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# Prior Training and Intermittent Retraining Attenuate Pimozide-Induced Avoidance Deficits

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BENINGER, R. J., A. G. PHILLIPS AND H. C. FIBIGER. *Prior training and intermittent retraining attenuate pimozide-induced avoidance deficits.* PHARMACOL BIOCHEM BEHAV **18**(4) 619–624, 1983.—Although the effects of neuroleptics on avoidance behavior have been studied extensively, no studies have systematically investigated the possible relationship between these effects and prior training. This paper reports the effects of prior training and intermittent retraining on pimozide-induced avoidance deficits. Experiment 1 investigated the effects of pimozide (0.5 or 1.0 mg/kg IP) on one-way avoidance responding in groups of rats (n=15 or 16) that had received 0, 2, 3, or 10 prior sessions of training (10 trials per session). All trained groups were more resistant to the disruptive effects of the drug than the groups receiving no prior training but the 2, 3, and 10 session pretrained groups did not differ significantly from one another. However, the avoidance responding of the pretrained groups eventually was impaired across the 15 sessions of testing under the drug condition; this effect was shown not to be attributable to an accumulation of the drug with repeated dosing. Experiment 2 showed that periodic retraining in the absence of pimozide reversed the cumulative session-to-session disruptive effects of the low and high dose of pimozide on avoidance responding, a finding not previously reported. The results suggest that dopaminergic systems play a role in the acquisition and maintenance of operant response learning. Avoidance learning fails to occur if training is conducted in untrained, pimozide-treated animals. Pretrained animals, when injected with pimozide, can maintain avoidance responding for several sessions but lose this ability in the continued absence of normal dopamine function; however, intermittent retraining can prevent this eventual loss of avoidance responding.

Pimozide Dopamine Avoidance Neuroleptics

NEUROLEPTIC drugs block dopamine receptors [9,12] and are well known to disrupt shock avoidance behavior [10]. However, prior experience with shock avoidance may play a critical role in determining the magnitude of this effect. Thus, avoidance responding of nonpretrained animals always is impaired by neuroleptics [2,3]. On the other hand, extended pretraining can reduce the disruptive effects of dopamine receptor blockade on avoidance behavior; when pretrained animals were injected with a neuroleptic and tested for 3 10-trial sessions they performed as well as controls [4]. However, when pretrained animals were followed for more trials after experimentally-induced reductions of dopamine function there was an eventual deterioration of avoidance responding [1, 8, 10]. This implies that, in addition to playing an important role in the acquisition of avoidance responses, the brain dopamine systems also may be involved in their long-term maintenance. However, the effects of avoidance pretraining on neuroleptic-induced deficits have not been studied systematically; not has the possibility that the gradual loss of avoidance responding in pretrained rats given neuroleptics in attributable to an accumulation of the drug with repeated dosing.

As pretraining clearly influences the observed effects of neuroleptics on avoidance responding, it is of interest to establish the limits of this effect. Previous studies have not tested the possibility that there is a systematic relationship between the amount of avoidance pretraining and the level of behavioral disruption produced by a neuroleptic. To test this possibility, animals in Experiment 1 received 0, 2, 3, or 10 sessions of avoidance training prior to extensive testing with pimozide. To test the possibility that the gradual loss of avoidance responding in these animals is due to an accumulation of drug with repeated dosing, half of each pretrained group received several home cage injections of pimozide prior to the first testing session with the drug. Experiment 2 extended the analysis of the effects of training in an undrugged state on subsequent performance in the presence of pimozide by interpolating two drug free retraining sessions between each avoidance session with pimozide.

## METHOD

### *Subjects*

One hundred and seventy-six male albino rats of the Wistar strain weighting 250 to 500 g were housed individually in a climatically controlled colony room kept on a 12 hr light-dark cycle. Food and water were available continuously.

*Apparatus*

A metallic gray shuttlebox (25×78×33 cm) was divided in half by a partition that could be opened by raising a 13 cm wide guillotine door. The grid floor on the shock side could be electrified by a scrambled 2.4 mA DC current (BRS/LVE). A 2900 Hz tone generator (Sonalert) was mounted below the grid floor on the shock side. Electromechanical relays and timers were used for environmental control and data collection.

*Procedure*

*Experiment 1: effects of prior training.* This experiment included 128 rats. As described below, there were 32 rats at each level of the pretraining variable; 2 doses of pimozide were used in the test sessions, 16 rats from each group of 32 receiving one dose; each dose group was further divided into subgroups, 8 receiving prior drug experience and 8 being drug naive in these sessions. Thus, four groups (n=32 in each group) of rats received 0, 2, 3 or 10 sessions of training with sessions occurring at about the same time each day seven days a week. Each session consisted of 10 trials with an intertrial interval of 30 sec. At the start of each session, the rat was placed into the safe side of the shuttlebox. After 30 sec the rat was placed into the shock side facing the end opposite the guillotine door. The trial then began with the onset of the tone which occurred simultaneously with the opening of the door. If the rat moved into the safe side during the 10-sec period, the tone was turned off, the door was lowered and an avoidance response was recorded. If the rat failed to avoid during the 10-sec period the offset of the tone was contiguous with the electrifying of the grid floor on the shock side. The subsequent movement into the safe side was followed by lowering of the door and an escape response was recorded. Any rat that failed to escape during the first 10 sec of shock was gently pushed to safety. Entry into the safe side always began the next intertrial interval of 30-sec after which the animal was replaced in the shock side by hand. Dependent variables were latency and number of avoidance and escape responses.

Pimozide injections (IP) were made 2 hr prior to the first session that followed the last training session. The drug was dissolved in hot tartaric acid (40 $\mu$mol/ml) and then cooled to room temperature prior to injection. Sixteen of the 32 rats in each group received 0.5 mg/kg and the remaining 16 received 1.0 mg/kg. Previous studies have reported a progressive day-to-day decline in avoidance responding of pretrained neuroleptic-treated rats. One possible interpretation of this result is that too little time was interpolated between injections to allow for total clearing of the previously injected drug from the system; the result would be that with each subsequent injection these residual amounts of drug would add to the new drug making the effective dose larger. This accumulation of drug could account for the progressive decline in avoidance responding. To test this possibility, 8 of the 16 rats in each dosage group received a home cage injection of pimozide on the 3 days prior to the first drug session. If the accumulation hypothesis is correct, these pretreated animals should show a more rapid loss of avoidance responding when given pimozide. When avoidance training occurred on a pretreatment day, pimozide injections were given at least 2 hr after the training session.

Drug sessions continued for a maximum of 15 days subject to the following criteria. The groups that received no training prior to drug sessions were followed for 5 sessions only; testing was terminated at this point because there was little evidence of avoidance acquisition. The remaining subgroups of 8 received daily drug sessions until the group completed two consecutive sessions with an average of 3.5 or less avoidance responses. At that time, all rats in the group that had reached an individual criterion of 2 consecutive sessions with 3 or fewer avoidance responses were no longer tested; the remaining rats continued to be tested until they reached this criterion or the 15 session limit.

*Experiment 2: effects of periodic retraining.* Six groups (n=7 or 8) of rats received 3 sessions of one-way avoidance training as described above. Two hr prior to the fourth session, 2 groups received vehicle, 2 groups received 0.5 mg/kg pimozide and 2 groups received 1.0 mg/kg. On each of the following 2 days, the 3 retest groups (one at each pimozide level) received avoidance sessions during which no drug was given. The control groups remained in their home cages during this time receiving neither avoidance sessions nor drug injections. All groups then were injected and tested on the following (third) day. This sequence was repeated for a total of 7 drug test sessions.

## RESULTS

Two dependent variables, number of avoidance responses per session and response latency, were recorded. In previous studies [2,3] each of these variables was analyzed separately and found to be affected similarly by the independent variables. For this reason, only response latencies were analyzed here; however, avoidance scores also are presented (see below).

*Experiment 1: Effects of Prior Training*

Mean (±SEM) response latencies for each group, collapsed over pretreatment conditions for the last training session and for the first 5 drug sessions are shown in Fig. 1. Analyses of variances included only the first 5 drug sessions because the 0 session training group was followed for only 5 sessions and because some of the groups receiving 1.0 mg/kg pimozide reached criterion by the fifth session. The effects of drug pretreatment, differences in baseline performance, drug dose, and amount of training prior to drug sessions are considered below.

*Drug pretreatment.* As described above, this condition was included to test the possibility that the gradual loss of avoidance responding in neuroleptic-treated rats is due to an accumulation of drug over repeated injections. The effects of 3 home cage injections prior to the initiation of drug sessions were analyzed using 8 separate two-way analyses of variance with repeated measures on the session variable, one analysis for each of the 2 groups at each level of the pretraining variable at each dose. The effect was not significant for the 0, 2, 3, and 10 session training groups receiving 0.5 mg/kg pimozide nor for the 2, 3 and 10 session training groups received 1.0 mg/kg ($p>0.05$ in each case). The only group revealing a significant pretreatment effect was the 0 session training group that received 1.0 mg/kg pimozide, $F(1,14)=30.09, p<0.01$. Because the pretreatment effect was insignificant for all pretrained groups and for one 0 pretrained group, the pretreated and non-pretreated rats were combined into 8 groups, one for each training condition at each dose (see Fig. 1).

*Baseline performance.* Baseline was defined as the last training session for the groups receiving 2, 3, or 10 sessions prior to drug tests. The groups that received 0.5 mg/kg dif-

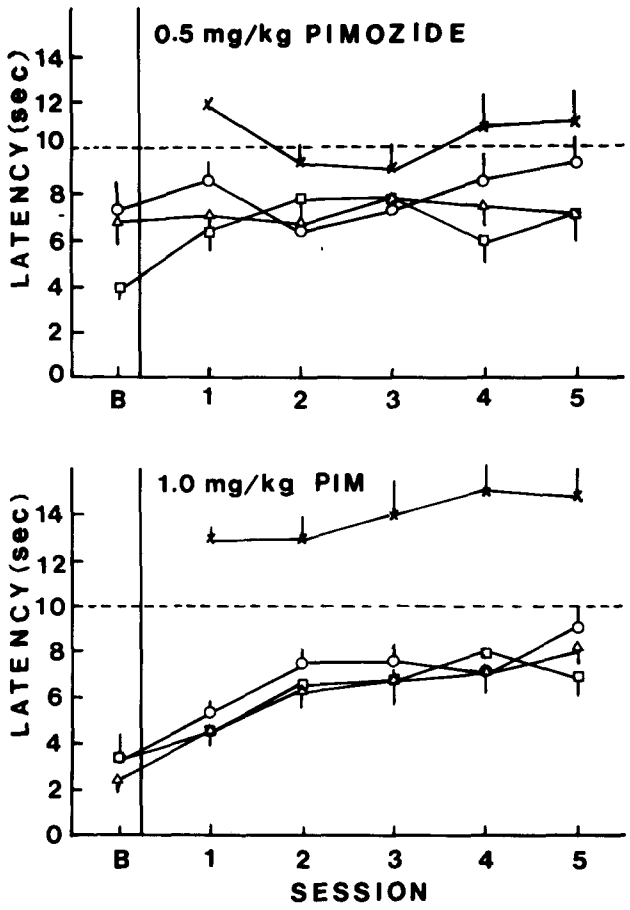


FIG. 1. Effect of pre-training (0 days (x), 2 days (△), 3 days (○), or 10 days (□)) on disruption of one-way active avoidance by pimozide (0.5, 1.0 mg/kg). Data points represent mean (±SEM) shuttle latencies on the last day of pre-training (B) and the first 5 daily pimozide sessions of Experiment 1.

fered in baseline latencies, $F(2,45)=6.36$, $p<0.005$; the groups that received 1.0 mg/kg showed no significant difference, $F(2,44)=3.35$, $p<0.05$. The difference in baseline latencies for the low dose groups was attributable to the longer latencies of the 2 and 3 session training groups (see Fig. 1).

*Drug dose.* The effect of dose of pimozide on shuttle latencies was analyzed separately for each of the 4 training conditions. Analyses of variance revealed a significant main effect of dose only for the 0 session training condition, $F(1,30)=7.47$, $p<0.01$. However, the dose by sessions interaction was significant for the 0 session, $F(4,120)=2.47$, $p<0.05$, 2 session, $F(4,120)=4.51$, $p<0.005$, 3 session, $F(5,150)=5.76$, $p<0.001$ and 10 session training groups, $F(8,232)=7.45$, $p<0.001$. These interactions occurred because the rate of decline in performance for the 1.0 mg/kg dose groups was steeper than for the 0.5 mg/kg dose. It should be noted that this effect, although significant, is not large for the first 5 sessions; however, when all 15 sessions are considered, the dose effect is striking (see below and Fig. 2). Thus, the performance of animals treated with 1.0 mg/kg pimozide was poorer than that of the groups receiving the 0.5 mg/kg dose.

*Amount of training prior to drug sessions.* Analyses of variance revealed a significant effect of training at both 0.5 and 1.0 mg/kg doses of pimozide, $F(3,60)=3.57$, $p<0.02$ and $F(3,59)=35.74$, $p<0.001$, respectively. Post hoc analyses revealed that the 2 and 10 session training groups differed from the 0 group at the lower dose, $F(1,30)=8.75$, $p<0.01$ and $F(1,30)=6.98$, $p<0.01$, respectively; however, the 3 session training group did not, $F(1,30)=2.70$, $p>0.05$. At the higher dose the 2, 3, and 10 session training groups differed from the 0 group, $F(1,30)=54.56$, $p<0.001$, $F(1,30)=47.17$, $p<0.001$ and $F(1,29)=50.48$, $p<0.001$, respectively. None of the other pairwise comparisons yielded significant group differences.

*Summary.* Animals that received 2, 3 or 10 sessions of avoidance training prior to the initiation of the drug trials were significantly less impaired by pimozide than animals receiving no prior training but did not differ from one another. However, in these previously trained animals, pimozide caused a dose-related progressive increase in shuttle latencies across daily drug sessions. This increase probably was not related to an accumulation of the drug since the latencies of animals receiving 3 home-cage injections prior to the first drug session did not differ significantly from those of nonpretreated groups.

As a result of the criteria employed for terminating the drug tests (see above), it was not possible to compare latencies across groups beyond the fifth test session because some rats had reached criterion. However, one measure was available for comparing the groups across the maximum of 15 drug sessions, viz., the total number of avoidance responses per group in each session. (Note that no individual rat was dropped from the test phase until it reached a criterion of 2 consecutive sessions with 3 or fewer avoidance responses with a maximum of 15 sessions.) These data are shown in Fig. 2. Clearly, avoidance responding of the rats receiving the high dose of pimozide deteriorated at a faster rate than responding of those receiving the low dose. After 15 sessisons with pimozide, the performance of the previously trained groups was about the same as that of the 0 session group for each dose.

### *Experiment 2: Effects of Periodic Retraining*

Mean (±SEM) response latencies for the retrained and no retraining groups for each drug dose are shown in Fig. 3. Only the 7 drug test sessions were included in the analyses of variance. Both the retrained and the no retraining groups showed a significant effect of drug dose, $F(2,20)=32.07$, $p<0.001$ and $F(2,20)=72.40$, $p<0.001$, respectively. For the retraining group, the vehicle latencies differed from those of the 0.5 and 1.0 mg/kg doses, $F(1,14)=4.74$, $p<0.05$ and $F(1,13)=39.91$, $p<0.001$, respectively and the 2 pimozide groups differed from each other, $F(1,13)=29.14$, $p<0.001$. For the no-retraining group the results were similar, $F(1,14)=12.27$, $p<0.005$ for vehicle vs. 0.05, $F(1,13)=288.04$, $p<0.001$ for vehicle vs. 1.0 and $F(1,13)=51.68$, $p<0.001$ for 0.5 vs. 1.0 mg/kg.

Periodic retraining did not produce a significant effect in the vehicle group, $F(1,14)=2.93$, $p>0.05$. However, 2 days of retraining in the no-drug condition significantly reduced the effects of 0.5 and 1.0 mg/kg pimozide on avoidance latencies. This was supported by a group effect in the analysis of variance comparing and retraining and no-retraining groups at each dose, $F(1,14)=13.38$, $p<0.005$ and

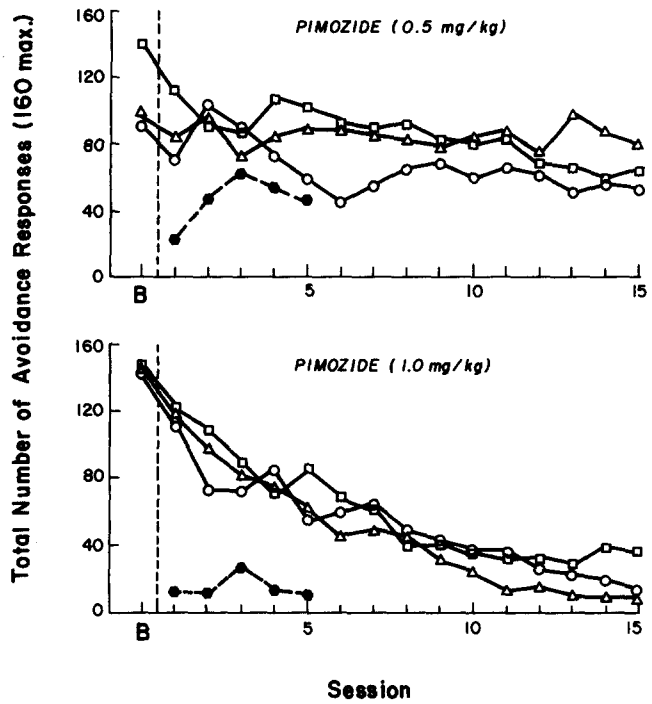


FIG. 2. Effect of pre-training (0 days (●), 2 days (△), 3 days (○), or 10 days (□)) on disruption of one-way avoidance by chronic treatment with pimozide (0.5, 1.0 mg/kg). Data points represent the total number of active avoidance responses per group on the last day of pre-training (B) and the 15 drug sessions of Experiment 1. Individual rats in the pre-trained groups were tested for a maximum of 15 sessions or until 3 or fewer avoidance responses were displayed on 2 consecutive sessions.

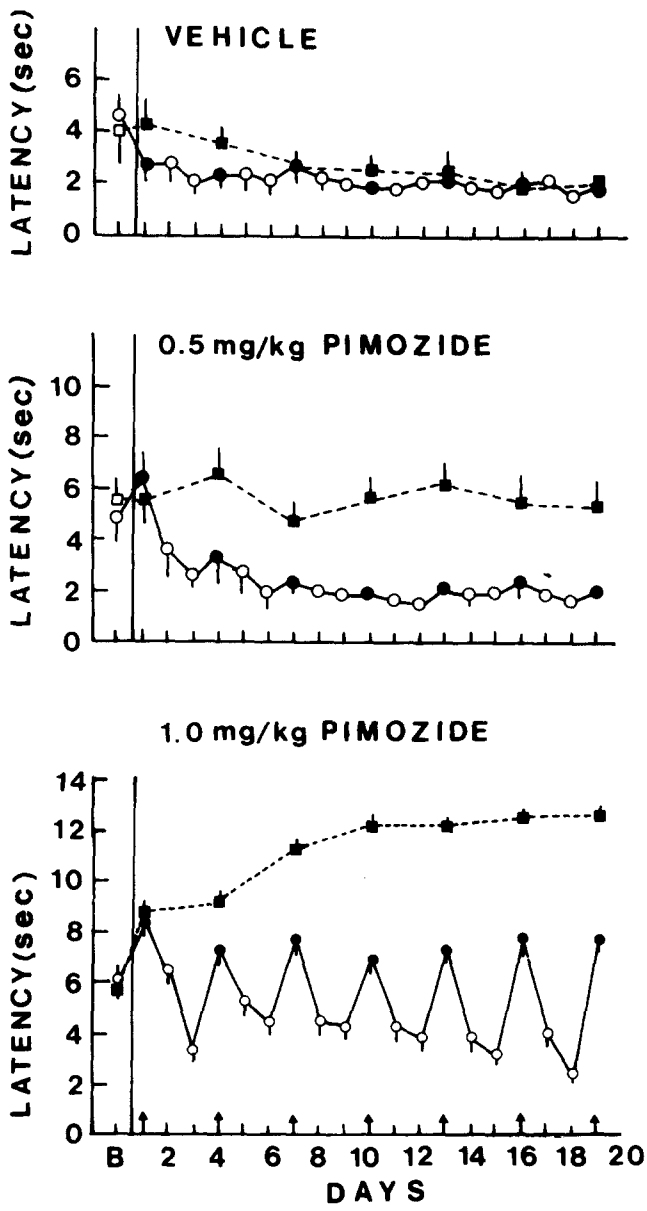


FIG. 3. Effect of retraining under no-drug condition on disruption of one-way active avoidance by pimozide (0.5, 1.0 mg/kg). Following 3 days of acquisition training, each group was tested with pimozide every third session (solid symbols). Half the subject (N=8) under each drug condition were retrained without pimozide for 2 days prior to each of the 7 drug sessions (○); the remaining subjects received no retraining (□). Data points represent mean (±SEM) shuttle latencies for each group in Experiment 2.

$F(1,12)=17.80$, $p<0.001$ for the 0.5 and 1.0 mg/kg dose, respectively. Thus, periodic retraining attenuated the effects of both doses of pimozide.

## DISCUSSION

The failure to avoid of non-pretrained animals treated with pimozide is in agreement with an extensive literature showing that neuroleptics block the acquisition of avoidance responding [2,3]. The eventual disruption of avoidance in pretrained animals also has been reported previously [1, 8, 10] as has the pretraining-produced attenuation of the effects of neuroleptics [4,6]. This latter finding is in agreement with the observation that pretraining of an operant response also results in an attenuation of the effects of neuroleptics [14,16]. The observation that 2, 3, or 10 sessions of pretraining did not result in a graded attenuation of the effects of pimozide is not in accord with a previous report in which animals pretrained for 9 sessions were significantly less affected by haloperidol than animals pretrained for only 2 sessions [4]. The reason for this discrepancy is unclear. However, in the previous study pretrained animals were followed only for 1–3 drug sessions; perhaps with a more extended test phase the effects of one dose of haloperidol [4] would have been observed not to differ in animals with 2 or 9 pretraining sessions. Observation of Fig. 2 provides some support for this suggestion. Thus, on drug day 1 for the 0.5 mg/kg dose of pimozide the group that received 10 pretraining sessions made more avoidance responses than the 2 or 3 day pretraining groups; on drug days 2 and 3 the high dose of pimozide impaired the avoidance responding of the 10 day pretraining group least. However, over 15 days of drug tests, amount of pretraining was shown not to affect significantly the ability to avoid.

The cumulative effect of repeated dosing with pimozide

on avoidance responding in pretrained animals cannot be attributed to an accumulation of the drug in the animals. According to this hypothesis, avoidance performance of pretrained animals is observed to deteriorate because insufficient time is allowed between doses for the complete clearing of the drug. As a result, the effective dose of drug increases with each injection. If this interpretation were correct, animals receiving home cage injections for 3 days prior to the initiation of drug sessions should show a faster deterioration of avoidance responding than nonpretreated animals. The present results showed that the effects of pretreatment were insignificant suggesting that the accumulation hypothesis is inadequate to account for the data.

The finding that intermittent retraining blocks the cumulative breakdown in avoidance responding of pretrained animals treated with neuroleptics has not been reported previously. This observation and the temporary resistance to the effects of neuroleptics seen in pretrained animals might suggest that during nondrug training sessions dopamine neurons mediate a plastic change in the brain that can influence avoidance responding for a time even when dopamine receptors subsequently are blocked.

There is now ample evidence that although nonpretrained animals under the influence of neuroleptics fail to learn operant avoidance responses, they do learn the association between a preshock conditioned stimulus (CS) and an unconditioned shock (US) stimulus. This was concluded when classical conditioning of a "fear" response (e.g., freezing, defecation) was found to be little affected by neuroleptics [2, 3, 5, 13]. Since this sensory-sensory associative learning is intact in animals treated with neuroleptics, failures to avoid must be related to some aspect of operant response learning. Previous observations of normal avoidance behavior by pretrained animals when tested under the influence of haloperidol led Fibiger *et al.* [4] to conjecture that dopamine ". . . is more critically involved in the acquisition of instrumental responses than in the expression of these responses." However, from the present results it is evident that dopamine plays an important role in operant response acquisition and also contributes to the long-term maintenance of operant responses.

Any attempt to summarize the role of dopamine in avoidance learning must focus on those neuronal events that permit a CS to elicit operant responses. When the activity of brain dopamine systems is blocked by dopamine receptor antagonists, non-pretrained animals are incapable of learning operant running in response to a CS, although learning of the classically conditioned CS-US association is unaffected [2]. Once the running response to the CS is acquired (by pretraining nondrugged animals) the CS may still elicit the avoidance response for a number of trials, despite the blockade of dopamine receptors by neuroleptic drugs. However, with repeated testing the avoidance response becomes progressively weaker until eventually it no longer is elicited by the CS.

The specific mechanism by which the avoidance response transiently is maintained in previously trained rats given neuroleptics remains to be resolved. Avoidance response learning in nondrugged animals would appear to be a dynamic process that requires dopamine systems to function normally for specific periods of time. From the present data, these critical periods can be defined as a minimum of 2 daily test sessions during acquisition and 2 sessions of retraining following acquisition of the avoidance response. As a consequence of this learning in the undrugged state, avoidance responses can be maintained over the short-term in the absence of normal dopamine function. One possible explanation of the maintenance of operant responding in the drugged state may involve classical conditioning of those neurochemical events necessary for initiation of the correct behavioral response. For example, repeated testing in a distinctive test environment could result in a classically conditioned increase in the activity of dopamine neurons. On subsequent tests, salient environmental stimuli would elicit a conditioned increase in the release of dopamine that in turn could offset at least transiently the effect of a particular level of dopamine receptor blockade and thereby facilitate normal response initiation. Recent reports of conditioned changes in the neurochemical activity of dopamine neurons [7, 11, 15] are consistent with this conjecture.

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