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ORIGINAL INVESTIGATION

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Stimulus conditioned to foot-shock stress reinstates alcohol-seeking behavior in an animal model of relapse

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Abstract *Rationale:* Stress and conditioned responses to drug cues have been implicated as critical factors in relapse to drug use. In the animal literature, both the conditioned effects of drug-related stimuli and the unconditioned effects of foot-shock stress have been well documented to reinstate extinguished drug-seeking behavior. What has remained largely unexplored, however, is the significance of stimuli conditioned to foot-shock stress for the resumption of drug seeking. Additionally, although relapse is often the result of several risk factors acting in combination, the possibility that interactions among risk factors such as conditioned stress and drug cues may intensify drug-seeking behavior has received little experimental attention. *Objectives:* The purpose of this study was to examine the individual and interactive effects of a stimulus conditioned to foot-shock stress (STRESS CS) and a stimulus conditioned to ethanol reward (EtOH CS) on the reinstatement of ethanol-seeking behavior following extinction. *Methods:* Male Wistar rats were trained to orally self-administer 10% ethanol on a fixed-ratio 3 schedule of reinforcement. The EtOH CS was established by response-contingently pairing 0.5 s illumination of a white cue light with each reinforced response. The STRESS CS was established by pairing a continuous white noise (70 dB) with intermittent foot shock (10 min; 0.5 mA; 0.5 s on; mean off period of 40 s). Ethanol dependence was induced by an ethanol vapor-inhalation procedure. After ethanol-maintained instrumental responding was extinguished by withholding ethanol and the EtOH CS, reinstatement tests were conducted. *Results:* Both exposure to the STRESS CS and response-contingent presentation of the EtOH CS reinstated extinguished responding at the previously active, ethanol-paired lever without further ethanol availability. When response-contingent availability of the EtOH CS was preceded by exposure to the STRESS CS, interactive effects of these stimuli on responding were observed. However, both the individual and interactive effects of the STRESS CS and the EtOH CS reached statistical significance only in rats with a history of ethanol dependence but not in ethanol-nondependent rats. *Conclusions:* The results confirm that both conditioned stress and ethanol cues elicit ethanol-seeking behavior and, more importantly, that these stimuli produce interactive effects resulting in an increased ethanol-seeking response. The findings also indicate that susceptibility to ethanol seeking induced by conditioned stress and alcohol cues depends significantly on the history of prior alcohol exposure.

Keywords Ethanol · Dependence · Self-administration · Reinstatement · Stress · Conditioned stimulus

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Introduction

The chronically relapsing nature of drug and alcohol addiction remains a considerable challenge for successful treatment of these conditions. Two major factors have been implicated as critically contributing to relapse risk: stressful life events and exposure to environmental stimuli previously associated with the subjective effects of drugs of abuse. Stress has an established role in the initiation and maintenance of drug abuse and is an important determinant of relapse in abstinent addicts (Marlatt and George 1985; Brown et al. 1995; Sinha et al. 2000). In humans, subjective reactivity to specific environments or situations associated with stressful events has been implicated in triggering drug seeking and relapse (Cooney et al. 1997; Sinha and O'Malley 1999; Sinha et al. 2000). Animal studies typically have employed foot shock to reinstate drug-seeking behavior (Shaham and Stewart 1995; Erb et al. 1996; Le et al. 1998; Buczek et al. 1999; Liu and Weiss 2002a). Intermittent foot shock contains both emotional and physical components of stress. Thus, foot-shock-induced reinstatement of drug seeking may be a result of the combined effects of emotional and physical

stress. However, specific environments or situations can acquire motivational significance by being paired with stressful events and thereby become conditioned to stress through Pavlovian conditioning. Exposure to such conditioned stress can elicit the same constellation of behaviors produced by the stressful events (Davis et al. 1993). Conditioned responses to trauma-related stimuli have been proposed as a factor in post-traumatic stress disorder (Grillon et al. 1996). However, it is not known whether conditioned stress has motivational significance for drug-seeking behavior. Therefore, the present study was designed to examine the response-reinstating effects of an auditory stimulus conditioned to foot shock (STRESS CS) rather than foot shock itself on ethanol-seeking behavior in an animal model of relapse.

Another important factor contributing to relapse is the conditioning of the subjective effects of drugs of abuse with environmental stimuli. Ethanol-associated environmental stimuli reinstate ethanol-seeking behavior after extinction (Meil and See 1996; Katner et al. 1999; Ciccocioppo et al. 2001; Liu and Weiss 2002a, 2002b) and can elicit craving in abstinent alcoholics (Kaplan et al. 1985; Cooney et al. 1997; O'Brien et al. 1998). It is important to note that during abstinence drug addicts are likely to confront situations that present multiple rather than only a single risk factor for relapse. In fact, resumption of addictive behavior during abstinence is often the result of multiple risk factors that act in combination (Cooney et al. 1997). However, the significance of interactions among such factors in increasing relapse risk has received little experimental attention. Thus, the purpose of the present study was to examine whether presentation of an ethanol-associated conditioned stimulus (EtOH CS) after exposure to the STRESS CS increases the recovery of ethanol-seeking behavior compared to the individual effects of these conditioned stimuli.

A final issue of importance with respect to relapse risk is the history of prior drug dependence. There is evidence showing that drug craving is positively correlated with the degree of previous alcohol consumption (Greeley et al. 1993; George et al. 2001; Streeter et al. 2002). However, the significance of dependence history for the reinstatement of ethanol-seeking behavior has been scarcely examined. Therefore, rats with a history of ethanol dependence were compared with ethanol-nondependent rats in terms of the reinstatement of ethanol-seeking induced by the STRESS CS, the EtOH CS, and the interactive effects of the STRESS CS and the EtOH CS.

Materials and methods

Subjects

Forty-two male Wistar rats (Charles River, Raleigh, N.C.), weighing 180–200 g at the beginning of the experiment, were used. Rats were housed three per cage in a temperature-controlled (22°C) vivarium on a normal 12-h/12-h light/dark cycle (on, 0600 hours; off, 1800 hours). Food and water were available ad libitum with the exception of the first 3 days of operant training (see *Ethanol self-administration training*). All training and test sessions were conducted during the dark phase at the same time each day (2000 hours to 2400 hours). All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute.

Self-administration stations

Training and testing were conducted in standard operant conditioning chambers (Coulborn Instruments, Allentown, Pa.) placed in sound-attenuating and air-ventilated cubicles as previously described (Weiss et al. 1993). The chambers contained two retractable levers located 4.5 cm to either side of a drinking reservoir. A white cue light (24 W) was located above the active lever. Responses at the active lever activated a syringe pump located outside the sound-attenuating cubicles that dispensed 0.1 ml of liquid into the drinking reservoir, while responses at the inactive lever had no scheduled consequences. Recording of responses, activation of the syringe pump (delivery of liquid) and presentation of the cue light were automatically controlled by an IBM compatible microcomputer.

Ethanol self-administration training and conditioning procedure

Rats were trained to orally self-administer 10% ethanol in daily 30-min sessions using previously described procedures (Weiss et al. 1993). Briefly, rats were placed initially on a 22-h water-restriction schedule for the first 3 days. During this time, animals were trained to respond for 0.2% (w/v) saccharin on a continuous reinforcement schedule. After operant responding was acquired successfully, water was made available again ad libitum in the home cage for the remainder of the experiment. For the following 5 days, lever responses were reinforced by a 5% (w/v) ethanol solution that contained 0.2% saccharin. Then, the concentration of ethanol was increased gradually from 5% to 8% and, finally, to 10% (w/v), while the concentration of saccharin was correspondingly decreased to 0%. During this period, an inactive lever was introduced and the reinforcement schedule at the active lever was switched to a fixed-ratio 3, where every third response resulted in delivery of the reinforcer. After saccharin was faded from the solution, a white cue light was illuminated response-contingently for 0.5 s with each rewarded response during all subsequent sessions (Fig. 1), and training continued until rats reached stable levels of ethanol intake.

Ethanol vapor inhalation procedure

After completion of self-administration training, ethanol dependence was induced by an ethanol vapor inhalation procedure modified from Rogers et al. (1979) as previously described (Macey et al. 1996). Briefly, rats were housed in groups of three in sealed Plexiglas chambers and continuously exposed to ethanol vapor for 12 days. Ethanol vapor was created by dripping 95% ethanol onto a 2000-ml Erlenmeyer vacuum flask kept at 50°C on a warming tray. Air was blown over the bottom of the flask at a rate of 11 l/min. Ethanol vapor was independently introduced into each chamber through a stainless-steel manifold. The concentration of the ethanol vapor was adjusted within the range of 22–27 mg/l to produce targeted blood alcohol levels (BAL) of 180–200 mg%.

At the end of the ethanol vapor inhalation procedure, animals were subjected to three cycles that consisted of a 12-h period during which rats were allowed to operantly self-administer 10% (w/v) ethanol followed by a 12-h period during which ethanol vapor inhalation was resumed. This procedure provided rats with an opportunity to establish an association between voluntary ethanol self-administration and alleviation of physical or affective with-

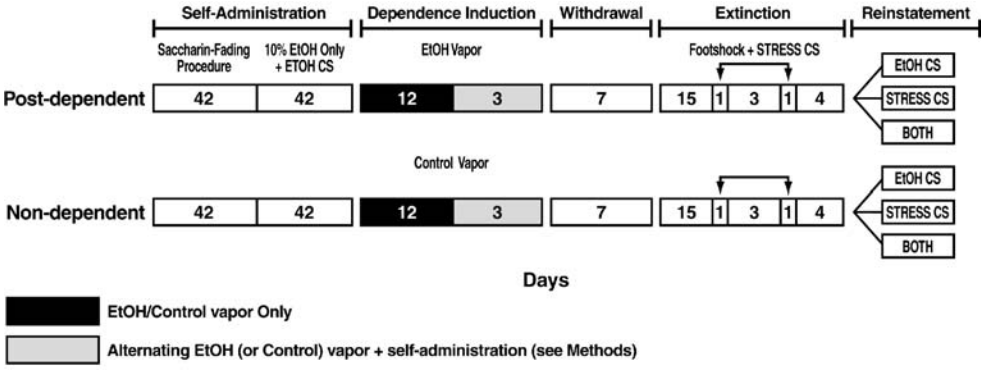

Fig. 1 Sequence of the experimental procedures

drawal symptoms. After this procedure, all animals were returned to the vivarium and left undisturbed for 7 days.

Ethanol-nondependent rats were subjected to the same procedures except that they were exposed to non-ethanol-containing air instead of ethanol vapor.

Blood alcohol determination

During the ethanol vapor inhalation period, tail blood samples (50 µl) were collected every other day for the measurement of BALs. Nondependent rats were subjected to sham bleeding procedures. Samples were centrifuged, and the serum was injected into an oxygen-rate alcohol analyzer (Analox Instruments, Lunenburg, Mass.) for blood alcohol determination.

Withdrawal sign rating

In a subset of rats that were not included in the subsequent reinstatement tests, withdrawal signs (including the ventromedial distal limb reflexion response, tail stiffness and abnormal body posture) were rated using a scale modified from Gothoni et al. (1983) 8–12 h after termination of ethanol vapor inhalation, as previously described (Macey et al. 1996). A subjective 0- to 2-point scale was used for each of these signs, with 0 representing undetectable, 1 representing moderate, and 2 representing severe withdrawal sign. An overall withdrawal severity score ranging from 0 to 6 was derived by adding rating scores for the three individual withdrawal signs. Ratings also were conducted in a subset of nondependent rats.

Extinction

One week after removal from ethanol vapor chambers, the rats were subjected to daily 30-min extinction sessions where responding was extinguished by withholding delivery of ethanol and presentation of the corresponding cue light. The extinction criterion was 6 or fewer responses per session at the active lever for three consecutive days.

Establishment of the STRESS CS

During the final stage of the extinction phase, a stimulus was conditioned to foot-shock stress (STRESS CS) by pairing a continuous white noise (70 dB) with foot shock. Two conditioning trials were conducted on extinction day 16 and day 20 immediately prior to the start of the extinction sessions. Inescapable intermittent foot shock (0.5 mA, 0.5-s duration, 40 s of mean inter-shock interval with range of 10–70 s) was administered for 10 min in separate chambers and delivered through a scrambler to the stainless-steel grid floor of the chambers.

Reinstatement tests

One day after the final extinction session (5 days after the last foot shock–white noise pairing), both post-dependent and nondependent rats were randomly separated into three groups. Thirty-minute reinstatement tests then were conducted under three conditions: after exposure to the STRESS CS, during response-contingent presentation of the EtOH CS, and during response-contingent availability of the EtOH CS following exposure to the STRESS CS. During the reinstatement tests, responses at the previously active lever were recorded but had no scheduled consequences, except for activation of the syringe pump motor (without delivery of ethanol).

Effects of the EtOH CS

Sessions were initiated by extension of the levers into the operant conditioning chambers, and each response at the previously active lever resulted in 0.5 s illumination of the cue light.

Effects of the STRESS CS

Rats first were placed in the foot-shock chambers where they were exposed for 10 min to the foot-shock-associated white noise stimulus without receiving foot shock. The rats then were immediately transferred to the operant conditioning chambers and tested for reinstatement without presentation of the EtOH CS.

Effects of the STRESS CS + EtOH CS

Rats first were exposed for 10 min to the foot-shock-associated white noise as described above, and then tested for reinstatement in the operant conditioning chambers with response-contingent presentation of the cue light.

Data analysis

Overall differences in the number of lever responses between post-dependent versus nondependent rats as well as among extinction (averaged across the final three sessions) versus reinstatement for post-dependent and nondependent rats were analyzed using mixed-factorial ANOVA with subsequent simple-effects tests. Differences among individual test conditions (STRESS CS versus EtOH CS versus STRESS CS + EtOH CS) were verified by Duncan post-hoc tests after one-way ANOVA for both post-dependent and nondependent rats.

Results

Ethanol self-administration

During ethanol self-administration training, rats developed stable levels of responding for 10% ethanol. The mean (±SEM) number of responses averaged across the final three sessions was 30.9±2.5, corresponding to a mean (±SEM) ethanol intake of 0.65±0.05 g/kg. There was no difference between post-dependent and nondependent rats as well as among subgroups designated for testing under different conditions in both post-dependent and nondependent rats (Fig. 2 and Fig. 3, Table 1).

Blood alcohol levels and withdrawal signs

The mean (±SEM) BALs in ethanol vapor-exposed rats were 189.6±16.3 mg% (range 123.3–245.6 mg%). Ethanol maintained operant responding during the three 12-h ethanol self-administration sessions at the end of the dependence induction period. The mean (±SEM) number of responses was 544.1±58.4 (dependent) versus 192.3±27.5 (nondependent) ($F_{1,40}$=29.71, P<0.0001), corresponding to a mean (±SEM) ethanol intake of 9.58±1.1 (dependent) versus 3.48±0.5 g/kg (nondependent) ($F_{1,40}$=27.40, P<0.0001). Ethanol vapor-exposed rats showed overt ethanol withdrawal signs 8–12 h after removal from ethanol vapor with a mean (±SEM) withdrawal severity score of 4.8±0.3, whereas nondependent rats manifested negligible withdrawal signs (0.9±0.2).

Extinction

In the first three extinction sessions, rats emitted average (±SEM) responses of 51.8±5.5 at the active lever and 4.6±0.7 at the inactive lever. During subsequent sessions, lever responding gradually decreased. All rats reached the extinction criterion within 24±3 sessions. There was no difference between post-dependent and nondependent rats as well as among subgroups designated for testing under the different reinstatement conditions in both post-dependent and nondependent rats (Table 1).

Reinstatement tests

In nondependent rats, neither the STRESS CS nor the EtOH CS increased responding over extinction performance (Fig. 2). However, in the STRESS CS + EtOH CS condition responding recovered with mean (±SEM) responses of 10.4±3.1 compared to extinction responses of 4.6±1.5 and this effect approached statistical significance (P≤0.059 after ANOVA: $F_{1,18}$=5.33, P<0.05). Lever responses at the inactive lever remained at extinction levels.

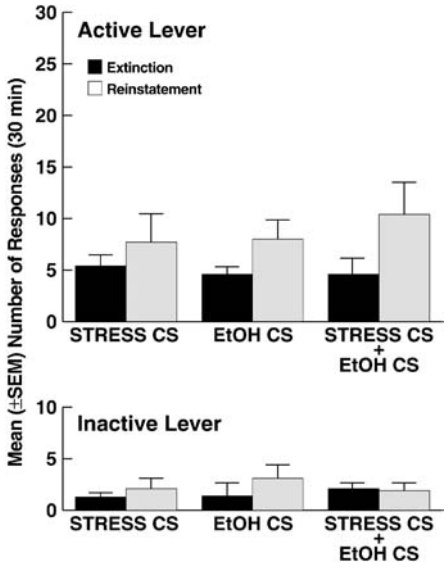

Fig. 2 Responses in ethanol-nondependent rats (n=7 in each test condition) after exposure to conditioned stress (STRESS CS), during response-contingent presentation of an ethanol-associated conditioned stimulus (EtOH CS), and during response-contingent availability of the EtOH CS preceded by exposure to the STRESS CS (STRESS CS + EtOH CS). Extinction data are presented as the mean (±SEM) across the final three extinction sessions

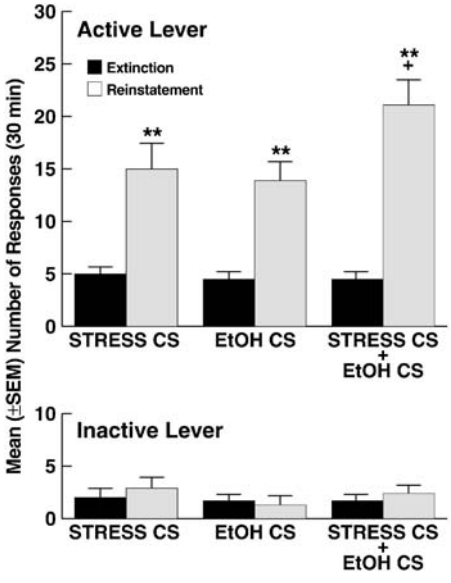

Fig. 3 Responses at a previously active and an inactive lever in rats with a history of ethanol dependence (n=7 in each test condition). Extinction responses were averaged across the final three sessions of the extinction phase. *STRESS CS*: Responses after exposure to conditioned stress; *EtOH CS*: responses during response-contingent presentation of an ethanol-associated conditioned stimulus; *STRESS CS + EtOH CS*: lever presses during response-contingent availability of the EtOH CS following exposure to the STRESS CS. **P<0.01, different from extinction; +P<0.05, different from STRESS CS and EtOH CS

Table 1 Mean (±SEM) responses at the active lever during the ethanol self-administration and extinction phases

Groups		Ethanol self-administration Across final three sessions	Extinction Across first three sessions
Post-dependent	Stress	34.6±6.5	48.3±10.0
	CS	32.5±4.2	56.1±15.3
	Stress + CS	35.1±7.0	50.1±5.6
Non-dependent	Stress	31.6±2.6	56.9±14.6
	CS	33.6±4.3	53.2±16.8
	Stress + CS	34.8±6.2	46.2±7.6

In post-dependent rats, responding recovered substantially after exposure to the STRESS CS, during response-contingent presentation of the EtOH CS or in the STRESS CS + EtOH CS condition, with mean (±SEM) responses at the active lever of 15.0±1.4 (STRESS CS), 13.9±2.3 (EtOH CS) and 24.1±2.4 (STRESS CS + EtOH CS) (Fig. 3). ANOVA revealed a significant main effect of sessions (extinction versus reinstatement) ($F_{1,18}$=99.43, P<0.0001) and subsequent simple-effects analysis verified significant (P<0.01) differences in the number of responses in all three test conditions over extinction responses. Moreover, there was a significant difference in the number of responses in the STRESS CS + EtOH CS condition versus responses in both the STRESS CS (P<0.05) and EtOH CS (P<0.05) conditions (Duncan post-hoc tests after one-way ANOVA: $F_{2,18}$=3.67, P<0.05). Lever responses at the inactive lever remained indistinguishable from extinction responses.

In a subset of post-dependent rats, the white noise that had not been paired previously with foot shock did not produce response-reinstating effects, and responses (6.3±1.8) at the active lever remained indistinguishable from extinction levels (data not shown).

ANOVA of the data from both post-dependent and nondependent rats revealed a significant main effect of ethanol dependence ($F_{1,36}$=14.76, P<0.001) and an interaction between the dependence × reinstatement test conditions $F_{1,36}$=15.03, P<0.001). Subsequent simple-effects analysis revealed significant differences between post-dependent and nondependent rats in the STRESS CS (P<0.05) and STRESS CS + EtOH CS (P<0.01) conditions. In the EtOH CS condition, the difference in the number of responses between post-dependent and nondependent rats failed to reach statistical significance.

Discussion

The results demonstrate that conditioned stress and ethanol cues reinstate ethanol-seeking behavior and, more importantly, that these stimuli can interact to increase ethanol-seeking responses in drug-free rats after extinction. Particularly important is the finding that the individual and interactive effects of conditioned stress and ethanol cues were observed only in rats with a history of ethanol dependence but not in nondependent rats.

The first major finding was that conditioned stress significantly reinstated ethanol-seeking behavior. Specifically, an auditory stimulus conditioned to foot shock significantly reinstated extinguished responding at the active, previously ethanol-paired lever in rats with a history of ethanol dependence. This effect cannot be attributed to the nonspecific arousal effects of conditioned stress because responding at the inactive lever remained indistinguishable from extinction responses. Nor can this effect be attributed to possible stressful properties of the white noise per se because, in a subset of rats, exposure to a similar white noise that had not been paired previously with foot shock did not induce recovery of responding. The present finding is in contrast with a previous observation where a tone or a tone/light compound stimulus conditioned to foot shock failed to reinstate heroin or cocaine seeking (Shaham et al. 2000). This disparity is likely to be accounted for, however, by substantial procedural differences as well as the use of different classes of drugs of abuse. Most importantly, in this earlier study (Shaham et al. 2000), conditioning sessions were conducted in drug-naive rats before the beginning of the operant drug self-administration training procedure; while, in the present study, the STRESS CS was established in drug-experienced rats just before the reinstatement tests. Thus, the recency of the association between foot-shock stress and STRESS CS may be an important factor determining the response-reinstating effects of conditioned stressors.

An important issue requiring consideration is whether the foot-shock-paired conditioned auditory stimulus served in fact as an effective stressor. No neuroendocrine measures of hypothalamo-pituitary-adrenal (HPA) axis activation were obtained because the blood sampling procedures likely would have interfered with the behavioral reinstatement measures. However, previous studies have demonstrated that auditory stimuli conditioned to foot shock activate the HPA axis and thus act as effective stressors (Shurin et al. 1995). An additional issue requiring consideration is whether the reinstating effects of the white noise are the result of conditioning of this stimulus with foot shock or accounted for by nonspecific effects. The present procedure lacked a condition where the auditory CS was presented in an unpaired manner with foot shock. It is highly unlikely that nonspecific arousal effects rather than conditioning effects explain the results obtained. First, the white noise stimulus per se did not produce response-reinstating effects in previously ethanol-dependent rats (n=5) that had been exposed to the same white noise without receiving foot shock (Liu and

Weiss, unpublished observation). Second, only an auditory CS paired with foot shock but not an unpaired stimulus was effective in reinstating cocaine-induced conditioned place preference (Sanchez and Sorg 2001).

The present finding extends the motivational significance of foot shock stress in inducing drug-seeking behavior to conditioned stress. In humans as well as animals, stress responses can be conditioned to contextual and discrete stimuli previously paired with aversive events (Davis et al. 1993). Moreover, responses to conditioned stress have been identified as an important factor in post-traumatic stress disorder (Grillon et al. 1996). Considering that emotional stress usually induces negative affect that may resemble a withdrawal-like internal state, the previous subjective experience of negative reinforcement by ethanol in post-dependent rats may have contributed importantly to ethanol-seeking responses elicited by conditioned stressors in these animals. This interpretation seems consistent with findings that alcohol abuse in humans often occurs in stressful situations where human addicts consume alcohol for its anxiolytic actions (Cappell and Greeley 1987; Pohorecky 1991). Moreover, the present finding is consistent with clinical observations that subjective reactivity to specific environments or situations associated with stressful events is implicated in drug craving and relapse in drug and alcohol addicts (Cooney et al. 1997; Sinha and O'Malley 1999; Sinha et al. 2000).

The results also confirm the motivational significance of ethanol-associated conditioned stimuli in eliciting ethanol-seeking behavior, replicating the results of a previous study (Liu and Weiss 2002a). However, in both of these studies, the response-reinstating effect of the ethanol CS was observed only in rats with a history of ethanol dependence. The lack of efficacy of the ethanol CS in nondependent rats is in contrast to other reports that have shown that ethanol-related cues reliably reinstate responding (Katner et al. 1999; Wilson et al. 2000; Liu and Weiss 2002b). There are at least two possible explanations for this discrepancy. First, in the present study ethanol reinforcement was paired with a light cue only rather than a compound stimulus, the latter class of stimuli being more effective in reinstating extinguished drug- and ethanol-seeking behavior than a single stimulus (Meil and See 1996). The sound of syringe-pump activation is unlikely to have become conditioned to ethanol reinforcement as a second CS along with the light cue because the syringe pump was located outside the sound-attenuating cubicles and, thus, was not audible inside the chamber. Moreover, association of the syringe pump sound with ethanol would have been extinguished during the extinction phase of the experiment. Second, the light cue was discretely and response-contingently paired with ethanol-reinforced responses rather than serving as a discriminative stimulus as in previous studies (Katner et al. 1999; Liu and Weiss 2002b). Discriminative stimuli have a predictive character and set the occasion for subjects to engage in a particular behavior, and may be more powerful in motivating behavior than the conditioned reinforcing effects of discrete ethanol-paired stimuli. Moreover, discretely drug-paired and discriminative stimuli may involve different modes of associative learning mediated via interconnected but distinct neural pathways (See 2002).

An important purpose of the present study was to ascertain whether conditioned stress and ethanol cues can interact to augment reinstatement. The results confirm this hypothesis. Response-contingent presentation of the EtOH CS after exposure to the STRESS CS produced an interactive effect reflected by a significant increase in the number of reinstatement responses over those produced by the STRESS CS and EtOH CS alone in post-dependent rats. In nondependent rats, even though the STRESS CS and the EtOH CS individually produced no response-reinstating effects, the recovery of responding in the STRESS CS + EtOH CS condition was marginally significant relative to extinction performance. This finding resembles significant interactive effects of foot shock per se and ethanol cues observed in a previous study (Liu and Weiss 2002a). One may speculate that emotional distress associated with stress induces a negative affective state in which the incentive salience of ethanol-related cues is increased possibly due to the anxiolytic aspect of ethanol's reinforcing actions. This interpretation would seem consistent with clinical findings that neither ethanol craving induced by an alcohol cue alone nor negative affect alone predicts relapse in alcoholics, whereas cue-induced craving in the presence of negative affect is a reliable predictor of relapse (Cooney et al. 1997).

The results demonstrate that prior ethanol dependence exacerbates the reinstatement of ethanol-seeking behavior. Specifically, both the STRESS CS and the EtOH CS produced significantly greater effects in dependent rats than in nondependent rats. Similarly, the availability of the EtOH CS following exposure to the STRESS CS produced a significant interactive effect in post-dependent rats but only a marginally significant effect in nondependent animals. These findings seem consistent with clinical observations showing that alcohol craving is positively correlated with the degree of alcohol dependence (Greeley et al. 1993; George et al. 2001; Streeter et al. 2002). The results, however, do not provide direct insight into the mechanisms underlying this phenomenon. The fact that post-dependent rats experienced a greater number of response–reinforcer pairings during the three 12-h self-administration sessions during withdrawal than nondependent rats (544 versus 192) is unlikely to account for the increased response–reinstatement in the post-dependent group. Results from ongoing studies (Liu and Weiss, unpublished observation) indicate that rats given three 12-h self-administration sessions (192 pairings) did not differ in the number of subsequent reinstatement responses from rats that received no access to ethanol (0 pairing) during the same time. Although these data were generated in nondependent rather than post-dependent rats, it seems reasonable to conclude that the number of additional response–reinforcer pairings during the three 12-h self-administration sessions similarly had little influence on

the degree of recovery of responding. However, as a possible explanation for the heightened response–reinstatement in post-dependent rats, one may speculate that in addition to acting as a positive reinforcer, ethanol serves as a negative reinforcer during the development of dependence by alleviating aversive withdrawal symptoms (Roberts et al. 2000). In fact, an ethanol CS can acquire more potent reinstating effects in previously ethanol-dependent rats when these animals are given the opportunity to self-administer ethanol after removal from chronic ethanol vapor inhalation (Liu and Weiss 2002a), as in the present experiment. Additionally, the enhanced ethanol-seeking response in post-dependent rats may have resulted from long-lasting neuroadaptative changes in that chronic high-dose ethanol exposure results in significant functional changes in the mesolimic dopamine system (Diana et al. 1996; Weiss et al. 1996; Nestby et al. 1999), corticotropin-releasing factor systems (Merlo Pich et al. 1995; Zorrilla et al. 2001), and opioid neurotransmission (Nestby et al. 1999; Lindholm et al. 2000). These neuroadaptative changes in stress-regulatory systems or the mechanisms that mediate the incentive motivational effects of ethanol may underlie the exacerbated drug-seeking responses in post-dependent rats.

In summary, the present results demonstrate that conditioned stress effectively reinstates ethanol-seeking behavior after extinction in previously ethanol-dependent rats. Additionally, the findings suggest that conditioned stress and ethanol-related environmental cues can produce interactive effects in eliciting relapse to alcohol-seeking behavior and that the vulnerability to relapse is significantly exacerbated in subjects with a history of alcohol dependence.

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