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Discontinuation of Diazepam and Sensitivity to a Shock Signal: Fear Conditioning Prior to Drug Treatment

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DAVIDSON, T. L. *Discontinuation of diazepam and sensitivity to a shock signal: Fear conditioning prior to drug treatment.* PHARMACOL BIOCHEM BEHAV **36**(3) 691–694, 1990.—The present experiment examined the disruptive capacity of a shock CS when Pavlovian training was completed prior to repeated daily treatment with diazepam (DZ). Two groups of rats were given 20 Pavlovian fear conditioning trials (two per day) to establish a light CS as a shock signal. Both groups were then trained for 15 daily sessions to bar press for food reinforcement. Before each session, one group was injected IP with 5 mg/kg DZ and the other with saline. All injections were discontinued after the last training session. Nine days later, the capacity of the CS to disrupt bar pressing was tested. The CS produced greater disruption of bar pressing for rats that had previously received chronic diazepam treatment. The results suggest that DZ discontinuation increases sensitivity to cues established as fear elicitors prior to initial administration of DZ.

Diazepam Discontinuation syndrome Conditioned fear Aversive conditioning Benzodiazepines Anxiolytic

BENZODIAZEPINE anxiolytics have been used extensively in the treatment of anxiety disorders [e.g., (12)]. However, "discontinuation syndromes" associated with the suspension of benzodiazepines may limit their clinical utility. In humans, withdrawal symptoms such as depression, anxiety, loss of appetite, tremor, and insomnia have been reported following suspension of treatment with benzodiazepines [for review see (12)]. In animals, increased motor activity [e.g., (9)] and increased sensitivity to benzodiazepine inverse agonists (e.g., FG-7142) have been reported following discontinuation of benzodiazepine administration [e.g., (11)].

In what may be another manifestation of a discontinuation syndrome, we have found that rats are hypersensitive to the behaviorally disruptive effects of a shock cue following discontinuation of treatment with diazepam [e.g., (2,3)] or lorazepam (2), two common benzodiazepines. We used a "transfer of control" design (7) in which rats were first trained to bar press for food, and were then given Pavlovian fear conditioning in which a conditioned stimulus (CS) signaled the delivery of a brief shock. The test phase assessed the capacity of the Pavlovian CS to suppress the previously learned instrumental response. No shocks were delivered during testing. We found that test phase suppression of bar pressing by the shock CS was greater for rats given benzodiazepines during initial bar press training than for saline controls. This effect of benzodiazepine treatment was obtained 12–14 days after benzodiazepine administration was discontinued.

One interpretation of our previous findings might be that the amount of fear conditioned to the CS during Pavlovian training was greater for rats previously treated with benzodiazepines than for saline controls. The notion is based, in part, on the finding of one experiment that, following discontinuation of administration of diazepam, rats showed stronger Pavlovian conditioning of freezing, an index of conditioned fear [e.g., (4)], to a shock CS (3). Hence, differences between benzodiazepine-pretreated rats and controls with respect to suppression of bar pressing by the CS could be attributable to differences in the strength of prior fear conditioning. If this is the case, the effects of benzodiazepine discontinuation on behavioral hypersensitivity to stimuli that elicit fear or anxiety may be specific to cues trained in the aftermath of benzodiazepine treatment.

The purpose of the present study was to evaluate this interpretation of our previous findings while further examining the range of conditions under which benzodiazepine discontinuation can intensify subsequent behavioral disruption by a shock signal. To accomplish this, the rats in the present experiment completed Pavlovian fear conditioning prior to the initiation of diazepam (DZ) administration. Since Pavlovian training took place prior to DZ treatment, discontinuation of DZ administration could not influence the strength of fear conditioning, nor could the effect of this discontinuation be specific to cues established as elicitors of anxiety or fear after treatment was suspended.

METHOD

Subjects

The subjects were 16 naive male Sprague-Dawley rats, about 100 days old at the beginning of the experiment. The rats were individually caged and maintained at 80% of their free-feeding weight. All rats had free access to water throughout the experiment except during experimental sessions.

Apparatus

All subjects were trained and tested in eight identical 21.6×

21.6 × 27.9 cm operant conditioning chambers. The chambers had stainless-steel end walls with ceilings and side walls made of clear Plexiglas. Each chamber had a food cup which was located near the bottom center of one wall. A removable bar, located to the left of the food cup, operated a microswitch whenever it was depressed. A 28 V DC jewelled light located directly above the bar served as a visual CS. The floor of each chamber was composed of 0.48 cm stainless-steel rods spaced 1.9 cm apart. The grid floor of each chamber could be electrified through a constant current shock source (Lafayette Instruments Co., Model No. 58006), and a neon bulb scrambler (Lafayette Instruments Co., Model No. 58020). Four of these chambers were housed in each of two separate rooms. In one experimental room, one set of four chambers was placed in separate shells which were constructed of wood and sound-attenuating material. The door of each shell was made of clear Plexiglas. A 6-W bulb, providing an additional source of illumination as a visual CS, was attached to the interior of each shell. A 60 cfm fan provided ventilation and masking noise within each shell. The remaining four chambers were housed in a different experimental room. White noise served to mask extraneous noises. In each room the chambers were arranged together such that a single low-light video camera could be used to monitor activity simultaneously in all boxes. Separate videotape recorders were used to record behavior in each set of four boxes. Experimental events in each room were controlled and recorded by relay and computer equipment located in adjoining rooms.

Procedure

All rats were assigned to two groups matched for free-feeding weight, and were assigned to squads of four. Conditioning chamber and conditioning room were counterbalanced with respect to group. The rats were then shaped to bar press to a criterion of 25 continuously reinforced responses. Throughout the experiment reinforcement consisted of one 45 mg Noyes pellet.

Beginning two days after all rats had attained this criterion, the bars were removed from each conditioning chamber, and all rats were given 10 days of Pavlovian fear conditioning. On each day the rats received two trials in which the offset of a 2-min steady light CS was immediately followed by a 0.9 mA shock of 0.5-sec duration. Mean intertrial interval (III) was 15 min.

The behavior of each rat was recorded once every 10 sec throughout each CS period. Freezing (i.e., the absence of all observable skeletal movement except for respiration and minimal vibrissae movements) served as the index of conditioning and was identified according to a classification scheme like that used by Fanselow and Bolles (4).

Beginning four days after the last fear conditioning session, the rats were given 30-min access to a VI 60-sec (VI60) reinforcement schedule. At 15 min prior to each VI training session rats in Group DZ (N = 8) received 5.0 mg/kg diazepam (Sigma Chemical Co., St. Louis, MO), the dose used in our previous studies [e.g., (2,3)]. This dose has been reported to have anxiolytic activity in rats [e.g., (5)]. The diazepam was prepared by moistening it with three drops of Tween 80 and this suspension was injected IP in 2 ml/kg of distilled water. Another group of controls (N = 8) was injected IP with 2 ml/kg of saline (0.9% NaCl in distilled water). Training sessions and injections continued for 15 days.

Injections were suspended at the conclusion of this bar press training phase. A period of nine days intervened between the last injection (i.e., the completion of bar press training) and the beginning of testing. During this period, the rats were weighed daily and fed enough to maintain them at 80% of their original free-feeding weight.

All rats were given one 30-min test session per day for four days. During each test session the rats were reinforced on a VI60 schedule. Superimposed on this schedule were two presentations of the 2-min visual CS (mean ITI = 15 min) which had been established as a signal for shock during fear conditioning. No shocks were administered during these test sessions. Injections were not given during the test phase of the experiment.

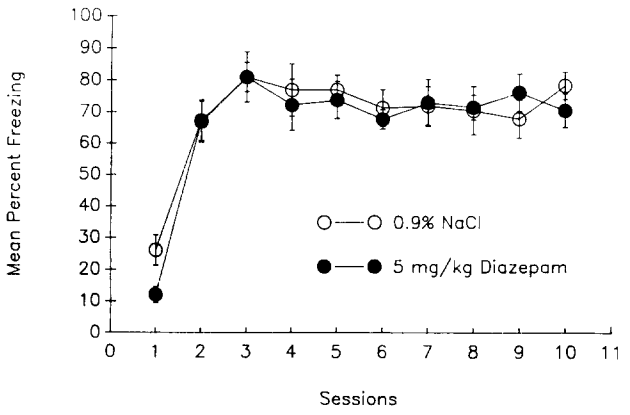

FIG. 1. Acquisition of Pavlovian conditioned fear to a signal (i.e., a light) for shock. Mean percent of freezing observed for each group during each session of Pavlovian training is depicted. The vertical bars represent standard error of the mean (SEM). Note: No injections of DZ or saline were given during this phase of the experiment. The legend indicates what the groups received in the next phase of the experiment.

RESULTS

The data collected during Pavlovian fear conditioning and during VI60 training were evaluated statistically with analyses of variance using Groups and Experimental Room as between subjects variables and Sessions as a within subjects variable. Trials was included as an additional within subjects variable for analysis of the results of the conditioned suppression test. Differences due to Groups did not differ dependent on Experimental room in any analysis. Hence, this variable will not be mentioned further.

Pavlovian Fear Conditioning

Figure 1 shows the mean percentage of freezing for each group during the light CS on each session of fear conditioning. There was little difference in the freezing behavior of the groups. Hence, there appeared to be no pretreatment differences in fear conditioning between rats scheduled to be treated with DZ and their saline controls. Analysis of variance obtained a significant main effect of Sessions, $F(9,108) = 30.12$, $p<0.01$, indicating that amount of freezing increased as a function of training. However, neither the main effect of Groups, $F(1,12)<1$, nor the Groups × Sessions interaction were reliable, $F(9,108)<1$.

Bar Press Training

Figure 2 depicts mean responses per minute for each group during each session of VI60 training. As can be seen in that figure, rats injected with DZ bar pressed at a rate comparable to that of saline controls. Although analysis of variance obtained significant main effect of Sessions, $F(14,168) = 11.98$, $p<0.01$, neither the main effect of Groups, $F(1,12)<1$, nor the Groups × Sessions interaction, $F(14,168) = 1.238$, $p>0.28$, attained significance.

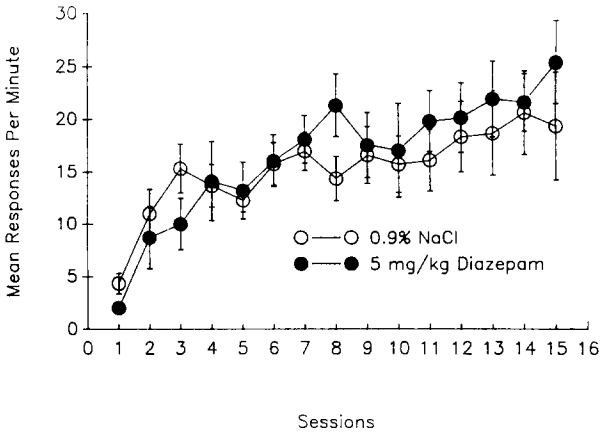

FIG. 2. Acquisition of food-reinforced bar pressing by rats injected with 5 mg/kg diazepam or 0.9% NaCl. Mean number of responses per min are depicted for each group. SEM is represented by the vertical bars.

Conditioned Suppression Test

Figure 3 shows the mean amount of conditioned suppression of bar pressing on each of the two test trials of each test session for each group. In order to attenuate the effects of individual differences in responding, the results of the conditioned suppression test are presented as a suppression ratio of the form A/(A+B) in which A is the rate of responding during the CS and B is the rate of responding during a 2-min period immediately prior to CS onset. Accordingly, a suppression ratio of 0 indicates complete suppression of responding during the CS, whereas a ratio of 0.5 indicates that amounts of responding during the CS and pre-CS periods were the same (i.e., no suppression by the CS).

Figure 3 shows mean suppression ratios for each group on each block of two test trials. Throughout testing, the shock CS suppressed bar pressing more for rats treated with DZ during original training than for saline controls. Statistical confirmation

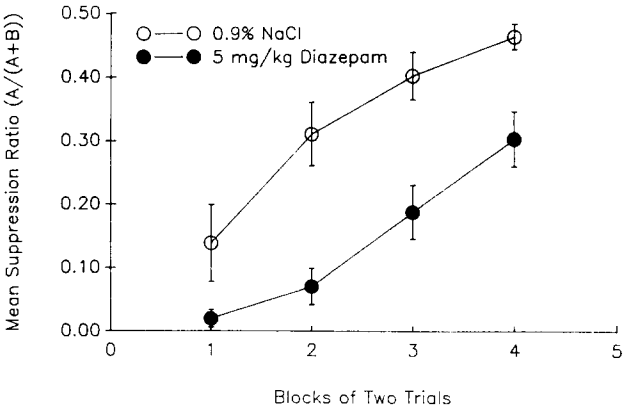

FIG. 3. Conditioned suppression of bar pressing to the tone previously established as a Pavlovian signal for shock. Mean suppression ratio during presentation of the tone is depicted for each group during each block of two test trials. Vertical bars represent SEM. Suppression ratios were calculated for each rat by dividing the number of bar presses during each 2-min light presentation (A) by the sum of this number plus the number of bar presses during the 2-min period which immediately preceded each light onset (B). See text for additional discussion of suppression ratios.

of this difference was provided by a significant main effect due to Groups, $F(1,11)=16.72$, $p<0.01$, over all of conditioned suppression testing. A reliable main effect of Test sessions was also obtained, $F(3,33)=45.40$, $p<0.01$, indicating that the capacity of the CS to suppress bar pressing decreased over the course of testing. The Groups × Test sessions interaction was not reliable, $F(3,33)=1.99$, $p>0.13$. The main effect of Trials, $F(1,11)=116.08$, $p<0.01$, and the Groups × Trials interaction, $F(1,11)=7.6$, $p<0.05$, were also reliable. Newman-Keuls tests revealed that differences in suppression ratios between trials was reliable ($p<0.05$) for controls (trial 1 = 0.256; trial 2 = 0.399) but not for rats previously treated with DZ (trial 1 = 0.103; trial 2 = 0.188).

The pattern of differences obtained for conditioned suppression to the CS were not the same as that obtained for pre-CS responding. During the two-minute period prior to CS onset, mean responses per min for rats treated in original training with DZ and with saline were 9.69 (SEM = 0.88) and 9.54 (SEM = 1.35), respectively. The difference between these response rates was not reliable, $F(1,11)<1$, for Groups.

DISCUSSION

The results of the present experiment confirmed that discontinuation of DZ administration increased the capacity of a CS for shock to disrupt food-reinforced bar pressing. This effect of drug discontinuation was obtained despite the fact that nine days intervened between the last administration of DZ and the first test trial. Furthermore, as reported previously (3), the effect of suspension of DZ administration appeared to be highly specific to the period of CS presentation. Bar press performance during "safe" pre-CS periods of testing did not differ between rats for which DZ treatment had been suspended and their saline controls.

However, unlike our previous results, the present findings were obtained under conditions where fear conditioning was completed prior to initiation of DZ treatment. Therefore, it can be concluded that suppression of instrumental performance in the presence of a shock signal did not depend on the effects of DZ discontinuation on the amount of fear conditioned to the CS. Furthermore, it appears that the disruptive capacity of a shock CS is strengthened following suspension of DZ administration whether that CS is established as a fear elicitor prior to (as in the present experiment) or after completion of drug treatment (2,3).

The present results and those of our previous studies may be accounted for in at least two ways. A hypothesis we proposed previously appealed to associative mechanisms. According to this view, the interoceptive stimulus consequences of DZ administration may have been conditioned to the bar press response during instrumental training. Disruption of test phase bar press performance could have resulted because internal cues produced by the CS were less similar to those originally conditioned DZ cues than they were to stimuli concomitant with administration of saline. In other words, the effect of DZ discontinuation observed in the present experiment may be the consequence of differential stimulus generalization.

The results can also be interpreted from a motivational perspective. It may be that termination of DZ treatment increases the vigor or strength of responses that can be elicited by a given amount of aversive conditioning. For example, BZ discontinuation may add directly to a central state of anxiety or fear that motivates responding to an aversive CS [see (1,10)]. Consistent with this view, subjective reports of increased anxiety have sometimes accompanied suspension of DZ treatment in humans [e.g., (8)]. In rats, DZ withdrawal has been reported to give rise to interoceptive stimuli like those resulting from administration of pentylenetetrazol, a suspected anxiogenic agent (6).

These two interpretations make different predictions with respect to whether or not the effects of BZ discontinuation on the behaviorally disruptive capacity of a fear cue would be specific to responses trained during administration of DZ. The associative hypothesis anticipates response specificity, the motivational account does not.

Our findings also have clear implications with respect to clinical concerns about the use and abuse of diazepam and other benzodiazepine anxiolytics. Our results caution that one consequence of DZ use may be increased sensitivity to the negative effects of eliciting strong anxiety or fear, once DZ use is discontinued. This increased sensitivity is contrary to the goals of treatment and may provide some of the basis for drug dependence.

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