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Chlordiazepoxide and Successive Discrimination: Different Effects on Acquisition and Performance

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McNAUGHTON, N. *Chlordiazepoxide and successive discrimination: Different effects on acquisition and performance.* PHARMACOL BIOCHEM BEHAV 23(3) 487–494, 1984.—Benzodiazepines have been reported to increase low rates of responding during a stimulus correlated with non-reinforcement while leaving prestimulus rates unaffected (successive discrimination). However, the results have been obtained by superimposition of the drug upon a discrimination which was learned in the absence of drug. The observed effects may therefore have been due to the *sudden change* in drug state (state-dependency) rather than to a specific action of the drug. The present experiments found that chronic administration of chlordiazepoxide (5 mg/kg, IP) impaired acquisition but not performance of successive discrimination. Intermittent administration of chlordiazepoxide impaired discrimination by increasing low rates of responding during the stimulus signalling non-reinforcement. This effect was obtained with saline-drug but not drug-saline state changes (asymmetric state-dependency). A final experiment showed that chronic administration of the drug did reduce well-learned inhibition resulting from signalled shock. It was concluded that chlordiazepoxide has not only pure anxiolytic but also state-dependent effects and that if successive discrimination depends on conditioned frustration it does so only while the discrimination is being learned.

Benzodiazepines Chlordiazepoxide Successive discrimination Frustration State dependency Nonreward Rat

GRAY [6] has proposed that anxiolytic drugs reduce behavioural inhibition when this results from stimuli which predict reward omission (frustration), stimuli which predict punishment and novel stimuli. This paper attempts to clarify a critical part of his evidence in relation to frustration. Gray states that anxiolytics "impair successive discrimination learning by causing overresponding in the presence of S−, but (do) not alter simultaneous discrimination learning" (op. cit. p. 35). For the specific case of the benzodiazepines Gray [7] cites seven studies which support this statement against only one discrepant study.

However, Dantzer's [3] review of the benzodiazepines presents a less clear picture. He cites two further discrepant reports and one report [4] in which there was a drug effect early in acquisition but not after the discrimination was well learned. Dantzer suggests that "differences in time of testing could therefore account for some of the contradictory results."

Dantzer's suggestion is not in conflict with Gray's hypothesis. During acquisition of successive discrimination an animal receives nonreward when it previously received reward and this could well generate frustration [1,2]. However, after considerable training the animal should be acquainted with the contingency and there is no reason to suppose that either conditioned or unconditioned frustration would necessarily be operative. Anxiolytics have been found to interact with nonreward in other behavioural tasks only if frustration has been involved [5,17].

At first glance, the idea that frustration is involved only during acquisition is untenable. Many of the studies cited by Gray test performance rather than acquisition [8,14]. However, they also deliver the drugs intermittently against a within-animal baseline control. This could well result in effects which are due not to anxiolytic action, but rather to the change in drug state—an example of what has been termed state-dependent learning [13].

The term state-dependency will be used in the rest of this paper to encompass any effect produced by a change in drug state where the same effect is not produced by constant administration of the drug. A number of points need to be noted: (1) A change in drug state can occur as either a drug-placebo shift *or* placebo-drug shift; (2) the effects of a drug-placebo and placebo-drug shifts may be similar (symmetric state-dependency) or different (asymmetric state-dependency); (3) state-dependency is often referred to in discussion of response rates—but other aspects of performance (e.g., discrimination) may also show state-dependency.

A recent study by Vachon *et al.* [15] suggests that when state-dependency is excluded, benzodiazepines affect acquisition but not performance of successive discrimination. In one experiment chlordiazepoxide was given throughout acquisition of a go/no-go black/white discrimination. In the second experiment chlordiazepoxide was again administered daily, but drug treatment was not started until acquisition had reached a criterion of 85% correct. The drug was effective in the first but not the second experiment. It could be

argued, however, that this study is not conclusive with respect to frustration. Both correct go and correct no-go trials were rewarded; whereas in the form of test discussed by Gray [6] there is an explicit S− during which no reward is available.

The present experiments investigate the following points: (1) whether chlordiazepoxide affects performance of successive discrimination based on nonreward if the drug is given continuously; (2) whether it affects acquisition when given continuously; (3) whether it affects performance when given intermittently; (4) whether it affects performance of a successive discrimination based on shock, when the drug is given continuously. This last experiment was included to discriminate between effects on simple learning and affects on conditioned 'emotional' processes.

EXPERIMENT 1

Introduction

The purpose of this experiment was to test the generality of the experiment of Vachon *et al.* [15] in which chlordiazepoxide, given continuously, did not affect performance of successive discrimination. The critical departure from their procedure was a total omission of reward during the S−. Other changes were: the use of rats instead of cats; the use of lever pressing rather than displacement of a food-well cover; and the use of stimulus lights separated from the reward magazine instead of coloured food-well covers as the discriminative stimulus.

Inspection of the figure presented by Vachon *et al.* [15] suggests that there may have been a marginal effect of the drug on the first few days of treatment—possibly due to state-dependency specific to S−. The present experiment, therefore, delivered the drug at an intermediate dose on day 1 of treatment before proceeding to the full dose.

Method

Subjects were 24 naive male Sprague Dawley rats weighing 200–300 g at the beginning of the experiment and housed in groups of four on a normal 12 hours on 12 hours off light cycle. They were gradually placed on a 23 hour food deprivation schedule and then maintained on this throughout the experiment.

The apparatus consisted of four Campden Instruments operant boxes fitted with one fixed and one retractable lever. The retractable lever was used throughout the experiment. Three 2.8 W stimulus lights were mounted above the levers and food magazine. All three were used together as S− for the discrimination. Recording of lever presses, control of the schedules and delivery of reward (one 45 mg Noyes pellet) were carried out with a computer.

Procedure

After two weeks of 23 hours food deprivation pretraining commenced. This consisted of a number of phases.

For the first two days each rat received one 15 minute session daily during which the lever was retracted and free food was delivered on an RT 30 second schedule (i.e., a schedule in which intervals are set up randomly with all values between 0–60 seconds having an equal probability of occurring. Food is delivered noncontingently at the end of each interval). Next they received 15 minute sessions during which the lever protruded into the box, no free food was available and reward was available on a continuous reinforcement schedule for lever pressing. Rats were rerun on this schedule until they had made a total of 12 lever presses within a session.

From this point on, all sessions lasted 48 minutes. All rats received eight sessions (one session per day) during which food was available on an RI 30 second schedule (as for RT 30 except food delivery is contingent) for lever pressing. After the eighth such session the final schedule was instituted.

For the final schedule, the RI 30 second schedule continued, essentially as before. However, superimposed on it were the S− (lighting of all three stimulus lights) during which no food was available and a time out during which the lever was retracted from the box. The sequence of events consisted of the following cycle. During one minute the RI 30 was operative; for the next minute the RI 30 was still operative and the number of responses (referred to below as preCS responses) made by the rat was recorded; for the next minute S− was presented, the RI 30 was not in operation and number of responses (referred to below as CS responses) was recorded; for the next two minutes the RI 30 was operative; and the final three minutes consisted of the time out. The entire cycle was repeated six times in each session.

After the first nine sessions of this schedule there was a two week break during which the rats were food deprived but not tested.

Drug Treatment

After 18 training sessions on the final schedule the rats were divided into two equal groups. On day 19 the drug group received 2.5 mg/kg chlordiazepoxide hydrochloride IP, controls received saline. From day 20 onwards the drug group received 5.0 mg/kg of the drug, controls received saline. All injections were given as 1 ml/kg 10 minutes before the start of the session. The dose and time interval were chosen since they were effective in previous behavioural and neurophysiological experiments [10, 11, 16].

Data Analysis

The number of CS responses and preCS responses (see above) were collected separately for each rat and trial. They were subjected to a square root transformation to produce normality of distribution and the mean over trials for each day was submitted to analysis of variance. Trends across days were assessed for orthogonal polynomial components.

Results and Discussion

The results of this experiment are shown in Fig. 1.

Throughout the period analysed there was highly significant suppression of responding during the S−, $F(1,242)=1190.0$, $p<0.0001$. There was a very slight trend for the discrimination to improve over days (days × discrimination linear $F(1,242)=5.8$, $p<0.05$). Drugged animals showed a tendency to increased response rates over days (days × drug linear $F(1,220)=17.4$, $p<0.01$) but this was equally evident in the CS and preCS scores and there was no sign of any reduction in discrimination by the drug (days × drug × discrimination all $F<1.0$).

This experiment supports the conclusion drawn from the Vachon *et al.* [15] study that chronic injection of chlordiazepoxide does not affect performance of a successive discrimination.

EXPERIMENT 2

Introduction

The dose of chlordiazepoxide used in the previous experiment is one which would be expected to be effective in rats

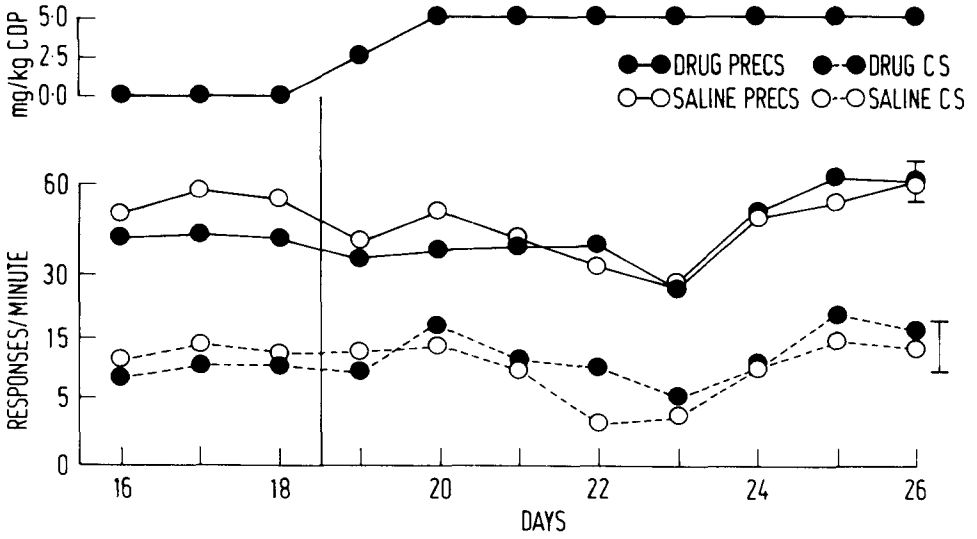

FIG. 1. Effect of gradual introduction of chlordiazepoxide, IP, on performance of a well learned successive discrimination. One group of animals (SALINE) received saline injections, a second group (DRUG) initially received no drug and then received one day at 2.5 mg/kg followed by a number of days at 5.0 mg/kg as indicated in the top of the figure. The vertical line of the figure indicates the transition from no drug to drug for this group. The data presented are response rates in the minute preceding the discriminative stimulus (preCS) and in the minute during the stimulus (CS) when no reward was available. The nonlinear response scale is the result of square root transformation. The smaller vertical bar represents 2 standard errors for within group comparisons, the larger vertical bar represents 2 standard errors for between group comparisons. There were no significant effects of the drug treatment.

[16]. Nonetheless, such a null result would be more convincing in comparison to a positive result obtained with similar rats, drug dose, and apparatus. Experiment 2 was carried out to provide such a result; and, for the reasons given in the introduction, to test whether the positive result of Vachon *et al.* [15] using the go/no-go paradigm could also be obtained with the successive discrimination schedule of Experiment 1.

Methods and Procedure

These were essentially as in Experiment 1.

Twenty naive male Sprague Dawley rats weighing between 200–300 g were maintained on 23 hours food deprivation for 10 days before the start of pretraining.

Lever press training was as in Experiment 1. After acquisition of lever pressing the rats were placed onto RI 30 second level pressing for food—unlike Experiment 1 sessions lasted 30 minutes and the S− (3 stimulus lights) was presented from the start to ensure that no suppression occurred to the S− presented alone. The schedule used was the same as for the final schedule of Experiment 1 except that the timeout was omitted and that initially the RI 30 second schedule continued during presentation of the S− (i.e., successive minutes were: RI 30 and preCS response collection; RI 30 and S− with CS response collection; RI 30; RI 30. The whole cycle was repeated six times per session). After eight sessions of acquisition of responding under this schedule, discrimination training began and the S− now signalled reward omission while RI 30 continued at all times when S− was not on.

Drug administration was as in Experiment 1 with the initial dose being given on session 7.

Data analysis was as in Experiment 1. Seventeen values were lost during data collection and were analysed as missing data.

Results

The results of this experiment are shown in Fig. 2.

There was a steady development of discrimination over the course of the experiment (days × discrimination linear $F(1,495)=273.8$, $p<0.0001$) with some nonlinearity (quadratic $F(1,495)=13.3$, $p<0.0025$). Drugged rats responded less than control rats at the beginning and end of training but responded as much as controls between sessions 10–20 (days × drug quadratic $F(1,478)=14.7$, $p<0.005$). The development of discrimination in the control animals was paralleled by a similar, but significantly smaller, amount of discrimination in the drugged animals. As with the overall discrimination data the reduction in discrimination produced by the drug showed both linear, $F(1,495)=24.2$, $p<0.0005$, and nonlinear, cubic $F(1,495)=7.3$, $p<0.01$, components.

Discussion

As in the experiment by Vachon *et al.* [15], the injection of chlordiazepoxide during acquisition impaired successive discrimination. Two points should be noted.

Firstly, the drugged animals in the present experiment show some development of discrimination. By contrast, those of Vachon *et al.* showed virtually no learning of the no-go component of the task. Given the differences between the experiments including species and task it is difficult to assess this difference. However, it is unlikely that number of sessions tested is the critical factor: in both experiments discrimination started to appear at about the 5th session in the controls and was essentially complete by the 15th session.

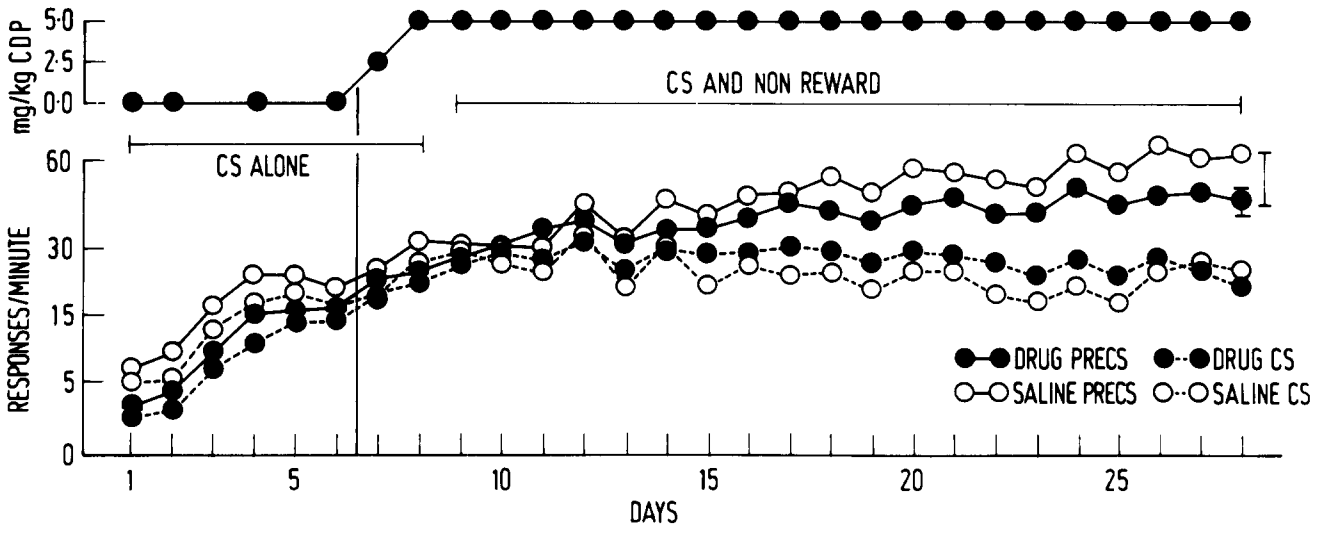

FIG. 2. Effect of chlordiazepoxide IP on acquisition of successive discrimination. All annotation and drug injection details as for Fig. 1. Day 1 represents the first day of RI 30 seconds training. From Day 1 to Day 8 the CS was presented but did not signal omission of reward (horizontal bar CS ALONE). From Day 9 onwards the CS was accompanied by omission of reward (horizontal bar CS AND NONREWARD). The progressive separation of the DRUG and SALINE scores, representing poorer discrimination in the drugged animals, was highly significant.

Secondly, the reduction in discrimination produced in the present experiment appeared by the end of acquisition to be as much through a reduction in S+ responding as an increase in S− responding. However, the drugged animals showed initially lower rates of response before the start of discrimination training. Further, higher doses of the drug can produce extensive muscle relaxation. It is just possible therefore that the present findings are due to a combination of a general response reduction produced by the drug in both S+ and S− periods coupled with interference with the discrimination producing an increase in responding only in S−.

Statistical evidence for this explanation is provided by analysis of the linear trend data across the experiment. The drug-preCS and saline-preCS data have very similar slope coefficients, differing by less than 1 standard error. This can be seen in Fig. 2 from the fact that the difference between groups on the last 5 days of testing is essentially the same as on the first 5 days. By contrast, the drug-CS values for the first 5 days are well below the saline-CS values, whereas the reverse is the case for the last 5 days. The cross-over for these two groups is reflected in a significant difference in linear trend across days, $t(495)=6.40$, $p<0.0005$.

However, an explanation of these effects in terms of muscle relaxation seems unlikely since acute injection of drugs such as chlordiazepoxide generally increase rather than decrease rates on schedules such as VI 30 (see also Experiment 4).

EXPERIMENT 3

Introduction

The results of the first two experiments show that continuous administration of chlordiazepoxide interferes with successive discrimination only when given during acquisition and not when it is given after acquisition is complete. This suggests that the effects previously found with intermittent administration of chlordiazepoxide after training are due to some form of state dependency.

In studies of the partial reinforcement extinction effect [5] it has been observed that effects of nonreward may be changed by anxiolytics in a state dependent manner even when the effects of reward in the same experiment are not. Further, this state dependency may be symmetric [9] or asymmetric [5] depending on the paradigm. (Asymmetric state dependency is said to occur when the transition from one drug state to a second affects behaviour in a way which the reverse transition does not.)

Experiment 3 investigates the effects of a brief transition from the drugged to the nondrugged state on performance of successive discrimination.

Method and Procedure

The animals, drug administration and behavioural schedule were the same as in the previous experiment. Having completed Experiment 2 the animals received a four-day rest period. During this time one of the control rats became ill and was excluded from Experiment 3.

The experiment was run for three days. On day 1 and day 3 all conditions were as for Experiment 2. On day 2 conditions were as for Experiment 2 except that rats in the chlordiazepoxide group received saline. Thus the experimental comparison was between a group receiving drug-saline-drug and a group receiving saline-saline-saline.

Analysis was performed as in Experiment 1.

Results

The results of the experiment are shown in Table 1. There is clear evidence of discrimination over all days and conditions, $F(1,51)=173.1$, $p<0.0001$, and a reduction in the discrimination in the drugged group (drug × discrimination $F(1,51)=11.3$, $p<0.0025$). Surprisingly there was no change in discrimination by the drug group on the day they received saline (days × discrimination × drug $F=0.2$). However, the shift to saline markedly reduced response rates in both the CS and preCS periods (days × drug $F(2,34)=8.4$, $p<0.0025$).

TABLE 1

MEANS OF TRANSFORMED RESPONSE RATES (SQUARE ROOT OF X+0.5) DURING THE MINUTE PRECEDING (preCS) AND THE MINUTE DURING (CS) A STIMULUS SIGNALLING OMISSION OF REWARD ON AN RI 30 SEC BASELINE

	preCS		CS	
	CDP	SAL	CDP	SAL
1	6.96 (48)	7.85 (61)	4.90 (24)	4.22 (17)
2	5.22 (27)	7.36 (54)	3.00 (8)	4.07 (16)
3	7.03 (49)	8.21 (67)	5.01 (25)	4.53 (20)

The rats had received either chlordiazepoxide, 5 mg/kg IP (CDP) or saline injections (SAL) throughout acquisition. Days 1 and 3 of the experiment were identical to acquisition. On Day 2 both CDP and SAL rats received saline. Response rates (reverse transform not mean of raw data) are given in brackets for comparison with figures and Table 2. Analysis of variance showed a highly significant days × drug effect with no sign of a days × drug × discrimination interaction.

TABLE 2

MEANS OF TRANSFORMED RESPONSE RATES (LOG OF X+1) DURING THE MINUTE PRECEDING (preCS) AND THE MINUTE DURING (CS) A STIMULUS SIGNALLING OMISSION OF REWARD ON AN RI 30 SECOND BASELINE

	preCS		CS	
	CDP	SAL	CDP	SAL
Pooled	1.69 (48)	1.58 (37)	1.21 (16)	0.93 (8)
Train-CDP	1.56 (35)	1.48 (29)	1.18 (14)	0.88 (7)
Train-SAL	1.82 (66)	1.70 (49)	1.25 (17)	0.99 (9)

Both rats previously trained with chlordiazepoxide and those previously trained with saline were used. No rat had received chlordiazepoxide during the previous nine days. Each rat received chlordiazepoxide, 5 mg/kg IP (CDP) and saline (SAL) injections in either the sequence CDP-SAL-SAL-CDP or SAL-CDP-CDP-SAL on four successive days. The data presented are means over rats and pairs of days for each drug condition (pooled) and the same data subdivided by original training drug condition. Response rates (reverse transform not mean of raw data) are given in brackets. Analysis of variance showed a significant main effect of drug and a significant drug × discrimination interaction. Pretreatment × drug × discrimination was totally without effect (F=0.28).

This experiment, therefore, demonstrates state dependency of responding but not state dependency of discrimination within the group which had received chlordiazepoxide throughout acquisition.

EXPERIMENT 4

Introduction

The above failure to reduce successive discrimination when switching animals from drug to no drug, taken together with the positive effects reported in the literature when giving previously undrugged animals chlordiazepoxide, suggest that this drug produces an asymmetric state dependence of successive discrimination, i.e., transfer from chronic drug to saline does not impair the discrimination but transfer from chronic saline to drug does.

Experiment 4 was run to check, within the animals and paradigm used in Experiment 3, that the impairment of discrimination usually reported in the literature could be obtained.

Method and Procedure

The animals, drug administration and behavioural schedule were the same as in the previous experiment. At the end of Experiment 3 the rats received a two-day rest period. This was followed by five days of successive discrimination training during which no chlordiazepoxide was given. All rats received two separate injections of 1 mg/kg melatonin as part of a separate study. There was then a second two-day rest period.

For Experiment 4 the animals previously trained under chlordiazepoxide and those trained under saline were assigned equally to two treatment groups. One of these groups received drug (D) and saline (S) over the four days of the experiment in the order DSSD and the other received them in the order SDDS.

The experiment therefore involved the following factors: (1) pretreatment: i.e., the acquisition drug condition of Experiment 2; (2) drug cycle: DSSD or SDDS; (3) discrimination: preCS vs. CS scores; (4) drug treatment: chlordiazepoxide or saline; (5) day pair type: each block of two days contained either a DS or an SD drug sequence. These factors were extracted in the analysis of variance. For this data a logarithmic rather than square root transform produced the better data distribution. Eight data were entered as missing values.

Results

There were no significant effects of the type of drug cycle. Despite the training under saline received in the previous week animals *originally trained* under chlordiazepoxide still showed poor discrimination (discrimination × pretreatment $F(1,56)=4.31$, $p<0.05$) and lower response rates (pretreatment $F(1,15)=5.40$, $p<0.05$). It should be emphasised that these statistics are based on the average for these rats of both chlordiazepoxide and saline treatment periods within the present experiment. Interestingly this lasting effect of the acquisition drug conditions did not interact with the effects of chlordiazepoxide administered acutely within the present experiment (pretreatment × drug $F=0.004$; pretreatment × drug × discrimination, $F=0.28$). Neither drug cycle nor day pair produced any significant effects.

On the basis of the above statistics, showing negligible contributions from drug cycle and day pair and negligible interaction with pretreatment, the critical results of the present experiment are the means for the drug × discrimination interaction.These are presented in Table 2 together with the means for the pretreatment × drug × discrimination interaction.

Acute administration of chlordiazepoxide produced an overall increase in response rates (drug $F(1,26)=16.74$, $p<0.0005$) which was significantly greater in the CS than the preCS period thereby reducing the discrimination (drug × discrimination $F(1,56)=6.00$, $p<0.025$). The apparent in-

crease in response rate within the preCS period alone does not achieve acceptable significance, $t(28)=1.73$, $p<0.10$.

Discussion

While the results of the present experiment could have been affected by the intervening injections of melatonin, they show a striking resemblance to the results previously reported for the effects of acute administration of chlordiazepoxide on the performance of successive discrimination.

It should particularly be noted that there is no tendency for preCS rates to be reduced and that hence, as emphasised by Gray [6] the reduction in discrimination observed is due to increase in CS rate.

Comparison of these results with those of Experiment 3 suggests that the transition from chronic drug treatment to saline affects overall response rates but not successive discrimination, while the transition from chronic saline treatment to drug affects successive discrimination but does not reduce response rates.

The acute injection of chlordiazepoxide increased preCS responding slightly. By contrast, chronic chlordiazepoxide given during acquisition decreased it (Experiment 2). It is unlikely, therefore, that this latter effect is due to any simple effect of the drug on performance. The lack of effect of essentially chronic injections of chlordiazepoxide on preCS rates in Experiment 1 reinforces this conclusion.

EXPERIMENT 5

Introduction

The results obtained in Experiments 1–4 are all consistent with the idea that chlordiazepoxide has an anxiolytic effect which is detectable with chronic treatment during acquisition of successive discrimination and a state-dependent effect which can be detected after acquisition is complete. The lack of an anxiolytic effect after training could be explained (see general introduction) by the idea that conditioned frustration is only important during acquisition.

An alternative possibility is that the chronic effect of chlordiazepoxide is not in any sense anxiolytic but is due to interference with some aspect of the learning process. One could then suppose that successive discrimination depended on frustration both during acquisition and afterwards. The most obvious difficulty with this suggestion is finding a candidate for the aspect of learning affected by the drug. Chlordiazepoxide does not affect simultaneous discrimination [7] nor the more sequential, inhibitory learning in a 16 arm maze [12]. It would be more satisfactory, however, to show that conditioned frustration were not involved in the performance as opposed to acquisition of successive discrimination. The fact that conditioned frustration is an intervening variable makes the design of such an experiment very difficult.

Experiment 5 represents an indirect approach to these questions. In this experiment a conditioned suppression paradigm is used to generate suppression during a CS. Loss of responding in this paradigm is not accompanied by omission of the shock. Clearly, if chlordiazepoxide is acting on some acquisition process it should have no effect on the performance of this task. Equally, if chlordiazepoxide is interfering with behavioural inhibition consequent on conditioned aversive stimuli then an effect should be seen.

Methods and Procedure

Subjects were 26 naive male Sprague Dawley rats weighing between 200–300 g at the start of the experiment. Apparatus and drug administration were as in Experiment 1 with the addition of Camden Instruments shock generators and scramblers connected to the grid floor of each skinner box.

After 10 days of 23-hour food deprivation pretraining commenced. The animals were shaped to lever press by presentation of an RT 62 second schedule of free reward with a simultaneously available continuous reinforcement schedule for bar pressing on the retractable lever. Throughout the experiment reward delivery was preceded by retraction of the lever and accompanied by illumination of the magazine for five seconds. After each rat had completed 10 rewarded presses the RT schedule terminated and the continuous reinforcement schedule was replaced by an incremental RI schedule which increased in value by 1.28 second for each reward received until RI 42 second was reached. Subsequent sessions lasted one hour and used RI 62 seconds.

After three days of RI 62 seconds, discrimination training commenced. The schedule was basically the same as that used in Experiment 1. During four minutes the RI 62 second schedule was operative; for the next 30 seconds it was still operative and responses were recorded. For the next 30 seconds the stimulus lights were on, and responses were recorded. Unlike Experiment 1 the RI schedule was still operative and also the CS period terminated in the delivery of a 0.2 second shock. As in Experiment 1 there then followed two minutes of RI, and three minutes of time out. The entire cycle repeated six times in each session.

For the first day of discrimination training all animals received a shock level of 0.15 mA. For the next 53 days shock level was adjusted individually for all animals to produce suppression ratios (CS responses/CS responses + preCS responses) in the region of 0.2–0.3. From day 55 onwards shock levels were kept constant.

On days 58, 59, 60 and 61 ten of the rats received injections of 2, 3.5, 5 and 5 mg/kg chlordiazepoxide respectively. The remaining 16 rats received saline injections.

Results and Discussion

The results for the three days of constant shock and four days of drug treatment are graphed in Fig. 3.

This shows an apparently clear release of responding by the drug during the CS period. However, there is also a slight trend to such an increase during the preCS period. As a result the loss of discrimination over days as assessed by linear regression is only marginally significant (days × discrimination × drug linear $F(1,168)=3.5$, $0.05<p<0.10$) and the main evidence for this loss rests on the drug × discrimination interaction, $F(1,168)=4.0$, $p<0.05$, which, by inspection of the figure, must be due to the action of the drug on days 58–61 rather than to a sampling bias present on days 55–57. Similarly, the significant increase in response rates (taking CS and preCS together) over days (drug × days linear $F(1,44)=6.1$, $p<0.025$) would appear from the figure to be due to a much greater contribution from CS rates than preCS.

It seems safe to conclude that despite a long period of training and despite gradual increments in drug dose chlordiazepoxide is interfering with conditioned suppression in the present paradigm by increasing CS rates in relation to preCS. Previous experiments have reported a greater interference with conditioned suppression. However, they have used intermittent administration of the drug and hence their results could be due largely to state-dependency. The state-

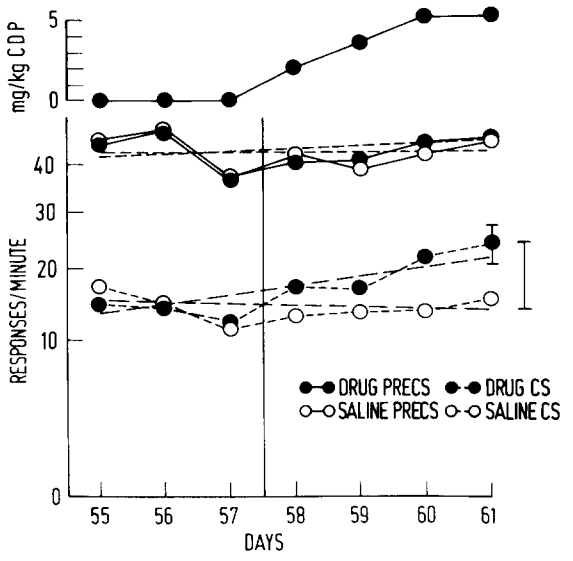

FIG. 3. Effect of chlordiazepoxide, IP, on well learned inhibition of responding on an RI 62 second baseline on which was superimposed a CS predicting shock. All annotation as in Fig. 1. DRUG animals received 2, 3.5, 5 and 5 mg/kg on Days 58–61 respectively. The broken straight lines superimposed on the graph are the linear regression lines from analysis of variance. This analysis detected a number of significant linear differences. It is argued in the text that these result mainly from the release of inhibition to the CS seen in response to the drug.

dependency observed in the present experiments with successive discrimination and reward omission make it highly likely that similar state-dependency will be observed in successive discriminations employing shock as a reinforcer.

GENERAL DISCUSSION

The above experiments demonstrate that chronic administration of chlordiazepoxide impairs acquisition of successive discrimination but has no effect if introduced after acquisition is complete. This result parallels that obtained by Vachon *et al.* [15] in a somewhat different go/no-go paradigm and may also be related to the similar results obtained by Dantzer [4] with more intermittent injections. The experiments also replicated the common result in the literature that acute injection of chlordiazepoxide impairs performance of successive discrimination. This was done using the same type of animal and apparatus as in the chronic experiments.

The difference between the effects of acute and chronic administration of chlordiazepoxide on performance can be attributed to state dependent effects of the former. However, the results of Experiment 3 and Experiment 4 show that this state dependency is asymmetric. The sudden introduction of saline on a drug baseline causes equivalent loss of responding to both CS and preCS leaving discrimination intact. By contrast, the introduction of drug on a saline baseline causes an increase in responding to CS with little change in responding to preCS. Thus a drug-saline shift produces state-dependency of response rates generally but not state-dependency of discrimination; while a saline-drug shift produces state-dependency of discrimination but not of response rates. Asymmetric state-dependency of reactions to the omission of reward (i.e., different effects of a saline-drug shift as opposed to drug-saline shift), though not of this particular type, has been observed previously with minor tranquillisers [5].

These results then reinforce Dantzer's [3] suggestion that time, and indeed exact method, of testing is important in determining the effect of benzodiazepines. They also suggest that more than one process needs to be invoked to account for the full range of effects of the drugs. It should also be noted that the present results could be due to an effect of the drug on the discrimination itself or from a release of inhibited responding independent of the capacity of the animal to form the discrimination. Theoretically [6] the latter appears more likely, but experiments with, e.g., successive conditional discrimination, would be required to settle this point.

If we exclude the results which can be explained in terms of state dependency there remains the question of what other process is acted on by chlordiazepoxide to impair acquisition of successive discrimination. Experiment 5 shows that chlordiazepoxide can affect inhibition of responding in a non-state-dependent manner, even after extensive training, in a situation where we might assume that training had not eliminated all aversive consequences for the animal. Gray [6] has proposed that chlordiazepoxide interferes with behavioural inhibition when this results from conditioned fear or conditioned frustration. He could, therefore, account for the present data by assuming that conditioned frustration is operative only during the early stages of acquisition of successive discrimination.

Other explanations are possible. However, if this suggestion is correct it implies that Gray's [6] theory may be right, but that the data on which he originally based his conclusions about successive discrimination are not strictly relevant.

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