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The role of drug-paired stimuli in extinction and reinstatement of ethanol-seeking behaviour in the rat

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Abstract

Male Wistar rats were trained to respond for ethanol (30 min/day) in an oral self-administration procedure. A single lever press resulted in presentation of 0.1 ml of 8% ethanol from a liquid dipper. When responding for ethanol stabilised, reinstatement sessions started. In the 30-min reinstatement session, lever pressing was first extinguished for 20 min by switching the dipper off. Then, different kinds of stimuli were non-contingently delivered and reinstatement of lever pressing was assessed. Fifteen random (random time = 15 s) presentations of the dipper containing 8% ethanol potently reinstated ethanol-seeking. The reinstatement of lever pressing was immediate and most responses were emitted during the time needed for the first five presentations to occur. Presentations of the empty dipper or delivery of a non-specific stimulus (high-amplitude tone) did not produce any reinstatement. These results indicate that non-contingent presentations of the ethanol-associated stimulus complex may reinstate operant behaviour previously reinforced with ethanol.

Keywords: Ethanol self-administration; Extinction; Reinstatement; Relapse; (Rat)

1. Introduction

There is a common belief that sustained abstinence should be a main goal of treatment programs addressed to alcohol addicts. Relapse to uncontrolled drinking is a major problem in the treatment of detoxified, abstinent alcoholics (Mann, 1996; Soyka, 1997). Continuous abstinence rates for placebo groups, that is, for motivated people receiving medical support during clinical trials, is low and ranges from 7 to 25% after one year (for review, see Zernig et al., 1997). This is why, from a clinical perspective, any new 'anti-alcohol' drugs should first of all promote abstinence, i.e., prevent relapse (Mann, 1996; Soyka, 1997; Zernig et al., 1997). However, a different approach is used in most animal studies. Typically, new drugs are tested in alcohol-preferring animals maintained on free-choice, long-term access to alcohol and water. Reduction of ethanol intake is thought to predict clinical utility of a given substance (for review, see Myers, 1994). Unfortunately, this approach neglects processes associated with abstinence and relapse (Carroll and Comer, 1996; De Wit, 1996).

Another animal model to study relapse to drug seeking is the reinstatement paradigm (for review, see Carroll and Comer, 1996; Self and Nestler, 1998). Recently, two groups have described extinction and reinstatement of operant responding for ethanol in rats. Chiamulera et al. (1995) have reported resumption of ethanol-seeking in rats receiving small amounts of ethanol solution contingent upon lever pressing. Lê et al. (1998) have shown that acute stress and, to a lesser extent, injections of ethanol, reinstated lever pressing for ethanol after extinction.

Both studies on reinstatement of ethanol-seeking used a between-session design with long-term extinction (4–10 daily sessions) preceding delivery of the priming stimuli. However, a within-session design is also possible. The within-session reinstatement paradigm has been used in several studies on reinstatement of cocaine-seeking (Carroll and Comer, 1996; Self and Nestler, 1998; Tran-Nguyen et al., 1998). Footshock stress or alcohol itself was used as the priming stimulus in the previous studies (Chiamulera et

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al., 1995; Lê et al., 1998). Clinical observations indicate that not only stress and alcohol itself, but also various alcohol-associated environmental stimuli increase the risk of relapse to uncontrolled drinking (De Wit, 1996). In the present study, we aimed to analyse reinstatement of ethanol-seeking evoked by ethanol-associated stimuli in the within-session paradigm.

2. Materials and methods

2.1. Subjects

Male Wistar rats (360–400 g at the beginning of the study) were housed two per cage in a room with controlled environmental conditions (temperature of 22 ± 1°C, ~ 60% humidity, a 12 h–12 h light–dark cycle with light on at 6:00 a.m.). The animals were supplied by a licensed breeder (HZL, Warsaw, Poland) at least 14 days before the start of experimental procedures. Standard lab chow (Bacutil, Poland) was always available in the home cages. Tap water was available ad libitum except as noted below. Treatment of the rats in the present study was in full accordance with the ethical standards laid down in respective European and local regulations.

2.2. Apparatus

Operant responding for ethanol (oral self-administration) was tested in standard operant conditioning chambers (Coulbourn Instruments, Allentown, PA, USA). The chambers (for details, see Bienkowski et al., 1997) consisted of test cages enclosed within sound-attenuating cubicles with fans for ventilation and background white noise. A white house light was centred near the top of the front of the cage. The start of sessions was signalled by turning the house light on. The cage was also equipped with two response levers, a liquid delivery system (the liquid dipper) and a high-amplitude tone generator (2.9 kHz; Sonalert, Coulbourn). Only one lever ('active' lever) activated the liquid dipper. Presses on the other lever ('inactive' lever) were recorded, but not reinforced. During a self-administration session, the liquid delivery system presented ethanol in 0.1-ml portion for 5 s. The availability of reinforcer was signalled by a brief audible click and a white light (4 W) located inside the liquid dipper hole. Programming of every session as well as data recording made use of the L2T2 Software package (Coulbourn) running on an IBM-compatible PC.

2.3. Operant responding for ethanol

The rats ($n = 12$) were trained to respond for ethanol according to Samson's sucrose-fading procedure (Samson, 1986) with some minor modifications (for details, see Piasecki et al., 1998). The animals were deprived of water for 22 h/day during the first 4 days of training and shaped to lever press for 10% sucrose solution on a fixed ratio (FR1) schedule of reinforcement. As soon as lever pressing was established, water started to be freely available in the home cages. All training sessions were 30 min long and one session was given each day. Starting on day 5, the animals received 2% ethanol–10% sucrose. Then, over the next 10–14 sessions, ethanol concentrations were gradually increased (from 2% to 8%; v/v) and sucrose concentrations were decreased (from 10% to 0%). The rats were allowed to stabilise their 8% ethanol consumption for at least 45 days. The criterion for stable responding was defined as ± 20% of the previous session's total number of responses for three consecutive sessions. When the self-administration behaviour stabilised, the rats were tested in reinstatement sessions with different kinds of priming stimuli (see below).

2.4. Reinstatement procedure

A within-session design was used to study reinstatement of ethanol-seeking after extinction. The reinstatement sessions lasted 30 min. The animals were first allowed to lever press in extinction for 20 min. The liquid delivery system was off during this period. Then, within the next 6–8 min, an ethanol-associated stimulus complex was repeatedly delivered (15×7.5 s) according to a random time 15 s schedule (RT15 s). The stimulus complex included a brief audible click associated with each activation of the liquid dipper and illumination of the light located inside the dipper hole. The dipper cup was either filled with 8% ethanol or remained empty. Following the non-contingent stimulus complex presentations, the extinction conditions were maintained to the end of the session. The effects of only seven deliveries of the dipper containing 8% ethanol were also examined. The rats tested in the 30-min extinction session (i.e., without any stimulus presentations) served as control animals. The ethanol intake during the reinstatement session was estimated by counting interruptions of a photocell located inside the dipper hole.

Other experiments tested the effects of the high-amplitude tone (15×7.5 s) in the reinstatement procedure. The high-amplitude tone was never paired with ethanol self-administration and thus served as a non-specific control cue.

In all the above experiments, randomly selected groups of 8–12 subjects were used. In order to be tested in each subsequent reinstatement session, the rat had to show stable ethanol intake in at least three consecutive self-administration sessions.

2.5. Statistics

A one-way analysis of variance (ANOVA) with repeated measures where appropriate, was used to analyse the data from the reinstatement sessions. Newman–Keuls

test was used for post-hoc comparisons. Student's *t*-test was employed when data from two groups were compared.

3. Results

All animals learned to self-administer 8% ethanol. The baseline number of lever presses ranged from 50 to 90 presses/30 min, with individual ethanol intakes of 0.5–0.8 g/kg/30 min. Typically, more than 75% of the ethanol solution was consumed within the first 10 min of the session.

Extinction of operant responding for ethanol was rapid (Fig. 1). In fact, approximately 80% of responses were emitted during the first 5 min of the 30-min extinction session. Importantly, spontaneous recovery of operant behaviour was not observed when sham stimuli (no stimulus presentations) were delivered during the session (Fig. 1).

Fifteen, but not seven, non-contingent presentations of the liquid dipper containing 8% ethanol produced significant reinstatement of ethanol-seeking [$F(2,22) = 4.38$, $P < 0.03$; Fig. 2A]. In contrast, 15 presentations of either the empty dipper or the high-amplitude tone did not reinstate operant behaviour ($P > 0.4$, *t*-test; Fig. 2A).

The distribution of responding induced by 15 deliveries of the dipper filled with ethanol was studied in detail. Fig. 2B shows the number of lever presses associated with consecutive blocks of presentations of the dipper. The number of responses declined with time [$F(2,21) = 4.17$, $P < 0.03$] and the highest rate of responding was generated by the first five stimulus deliveries. A representative individual record of extinction and reinstatement of lever pressing behaviour is shown in Fig. 3.

The total ethanol intake associated with 15 dipper deliveries reached 0.13 g/kg of absolute alcohol. The ethanol consumption associated with the first five dipper presentations was as low as 0.034 g/kg. Typically, the subjects skipped (i.e., did not explore the dipper hole) the first one to two presentations.

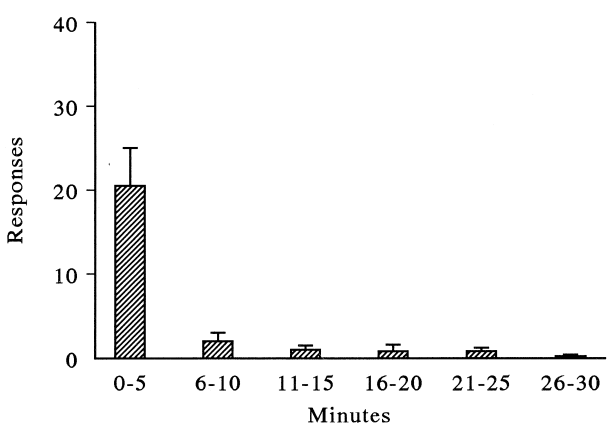

Fig. 1. Extinction of lever pressing for ethanol in the 30-min extinction session. Bars represent mean (with S.E.M.) numbers of responses on the previously 'active' lever in six consecutive 5-min periods; $n = 12$ rats.

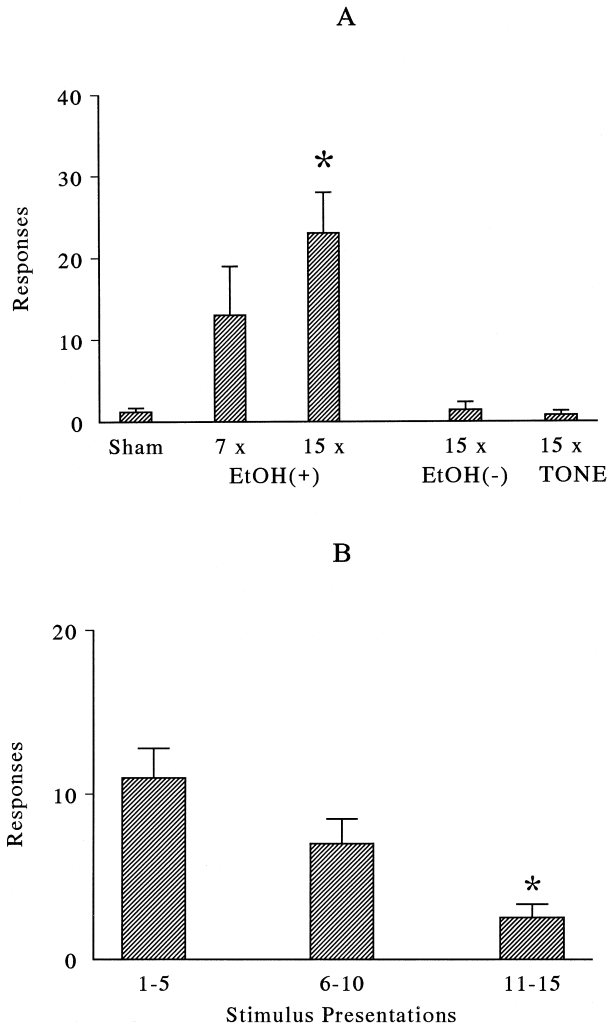

Fig. 2. (A) Reinstatement of ethanol-seeking induced by 7 or 15 non-contingent, random presentations of the liquid dipper containing 8% ethanol [EtOH(+)]. For comparison, the effects of 15 deliveries of either the empty dipper [EtOH(−)] or the high-amplitude tone (TONE) is shown. The dipper or tone deliveries started after 20 min of extinction. Bars represent mean (with S.E.M.) numbers of responses on the previously 'active' lever in the last 10 min of the 30-min reinstatement session; $^*P < 0.03$ vs. sham presentations. (B) Mean (with S.E.M.) numbers of responses on the previously 'active' lever shown as a function of consecutive presentations of the dipper containing 8% ethanol. $^*P < 0.03$ vs. the operant behaviour induced by the first five stimulus deliveries.

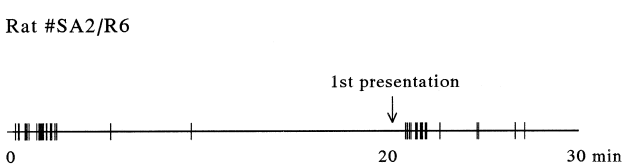

Fig. 3. Pattern of responding from a representative subject tested in the 30-min reinstatement session. Reinstatement of ethanol-seeking was induced by 15 non-contingent, random (RT15 s) presentations of the liquid dipper filled with 8% ethanol. The first stimulus delivery (*arrow*) occurred after ~20 min of extinction and the last (15th) delivery occurred 6 min 45 s later. Hatchmarks represent the times of each lever press.

The mean number of 'inactive' lever presses in all the above experiments was negligible (< 1.4 presses/30 min; data not shown).

4. Discussion

The pattern of both ethanol self-administration and extinction of ethanol-seeking was fully comparable with that found in our previous study (Piasecki et al., 1998).

The non-contingent, random presentations of the high-amplitude tone, which was never paired with ethanol self-administration, did not lead to any reinstatement of ethanol-seeking. This finding excludes the possibility that any non-specific stimulus might reinstate lever pressing behaviour in our procedure. Similarly, non-contingent activation of the empty dipper did not lead to any significant resumption of responding. Thus, the auditory and visual cues associated with each dipper activation were not able to prime ethanol-seeking in the absence of ethanol in the dipper cup.

The non-contingent, repeated activation of the liquid delivery system filled with ethanol produced robust reinstatement of responding previously reinforced with ethanol. The magnitude of reinstatement depended on the number of presentations, reaching statistical significance after 15 deliveries. Because the deliveries of the empty dipper did not produce any reinstatement, one could hypothesise that it was mainly taste and/or smell of 8% ethanol which primed operant responding after extinction.

Although the consumption of ethanol during the reinstatement sessions was very low (0.13 g/kg), the possibility exists that the central effects of ethanol were sufficiently strong to prime ethanol-seeking. However, this assumption is rather unlikely. First, the number of lever presses associated with the consecutive dipper presentations declined. In contrast, the cumulative intake of ethanol (and possibly also its central effects) increased with each dipper presentation. Second, although the ethanol intake produced by the first five deliveries was negligible (0.034 g/kg), these initial presentations were associated with strong and almost immediate reinstatement of lever pressing behaviour.

In a more recent series of experiments employing the reinstatement paradigm, we have tested the effects of non-contingent deliveries of different concentrations of ethanol. Surprisingly, priming effects of tap water and 8% ethanol were fully comparable (Bienkowski et al., unpublished). This latter finding finally excludes the possibility that any central effects of ethanol and/or its sensory properties play a critical role in the reinstatement of ethanol-seeking in our procedure. Generally, it appears that the reinstatement of ethanol-seeking in the present paradigm is evoked by the compound stimulus including the visual/auditory cues emitted by the dipper and some sensory properties of liquids available in the dipper cup.

Discriminative stimuli set the occasion when behavioural responses are followed by reinforcement. These stimuli may produce relapse in drug-seeking after extinction (Bouton and Swartzentruber, 1991; McFarland and Ettenberg, 1997). In the present study, the ethanol-associated stimuli could act as discriminative stimuli and reinstate responding after extinction. This problem might be further addressed in future experiments analysing the exact distribution of lever pressing as a function of different times and frequencies of non-contingent dipper activation.

In contrast to the previous reinstatement studies with ethanol (Chiamulera et al., 1995; Lê et al., 1998), prolonged (4–10 daily sessions) periods of extinction were not used in the present study. Obviously, long-term extinction of operant responding for alcohol in the natural environment is not included in typical treatment programs addressed to alcoholics. However, detoxified addicts are 'forced' to keep abstinence in the initial in-patient phase of treatment (Batel, 1996; Mann, 1996). In our current studies, we try to determine how different periods of forced abstinence alter extinction and reinstatement of ethanol-seeking. Recently, Tran-Nguyen et al. (1998) have shown time-dependent enhancement of cocaine-induced reinstatement of cocaine-seeking. Although preliminary, these results are particularly interesting as they suggest that forced abstinence may actually increase the magnitude of cocaine-seeking behaviour.

Acknowledgements

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