth (1990) reported EtOH-induced CPP after 15 drug-compartment pairings (biased design, without saline injections on alternate days), suggesting that a longer conditioning period might be necessary for associating weak positive reinforcing properties of EtOH with a distinct compartment.

Recently, we observed EtOH-induced CPP after repeated injections of EtOH. Animals injected i.p. with 0.5 g/kg of EtOH during pre-exposure period (20 days) and CPP procedure showed significant CPP to the compartment paired with EtOH. On the other hand rats receiving higher EtOH dose (1.0 g/kg) failed to develop the CPP (Bienkowski et al., 1995). These results suggested a development of sensitization to EtOH rewarding properties after administration of lower doses. Alternative explanations, e.g. development of tolerance to aversive properties of EtOH, might be considered since repeated injections of EtOH (usually higher dose) are known to attenuate subsequent conditioned taste aversion (CTA) (for review see Baker and Cannon, 1982). However, we had considered these results with caution since a non-specific effect of
repeated handling procedure and stressful stimuli associated with daily i.p. injections had not been ruled out in our preliminary report (Bienkowski et al., 1995).

In the present paper, we report the results of place conditioning studies with morphine (as a reference compound), ethanol and ethanol after an additional pre-exposure or conditioning period.

**MATERIALS AND METHODS**

**Animals**

Adult male Wistar rats (240–280 g at the start of each study) were used. The rats were housed 3–4 per cage, in the room with controlled environmental conditions (temperature of 21 ± 1°C, 50% relative humidity and 12:12 h light:dark cycle, light on at 0600). Standard laboratory food (Bacutil, Poland) and tap water were available ad libitum. All procedures were carried out between 1300 and 1800 h.

**Apparatus and conditioning procedure**

The CPP apparatus contained five rectangular, wooden boxes. Each box consisted of two end compartments (25 × 34 × 38 cm) and a middle area (25 × 11 × 38 cm). One of the end compartments had brown-painted walls and the floor made of black Plexiglas, whereas the second end compartment had its walls painted white and the floor made of rough naturally coloured plank. The middle area was all painted grey. Removable guillotine doors separated the middle area from the end compartments. The apparatus was enclosed in the temperature-controlled, dimly-lit room. A video camera and video tape recorder were used for acquisition of data. The data were subsequently scored by a 'blind' experimenter. The CPP procedure consisted of three phases and lasted for 13 consecutive days. During phase 1 (pre-conditioning phase; 2 days), the animals were allowed to explore freely the entire box for 10 min. On day 2 (pre-test), the time spent by the rat in each of the two end compartments was recorded and this provided a measure of pre-conditioning preference for the white or black compartment.

In phase 2 (conditioning phase; 10 days) the animals were injected daily with ethanol (0.5 or 1.0 g/kg, 10% v/v) or saline i.p., and after 5 min they were then confined to the non-preferred or preferred compartment, respectively. The conditioning sessions (five with the drug and five with saline) lasted for 20 min. In the place conditioning study with morphine, the rats received 2.0 mg/kg of morphine or saline and after 10 min were confined to the non-preferred or preferred compartment, respectively. The conditioning session lasted 30 min. The order of injections (drug–saline–drug–saline . . . or saline–drug–saline–drug . . .) was counterbalanced across all groups. Saline-treated control groups received 0.9% NaCl every day. During phase 3 (post-test; 1 day), the animals were allowed to explore the entire box for 10 min and the time spent in each of the two end compartments was recorded.

**Experiment 1: place conditioning with morphine**

In experiment 1, morphine sulfate (Polfa, Warsaw, Poland) was used to show that place preference conditioning might be obtained in our apparatus after five morphine-non-preferred compartment pairings. Morphine (2.0 mg/kg; salt form) was dissolved in saline and given s.c. in a volume of 1.0 ml/kg. The naive animals were randomly assigned to two experimental groups: a saline-treated control group (n = 10) and a morphine-treated group (n = 10). The procedure of conditioning and testing was as described above.

**Experiment 2: place conditioning with ethanol**

In this experiment, the ability of ethanol to induce place conditioning was assessed in completely naive animals. The rats were randomly assigned to three experimental groups: a saline-treated control group, a 0.5 g/kg ethanol-treated and a 1.0 g/kg ethanol-treated group (n = 8 rats per group). The procedure of conditioning and testing was as described above.

**Experiment 3: prolonged conditioning with ethanol**

In experiment 3, lasting for 35 days, after the pre-conditioning phase (2 days; phase 1), three conditioning phases, 10 days each, ended by post-tests, were conducted (post-test 1, 2, 3). The rats were randomly assigned to two experimental groups: a saline-treated control group (n = 8) and a 1.0 g/kg EtOH-treated group (n = 8). The procedure of conditioning and testing was as described above.
Experiment 4: conditioning with ethanol after a pre-exposure period

In experiment 4, lasting for 33 days, the conditioning procedure (described above) started after the pre-exposure period. During the pre-exposure period, the animals received 20 daily injections of saline or EtOH. The rats were randomly assigned to five groups differing in the treatment during the pre-exposure period and the conditioning phase: saline–saline, saline–0.5 g/kg EtOH, saline–1.0 g/kg EtOH, 0.5 g/kg EtOH–0.5 g/kg EtOH and 1.0 g/kg EtOH–1.0 g/kg EtOH (n = 8–10 rats per group).

Experiment 5

In this experiment, designed to replicate some findings of experiment 4, larger groups (n = 12–15 rats each) treated with saline during the pre-exposure period were studied. The rats were randomly assigned to three groups differing in the treatment during the conditioning phase (0.5 or 1.0 g/kg of EtOH, or saline). All groups received 20 daily injections of saline during the pre-exposure period. The conditioning procedure (described above) started after the pre-exposure period.

Statistics

All the results [i.e. mean time in seconds (±SEM) spent in the non-preferred compartment] were analysed by two-way ANOVA (group x phase) with repeated measure, and by Newman–Keuls test for individual post-hoc comparison.

RESULTS

Experiment 1

Five conditioning sessions with morphine resulted in a significant conditioned place preference to the compartment paired with the drug (Fig. 1). Two-way ANOVA showed significant effect of group: $F(1.18) = 5.95$, $P = 0.025$; phase: $F(1.18) = 14.87$, $P < 0.001$; and group x phase interaction: $F(1.18) = 8.64$, $P < 0.01$. Post-hoc analysis showed that the morphine-treated group differed significantly ($P < 0.01$) from the saline-treated group during post-test (phase 3).

Experiment 2

A short period of conditioning (five sessions) with ethanol (0.5 or 1.0 g/kg) was not sufficient to induce any significant place conditioning (Fig. 2). Two-way ANOVA did not show significant effect of group, phase or group x phase interaction (all $F$ values <1).

Experiment 3

Fifteen pairings of 1.0 g/kg EtOH dose with the

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Fig. 1. Results of place conditioning with 2.0 mg/kg of morphine (experiment 1). The data are expressed as the mean time (±SEM) spent by the rats in the non-preferred compartment during pre-and post-test. Numbers in parentheses indicate size of the groups. *$P < 0.01$ vs saline–saline group.

Fig. 2 Results of place conditioning with 0.5 or 1.0 g/kg of ethanol (experiment 2). The data are expressed as the mean time (±SEM) spent by the rats in the non-preferred compartment during pre- and post-test. Numbers in parentheses indicate size of the groups.
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non-preferred compartment (experiment 1) did not result in a place preference conditioning (Fig. 3). Two-way ANOVA did not show significant effect of group, phase or group × phase interaction (all F values < 1). The time spent in the non-preferred compartment during the subsequent post-tests remained relatively stable for every group.

Experiment 4
Two-way ANOVA showed significant effect of group: $F(4,39) = 3.93, P < 0.01$; phase: $F(1,39) = 6.28, P < 0.001$; and group × phase interaction: $F(4,39) = 2.66, P < 0.05$ (Fig. 4A). Lower dose of EtOH (0.5 g/kg) was capable of inducing significant CPP in the group receiving 0.5 g/kg EtOH during pre-exposure period ($P < 0.01$ vs saline–saline group during post-test). Post-hoc analysis did not reveal any other significant differences vs saline–saline group, though marked increase in preference to the drug-paired compartment was observed in saline–0.5 g/kg EtOH group.

Experiment 5
Two-way ANOVA revealed significant effect of phase: $F(1,38) = 5.20, P < 0.001$; and interaction: $F(2,38) = 4.29, P < 0.025$. The effect of
DISCUSSION

The results of experiment 1 indicate that, using our apparatus, it is possible to produce significant place preference conditioning with a standard dose of morphine. These results are in line with many previous studies demonstrating conditioned place preference after morphine injections (Mucha and Iversen, 1984; Barr et al., 1985).

In accordance with many other reports (Cunningham, 1979; Van Der Kooy et al., 1983; Asin et al., 1985), ethanol did not induce place preference conditioning after five conditioning sessions (experiment 2). The CPA reported in several studies with higher ethanol doses (Cunningham, 1979; Van Der Kooy et al., 1983) was not observed in the present study. The procedure used by us (the biased design) may be a possible explanation of an apparent lack of aversive conditioning with 1.0 g/kg dose of ethanol.

Furthermore, three conditioning phases [i.e. 15 ethanol (1.0 g/kg)-compartment pairings] did not result in any significant place conditioning effect (experiment 3). Our results thus do not support a previous finding (Bozarth, 1990), but an obvious procedural difference makes direct comparison between the studies difficult.

Data from experiments 4 and 5 suggest a development of sensitization to EtOH rewarding effects, detected in biased CPP procedure, after prolonged administration of relatively small dose (0.5 g/kg), but not after prolonged pre-exposure and conditioning with a higher dose (1.0 g/kg). The results of the present study are in accordance with our previous findings (Bienkowski et al., 1995) and, in addition, suggest a role for repeated non-specific handling and injections, as the animals treated daily with saline showed CPP after administration of lower but not higher, dose of EtOH. Thus, prolonged pre-exposure to low EtOH dose as well as repeated stress induced by handling and i.p. injections were capable of sensitizing the rats to the rewarding effects of a 0.5 g/kg dose of EtOH. In addition, our results are in line with previous findings that prolonged pre-exposure to EtOH was necessary for producing CPP response (Reid et al., 1985; Gauvin and Holloway, 1991), facilitating i.v. self-administration (Numan, 1981) and attenuating CTA induced by EtOH (Baker and Cannon, 1982; Stewart et al., 1991). Recently, similar findings were reported for another weak primary reinforcer in rats, nicotine. Only the rats with a history of nicotine exposure showed CPP to the compartment paired with the drug (Shoaib et al., 1994).

The higher dose of EtOH (1.0 g/kg) did not produce CPP either after prolonged conditioning (experiment 3) or pre-exposure (experiment 4). These results are in contrast with findings by Bozarth (1990), though strain differences in aversive threshold for EtOH and procedural variations (lack of saline injections on the alternated days in the Bozarth study) may partially explain the discrepancy. Davies and Parker (1990) reported lack of effect of nine EtOH injections (2.0 g/kg) on subsequent place conditioning, suggesting that lower, less aversive, doses of EtOH might be more appropriate.

Although behavioural sensitization has been well described for psychostimulants (Shippenberg and Heidbreder, 1995) or nicotine (Shoaib et al., 1994), the literature on sensitization to EtOH's behavioural or rewarding effects is sparse. Cunningham and Noble (1992) demonstrated that repeated injections of EtOH produce sensitization to its activating effect in DBA/2J inbred mice during conditioning trials that produce preference for EtOH-paired environmental stimuli. Other work demonstrated positive effects of nicotine or amphetamine injections on EtOH drinking behaviour in rats, and reported the potentiation of EtOH-induced locomotor stimulation and dopamine turnover after nicotine pre-exposure in mice (Soderpalm et al., 1993; Fahlke et al., 1994; Johnson et al., 1995).

There is general agreement that the mesolimbic dopaminergic system is the anatomical locus for reinforcing effects of drugs of abuse (Di Chiara, 1995). EtOH, like other abused drugs, has been consistently reported to stimulate dopamine (DA) neurotransmission in this brain region (Gessa et al., 1985; Yoshimoto et al., 1982). Although the neuropharmacological mechanisms underlying the sensitization of EtOH rewarding effects by repeated stressful stimuli remain unknown, the
most probable explanation involves the changes in DA neurotransmission. Release of DA occurs after exposure to different kinds of stimuli including those possessing negative properties. Importantly, not only strong negative stimuli (e.g. the foot-shock), but also mild stressors such as restraint of conscious rats enhances the release of DA from the nucleus accumbens and prefrontal cortex (for review, see Salamone, 1994). Thus, it may well be that DA mechanisms underlie the sensitization of reinforcing effects of EtOH after prolonged pre-exposure to EtOH as well as chronic injection stress. Recently, Persico et al. (1995) showed that pre-exposure to repeated i.p. saline injections profoundly changes transcription factor gene expression, DA turnover and locomotor activity, particularly in a novel environment. In addition, by comparing data from their different studies (Persico et al., 1993, 1995), the above authors suggested development of 'cross-sensitization' between amphetamine and injection stress.

Another plausible mechanism that may underlie the stress-induced sensitization for rewarding properties of EtOH may relate to GABA-ergic neurotransmission. Stress decreases the function of chloride channel coupled to the GABA-A receptor due to reduction in density of benzodiazepine receptors and increases in the density of so-called inverse benzodiazepine agonist binding (e.g. Mennini et al., 1988). Notably, these latter authors reported the down-regulation of [3H]-flunitrazepam binding sites in the rat cortex following repeated handling.

With regard to the dosage, it seems that the higher dose of EtOH (1.0 g/kg) was unable to produce CPP because of its aversive properties. It is noteworthy that low doses of EtOH have been consistently reported as ineffective in inducing the CTA in rats, whereas a higher dose produced clear-cut aversive effects (Cannon and Carrell, 1987; Froehlich et al., 1988). Thus, low EtOH dose is likely to be a critical factor in producing the CPP, as higher doses may evoke a variety of unpleasant effects which can mask the true rewarding effect (Nadal et al., 1992). We cannot offer, at present, a more precise explanation of the differences in effects of the two doses of EtOH used in this study.

Given that background, the present findings suggest that factors such as drug pre-exposure or stress might be necessary to bring out the rather weak primary reinforcing properties of EtOH in the CPP paradigm.

Acknowledgements—This study was partially supported by a grant from KBN, Warsaw (GP 20701907). The authors wish to thank Dr Mariusz Papp for helpful comments and advice.

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