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The Effect of External Stimulus Change on Ethanol-Produced Dissociation[1]

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DUNCAN, P. M. *The effect of external stimulus change on ethanol-produced dissociation.* PHARMAC. BIOCHEM. BEHAV. 11(4) 377–381, 1979.—Rats which were subjected to aversive Pavlovian conditioning while in a state of ethanol-produced intoxication showed significantly less conditioned suppression of water drinking in the non-drugged state only if the external stimulus situation was also changed between conditioning and testing. This interaction between internal and external stimulus change supports a generalization-decrement explanation of drug-produced dissociation of memory. The number of conditioning trials was also manipulated, but this variable had no significant effect on the conditioned responses. In a second experiment, ethanol injections were given again shortly before testing. This treatment caused a normal degree of conditioned drinking suppression in rats conditioned in the ethanol state, indicating that state-dependent learning was responsible for the conditioned response deficit seen in the first experiment. The dissociation was asymmetrical since rats injected with ethanol before testing only showed a high degree of conditioned suppression.

Ethanol State-dependent learning Drug-produced dissociation Generalization Conditioned drinking suppression

HUMANS [8] and animals [7,15] may exhibit impaired memory of events which occur while they are intoxicated with ethanol if the memory is tested during the non-drugged state. However, this memory impairment may be reversed upon reinstatement of the original drug condition, thereby demonstrating that the lack of responding (dissociation) seen in the nondrugged state was actually a failure of memory retrieval. This phenomenon is known as state-dependent learning [12].

It is well known that psychoactive drugs can act as stimuli, and a large body of literature exists which demonstrates that animals can learn to discriminate various drug states (cf. [1]). Overton [10] maintained that the memory dissociation effect of some drugs was also due primarily to the stimulus state produced by drug treatment. Overton [11] later demonstrated that a change in external contextual stimuli (stimuli which persist throughout a learning period, as opposed to stimuli which are presented only prior to an unconditioned stimulus, or are contingent on a response) between a training and a test session can produce dissociation of memory. This "generalization decrement" theory of drug-produced dissociation has been discussed by several investigators [3, 4, 14, 17, 18]. According to this theory, a drug state acts as a very salient internal contextual stimulus. When this stimulus state changes between a conditioning period and a testing period, a sharp generalization gradient renders the memory less retrievable, and results in dissociation.

The first experiment presented here tested a prediction that was derived from the generalization decrement theory of drug dissociation. If a change in an internal contextual stimulus state acts in a similar manner as does a change in an external contextual stimulus, these two sources of generalization decrement should interact in their production of memory dissociation. More specifically, there should be degrees of both external and internal stimulus change which would not produce dissociation unless both types of change were experienced in combination.

EXPERIMENT 1

Ward (unpublished MA thesis research conducted in the author's laboratory) investigated ethanol-produced memory dissociation by conditioning rats in the intoxicated state, and later testing them for degree of suppression of water drinking in response to the shock-predicting CS. In that experiment, external stimulus conditions were changed between conditioning and test sessions. An ethanol dose of 800 mg/kg produced no dissociation, but a 1600 mg/kg dose caused a significant reduction of conditioned suppression. In the present experiment an ethanol dose of 1400 mg/kg was chosen in order to determine the possible interaction of external stimulus change with a moderate degree of ethanol intoxication that could be expected to produce memory dissociation in the drinking suppression paradigm. Since the generalization decrement resulting from changing the external stimulus conditions might also cause changes in degrees of conditioned fear in the absence of any drug manipulations, the number of conditioning trials was also varied in this experiment. This manipulation was done in an attempt to deter-

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mine whether any drug effects seen were specific to a certain degree of conditioned fear.

A two (1400 mg/kg ethanol vs saline) by two (external stimulus conditions shifted vs external conditions held constant) by three (two, four, or six CS-shock pairings) between-groups factorial experimental design was used to investigate the effects of these three variables on conditioned drinking suppression resulting from aversive Pavlovian conditioning.

METHOD

Animals

One hundred and twenty-two male Long-Evans rats, about 120 days old, were housed individually on the day prior to the start of experimental procedures. Mean body weight was 330 g. Free access was given to food and water, except for periods of water deprivation as described below.

Apparatus

The rats were conditioned and tested in a metal and Plexiglas box which measured 30 cm in each dimension. This chamber had a grid floor, a loudspeaker, and a drinking tube which protruded through a wall. The chamber was located inside a larger sound-attenuating enclosure which contained a 30 W light for illumination. Footshock was provided by a Grason-Stadler shock generator-scrambler. Auditory stimulus presentations and shocks were programmed, and drinking was recorded via a drinkometer circuit and a system of electromechanical and solid-state components.

Ninety-five percent ethanol solution was diluted with 0.9% saline (sodium chloride) to obtain an injection solution of 25% (volume/volume) ethanol.

Procedure

Day 1. This experiment was designed to investigate conditioned heart-rate response as well as drinking suppression, so EKG electrodes were implanted on the first day. While under ether-produced anesthesia, all rats had two steel wire loops implanted on either side of the chest. Water bottles were removed from the rats' home cages.

Days 2 and 3. The rats were given 15 minutes access to water in the conditioning-testing chamber. A 70-dB intensity white noise background was present during this and all subsequent phases of the experiment.

Day 4. Each rat was placed in the chamber, and 15 sec after water drinking commenced, an auditory stimulus was presented. This stimulus was a 28-sec duration of 5-Hz interruption in the white noise background, and was presented at this time for adaptation purposes. The latency for each rat to emit 50 licks after stimulus onset was recorded. Rats with high, intermediate, and low suppression latencies to this initial stimulus presentation were distributed evenly among the experimental conditions. After adaptation, water bottles were replaced in the home cages.

Day 5. The rats were subjected to aversive Pavlovian conditioning in the chamber. A conditioning trial consisted of a presentation of the 28-sec auditory stimulus (the conditioned stimulus), the final second being accompanied by an unavoidable 1-mA footshock. The first trial started five minutes after each rat was placed in the chamber, and the intertrial interval was four minutes. Five minutes after the final footshock, the rats were removed from the chamber. The drinking tube was not present in the chamber during conditioning.

The rats were divided into 12 treatment groups of nine animals each, according to the 2×2×3 factorial design. Rats in six of the groups received a 1400 mg/kg intraperitoneal ethanol injection 15 min before the start of the conditioning session. The other six groups received saline injections of equivalent volume.

Three of the ethanol and three of the saline groups were conditioned with the conditioning chamber inside the larger enclosure, and with the house light illuminated. Thus, the external stimulus conditions were essentially identical to those of the adaptation and test days. These groups will be referred to as the no stimulus change groups. The remaining three ethanol and three saline groups were conditioned with the stimulus situation markedly different from the adaptation and test days. This difference was accomplished by removing the conditioning chamber from the enclosure, moving it about three m away to a table top, and turning off the room lights so conditioning was done in near-total darkness. These groups will be referred to as the stimulus change groups.

Four groups of rats (one from each combination of the ethanol-saline variable and the stimulus change variable) were given two conditioning trials. Four groups received four, and four groups received six conditioning trials. An additional two groups of rats (n=7 each) were given saline injections and four CS presentations, but no footshock. One of these nonshock control groups was in the no stimulus change condition, and the other group was in the stimulus change condition.

Day 6. The rats were left undisturbed in their home cages with free access to water.

Day 7. Water bottles were removed from the home cages.

Day 8. The water-deprived rats were placed in the conditioning-testing chamber and polygraph leads were attached to the EKG electrodes. The leads restricted the rats' movement only slightly. The drinking tube was inserted into the chamber, and if drinking occurred, the rat was given 10 min of access to the drinking tube. If no drinking occurred within 5 min, the rat was returned to its home cage and allowed to drink for 10 min. This day's procedure was included to reduce generalized fear of the test apparatus and thus promote prompt onset of drinking on the subsequent day.

Day 9. Each rat was placed in the test chamber and the EKG leads were attached. Five minutes later, the drinking tube was presented. Fifteen seconds after reliable drinking started, the CS was presented for 28 sec. The latency of the rat to emit the first 50 licks after CS onset was recorded, as were the total number of licks emitted during the CS presentation. After 100 post-CS licks were emitted, the drinking tube was removed. Five minutes later the drinking tube was presented again to initiate to a second test trial. No footshock was administered on the test day.

RESULTS

Very similar results were seen in both the total-licks-emitted measure and in the latency measure. Since these two sources of data were redundant, only the latency data will be presented.

The pattern of differences among conditioned heart-rate changes was generally similar to that seen in the drinking

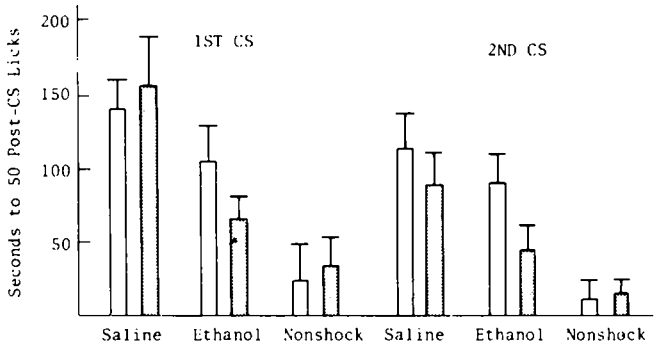

FIG. 1. Mean latency to emit 50 post-CS licks (±SEM) to the first and second CS presentations on test day. Labels on horizontal axis indicate drug treatment during conditioning period. Ethanol dosage was 1400 mg/kg. Nonshocked rats received saline injections. Shaded bars indicate external stimuli changed between conditioning and testing sessions. Nonshaded bars indicate no change in external stimuli.

suppression. However, variability among rats in the direction of heart-rate change from baseline rates made meaningful analysis of heart-rate data difficult or impossible. Therefore, the heart-rate data will not be presented here.

Animals with latencies in excess of 5 min for 50 post-CS licks were given a score of 300 sec. This truncation was done to reduce skewness of the data, and was required for only seven rats, all from saline groups. A 2×2×3 factorial analysis of variance was conducted for the latency scores of each CS presentation. These analyses showed that there were no significant main or interaction effects of the number-of-conditioning-trials variable, so the data were pooled across this variable for further analysis. The means of the combined groups are presented in Fig. 1.

The pattern of mean values and the results of the statistical analyses indicate that a significant degree of dissociation was seen only in the group which underwent a change in both internal (drug-produced) and external stimuli between the conditioning and test periods; i.e., neither the drug state change only, nor the external stimulus change only was sufficient to produce a significant generalization decrement (dissociation).

These conclusions are drawn from the following statistical results ($p<0.05$ and $df=1,96$ in all significant comparisons mentioned): The overall ANOVA showed that for the first CS presentation, the main effect of ethanol was significant ($F=8.07$), as was the external stimulus change by ethanol interaction effect ($F=3.96$). The main effects of external stimulus change, number of conditioning trials, and the remaining interaction effects were not statistically significant.

For the second CS presentation, the main effect of ethanol was again significant ($F=4.00$), as was the external stimulus change effect ($F=4.20$). The ethanol by external stimulus change interaction effect was not significant. The number of conditioning trials, and the remaining interaction effects were also not significant for the second CS presentation.

For further analysis of the data individual comparisons among the four groups (pooled across the conditioning trials variable) were conducted by means of Duncan's Multiple Range Test. These analyses showed that within the condition of no external stimulus change, the ethanol effect was not significant for either CS presentation (the ethanol no-shift group compared to the saline no-shift group). In contrast, significant differences were found for these comparisons within the external stimulus change condition (saline shift group compared to the ethanol shift group, $p<0.05$). Specific comparisons between the two groups which received saline (external stimulus change vs no external stimulus change) revealed no significant differences. When the two groups which had received ethanol during conditioning were compared, no significant differences were found for the first CS presentation, but such a difference ($p<0.05$) was found between these groups for the second CS. Finally, the ethanol-external shift group was significantly different from the saline-no external shift group for both CS presentations ($p<0.01$).

DISCUSSION

Under the conditions of the present experiment, neither the drug-state change alone nor the external stimulus change alone produced a significant degree of dissociation. However, when the two types of stimuli were both changed between conditioning and test periods, significantly less conditioned drinking suppression was produced. The ethanol state during conditioning produced a tendency for dissociation in the no-external change condition, and this tendency was enhanced (and made significant) by the addition of the external stimulus change. The interaction of the two stimulus changes appears to be of a synergistic nature, because upon presentation of the first CS the external change alone did not produce even a tendency for a lessened degree of conditioned response. External contextual stimulus changes have been previously reported to impair memory retrieval in other paradigms and with other species [6, 9, 11]. Apparently the simple association of a distinct auditory stimulus with an unavoidable footshock is resistant to generalization decrement effects caused by changes in external contextual stimuli. A tendency for a generalization decrement resulting from only the external stimulus change was seen on the second CS presentation, and this effect was also enhanced by the combination of both sources of stimulus change.

The interaction seen here is no doubt dependent on the drug dosage, as well as the type of drug and the type and degree of external stimulus change. In the previous study of this nature (Ward's MA thesis) 800 mg/kg ethanol did not produce a significant degree of dissociation in conjunction with a dark-to-light external stimulus change. Conversely, higher doses of ethanol can produce memory deficits in rats [5] and in humans [8] without external stimulus change. Finally, the interaction apparently also depends on the experimental paradigm, since Holloway [7] found ethanol-produced dissociation at a dosage very similar to that used here, when rats were required to learn active or passive avoidance.

The results of this experiment can be interpreted as indicating that the two sources of stimulus change interact synergistically or additively, as described above. Ethanol's stimulus properties are obviously critical to this interpretation. However, since all groups conditioned drugged were tested nondrugged, ethanol's stimulus effect (or the effect of changing this stimulus) was confounded with other possible ethanol effects. These additional (non-stimulus) ethanol ef-

fects could alter associations formed in the drugged state, making them weaker, or more susceptible to the effects of changing the external stimulus situation. This possibility was investigated in Experiment 2, in which the effects of ethanol treatment during the test period, as well as during the conditioning period, were determined.

EXPERIMENT 2

Investigation of the effect of changing the ethanol state versus not changing it, including a change from the nondrugged to the drugged state, was done by using a standard "transfer" design which has frequently been used to study the state-dependent properties of drugs (cf. [14]). Thus, an added benefit of this experiment was to ensure that state-dependent learning actually was involved in the ethanol effects seen in Experiment 1.

METHOD

Animals

Forty-eight additional male Long-Evans rats, similar in age, weight, and obtained from the same supplier as were those used in Experiment 1, were used in Experiment 2. Housing, feeding and watering procedures were also identical to those of Experiment 1.

Apparatus

The apparatus was that described in Experiment 1.

Procedure

A 2×2 factorial design was used, with ethanol or saline injections shortly prior to conditioning or testing being the two conditions of each variable. Four groups of nine rats each were used, plus two additional non-shocked groups, each containing six rats. Preliminary water deprivation, habituation to the apparatus, and establishment of reliable drinking were as described in Experiment 1, as were all other procedures, except where differences are described below. EKG electrodes were not implanted.

The four experimental groups were designated ethanol-saline (ES), SE, SS and EE. On the conditioning day, all rats were subjected to four CS-shock pairings in the stimulus change (dark) condition of Experiment 1. Rats in Groups ES and EE received ethanol injections (1400 mg/kg) 15 min prior to the start of the conditioning period, whereas groups SE and SS received saline injections. Rats in both nonshocked groups received saline injections prior to four CS-only presentations.

On test day, all rats received injections of either saline or ethanol 15 min prior to the suppression-test session. Pilot work with non-shocked rats revealed that exact duplication of the ethanol treatment given on conditioning day (1400 mg/kg) resulted in performance effects which sometimes interfered with reliable drinking in the test situation. To avoid this problem, rats in Groups EE, SE, and one nonshocked control group were given IP injections of 1000 mg/kg ethanol in order to produce the ethanol state (similar to that of the conditioning day for Group EE). Groups ES, SS, and the other nonshocked group received injections of equivalent volumes of saline. With the exceptions of pretest injections, test-day procedures were as described in Experiment 1.

RESULTS

The mean latencies to emit 50 post-CS licks are presented in Table 1. The Ethanol treatment just prior to conditioning (plus the external stimulus change) again resulted in significantly less conditioned suppression when the rats were tested later in the non-ethanol state (comparison of Groups ES and SS). However, EE group rats, both conditioned and tested in the ethanol state, exhibited a degree of suppression very similar to that of the SS group. The drinking suppression of the EE group was not due to a nonspecific performance-impairing effect of ethanol, in that the nonshocked control group which received ethanol prior to testing showed drinking onset and persistence which was very similar to that of the saline-injected nonshocked group. Finally, the SE group exhibited a degree of suppression very similar to that of the SS control group.

TABLE 1

MEAN LATENCY AND SEM (SEC) TO EMIT 50 POST-CS LICKS

Group	1st CS	2nd CS
Saline-Saline	160 (± 19)	147 (± 16)
Ethanol-Saline	73 (± 24)	51 (± 13)
Saline-Ethanol	152 (± 16)	139 (± 12)
Ethanol-Ethanol	178 (± 22)	130 (± 20)
Nonshock Saline	47 (± 8)	45 (± 8)
Nonshock Ethanol	53 (± 9)	44 (± 8)

Analyses of variance were conducted on the data from the four experimental groups. Latencies were truncated at 300 seconds, and separate analyses were conducted for each CS presentation. Since the patterns of significant differences were very similar for both CS presentations, the statistical results of only the first-CS analysis will be presented. The main effect of the ethanol treatment during neither conditioning nor test periods was significant ($p>0.10$). However, the ethanol-saline by conditioning-testing interaction effect was significant, $F(1,32)=4.30$, $p<0.05$ (as are all subsequent significant p values).

Further analyses via Duncan's Multiple Range Test showed that the ES group was significantly different from each of the other three groups, but that no other differences among the four experimental groups were significant.

A t-test showed that the two nonshocked control groups were not significantly different ($p>0.10$) in their drinking suppression.

DISCUSSION

The results of this experiment indicate that the dissociative effect of ethanol seen in the first experiment was a state-dependent learning phenomenon. When an ethanol state similar to that of conditioning was reproduced during the test situation (EE group), drinking suppression very similar to that of the saline control group was seen. The EE group's suppression was significantly greater than that of the ES group, which displayed dissociation of memory as was seen in Experiment 1.

An important criticism of the transfer design used here [13] mainly involves the difficulty sometimes encountered in

interpreting "performance effects" of drug treatments. However, the EE group rats' high degree of drinking suppression was not due to a nonspecific depression of behavior (which might have resulted from the ethanol treatment during the suppression test) because the CS was not presented until reliable drinking commenced, and the ethanol-nonshock group's drinking behavior was very similar to that of the saline-nonshock group. Therefore, the data seem to be best interpreted via state-dependent learning, i.e., reinstatement of a drug state similar to that present during conditioning permitted memory retrieval and resulted in a high degree of conditioned drinking suppression.

These results also indicate that ethanol's stimulus properties were involved in the dissociation effect (and presumably also contribute to the dissociation seen in Experiment 1). The EE group's high degree of suppression, even though these results were conditioned drugged and underwent an external stimulus change, shows that not just ethanol during conditioning, but the change from the drugged state to the nondrugged state is necessary to produce dissociation. The pattern of suppression is also consistent with the results of Experiment 1 in which any one source of stimulus change did not produce dissociation, but so long as the rats were conditioned drugged, two sources of stimulus change did result in a marked generalization decrement. External change only was not sufficient to produce generalization decrement when there was no drug treatment in both conditioning and testing periods (SS group), or when the rats were drugged during both periods (EE group).

Finally, however, there was obviously some type of ethanol effect in addition to its stimulus properties involved in the dissociation. The SE group, which underwent both types of stimulus change between the conditioning and test sessions, displayed a high degree of drinking suppression, quite similar to that of the rats which received no ethanol treatment. This asymmetrical pattern of dissociation is frequently seen in state-dependent learning experiments, and presents a definite problem for a simple version of the generalization decrement theory of drug-produced dissociation [4,12]. It is apparent that the change from ethanol-state during the conditioning session to the normal state during the test period is not equivalent to the reversal of the sequence, and such equivalence should be seen if only a stimulus-state change is responsible for the dissociation.

Several previous investigators have suggested that asymmetrical state-dependency may result from some interaction of the stimulus properties of the drug state and the drug's effect on learning or performance [2, 12, 16, 17]. There seem to have been few experiments directed toward actually demonstrating such a basis for asymmetrical dissociation. Holloway [7] did conduct this type of investigation, and found that an interaction between ethanol's disinhibitory action and its dissociative properties generally could not account for his observation of asymmetrical dissociation of active and passive avoidance in rats.

Perhaps ethanol impairs information acquisition or storage processes so that a closer match with contextual stimuli which were present during learning is necessary for effective information retrieval. In other words, ethanol's CNS depressant function during conditioning might render the animal even more susceptible to a stimulus generalization decrement effect, and the stimulus properties of the drug would thus become much more critical for assisting in the later recall of memories acquired and stored during the drug state. Such a combination of effects could result in asymmetrical dissociation, because the drug state (stimulus) change would not be nearly so debilitating to memory retrieval for animals conditioned in the normal state and tested drugged.

In summary, the results presented here support the notion that ethanol's dissociative action is based partially on its stimulus properties, but also indicate that some effect other than the drug's stimulus function is involved.

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