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Learning and Tolerance to the Ataxic Effect of Ethanol

SUSAN J. LARSON AND SHEPARD SIEGEL

McMaster University, Department of Psychology, Hamilton, Ontario, L8S 4K1, Canada

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LARSON, S. J. AND S. SIEGEL. *Learning and tolerance to the ataxic effect of ethanol.* PHARMACOL BIOCHEM BEHAV **61**(1) 131–142, 1998.—It has been well documented that drug-associated cues are important for the development and expression of drug tolerance. The Pavlovian conditioning analysis of tolerance emphasizes the importance of a drug-associated cues to tolerance by equating a drug administration with a learning trial. According to this analysis, tolerance should be subject to external inhibition, the disruption of a conditional response by a novel stimulus. We previously reported that tolerance to the ataxic effect of ethanol was attenuated by a novel strobe/noise presentation (31). In this article we report evidence of a compensatory CR in rats tolerant to the ataxic effect of ethanol as tested on the tilting plane. Both the compensatory CR and tolerance were disrupted by the presentation of a novel strobe/noise stimulus providing converging evidence that the attenuation of tolerance by a novel stimulus results from external inhibition of Pavlovian conditioning. The disruption of ethanol tolerance and the conditional response mediating tolerance was also apparent when the novel omission of the strobe/noise stimulus was used as the external inhibitor in rats made tolerant to ethanol with the stimulus on. Finally, we have shown that the disruptive effect of a novel stimulus on ethanol tolerance is decreased when there is a 10-day delay between the final tolerance development session and testing, demonstrating that the interval between training and testing is important when assessing associative tolerance. © 1998 Elsevier Science Inc.

Alcohol tolerance Ataxia Conditioning Ethanol tolerance External inhibition

TOLERANCE, a decrease in responsivity to a drug over repeated administrations, can be affected by drug-associated cues. The contribution of drug-associated cues to tolerance is emphasized in a Pavlovian conditioning analysis of drug tolerance [reviewed in (28)]. Using the usual conditioning terminology, cues accompanying the primary drug effect function as conditional stimuli (CSs). The direct effect of the drug constitutes the unconditional stimulus (UCS). Prior to any learning, this pharmacological stimulation elicits responses that compensate for the drug-induced disturbances (20). These responses that compensate for the drug effect are unconditional responses (UCRs). After some pairings of the predrug CS and pharmacological UCS, a drug-compensatory response is elicited as a conditional response (CR). When the conditioning analysis of tolerance was first presented over 20 years ago, pharmacological CRs were described as opposite in direction to the pharmacological UCR, the UCR being defined as the measured drug effect (24). However, "there has been an important revision of the Pavlovian analysis of tolerance since its initial formulation" (18), p. 392; see also (6,8,20). The CR, although typically opposite in direction to the drug effect, is not opposite to the unconditional response elicited by the drug. This primary drug effect is the UCS—it unconditionally elicits responses that typically—but not always, see (20)—attenuate this primary effect. Conditional responses that mediate tolerance are in the same direction as this drug-elicited compensatory response; thus, they are compensatory to the drug effect. These drug compensatory CRs mediate the development of tolerance by counteracting the drug effect.

In many experiments demonstrating the role of conditioning in drug tolerance, the importance of drug-associated cues is assessed in the absence of the CS. By testing subjects for drug tolerance with no CS present, no CR should be elicited. The absence of a CR attenuates tolerance because drug anticipatory responses that cancel out the drug effect are not elicited. Tests of tolerance in the absence of drug-associated cues have demonstrated the importance of Pavlovian conditioning in the expression of tolerance [e.g., (24)]. Further research has

Requests for reprints should be addressed to Shepard Siegel, Department of Psychology, McMaster University, Hamilton, Ontario L8S 4K1, Canada.

shown that tolerance is subject to such Pavlovian conditioning manipulations as extinction (27), latent inhibition (25,33), sensory preconditioning (3), as well as many others [for a review, see (28)]. In addition, external inhibition, or the disruption of a CR by a novel stimulus (16), has been used to assess the robustness of the conditioning analysis of tolerance (19,31,32).

Evidence of external inhibition of tolerance was first presented by Siegel and Sdao-Jarvie (32). They demonstrated that a novel strobe light attenuates tolerance to the hypothermic effect of ethanol. Their demonstration of external inhibition of tolerance was taken as support for the conditioning analysis of tolerance. Shortly after Siegel and Sdao-Jarvie (32) reported external inhibition of tolerance, Cunningham and colleagues (2,17) demonstrated that a variety of stressful stimuli, including a strobe light, would enhance ethanol-induced hypothermia in rats receiving ethanol for the first time. This nonassociative enhancement of hypothermia provided an alternative explanation for Siegel and Sdao-Jarvie's (32) findings. Because Siegel and Sdao-Jarvie did not test a drug-naive group with the strobe light in their experiment, it is possible that the attenuation of tolerance they report could have resulted from a hypothermia-enhancing effect of the strobe light, rather than from the disruption of an hypothesized CR. Thus, Siegel and Sdao-Jarvie's (32) results do not provide an unequivocal demonstration of external inhibition of ethanol tolerance.

Recently, Siegel and Larson (31) investigated external inhibition of ethanol tolerance in a preparation not confounded by the nonassociative effect of the stimulus. They studied external inhibition of tolerance to the ataxic effect of ethanol. Siegel and Larson (31) found that a novel strobe light/white noise combination disrupted tolerance to the ataxic effect of ethanol by augmenting ethanol-induced ataxia. Importantly, no augmentation of ataxia resulted when rats were presented with a novel stimulus during their first ethanol administration. Because the novel stimulus did not enhance ethanol-induced ataxia in the absence of tolerance, Siegel and Larson (31) concluded that tolerance to the ataxic effect of ethanol is subject to external inhibition. They speculated that the disruption of tolerance by a novel stimulus resulted from the disruption of a compensatory CR mediating ethanol tolerance; however, no observation of a CR was made to support this speculation.

The first experiment in the present series was designed to assess whether the disruption of tolerance to the ataxic effect of ethanol by a novel stimulus resulted from the disruption of a CR. Evidence that a novel stimulus disrupts a CR mediating tolerance would provide additional support that the disruption of tolerance by a novel stimulus is, in fact, external inhibition of tolerance. In Experiment 1, a placebo was given to ethanol-tolerant rats in a drug predictive environment. A compensatory CR—"hypertaxia," or enhanced behavioral functioning—was expected. It was also predicted that both ethanol tolerance and the CR mediating tolerance would be disrupted by the presentation of a novel stimulus.

GENERAL METHOD

Subjects

Subjects were male Sprague–Dawley rats, weighing 275–335 g at the start of experimentation. Rats were housed in pairs in clear plastic cages with wood chips on the floor. They were on a 12 D:12 L cycle (lights on at 0700 h). All experimental sessions occurred during the light portion of the cycle. Food and water were available ad lib.

Experimental Setting and Apparatus

All experimental sessions occurred in a 3 × 2.7 m room distinct from the colony. The experimental room was lit with overhead fluorescent lighting, unless otherwise noted. Subjects were assessed for ataxia using a tilting plane (1,31). The tilting plane consisted of an alley, constructed from Plexiglas, 60 cm long × 18 cm wide × 30 cm high. The alley was open at the top and hinged at one end. A crank and pulley system was operated to raise the unhinged end of the alley. Inclination occurred at about 2° for one rotation of the handle and elevation occurred at approximately 4° per second. A protractor built into the hinged end of the alley provided an indication of the angle of inclination when the plane was tilted.

Injected Substances

Injected substances were either 1.5 g/kg of ethanol or physiological saline. Ethanol injections were made from 95% ethanol diluted in saline to form a 15% solution. Control rats were injected with physiological saline equated volumetrically with ethanol. All injections were given intraperitoneally.

Extraneous Stimulus

The extraneous stimulus consisted of a strobe light and white noise presented simultaneously. The strobe light was generated by a Grass Photostimulator (Model PS2). The photostimulator used a Xenon flash tube to generate 10-μs flashes. It was set at a flash rate of 4 Hz at maximum intensity. The photostimulator was placed at ceiling height, approximately 2 m from the tilting plane. Moderate level white noise was generated by an overhead room speaker. When the strobe/noise combination was presented, the overhead room lights were off and a red light was turned on.

Experimental Sessions

At the start of an experimental session, rats were placed singly in plastic cages (identical to their home cage) and transported to the experimental room where they remained for the duration of the session. Within 2 min of transport to the experimental setting an initial preinjection slip angle was determined for each animal. Slip angle was measured by the experimenter, who, after placing the rat in the alley, turned the crank and noted the angle of inclination at which the rat began to slip. Rats were injected with the appropriate solution within 2 min of the first slip angle assessment. After injection, slip angle was measured every 2 min for seven additional slip angle assessments. Within 2 min of the final slip angle assessment, subjects were returned to the colony and placed in their home cage.

Data Treatment

A measure of maximum impairment was computed for each subject for each session by taking the difference, in degrees, between the smallest postinjection slip angle and that subject's preinjection slip angle for that session (1,31). Thus, increasing ataxia is indexed as increasingly negative impairment scores. The measure of maximum impairment has been used by ourselves (31) and others (1) when measuring the behavioral impairing effect of ethanol with the tilting plane. Two other methods of data treatment were used to confirm that maximum impairment was an appropriate method of compiling the data. In one case, the smallest post injection slip angle/the preinjection slip angle × 100 computed a percent of

baseline impairment score. Results obtained in this analysis were similar to results obtained using maximum impairment. In addition, analyses were conducted that took into consideration Newton's of force (mass × gravity × sin of the slip angle). Once again, this data treatment produced results similar to maximum impairment scores. Because maximum impairment is consistent with other reports in the literature, all analyses in this article will use a maximum impairment score to index ataxia.

Data were analyzed using analysis of variance (ANOVA) and planned comparisons were conducted using an *F*-test. A significance level of $p < 0.05$ was used for all statistical tests.

Experiment 1

As indicated above, the purpose of this experiment was to test for a compensatory CR in rats tolerant to the ataxic effect of ethanol. During the CR (placebo) test, some subjects were presented with a novel stimulus to assess the effect of novelty on the CR. In addition, to replicate findings reported by Siegel and Larson (31), a tolerance test was conducted to assess the effect of a novel stimulus on ethanol tolerance.

PROCEDURE

Tolerance Development

During tolerance development subjects were injected with ethanol (group ETH, $n = 20$) or saline (group SAL, $n = 20$) during daily experimental sessions. The tolerance development phase of the experiment lasted for 22 consecutive days.

CR Test

On day 23 all rats were injected with saline for a CR test. Half of the rats from both group ETH and group SAL were presented with the novel strobe light/white noise stimulus (ETH/ON, $n = 10$; SAL/ON, $n = 10$). The stimulus was turned on within 90 s of injections and remained on for the duration of the experimental session. Remaining rats from both group ETH and group SAL were not presented with the novel stimulus (ETH/OFF, $n = 10$; SAL/OFF, $n = 10$). The test environment for these subjects was identical to their tolerance development environment.

Tolerance Reacquisition

Reacquisition of tolerance occurred for 4 days following the CR test (days 24–27). During these sessions rats were run according to their tolerance development schedule—subjects from group ETH were injected with ethanol and subjects group SAL were injected with saline during daily experimental sessions.

Tolerance Test

On day 28 all rats were injected with ethanol for a tolerance test. Half of the rats from both group ETH and group SAL were presented with the novel strobe/noise stimulus (ETH/ON, $n = 10$; SAL/ON, $n = 10$). Once again, the stimulus was turned on within 90 s of injections and remained on for the remainder of the experimental session. Remaining rats from both group ETH and group SAL were not presented with the novel stimulus (ETH/OFF, $n = 10$; SAL/OFF, $n = 10$). All of the subjects presented with the novel stimulus during the CR test were not presented with it during the tolerance test.

RESULTS AND DISCUSSION

Tolerance Development

Figure 1 depicts the mean impairment scores for group ETH and group SAL subjects over two-session blocks during the tolerance development phase of the experiment.

The ataxic effect of ethanol was present at the start of the experiment—group ETH subjects showed greater impairment than group SAL subjects. Over the course of the toler-

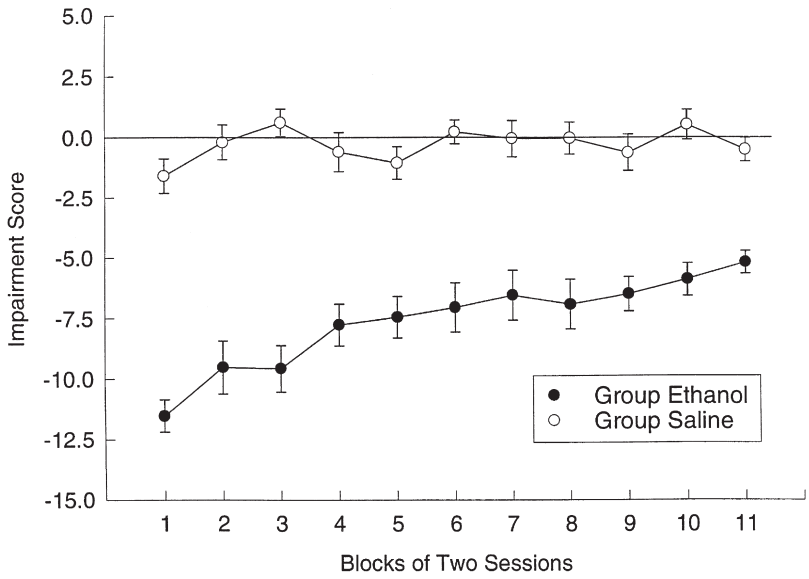

FIG. 1. Mean impairment scores (±1 SEM) for ethanol- and saline-injected rats over two-session blocks during tolerance development (Experiment 1).

ance development sessions, group ETH subjects displayed increasingly less impairment, while there was little change in impairment for group SAL subjects. A mixed-design ANOVA conducted on the data summarized in Fig. 1 indicated a significant tolerance development solution (ETH vs. SAL) × block interaction, $F(10, 380) = 3.08$, $p < 0.001$. Further one-way repeated measures analyses indicated a significant block effect for group ETH, $F(1, 190) = 6.09$, $p < 0.001$, but not for group SAL.

CR Test

For the CR test all rats were injected with saline. Positive impairment scores were expected in rats experiencing a CR, reflecting hypertaxia, or an enhanced ability to stay on the tilting plane. Figure 2 (top panel) depicts the mean impairment scores of group ETH and group SAL subjects that were, or were not, presented with the novel stimulus.

As can be seen in this figure, ethanol-experienced subjects tested with the stimulus off had larger positive impairment scores than all other groups. Group ETH subjects tested with the stimulus on had impairment scores similar to the subjects in both saline groups. A one-way ANOVA indicated that the groups differed significantly, $F(3, 36) = 9.66$, $p < 0.001$. A planned comparison of the difference between groups ETH/ON and ETH/OFF was significant, $F(1, 36) = 13.09$, $p < 0.001$. Group SAL/ON did not differ significantly from group SAL/OFF. The results of the CR test indicate that rats tolerant to the ataxic effect of ethanol exhibit a compensatory CR when tested with a placebo in the presence of ethanol-associated cues, and this CR was disrupted by the novel stimulus. The results are consistent with a conditioning analysis of ethanol tolerance.

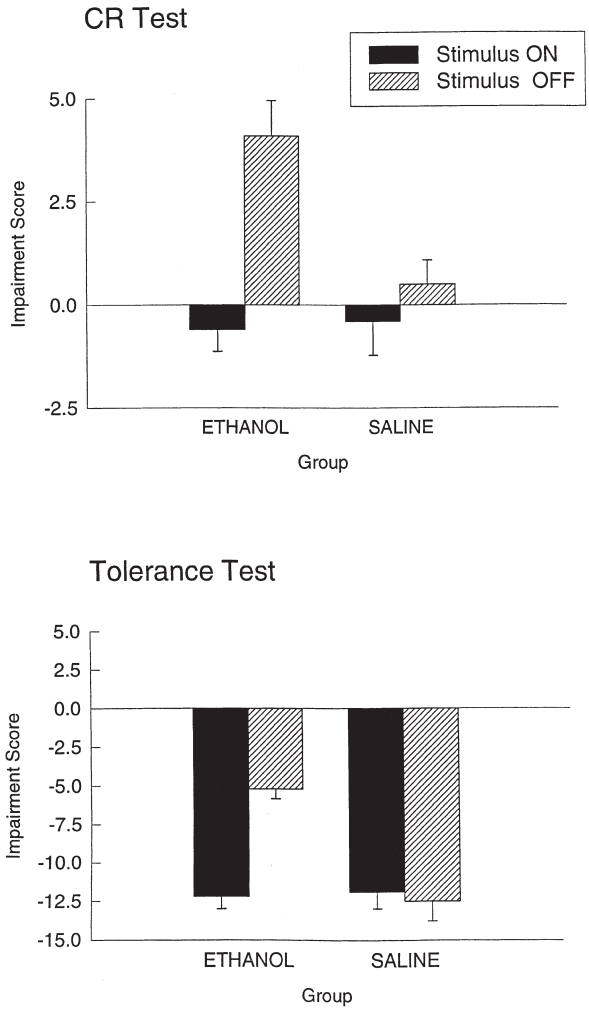

FIG. 2. Top panel: Mean impairment scores (+1 SEM) displayed by saline-injected rats that were, or were not, presented with the strobe/noise on the CR test (Experiment 1). Group SAL rats received saline throughout the experiment; group ETH rats received saline for the first time on this test. Bottom panel: Mean impairment scores (+1 SEM) displayed by ethanol-injected rats that were, or were not, presented with the strobe/noise on the tolerance test (Experiment 1). Group SAL rats received ethanol for the first time on this test; group ETH rats received ethanol throughout the experiment.

Tolerance Test

For the tolerance test all subjects were injected with ethanol. Figure 2 (bottom panel) depicts the mean impairment scores of group ETH and group SAL subjects that were, or were not, presented with the novel stimulus. As can be seen in this figure, ethanol-experienced subjects presented with the stimulus were more impaired than ethanol-experienced subjects not presented with the novel stimulus. Group SAL subjects, receiving ethanol for the first time, were similarly impaired whether they were, or were not, presented with the stimulus. A one-way ANOVA indicated that the groups differed significantly, $F(3, 36) = 12.86$, $p < 0.001$. The difference between groups ETH/ON and ETH/OFF was significant, $F(1, 36) = 25.59$, $p < 0.001$. Group SAL/ON did not differ significantly from group SAL/OFF.

The novel stimulus reinstated impairment at pretolerance levels in ethanol-experienced subjects while having no effect on saline-experienced subjects receiving ethanol for the first time. Because the stimulus did not enhance ethanol-induced impairment in the ethanol-naive subjects, the disruption of tolerance by a novel stimulus did not result from a nonassociative enhancement of impairment by the novel stimulus (2).

Experiment 2

In Experiment 1, we investigated the effect of a novel stimulus on ethanol tolerance and on the CR mediating tolerance. We found that the addition of a novel, extraneous cue attenuated both tolerance and the CR mediating tolerance. Just as the addition of a novel stimulus can disrupt tolerance, the novel omission of an extraneous cue should also disrupt tolerance. In fact, this was shown in an experiment concerning external inhibition of morphine tolerance (19). In this experiment, two groups of rats were made tolerant to the analgesic effect morphine. One of these groups received a small injection of ethanol 15 min after the morphine injection. External inhibition of tolerance was investigated either by the introduction of a novel postmorphine ethanol cue or by the novel omission of the usual postmorphine ethanol cue. Both novel conditions attenuated tolerance. Based on the Poulos et al. (19) finding, we predicted that the novel omission of an extraneous cue would disrupt ethanol tolerance, and this prediction was assessed in Experiment 2.

Experiment 2 was similar to Experiment 1 except that tolerance development sessions were conducted with the strobe light/white noise stimulus on. On test days, the omission of the strobe light/white noise was used as the external inhibitor.

PROCEDURE

Pretolerance Test

Day 1 of the experiment was a pretolerance test. Two groups of rats (n = 20/group) received different injected substances—either ethanol or saline—during this test. Half of the rats injected with each substance were presented with the strobe light/white noise and remaining rats were not. The pretolerance test was included to evaluate the effect of the novel stimulus in rats receiving the drug for the first time.

Tolerance Development

During tolerance development (days 2–23) subjects were injected with either ethanol (group ETH, n = 20) or saline (group SAL, n = 20) during daily experimental sessions. Subjects were injected with the same substance during tolerance development sessions as they were during the pretolerance test. Throughout tolerance development sessions the strobe/noise stimulus was presented to all rats.

Tolerance Test

On day 24 all rats were injected with ethanol for a tolerance test. Half of the rats from both group ETH and group SAL were presented with the strobe/noise stimulus for this test (ETH/ON, n = 10; SAL/ON, n = 10). Remaining rats were tested with the novel omission of this stimulus (ETH/OFF, n = 10; SAL/OFF, n = 10). The strobe/noise stimulus was turned off within 90 s of injections and remained off for the duration of the experimental session. Half of the group ETH and group SAL subjects tested with the strobe/noise stimulus during this test were previously tested with it during the pretolerance test and half were not.

Tolerance Reacquisition

Reacquisition of tolerance occurred for 4 days following the tolerance test (days 25–28). During these sessions rats were run according to their tolerance development schedule—subjects from group ETH were injected with ethanol and subjects from group SAL were injected with saline during daily experimental sessions. All tolerance reacquisition sessions occurred with the strobe/noise stimulus on.

CR Test

On day 29 all rats were injected with saline for a CR test. Half of the rats from both group ETH and group SAL were presented with the strobe/noise stimulus (ETH/ON, n = 10; SAL/ON, n = 10). Remaining subjects were tested with the novel omission of the strobe/noise stimulus (ETH/OFF, n = 10; SAL/OFF, n = 10). The strobe/noise stimulus was turned off within 90 s of injections and remained off for the duration of the experimental session. All subjects presented with the stimulus during the tolerance test were not presented with it during the CR test.

RESULTS AND DISCUSSION

Pretolerance Test

The ataxic effect of ethanol was apparent during the pretolerance test; ethanol-injected subjects showed greater impairment than saline-injected subjects. The impairment of both ethanol- and saline-injected subjects was not augmented by the presentation of the strobe/noise stimulus (mean impairment scores: group ETH/ON: −10.4, group ETH/OFF: −10.9, group SAL/ON: −2.6, group SAL/OFF: −1.5). A one-way ANOVA indicated that the groups differed significantly, $F(3, 36) = 36.40$, $p < 0.001$. No planned comparisons (group ETH/ON vs. group ETH/OFF; group SAL/ON vs. group SAL/OFF) were significant. There was, however, a significant effect of treatment when comparing groups ETH and SAL ignoring the stimulus presentation, $F(1, 38) = 110.87$, $p < 0.001$. The pretolerance test demonstrated that the novel stimulus did not enhance impairment in either the ethanol- or saline-injected subjects, but that ethanol-injected subjects were more impaired than saline-injected subjects.

Tolerance Development

The results of the tolerance development phase in this experiment were similar to the results obtained in Experiment 1. The ataxic effect of ethanol was present at the start of the experiment—group ETH subjects showed greater impairment than group SAL subjects (mean impairment score for block 1: group ETH: −10.3, group SAL: −1.1). Over the course of tolerance development sessions, group ETH subjects displayed increasingly less impairment, while there was little change in impairment for group SAL subjects (mean impairment score for block 11: group ETH: −4.2, group SAL: −0.2). A mixed-design ANOVA conducted on the tolerance development data indicated a significant tolerance development solution (ETH vs. SAL) × block interaction, $F(10, 380) = 8.10$, $p < 0.001$. Further one-way repeated measures analyses indicated a significant effect of block for group ETH, $F(10, 190) = 16.71$, $p < 0.001$, but not for group SAL.

Tolerance Test

For the tolerance test all subjects were injected with ethanol. Recall that the novel cue was the omission of the strobe light/white noise stimulus. Figure 3 (top panel) depicts the mean impairment scores of group ETH and group SAL subjects that were, or were not, presented with the stimulus.

As can be seen in this figure, ethanol-experienced subjects tested with the novel omission of the stimulus were more impaired than ethanol-experienced subjects tested with the stimulus on. Group SAL subjects, receiving ethanol for the first time, were similarly impaired whether they were, or were not, presented with the stimulus. A one-way ANOVA indicated that the groups differed significantly, $F(3, 36) = 22.30$, $p < 0.001$. The difference between groups ETH/ON and ETH/OFF was significant, $F(1, 36) = 16.08$, $p < 0.001$. Group SAL/ON did not differ significantly from group SAL/OFF. External inhibition of tolerance was apparent—group ETH subjects tested with the novel omission of an extraneous stimulus were more impaired than group ETH subjects tested with the stimulus. The novel omission of the stimulus had no effect on subjects from group SAL receiving ethanol for the first time.

CR Test

For the CR test all rats were injected with saline and positive impairment scores would be expected in rats experiencing a CR. Figure 3 (bottom panel) depicts the mean impairment scores of group ETH and group SAL subjects that were, or were not, presented with the stimulus. As can be seen in this figure, ethanol-experienced subjects tested with the stimulus on (drug predictive environment) had larger positive impair-

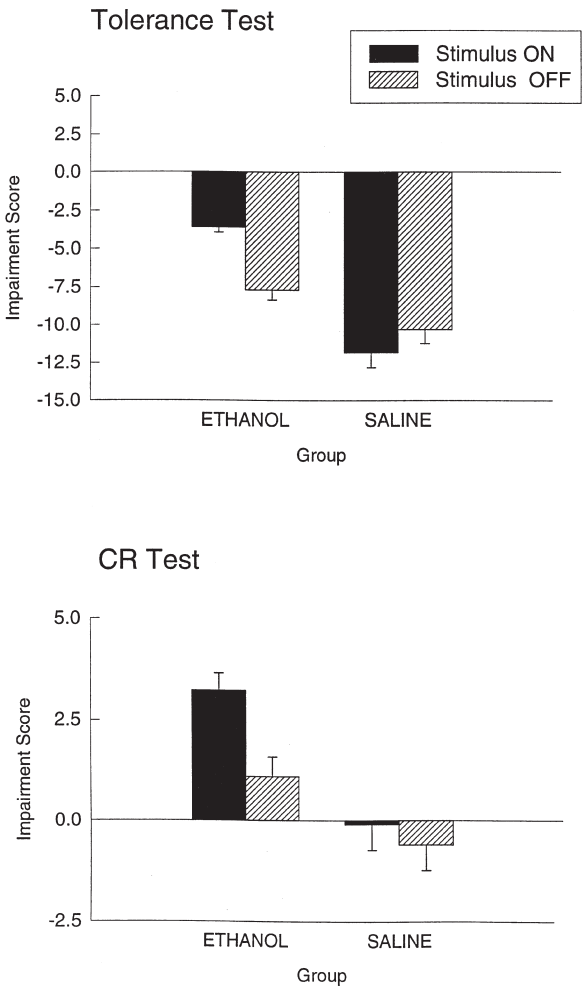

FIG. 3. Top panel: Mean impairment scores (+1 SEM) displayed by ethanol-injected rats that were, or were not, presented with the strobe/noise on the tolerance test (Experiment 2). Group SAL rats received ethanol for the first time on this test; group ETH rats received ethanol throughout the experiment. Bottom panel: Mean impairment scores (+1 SEM) displayed by saline-injected rats that were, or were not, presented with the strobe/noise on the CR test (Experiment 2). Group SAL rats received saline throughout the experiment; group ETH rats received saline for the first time on this test.

ment scores than all other groups. Group ETH subjects tested with the novel omission of the stimulus had impairment scores very similar to the subjects in both saline groups. A one-way ANOVA indicated that the groups differed significantly, $F(3, 36) = 10.53$, $p < 0.001$. A planned comparison of the difference between groups ETH/ON and ETH/OFF was significant, $F(1, 36) = 8.12$, $p < 0.01$. Group SAL/ON did not differ significantly from group SAL/OFF. The results of the CR test indicate that the novel omission of an extraneous stimulus disrupted the CR in group ETH subjects but did not affect impairment for group SAL subjects. The disruption of tolerance and the CR by both the novel omission of an extraneous stimulus and the novel addition of an extraneous stimulus is evidence that external inhibition of tolerance results from a general disruption of Pavlovian conditioning and is not related to the specific stimulus used for testing.

Experiment 3a

In Experiments 1 and 2, tolerance and CR tests occurred with a 24-h interval between the final tolerance development session and testing. Experiment 3 was designed to evaluate the effect of a novel stimulus on tolerance and the CR when the delay between the final tolerance development session and testing was increased. This experiment was conducted to study the relevance of stimulus generalization to the disruption of tolerance by a novel stimulus.

In Pavlovian conditioning, after repeated CS-US pairings, the CS will elicit a CR. In addition to the CS, other stimuli can elicit the CR and this is called stimulus generalization (16). Stimulus generalization is most pronounced when test stimuli are similar to the CS; therefore, the greater the disparity between the CS and test stimuli, the less generalization observed. Generalization gradients can be constructed that reflect peak responding to the CS, with least responding to test stimuli most dissimilar to it. It is well documented that generalization gradients flatten over time (21). That is, as the interval between training and testing is increased, stimuli that previously did not elicit a response shortly after training will do so. Riccio and colleagues have suggested that flattening of generalization gradients reflects a loss of memory for stimulus attributes (21,22). If an organism detects the disparity between training and test stimuli, no learned response will be elicited. If, over time, the organism fails to discriminate between training and test stimuli, a CR will be elicited by the test stimuli. Thus, the failure to discriminate between training and test stimuli modulates flattening of the generalization gradient.

Flattening of the generalization gradient may be relevant to conditioned tolerance. Riccio and colleagues suggest that the "loss of tolerance resulting from a change in context can be seen as directly analogous to stimulus generalization decrement" [(9), p. 265]. When assessing the importance of drug-associated cues, tolerance tests often occur in an environment distinct from the drug predictive environment, and attenuation of tolerance is evidenced. If the interval between tolerance development and testing increases, then the disruptive effect of a context change on tolerance should be minimized, because generalization gradients flatten over time. Feinberg and Riccio (9) investigated this prediction. Two groups of rats were made tolerant to morphine's analgesic effect in a drug-predictive environment (CS). One group of rats was tested for environmental control of morphine tolerance with a short (2-day) delay between the tolerance development phase of the experiment (training) and testing. A second group of rats were tested with a long (7-day) delay between training and testing. Half of the rats from both the long- and short-delay groups received a morphine tolerance test in the drug-predictive environment. Remaining rats were tested in an alternative environment not associated with morphine. Morphine tolerance was attenuated if testing occurred in the alternative environment after a short delay replicating previous demonstrations of environmental control of tolerance. Morphine tolerance was not attenuated when testing occurred in the alternative environment after a long delay—rats remained tolerant to morphine despite the context switch. Based on the results of this experiment, Feinberg and Riccio (9) conclude that conditioned tolerance is subject to flattening of the generalization gradient because rats did not discriminate between the train-

ing and testing context if tested with a long interval between training and testing.

Feinberg and Riccio (9) demonstrated that environmental control over tolerance is minimized as the interval between training and testing is increased. To the extent that drug-associated cues are less relevant to the expression of tolerance after a long delay between training and testing, the disruption of tolerance by a novel stimulus should also be less pronounced after a long delay. This prediction was tested in Experiment 3. In this experiment, the disruption of tolerance and conditional responding was tested in ethanol-tolerant rats either with a short (24-h) delay between the last tolerance development session (training) and testing, or with a long (10-day) delay between training and testing. We predicted that, replicating previous findings, the novel stimulus would attenuate tolerance and disrupt the CR when rats were tested with a short delay between training and testing. We also predicted that the effect of the novel stimulus would be minimized when rats were tested with a long delay between training and testing.

PROCEDURE

Tolerance Development

During tolerance development (days 1–22) subjects were injected with ethanol (group ETH, $n = 32$) or saline (group SAL, $n = 16$) during daily experimental sessions. The strobe/noise stimulus was not presented during experimental sessions.

Short Delay

Sixteen rats from Group ETH were given a tolerance and a CR test within 2 days of the last tolerance development session.

Tolerance test. On day 23, 16 group ETH rats, tested with the short delay [ETH(S)], were injected with ethanol for a tolerance test. Half of the rats were presented with the novel stimulus, as per Experiment 1 [ETH(S)/ON, $n = 8$]. Remaining rats were not presented with the novel stimulus [ETH(S)/OFF, $n = 8$].

CR test. On day 24, the same 16 ETH(S) rats were injected with saline for a CR test. Half of the rats were presented with the novel stimulus [ETH(S)/ON, $n = 8$]. Remaining rats were not presented with the novel stimulus [ETH(S)/OFF, $n = 8$]. All rats presented with the stimulus during the tolerance test were not presented with it during the CR test.

Long Delay

The 16 remaining subjects from group ETH were given a tolerance and a CR test with a 10-day delay between the last tolerance development session and the first test. All subjects from group SAL ($n = 16$) were also tested with this 10-day delay. These rats were left undisturbed in the colony room during this 10-day interval.

Tolerance test. On day 33, 16 group ETH rats, tested with the long delay [ETH(L)], were injected with ethanol for a tolerance test. Half of the rats were presented with the novel stimulus [ETH(L)/ON, $n = 8$]. Remaining rats were not presented with the novel stimulus [ETH(L)/OFF, $n = 8$]. The 16 group SAL rats, also tested with the long delay, were injected with ethanol for this test. Half of these rats were presented with the novel stimulus [SAL(L)/ON, $n = 8$] and half were not [SAL(L)/OFF, $n = 8$].

CR test. On day 34, the 16 ETH(L) and 16 SAL(L) rats were injected with saline for a CR test. Half of the rats from groups ETH and SAL were presented with the novel stimulus [ETH(L)/ON, $n = 8$; SAL(L)/ON, $n = 8$]. Remaining rats were not presented with the novel stimulus [ETH(L)/OFF, $n = 8$; SAL(L)/OFF, $n = 8$]. All rats presented with the novel stimulus for the tolerance test were not presented with it for the CR test.

RESULTS AND DISCUSSION

Tolerance Development

The results of the tolerance development phase in Experiment 3a replicated previous findings reported in this article. The ataxic effect of ethanol was present at the start of the experiment—group ETH subjects showed greater impairment than group SAL subjects (mean impairment score for block 1: group ETH: −9.0, group SAL: −0.4). Over the course of tolerance development sessions, group ETH subjects displayed increasingly less impairment, while there was little change in impairment for group SAL subjects (mean impairment score for block 11: group ETH: −4.1, group SAL: −0.1). A mixed-design ANOVA conducted on the tolerance development data revealed a significant tolerance development solution (ETH vs. SAL) × block interaction, $F(10, 460) = 4.83$, $p < 0.001$. Further one-way repeated measures analyses indicated a significant effect of block for group ETH, $F(10, 310) = 15.77$, $p < 0.001$, but not for group SAL.

Tolerance Test

For the tolerance test all rats were injected with ethanol. Figure 4 (top panel) depicts the mean impairment scores for group ETH and group SAL subjects that were, or were not, presented with the novel stimulus, either 1 day after [ETH(S)], or 11 days after [ETH(L) and SAL(L)], the last tolerance development session.

As can be seen in this figure, group ETH(L) subjects remained tolerant to the ataxic effect of ethanol despite the 10-day interval with no ethanol injections. These subjects were similarly impaired whether they were, or were not, presented with the novel stimulus. Group ETH(S) subjects presented with the novel stimulus were more impaired than group ETH(S) subjects not presented with the novel stimulus. Group SAL(L) subjects, receiving ethanol for the first time, were similarly impaired whether they were, or were not, presented with the novel stimulus. A one-way ANOVA indicated that the groups differed significantly, $F(5, 42) = 6.57$, $p < 0.001$. A planned comparison of the difference between groups ETH(S)/ON and ETH(S)/OFF was significant, $F(1, 42) = 13.36$, $p < 0.001$. Group ETH(L)/ON did not differ significantly from group ETH(L)/OFF nor did group SAL(L)/ON differ from group SAL(L)/OFF. These comparisons demonstrate that group ETH(S) subjects presented with the novel stimulus were more impaired than group ETH(S) subjects not presented with the novel stimulus. The novel stimulus had no effect on groups SAL(L) or ETH(L). These results confirm the prediction that external inhibition of tolerance is sensitive to the interval between training and testing. The failure to disrupt tolerance by a novel stimulus when tested with a 10-day delay between training and testing presumably resulted because environmental control over tolerance was less pronounced as the interval between training and testing increased.

CR Test

For the CR test all rats were injected with saline and positive impairment scores were expected in rats experiencing a

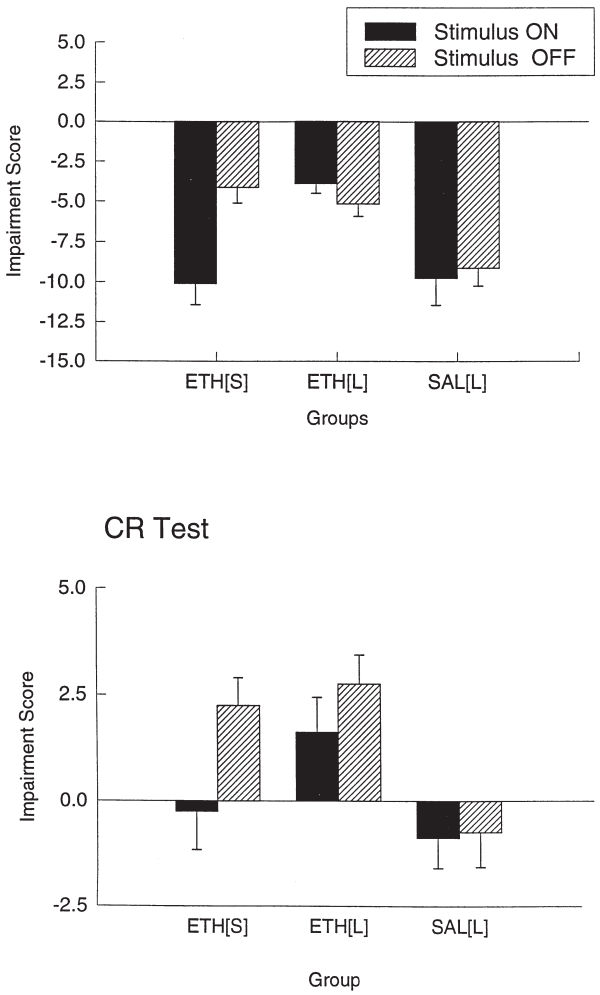

FIG. 4. Top panel: Mean impairment scores (+1 SEM) displayed by ethanol-injected rats that were, or were not, presented with the strobe/noise on the tolerance test with either a short [ETH(S)] or long [ETH(L) and SAL(L)] delay between tolerance development and the first test (Experiment 3a). Group SAL rats received ethanol for the first time on this test; group ETH rats received ethanol throughout the experiment. Bottom panel: Mean impairment scores (+1 SEM) displayed by saline-injected rats that were, or were not, presented with the strobe/noise on the CR test with either a short [ETH(S)] or long [ETH(L) and SAL(L)] delay between tolerance development and the first test (Experiment 3a). Group SAL rats received saline throughout the experiment; group ETH rats received saline for the first time on this test.

CR. Figure 4 (bottom panel) depicts the mean impairment scores of group ETH and group SAL subjects that were, or were not, presented with the novel stimulus, either 2 days after [ETH(S)], or 12 days after [ETH(L) and SAL(L)], the last tolerance development session. As can be seen in this figure, group ETH(S)/OFF subjects had larger positive impairment scores than group ETH(S)/ON subjects. This difference, although smaller, was also observed for the ethanol-experienced subjects tested with the long delay [i.e., ETH(L)/OFF had larger positive impairment scores than ETH(L)/ON]. Group SAL(L) subjects that were, or were not, presented with the novel stimulus were similarly impaired. A one-way ANOVA indicated that the groups differed significantly, $F(5, 42) = 4.40$, $p < 0.01$. A planned comparison of the difference between groups ETH(S)/ON and ETH(S)/OFF was significant, $F(1, 42) = 5.34$, $p < 0.05$. Group ETH(L)/ON did not differ significantly from group ETH(L)/OFF nor did group SAL(L)/ON differ from SAL(L)/OFF. These comparisons demonstrate that the novel stimulus decreased the CR in group ETH(S). The nonsignificant attenuation of the CR in ETH(L) subjects provides evidence that the disruptive effect of a novel stimulus is decreased as the interval between training and testing is increased.

Experiment 3b

In Experiment 3a the effect of a novel stimulus was assessed in rats tested with a short and long delay between training and testing. In Experiment 3a, all tolerance tests were conducted first (i.e., tolerance tests occurred 24 h before CR tests). Experiment 3b also assessed the disruption of tolerance and the CR with both a short and long delay between training and testing. In this experiment, the order of the tests was reversed—CR tests were conducted before tolerance tests. Other than the order of tests, Experiment 3b was identical to Experiment 3a.

PROCEDURE

Tolerance Development

During tolerance development sessions (days 1–22), subjects were injected with ethanol (group ETH, $n = 30$) or saline (group SAL, $n = 16$) during daily experimental sessions. During experimental sessions the strobe/noise stimulus was off.

Short Delay

Fourteen rats from group ETH were given a tolerance and a CR test within 2 days of the last tolerance development session.

CR test. On day 23, 14 group ETH rats, tested with the short delay [ETH(S)], were injected with saline for a CR test. Half of the rats were presented with the novel stimulus [ETH(S)/ON, $n = 7$]. Remaining rats were not presented with the novel stimulus [ETH(S)/OFF, $n = 7$].

Tolerance test. On day 24, the 14 ETH(S) rats were injected with ethanol for a tolerance test. Half of the rats were presented with the novel stimulus [ETH(S)/ON, $n = 7$] and half were not [ETH(S)/OFF, $n = 7$]. All of the rats presented with the stimulus during the CR test were not presented with it during the tolerance test.

Long Delay

The 16 remaining subjects from group ETH were given a tolerance and a CR test with a 10-day delay between the last tolerance development session and the first test. All subjects from group SAL ($n = 16$) were also tested with this 10-day delay. These rats were left undisturbed in the colony room during this 10-day interval.

CR test. On day 33, 16 group ETH rats, tested with the long delay [ETH(L)], were injected with saline for a CR test. Half of the rats were presented with the novel stimulus [ETH(L)/ON, $n = 8$]. Remaining rats were not presented with the novel stimulus [ETH(L)/OFF, $n = 8$]. The 16 group SAL rats, also tested with the long delay, were injected with saline for this test. Half of these rats were presented with the novel

stimulus [SAL(L)/ON, $n = 8$] and half were not [SAL(L)/OFF, $n = 8$].

Tolerance test. On day 34, the 16 ETH(L) and 16 SAL(L) rats were injected with ethanol for a tolerance test. Half of the rats from groups ETH and SAL were presented with the novel stimulus [ETH(L)/ON, $n = 8$; SAL(L)/ON, $n = 8$]. Remaining rats were not presented with the novel stimulus [ETH(L)/OFF, $n = 8$; SAL(L)/OFF, $n = 8$]. All rats presented with the novel stimulus for the CR test were not presented with it for the tolerance test.

RESULTS AND DISCUSSION

Tolerance Development

The ataxic effect of ethanol was present at the start of the experiment—group ETH subjects showed greater impairment than group SAL subjects (mean impairment score for block 1: group ETH: −8.9, group SAL: −0.6). Over the course of tolerance development sessions, group ETH subjects displayed increasingly less impairment, while there was little change in impairment for group SAL subjects (mean impairment score for block 11: group ETH: −4.0, group SAL: −0.9). A mixed-design ANOVA conducted on the tolerance development data indicated a significant tolerance development solution (ETH vs. SAL) × block interaction, $F(10, 440) = 4.70$, $p < 0.001$. Further one-way repeated measures analyses indicated a significant effect of block for group ETH, $F(10, 290) = 13.64$, $p < 0.001$, but not for group SAL.

CR Test

For the CR test all rats were injected with saline. Figure 5 (top panel) depicts the mean impairment scores of group ETH and group SAL subjects that were, or were not, presented with the novel stimulus either 1 day after [ETH(S)], or 11 days after [ETH(L) and SAL(L)], the last tolerance development session. As can be seen in this figure, group ETH(S)/OFF subjects had larger positive impairment scores than group ETH(S)/ON subjects. This difference, although smaller, was also observed for the ethanol-experienced subjects tested with the long delay [i.e., ETH(L)/OFF had larger positive impairment scores than ETH(L)/ON]. Group SAL(L) subjects that were, or were not, presented with the novel stimulus were similarly impaired. A one-way ANOVA indicated that the groups differed significantly, $F(5, 40) = 3.67$, $p < 0.01$. A planned comparison of the difference between groups ETH(S)/ON and ETH(S)/OFF was significant, $F(1, 40) = 5.97$, $p < 0.05$. Group ETH(L)/ON did not differ significantly from group ETH(L)/OFF, nor did group SAL(L)/ON differ from SAL(L)/OFF. These comparisons demonstrate that the novel stimulus significantly attenuated the CR in group ETH(S) subjects, while it only slightly attenuated the CR in group ETH(L) subjects. Thus, the CR is less affected by the novel stimulus if a long delay occurs between training and testing. These findings were similar to the results from the CR test in Experiment 3a.

Tolerance Test

For the tolerance test all rats were injected with ethanol. Figure 5 (bottom panel) depicts the mean impairment scores for group ETH and group SAL subjects that were, or were not, presented with the stimulus either 2 days after, or 12 days after, the last tolerance development session. As can be seen in this figure, group ETH(L) subjects remained tolerant to the ataxic effect of ethanol and were similarly impaired whether they were, or were not, presented with the novel stimulus. Group ETH(S) subjects presented with the novel stimulus were more impaired than group ETH(S) subjects not presented the novel stimulus. Group SAL(L) subjects, receiving ethanol for the first time, were similarly impaired whether they were, or were not, presented with the novel stimulus. A one-way ANOVA indicated that the groups differed significantly, $F(5, 40) = 10.95$, $p < 0.001$. A planned comparison of the difference between groups ETH(S)/ON and ETH(S)/OFF was significant, $F(1, 40) = 6.27$, $p < 0.02$. Group ETH(L)/ON

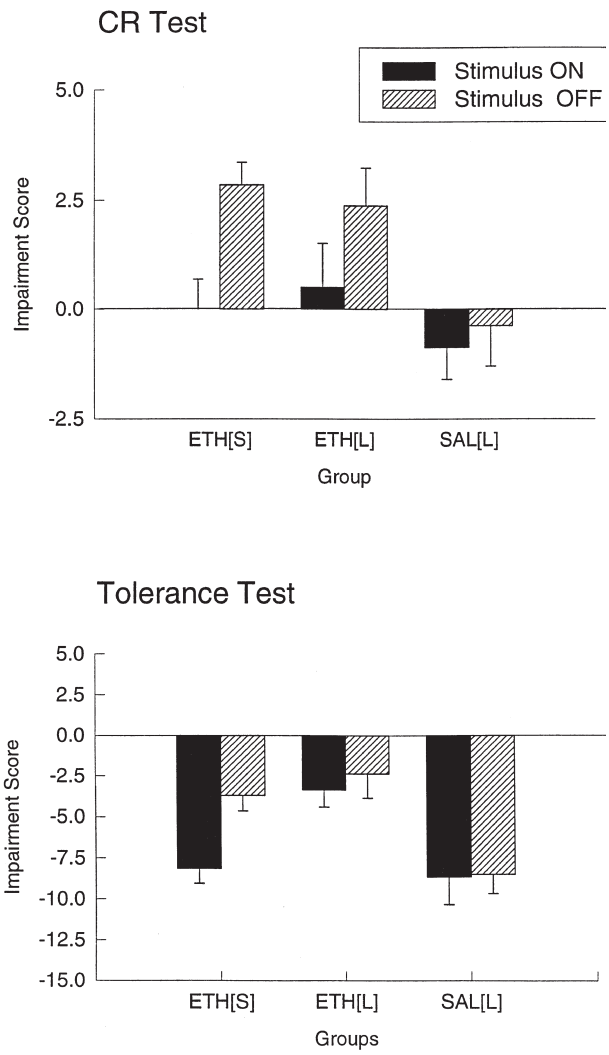

FIG. 5. Top panel: Mean impairment scores (+1 SEM) displayed by saline-injected rats that were, or were not, presented with the strobe/noise on the CR test with either a short [ETH(S)] or long [ETH(L) and SAL(L)] delay between tolerance development and the first test (Experiment 3b). Group SAL rats received saline throughout the experiment; group ETH rats received saline for the first time on this test. Bottom panel: Mean impairment scores (+1 SEM) displayed by ethanol-injected rats that were, or were not, presented with the strobe/noise on the tolerance test with either a short [ETH(S)] or long [ETH(L) and SAL(L)] delay between tolerance development and the first test (Experiment 3b). Group SAL rats received ethanol for the first time on this test; group ETH rats received ethanol throughout the experiment.

did not differ significantly from group ETH(L)/OFF, nor did group SAL(L)/ON differ from group SAL(L)/OFF. These comparisons demonstrate that group ETH(S) subjects tested with the novel stimulus were more impaired than group ETH(S) subjects not tested with the novel stimulus. The novel stimulus had no effect on group SAL(L) or group ETH(L) subjects.

These results from the tolerance test in Experiment 3a and 3b confirm that tolerance to ethanol-induced ataxia is retained over a 10-day period with no ethanol injections. Tolerance was not significantly disrupted by the novel stimulus for the subjects tested with a long delay between training and testing. The novel stimulus did disrupt tolerance in subjects tested after a short delay. Based on these findings, it is evident that the disruption of tolerance by a novel stimulus is decreased as the interval between training and testing is increased.

GENERAL DISCUSSION

Disruption of Tolerance by a Novel Stimulus

The results obtained in the tolerance development phases of the experiments reported in this article demonstrate that tolerance to ethanol-induced ataxia is apparent within 22 tolerance development sessions. These findings are consistent with those of Siegel and Larson (31), who also reported tolerance to ethanol-induced ataxia within 22 days.

In these experiments we demonstrate that a novel stimulus reinstates ataxia in ethanol-tolerant rats if tested shortly after the final tolerance development session. We have also shown that the novel stimulus does not enhance ethanol-induced ataxia in rats receiving ethanol for the first time, either on the first day of experimentation (Experiment 2) or after 22 saline sessions (Experiments 1, 2, and 3). It was important to demonstrate that the strobe/noise stimulus did not enhance ataxia in subjects receiving ethanol for the first time to confirm that the attenuation of tolerance by a novel stimulus was not resulting from a nonassociative enhancement of ethanol's effect (2,17). Because the novel stimulus only enhanced impairment in subjects tolerant to the ataxic effect of ethanol, it appears that the novel stimulus was disrupting Pavlovian conditioning mediating tolerance. The observation that a novel stimulus will disrupt tolerance without enhancing ataxia in rats receiving ethanol for the first time is consistent with previous findings by Siegel and Larson (31).

In Experiment 2, we demonstrated the attenuation of tolerance by the omission of an extraneous cue. This finding is similar to reports that the novel omission of an interoceptive (ethanol) cue will attenuate morphine tolerance (19). Evidence that both the novel presentation of a stimulus and the novel omission of a stimulus attenuates tolerance provides converging evidence that the disruption of tolerance by a novel stimulus is attributable to a disruption of Pavlovian conditioning.

A more complete attenuation of tolerance occurred when the novel stimulus was the novel addition of the strobe/noise stimulus (Experiment 1) than when it was the novel omission of the stimulus (Experiment 2). These differences may relate to the strength of the novel stimulus. Previous research has demonstrated that external inhibition depends, in part, upon the intensity of the external inhibitor (37). Because the novel omission of the strobe/noise stimulus involved turning on the overhead fluorescent lights and turning off the strobe light/white noise, this created an environment similar to the animal colony in terms of noise and lighting. On the other hand, the addition of the novel stimulus involved introducing a stimulus (strobe light/white noise) to the animals unlike any they had previously experienced. It is conceivable, then, that the omission of the strobe light/white noise may not have been as novel or as intense a stimulus as the addition of the strobe light/white noise. Such a difference in the strength of the novel stimulus between experiments may account for the less complete disruption of tolerance seen in Experiment 2.

Disruption of Conditional Responding

In these experiments we have demonstrated a "hypertaxic" compensatory CR. That is, ethanol-tolerant rats injected with saline in the drug-associated environment displayed an enhanced ability to stay on the tilting plane. This finding provides evidence in support of Siegel and Larson's (31) speculation that tolerance to ethanol's ataxic effect is mediated by a compensatory response. The CR observed in these experiments, enhanced behavioral functioning, is similar to reports that people demonstrate enhanced performance in a behavioral task when expecting alcohol but receiving a placebo (23).

The conditional hypertaxic response was disrupted by both the addition of a novel stimulus and the novel omission of a stimulus if testing occurred shortly after the final tolerance development session. This finding is important because it provides converging evidence that the attenuation of tolerance by a novel stimulus results from the disruption of a CR. External inhibition was indeed occurring.

Although many experiments evaluating the Pavlovian conditioning analysis of tolerance have demonstrated compensatory CRs, some have not (7,28). Evidence that the novel omission of an event can disrupt a CR has implications for understanding failure to observe compensatory CRs during placebo tests in drug-tolerant subjects. Evidence presented here (Experiment 2) and by Poulos et al. (19), demonstrates that the nonoccurrence of an event disrupts a CR. This, coupled with evidence that the early drug effect can signal the later drug effect (11,35) suggests that a placebo test may cause external inhibition. It has been shown that interoceptive pharmacological events, acting as a CS, can signal an oncoming drug effect (11). In the case of slow-acting drugs, it is possible that the early drug effects signal the later, peak, drug effects, just as a CS signals a US. During a placebo test, normally used to test for a CR, the early drug effect is not present. The novel omission of this early drug effect may attenuate the expected CR. Indeed, "it is not surprising that the omission of all drug cues in a placebo test occasionally result in a failure to detect CRs" [(19), p. 415].

Relevance of Interval Between Training and Testing

Tolerance to ethanol-induced ataxia was still apparent when testing occurred with a 10-day delay between the last tolerance development session and the first test. The retention of tolerance to the ataxic effect of ethanol is compatible with other observations that tolerance to the behavioral impairing effect of ethanol can be retained for a number of weeks in the absence of continued ethanol administration [e.g., (12)]. According to the Pavlovian conditioning analysis, once tolerance has developed it should be retained in the absence of drug administrations as long as it is not extinguished, i.e., as long as the CS (drug predictive stimulus) is not presented (5,16).

The novel stimulus did not attenuate ethanol tolerance in subjects tested with a long delay between training and testing,

illustrating that the interval between training and testing is important for the demonstration of external inhibition of tolerance. This result is compatible with findings of Feinberg and Riccio (9), who demonstrated that rats fail to show environmental control of tolerance if tested with a long interval between training and testing.

A CR was observed after a 10-day delay between the tolerance development phase of the experiment and testing. Evidence of a CR after this long delay supports that suggestion that the retention of tolerance is mediated by the retention of Pavlovian conditioning. The CR, evident after this 10-day delay, was disrupted slightly, but not significantly, by the novel stimulus. The failure of the novel stimulus to significantly attenuate ethanol tolerance and the CR mediating tolerance if a long delay between training and testing occurred is predicted if environmental control over tolerance is diminished as the interval between training and testing is increased (9,22). Our findings reinforce the importance of considering the interval between training and testing when studying environmental control of tolerance.

A Pavlovian vs. an Instrumental Analysis

The results presented in this article are consistent with an analysis of tolerance that emphasizes the contribution of Pavlovian conditioning to tolerance. The results may also be interpreted as illustrating the contribution of instrumental learning to tolerance. That is, tolerance to the behavioral impairing effect of ethanol may be mediated by an instrumental strategy learned as a result of intoxicated practice on the tilting plane. Dews (4) suggested that the experienced drinker is less impaired by alcohol than the inexperienced drinker because the experienced drinker has acquired a behavioral strategy (e.g., a broad-based gait) as a result of intoxicated practice that would assist him/her in coping with drug-induced impairments. An instrumental strategy similar to the one described in humans may have been acquired in the subjects in these experiments (e.g., the rats may have changed their stance or altered their position in the tilting plane). Such an instrumental response would delay sliding down the tilting plane and may restore balance to the animal. Alternatively, the CR observed in rats tolerant to the ataxic effect of ethanol may reflect a classically conditioned response elicited reflexively by the US (e.g., a change in muscle control), which restores homeostasis to the organism (18). Because instrumental conditioning, like Pavlovian conditioning, is disrupted by a novel stimulus (10,36,38), the findings reported in this article are consistent with both interpretations.

Human Implications

Regardless of the nature of the conditional response (i.e., instrumental or classical), the attenuation of tolerance by a novel stimulus has important implications. The conditioning analysis of tolerance has been shown to be important in understanding drug overdose (14,15,30,34). Based on findings of external inhibition of tolerance, we would predict that if people consistently administer a drug under one set of circumstances, a novel stimulus presentation or the novel omission of a stimulus should disrupt tolerance and result in an exaggerated drug effect. Indeed, anecdotal evidence supports such a suggestion (26,29). Finally, the disruption of tolerance by a novel stimulus may have forensic implications. For instance, a drug effect may be enhanced in an apparently tolerant person by a novel stimulus, such as the arrival of the police (13).

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