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Reinstatement of Ethanol Seeking in Rats: Behavioral Analysis

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BIENKOWSKI, P., E. KOROS, W. KOSTOWSKI AND A. BOGUCKA-BONIKOWSKA. *Reinstatement of ethanol seeking in rats: Behavioral analysis.* PHARMACOL BIOCHEM BEHAV **66**(1) 123–128, 2000.—The reinstatement model has been repeatedly used to study relapse to heroin- or cocaine-seeking behaviour in rats. The aim of the present study was to evaluate basic behavioral parameters of cue-induced reinstatement of ethanol seeking in a within-session paradigm. Rats were trained to respond for ethanol in an oral self-administration procedure where each lever press resulted in presentation of 0.1 ml of 8% ethanol from a liquid dipper. In the reinstatement paradigm operant behaviour was first extinguished for 20 or 60 min by switching the dipper off. Then, ethanol-associated stimuli were noncontingently delivered and reinstatement of responding was assessed. Deliveries of the empty dipper, i.e., visual/auditory cues only, did not result in any reinstatement. In contrast, 15 random presentations of the dipper containing either ethanol (4–8%; v/v) or water significantly reinstated ethanol seeking. In a control self-administration experiment responding dropped to nonsignificant levels when water was substituted for ethanol. The magnitude of reinstatement did not depend on the duration of the extinction phase. These results seem to indicate that in the present paradigm reinstatement of ethanol seeking is driven by a compound stimulus including the visual/auditory cues and some nonspecific sensory properties of liquid available in the dipper. © 2000 Elsevier Science Inc.

Ethanol self-administration Extinction Reinstatement Relapse Craving

THE high probability of relapse still remains the major challenge for treatment programs addressed to detoxified alcoholics (17,27,31). Thus, more preclinical studies on relapse to alcohol-seeking and alcohol-taking behaviour are clearly needed. An animal model to study relapse to drug seeking is the reinstatement paradigm (3,7,11,25,26,28). Subjects tested in the reinstatement model must present stable self-administration behaviour for a long period of time. In the reinstatement phase, operant behaviour is first extinguished (drug reinforcement absent). Then, different kinds of stimuli are noncontingently delivered and resumption of operant behaviour is assessed [for reviews, see (7,25)]. Reinstatement of cocaine, heroin, and nicotine seeking has been extensively studied by several laboratories. Both within-session (7,11,24,25,29) and between-session design (10,19,26) has been used in these experiments. Importantly, all major categories of stimuli that have been proposed to increase probability of relapse in human addicts, i.e., priming dose of abused substance, stressful events, and drug-associated environmental cues (12,13,16), produce reinstatement in animal subjects (7,9–11,19,20,25,28).

Two groups have recently studied reinstatement of ethanol seeking in rats trained to respond for ethanol in the operant oral self-administration procedure (8,15). Chiamulera et al. (8) have reported resumption of extinguished ethanol seeking in rats allowed to consume small amounts of ethanol. Another group (15) has shown that acute foot shock stress, and to a lesser extent ethanol administration, reinstated ethanol-seeking behaviour. The between-session design with long-term extinction (4–10 daily sessions) preceding delivery of the priming stimuli was employed in the above studies (8,15).

More recently, we have described the within-session reinstatement paradigm (3,4) based on the oral ethanol self-administration procedure introduced by Samson (23). In a 30-min reinstatement session, lever pressing was first extin-

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guished for 20 min by switching a liquid dipper off. Noncontingent, random presentations of ethanol-associated discriminative stimuli (i.e., the dipper filled with 8% ethanol) produced robust reinstatement of responding previously reinforced with ethanol. In contrast, repeated deliveries of a nonspecific control cue (high-amplitude tone), which was never paired with ethanol self-administration, did not lead to any reinstatement.

The magnitude of reinstatement depended on the number of dipper presentations reaching statistical significance after 15 deliveries (3). As in the reinstatement phase animals were allowed to consume small amounts of ethanol from the dipper one could hypothesise that some central effects of ethanol were sufficiently strong to prime ethanol seeking. This assumption seemed to be unlikely for two reasons. First, the ethanol intake during the reinstatement phase was low, and did not exceed 0.15 g/kg. Second, although the ethanol intake produced by the first five dipper deliveries was negligible ($<$0.04 g/kg) these initial presentations were associated with the strongest reinstatement. Because deliveries of the empty dipper did not produce any reinstatement, we assumed that it was mainly taste and/or smell of 8% ethanol that primed operant behaviour after extinction (3). This problem was further analysed in the present study. The main goal of the present study was to evaluate whether any sensory or pharmacological effects of ethanol might be involved in resumption of ethanol seeking in our procedure. For this reason, reinstatement induced by deliveries of the dipper containing either water or different ethanol concentrations was studied. In addition, longer duration of extinction [comparable to that used in previous studies on reinstatement of cocaine seeking (7,11,,24,25)] was introduced and compared with 20-min extinction used in our previous experiments (3,4). To confirm that ethanol, but not water, served as a reinforcer in the present study, responding for water was assessed in rats trained to self-administer ethanol.

METHOD

Subjects

Thirty-two male Wistar rats (HZL, Warsaw, Poland) were housed two per cage in a temperature- and humidity-controlled room on a 12 L:12 D cycle (lights on at 0600 h). The subjects (200–220 g) were supplied by the breeder at least 14 days before the start of experimental procedures. Standard lab chow (Bacutil, Warsaw, Poland) was always available in the home cages. Tap water was available ad lib, except as noted below. All procedures were conducted in full accordance with respective Polish and European (directive No. 86/609/EEC) regulations and were approved by a local Ethics Committee.

Apparatus

Responding for ethanol (oral self-administration) was tested in eight standard operant conditioning chambers (Coulbourn Instruments, Inc., Allentown, PA). The chambers [for details, see (2,3)] 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 and a liquid delivery system (the liquid dipper). 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 a 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.

Operant Responding for Ethanol

The rats were trained to respond for ethanol according to Samson's sucrose-fading procedure (23) with some minor modifications [for details, see (3,22)]. 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 1 (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% v/v ethanol–10% w/v sucrose. Then, over the next 12–15 sessions ethanol concentrations were gradually increased (from 2 to 8%) and sucrose concentrations were decreased (from 10 to 0%). The rats were allowed to stabilise their 8% ethanol consumption for at least 30 days. The criterion for stable responding was defined as $\pm$20% of the previous session's total number of responses for three consecutive sessions. Only subjects that consistently emitted more than 20 responses on the "active" lever/30 min were used in experiments described below.

Extinction of Ethanol Seeking

To evaluate a within-session pattern of responding in extinction some rats (n = 8) were tested in 90-min extinction sessions. In the extinction session the liquid delivery system was off, and responding on either lever had no consequences. The 90-min extinction duration was used to assess any spontaneous recovery of operant behaviour that might occur after initial extinction.

Reinstatement of Ethanol Seeking: Role of Ethanol Concentration

A within-session design was used to study reinstatement of ethanol seeking after extinction (3). The reinstatement sessions lasted 30 min. The animals were first allowed to lever press in extinction for 20 min. Then, within the next 6–8 min an ethanol-associated discriminative stimulus complex was repeatedly delivered (15 $\times$ 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 filled with water, ethanol (4 or 8%; v/v), or remained empty. Following the noncontingent stimulus complex presentations, the extinction conditions were maintained to the end of the session. The rats tested in the 30-min extinction session (i.e., without any stimulus presentations) served as control animals.

Groups of six or eight randomly selected subjects were tested in each stimulus condition. These animals emitted $\leqslant$1 response in the 10 min preceding the start of the dipper deliveries. The stimulus conditions (presentations of the empty dipper; presentations of the dipper filled with different ethanol concentrations; no dipper deliveries) were studied in a counterbalanced order. In general, most of the subjects were tested in more than one stimulus condition. To be tested in each subsequent reinstatement session the rat had to show stable (see above) ethanol intake in at least four consecutive self-administration sessions.

Water Reinforcement in Rats Trained to Respond for Ethanol

In the experiment described above, noncontingent deliveries of the dipper filled with water produced significant reinstatement of ethanol seeking. Thus, we decided to analyse water reinforcement in the subjects ($n = 4$) trained to self-administer ethanol. For this reason, water self-administration in six consecutive 30-min sessions was assessed.

Reinstatement of Ethanol Seeking: Role of Duration of Extinction Phase

The effect of extinction duration (20 vs. 60 min) on reinstatement of ethanol seeking was studied in another group of subjects. The extinction conditions were tested in a counterbalanced order. The reinstatement sessions lasted 30 or 70 min, and the animals ($n = 6$–7) were first allowed to lever press in extinction for 20 or 60 min, respectively. All animals emitted ⩽1 response in the 10 min preceding the start of the dipper deliveries. After extinction, the ethanol-associated stimulus complex was repeatedly delivered according to the RT15-s schedule (see above). The dipper cup contained 8% ethanol. Following the noncontingent stimulus complex presentations, the extinction conditions were maintained to the end of the session. The rats ($n = 6$) tested in the 30- or 70-min extinction session (i.e., without any stimulus presentations) served as control subjects.

Data Analysis

A one- or two-way analysis of variance (ANOVA) with repeated measures where appropriate was used to analyse the data. Newman–Keuls test was chosen for post hoc comparisons. Multiple regression was employed to assess possible correlations between parameters of ethanol self-administration and ethanol-seeking behaviour. A probability level (p) less than 0.05 was considered significant.

RESULTS

Operant Responding for Ethanol

Twenty-four out of 32 rats learned to self-administer 8% ethanol. Typically, the baseline number of lever presses ranged from 40 to 90 responses/30 min, with absolute alcohol intakes of 0.5–0.8 g/kg/30 min. The pattern of ethanol self-administration in the present experiments was comparable with that described in our previous studies (3–5,22).

Extinction of Ethanol Seeking

Extinction of responding for ethanol was rapid. As shown in Fig. 1, more than 85% of responses were emitted during the first 10 min of the 90-min extinction session. Importantly, after the initial burst of lever pressing there were no spontaneous recovery of operant behaviour.

Reinstatement of Ethanol Seeking: Role of Ethanol Concentration

Fifteen noncontingent presentations of the liquid dipper containing either water or ethanol (4 or 8%) produced significant reinstatement of ethanol seeking, $F(4, 29) = 5.84$, $p < 0.01$. In contrast, 15 deliveries of the empty dipper did not reinstate operant behaviour (Fig. 2).

Correlational analysis did not reveal any significant relationship between individual ethanol self-administration and extinction/reinstatement of lever pressing behaviour (rs < 0.4, ps $>$ 0.2). Similarly, number of responses emitted in extinction did not predict the magnitude of reinstatement ($r = 0.43$, $p = 0.15$).

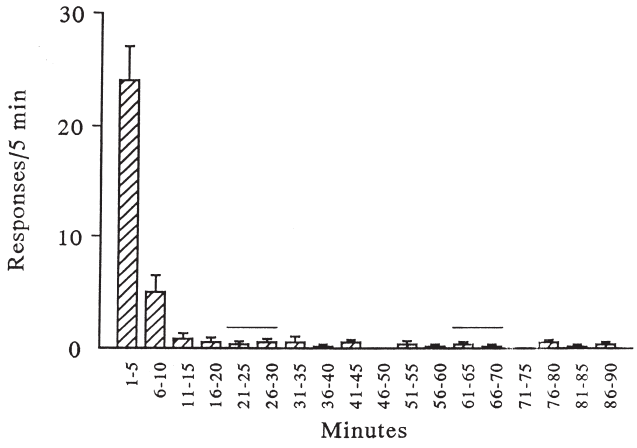

FIG. 1. Responding for ethanol in the 90-min extinction session. In the extinction session the liquid dipper was inactive and lever presses had no consequences. Bars represent mean (±SEM) numbers of responses on the previously "active" lever in consecutive 5-min periods ($n = 8$ rats). Horizontal lines indicate periods when in the subsequent reinstatement experiments the stimulus complex was noncontingently presented (see Figs. 2, 4, and 5).

Water Reinforcement in Rats Trained to Respond for Ethanol

When water was substituted for ethanol in the six consecutive self-administration sessions the number of lever presses progressively decreased, $F(7, 24) = 8.86$, $p < 0.001$. Operant responding dropped below five responses/30 min on the sixth day of water self-administration period (Fig. 3). When water was replaced with 8% ethanol lever pressing immediately reached its baseline value (see days 1 and 8; Fig. 3).

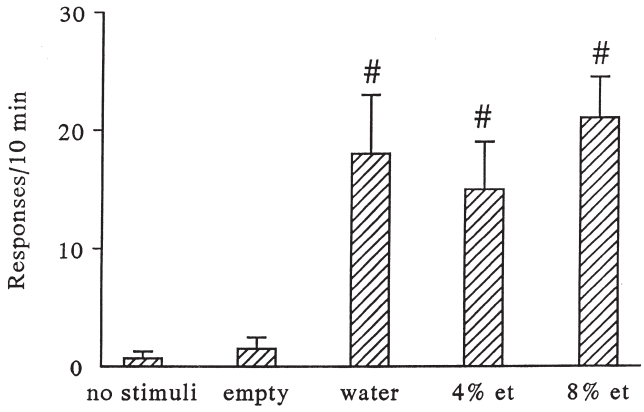

FIG. 2. Reinstatement of ethanol seeking by 15 random (RT15 s), noncontingent presentations of the liquid dipper containing different concentrations of ethanol or water. For comparison, the effect of 15 deliveries of the empty dipper is shown. The dipper deliveries started after 20 min of extinction. Bars represent mean (±SEM) numbers of responses on the previously "active" lever in the last 10 min of the 30-min reinstatement session. $^{\#}p < 0.05$ vs. the control group tested in the extinction session without any dipper deliveries ("no stimuli"); $n = 6$–8 rats.

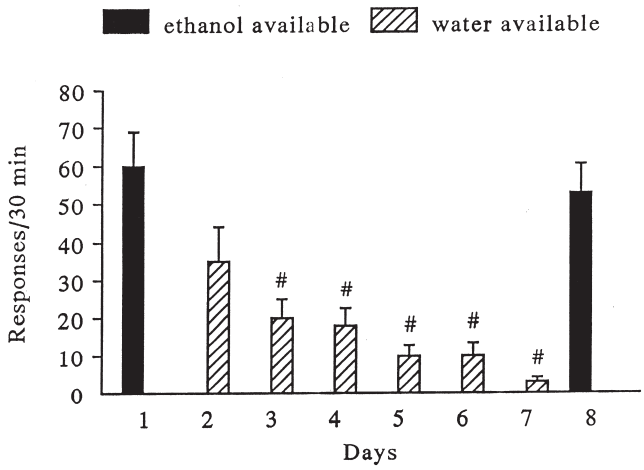

FIG. 3. Responding for water in six consecutive 30-min sessions (days 2–7) in the rats trained to self-administer ethanol. For comparison, results of the ethanol self-administration session performed on the day before (day 1) and on the day after (day 8) the water self-administration period are shown. Bars represent mean (±SEM) numbers of responses on the "active" lever. $^{\#}p < 0.05$ vs. the ethanol self-administration condition; $n = 4$ rats.

Reinstatement of Ethanol Seeking: Role of Duration of Extinction Phase

Extinction duration (20 vs. 60 min) did not alter the magnitude of reinstatement, $F(1, 21) = 0.003$, $p = 0.98$. Presentations of the ethanol-paired stimuli induced significant resumption of responding, $F(1, 21) = 27.09$, $p < 0.001$, after both 20- and 60-min extinction (Fig. 4). Representative individual patterns of responding in the reinstatement paradigm are shown in Fig. 5.

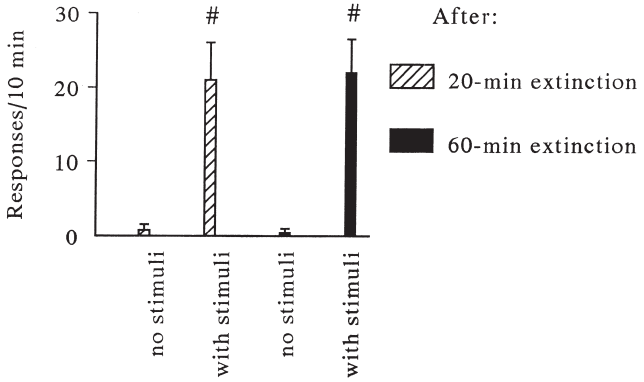

FIG. 4. The effect of extinction duration on reinstatement of ethanol seeking. Reinstatement was produced by 15 noncontingent, random (RT15 s) presentations of the liquid dipper containing 8% ethanol ("with stimuli"). The dipper deliveries in the 30- or 70-min reinstatement session started after 20 or 60 min of extinction, respectively. Bars represent mean (±SEM) numbers of responses on the previously "active" lever in the last 10 min of the respective reinstatement session. $^{\#}p < 0.05$ vs. the control groups tested in the respective extinction session without any dipper deliveries ("no stimuli"); $n =$ 6–7 rats.

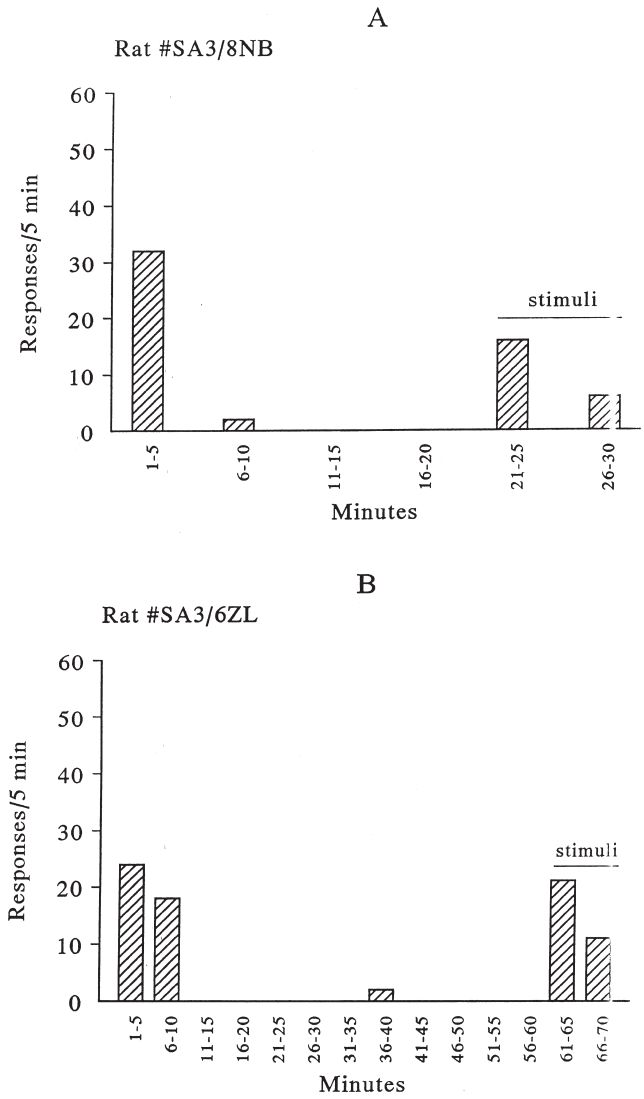

FIG. 5. Individual patterns of responding from the representative subjects tested in the 30- (A) or 70-min reinstatement session (B). Reinstatement of ethanol seeking was induced by 15 random presentations of the liquid dipper filled with 8% ethanol. Bars represent mean numbers of responses on the previously "active" lever in 6 (A) or 14 (B) consecutive 5-min periods. Horizontal lines indicate periods when the stimulus presentations occurred.

The mean number of "inactive" lever presses in all the above experiments was negligible (<0.8 responses/30 min), and did not vary between the experimental groups (data not shown).

DISCUSSION

In general, the present findings are consistent with previous reports that noncontingent presentations of drug-associated environmental stimuli, after a period of extinction, may reinstate cocaine, morphine, and alcohol seeking in rats [(3,4,9,11,19,20), but see also (30)].

The concentration of ethanol available in the dipper did not determine the magnitude of reinstatement. In fact, the

priming effect was similar regardless of the fact that the dipper was filled with 8% ethanol or tap water. The above findings do not necessarily mean that sensory properties of ethanol cannot prime ethanol seeking. For example, it is possible that evidence for a concentration–response relationship might have been obtained if the reinstatement sessions had been run repeatedly. However, our findings seem to exclude the possibility that pharmacological effects of ethanol and/or its sensory properties play any specific role in reinstatement of drug seeking in the present procedure (see Introduction).

In agreement with our previous report (3), noncontingent deliveries of the empty dipper did not lead to resumption of lever pressing. Thus, the ethanol-paired auditory and visual cues alone were not able to prime ethanol seeking in the absence of liquid in the dipper cup. It seems that reinstatement of ethanol seeking in the present study was due to a compound stimulus including the visual/auditory cues and some nonspecific sensory properties of liquid available in the dipper cup. Notably, it has been recently shown (19) that only a compound stimulus (visual cues + sound of the micropump) was able to reinstate cocaine-seeking after long-term extinction.

Rats working for ethanol in our paradigm have free access to water for 23.5 h per day. However, water is not accessible during the 30-min self-administration session. Because presentation of the dipper containing water led to reinstatement of ethanol seeking, it was of particular importance to determine whether water might have any reinforcing properties in the subjects trained to self-administer ethanol. Importantly, the results of the control experiment clearly indicated that the rats trained to respond for ethanol did not maintain responding for water [for similar findings, see (21,22)].

Discriminative stimuli set the occasion when behavioural response are followed by reinforcement (6). For example, it has been demonstrated that odour of ethanol may serve as a discriminative stimulus for ethanol-reinforced lever pressing (21). Discriminative stimuli may also produce relapse to drug seeking after extinction (6,18). Presumably, in the present study the ethanol-associated stimuli acted as discriminative stimuli, and thus reinstated operant behaviour. However, it is also possible that in the oral self-administration paradigm cues paired with liquid dipper activation signal the so-called time-out period. Responding during that period does not lead to additional reinforcement. Not surprisingly, we have observed (Bienkowski et al., unpublished) that most of the well-trained subjects having consumed alcohol from the dipper cup still refrained from responding when the dipper was active. Thus, it is not clear whether resumption of lever pressing in the present reinstatement paradigm was cued by the appearance or rather disappearance of the ethanol-associated stimuli. This problem is currently addressed in a series of experiments analysing exact distribution of lever pressing as a function of different times and frequencies of noncontingent dipper presentations.

Extinction time (20 vs. 60 min) did not influence the magnitude of reinstatement. Other authors have found no substantial difference in the magnitude of reinstatement of cocaine seeking induced by priming cocaine administration after 30- or 60-min extinction (11). However, these investigators have also shown a downward trend in the reinstatement magnitude as the extinction duration increased to 120 and 180 min.

In contrast to some previous studies on reinstatement of ethanol seeking (8,15), prolonged (days) withdrawal periods associated with between-day extinction procedure were not used in the present experiments. However, long-term extinction of operant responding for ethanol is not involved in typical treatment programs addressed to recovering alcoholics. Detoxified alcohol addicts are "forced" to keep abstinence at least during the in-patient phase of treatment (1,17). Accordingly, in our ongoing project, we try to determine how different periods (7–30 days) of abstinence alter resumption of ethanol seeking in the reinstatement paradigm. Notably, it has been reported that prolonged cocaine withdrawal (days or weeks) may potently increase cocaine-seeking behaviour in the within-session reinstatement paradigm (24,29). Time-dependent enhancement of alcohol-seeking behaviour might have important implications for clinical strategies of treatment of alcoholism.

Interestingly, correlational analysis indicated that lever pressing in extinction did not predict magnitude of cue-induced reinstatement of ethanol seeking. This finding would indicate that ethanol-seeking behaviour induced by nonspecific context stimuli (extinction phase) and discrete cues (reinstatement phase) may be mediated by different neural mechanisms. Results of our recent pharmacological studies seem to support the above hypothesis. We have shown that a low-affinity NMDA receptor antagonist, 1-amino-1,3,3,5,5-pentamethyl-cyclohexane (MRZ 2/579) may potently alter extinction but not reinstatement of ethanol seeking [(4); Bienkowski et al., unpublished].

Lever pressing for ethanol predicted neither extinction nor reinstatement of ethanol seeking in the present study. Recently, Koros et al. (14) have reported no relationship between nonoperant, voluntary ethanol consumption, and extinction of operant responding for ethanol. Moreover, our pharmacological experiments have revealed that ethanol self-administration and extinction of ethanol seeking may be differentially modulated by NMDA receptor antagonists (5) and an opioid receptor antagonist, naltrexone (4). Taken together, the results of our previous (4,5,14) and present experiments would suggest that diverse neural processes mediate maintenance, extinction, and reinstatement of ethanol-reinforced behavior in rats.

Considering relevance of our results for human alcoholism, one should bear in mind that the maintenance phase of ethanol self-administration was limited to thirty 30-min daily sessions, while human alcoholics drink ethanol for years. Further studies (e.g., mapping of metabolic brain activity) should determine neural substrate of ethanol seeking in the present paradigm and compare it with available human data.

Concluding, it appears that resumption of ethanol seeking in the present paradigm is evoked by the compound stimulus including the visual/auditory cues emitted by the dipper and some basic sensory properties of liquid available in the dipper cup. Clearly, more experiments are needed to explain neuropharmacological mechanisms involved in cue-induced reinstatement of ethanol seeking. The results of our recent study would suggest that conditioned opioid activation may play a role in this phenomenon (4).

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