Pharmacology Biochemistry & Behavior, Vol. 14, pp. 101–105. Printed in the U.S.A.

The Effects of Pimozide During Pairing on the Transfer of Classical Conditioning to an Operant Discrimination[1]

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Received 21 June 1980

BENINGER, R. J. AND A. G. PHILLIPS. *The effects of pimozide during pairing on the transfer of classical conditioning to an operant discrimination.* PHARMAC. BIOCHEM. BEHAV. 14(1) 101–105, 1981.—Transfer of classical conditioning to operant learning was demonstrated by showing enhanced acquisition of an operant discrimination in a group of rats (n=6) previously exposed to pairings of the discriminative stimulus with food as compared to control animals (n=6). A group (n=6) that received the classical conditioning sessions while under the influence of the neuroleptic, pimozide (1.0 mg/kg, IP) also showed enhanced acquisition of the discrimination when tested while undrugged but their performance was intermediate between that of the other groups for the first seven sessions. For the remaining sessions, the two groups that had received classical conditioning did not differ from each other and both groups discriminated better than the controls. These data may indicate a role for dopaminergic neurons in the mechanism by which the effects of classical conditioning influence operant responding.

Classical-operant transfer Pimozide Discrimination learning Rats Dopamine Two-process learning theory

IT is well established that animals treated with neuroleptic drugs fail to acquire or perform avoidance responses. This effect was reported for animals treated with chlorpromazine [25], haloperidol [12], pimozide [5] and a wide range of similarly acting drugs [18]. Neuroleptic drugs produce hypokenesia in a dose-related fashion (see [29]) making it difficult to know whether the observed deficits in avoidance responding are related to motor effects or impairments in learning the association between the preshock conditioning stimulus (CS) and the unconditioned stimulus (US) shock. The observation that neuroleptic-treated rats, although failing to avoid, escape readily from footshock might suggest that a sensory-sensory (S-S) associative learning deficit underlies this disruption of avoidance responding. However, a number of investigations showed that CS-US associations were learned by neuroleptic-treated rats: Posluns [25] showed that under some conditions avoidance responses occurred in rats treated with chlorpromazine indicating that the association of the CS and shock had been learned. Beninger *et al.* [5] showed subsequently that conditioned suppression of food-reinforced leverpressing occurred during presentation of a CS that had failed to control avoidance responding in the same rats when treated with pimozide; control animals showed significantly less suppression to the CS. Since CS-US pairings occurred only during sessions when the animals were under the influence of pimozide, the association must have been learned by the drugged rats.

These results suggest that neuroleptic drugs do not block the learning of associations between stimuli. This conclusion is consistent with the recent observation that pimozide did not disrupt the acquisition of defensive burying [23,24] of a prod paired with shock [4]. Similarly, this conclusion is supported by reports that neuroleptics do not block classical conditioning [13], latent learning [1], or short term memory [3].

Recently, Beninger and Phillips [7] reported that animals treated with pimozide failed to show conditioned reinforcement. This observation cannot be attributed to the effect of the drug on performance because the critical comparisons were made with undrugged animals. The procedure first involved classical pairings of a tone with food over several sessions. The effectiveness of the tone as a conditioned reinforcer was assessed in extinction by comparing the rate of pressing two levers, one of which produced the tone. Animals injected with pimozide during classical conditioning failed to respond more on the tone lever when tested while undrugged.

The failure to establish conditioned reinforcement in

[1]This research was supported by a Medical Research Council grant to A.G.P. R.J.B. is a Medical Research Council postdoctoral fellow. Pimozide was generously supplied by Dr. A. Wauquier, Jansen Pharmaceutica.

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pimozide-treated animals need not imply the absence of S-S associative learning in an appetitive task. Possibly, evidence of S-S associative learning was not seen because pimozide interfered with the ability of the conditioned reinforcer (CS) to maintain responding when the operant testing phase occurred in extinction. To test this interpretation, the present experiment employed a combination of classical and operant conditioning procedures in which reinforcement still occurred in the operant phase. Studies have shown faster learning of an instrumental discrimination when the discriminative stimulus has been paired previously with food using a classical conditioning procedure [9, 16, 28]. A variation of this procedure was employed in the present experiment to assess the effects of pimozide on S-S associative learning involving appetitive stimuli. Two groups of rats, one injected with pimozide, received Pavlovian conditioning consisting of pairings of a tone (CS) and food pellet (US). A third group received no Pavlovian conditioning. In the second phase of the experiment, all three groups were trained on an operant discrimination in which the tone signalled food reinforcement. The hypothesis that pimozide does not block learning of the CS-US association would be confirmed if the pimozide group, like the control group receiving Pavlovian conditioning, acquired the operant discrimination faster than the group with no previous tone food pairings.

METHOD

Subjects

Eighteen male albino rats of the Wistar strain were housed individually in a climatically controlled colony room kept on a twelve hr light/dark cycle. The rats weighed from 235 to 275 g and were maintained at 80% of these initial free-feeding weights throughout the experiment by daily feeding with measured rations.

Apparatus

The two separate phases of the experiment were carried out in different apparati. For the classical conditioning phase, the experimental environment consisted of four similar Plexiglas chambers (30.0×21.5×46.5 cm high) with a feeder cup located in the middle of one of the 30.0 cm sides at a height of 1.5 cm. The operant conditioning sessions were carried out in three similar chambers (23.4×20.4×19.5 cm) with Plexiglas sides and top, aluminum plate ends and a grid floor. A lever (Scientific Prototype) that was 5.0 cm wide was positioned in the middle of one of the end walls at a height of 5.5 cm and had a force requirement of about 0.11 N. A feeder cup was located to the left of the lever at a height of 1.5 cm. Each of the seven chambers was located in a ventilated, sound-attenuating box illuminated by an overhead light and fitted with a 2900 Hz tone generator (Sonalert). Environmental control and data collection was performed by a Data General Nova 3 computer.

Procedure

The eighteen rats were assigned randomly to three groups (n=6). Two groups received four classical conditioning sessions similar to those in the conditioned reinforcement experiment discussed above [7]. Thus, during the first session the Tones-Pellets group received 80 randomly presented 3-sec tones with an average intertone interval of 45 sec and each terminating with the presentation of one 45 mg Noyes Precision Food Pellet. For the next three sessions, pellets followed tones 33% of the time. This partial pairing procedure was employed because it has been demonstrated to produce more durable conditioning [14, 32, 33]. The Tones-Pellets-Pimozide group received the same treatment but was injected (IP) with 1.0 mg/kg pimozide (dissolved in boiling tartatic acid and then cooled to about 40° before injection) 90 min prior to each session. This dose was used because it successfully blocked conditioned reinforcement in the Beninger and Phillips [7] study but failed to block associative learning in several other experiments [5,6]. All rats in the pimozide group were observed to eat readily all pellets presented during the four conditioning sessions. The Pellets group received pellets according to the same schedule for the four conditioning sessions but tones never were presented.

Thirty-min lever-press training sessions began after conditioning with each rat receiving three sessions of continuous reinforcement. A multiple random interval (RI) 32-sec, extinction (EXT) schedule then was initiated. During the RI, a response-contingent pellet became available every 32 sec on the average. One-min components simply alternated with the RI being signalled by the tone (3 sec on, 3 sec off, etc.) and EXT by the absence of the tone. Discrimination sessions were 30 min in duration and occurred once a day at approximately the same time each day, 4 or 5 days a week for a total of 18 sessions. No drugs were given during these sessions.

RESULTS

Discrimination ratios were calculated for each cycle of the multiple schedule (i.e., each pair of components) for each rat by dividing the rate (lever-presses per min) in RI by the sum of the RI and EXT rates. A ratio of 0.5 would indicate no discrimination with values greater than 0.5 indicating that a larger proportion of responses occurred in the RI component. These ratios were combined into blocks, each block consisting of the mean of the ratios for 5 cycles (thus, there were 3 blocks per session). The group means are shown in Fig. 1 (note that Fig. 1 shows within session data; every 3 points constitute one session). The data from daily sessions 2 to 18 were analyzed using a two variable analysis of variance with repeated measures on one variable (see [30]) followed by post hoc tests where appropriate. The first discrimination session (blocks 1 to 3) was treated as a warmup and the data were not included in the overall analysis.

Overall, the groups differed significantly, $F(2,15)=10.51$, $p<0.001$. Comparison of the discrimination ratios of the Pellets and Tones-Pellets groups clearly indicated that prior association of the tone with pellets resulted in enhanced performance. This was reflected in the significant difference between these groups, $F(1,10)=17.78$, $p<0.002$. The Tones-Pellets-Pimozide group differed from the Pellets group, $F(1,10)=10.24$, $p<0.01$, but not from the Tones-Pellets group, $F(1,10)=2.11$, $p>0.05$, although the groups by blocks interaction with the latter group was significant, $F(50,500)=2.05$, $p<0.001$. The interaction indicates that the relationship between the Tones-Pellets and Tones-Pellets-Pimozide groups changed over blocks. From Fig. 1 it appears that initially the two groups differed but over time they converged. Post hoc analysis of variance confirmed the significance of this effect, the two groups differing significantly over sessions 2 to 4 (blocks 4 to 12), $F(1,10)=16.19$, $p<0.002$, marginally on sessions 5 to 7 (blocks 13 to 21), $F(1,10)=4.68$, $p<0.056$, and not on sessions 8 to 10 (blocks 22 to 30), $F(1,10)=0.34$, $p>0.05$, or thereafter.

The mean number (±SEM) of blocks required to reach a

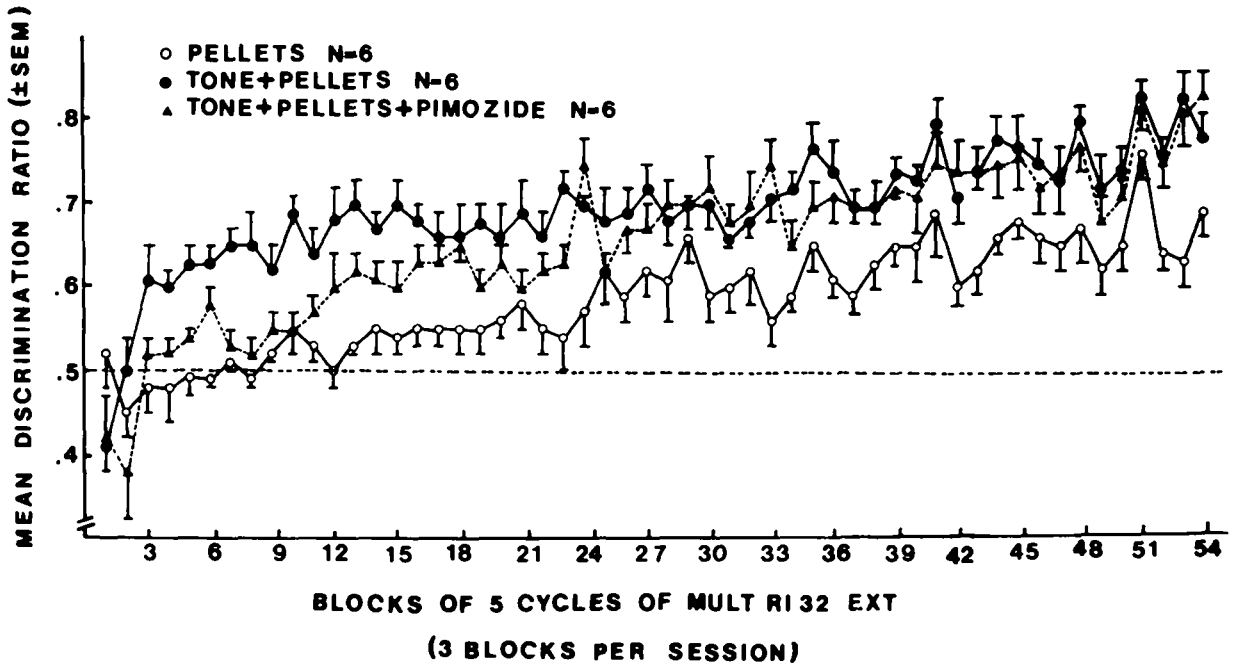

FIG. 1. Mean discrimination ratios for each group for each block (3 blocks per session) for the 18 sessions of training on the multiple RI 32 sec EXT schedule. Vertical lines from each point indicate SEMs. The broken horizontal line at 0.5 indicates equal distribution of responding in each component of the multiple schedule while values above 0.5 indicate a greater proportion of responding in the RI component. Note that no drugs were given during these sessions.

criterion of two consecutive blocks with a discrimination ratio of 0.65 or higher for the Pellets, Tone-Pellets, and Tone-Pellets-Pimozide groups was 39.5(±6.2), 12.2(±3.6), and 23.7(±3.6), respectively. Comparison of these values further indicates that the performance of the Tones-Pellets-Pimozide group was intermediate between that of the other two groups. Analysis of variance showed that these differences were significant, $F(2,15)=8.80$, $p<0.003$.

Discrimination ratios do not allow comparison of absolute rate of responding in the RI and EXT components from group to group. Therefore, Fig. 2 shows the response rate (lever presses per min) in each component combined into 54 blocks for the 18 sessions. There was no significant difference among groups in EXT (S−) rates, $F(2,15)=0.41$, $p>0.05$, but the groups by blocks interaction was significant, $F(100,750)=1.27$, $p<0.05$. However, no systematic change in group differences could be found to account for this interaction. For example, separate analysis of sessions 2 to 4 (blocks 4 to 12) yielded no significant group effect, $F(2,15)=1.51$, $p>0.05$.

Comparison of the S+ rates also resulted in an insignificant group effect, $F(2,15) + 2.48$, $p>0.05$ and a significant interaction $F(100,750)=1.51$, $p<0.001$. In this case, however, the significant interaction was produced by systematic changes in group differences. Thus, groups did not differ in sessions 2 to 4 (blocks 4 to 12), $F(2,15)=0.77$, $p>0.05$ and sessions 5 to 7 (blocks 13 to 21), $F(2,15)=2.06$, $p>0.05$, differed marginally in sessions 8 to 10 (blocks 22 to 30), $F(2,10)=3.18$, $p=0.07$ and significantly in sessions 11 to 13 (blocks 31 to 39), $F(2,10)=3.75$, $p<0.05$.

In summary, the S− rates of the three groups did not differ significantly; the S+ rates of the Tones-Pellets and Tones-Pellets-Pimozide groups were initially similar to the S+ rates of the Pellets group but after 10 sessions of discrimination training, the former two groups were responding at a higher rate than the latter (see Fig. 2).

DISCUSSION

The group that received tone-pellet pairings prior to instrumental training performed better on the discrimination than the Pellets group. This result is consistent with previous experiments showing transfer from classical to instrumental conditioning when the classical CS is used as the S+ in the operant discrimination [9, 16, 28]. The group receiving pimozide injections during the classical conditioning phase also showed enhanced acquisition of the discrimination when compared to the Pellets group. These data confirm, in an appetitive as compared to an aversive task, that neuroleptic drugs do not block S-S associative learning [1, 5, 13].

Initially the Tones-Pellets-Pimozide group did not discriminate as well as the Tones-Pellets group but this effect was short lived as these groups did not differ significantly from blocks 22 to 54 (sessions 8 to 18). There are several possible interpretations of the initial deficit of the Tones-Pellets-Pimozide group. One possibility is that pimozide *weakened* but did not block the formation of the association between the tone and food during classical conditioning. State dependent learning also may have been a factor if possible stimulus effects of pimozide were associated with tone and food during classical conditioning (see [19]). Alternatively, it is possible that pimozide did not affect the tone-food association but instead affected the ability of this learning subsequently to influence discriminated responding. Each of these possibilities will be examined in detail.

Evidence against the possibility that pimozide produced a weakening of S-S associative learning comes from several sources. In one study, groups of saline- and pimozide-

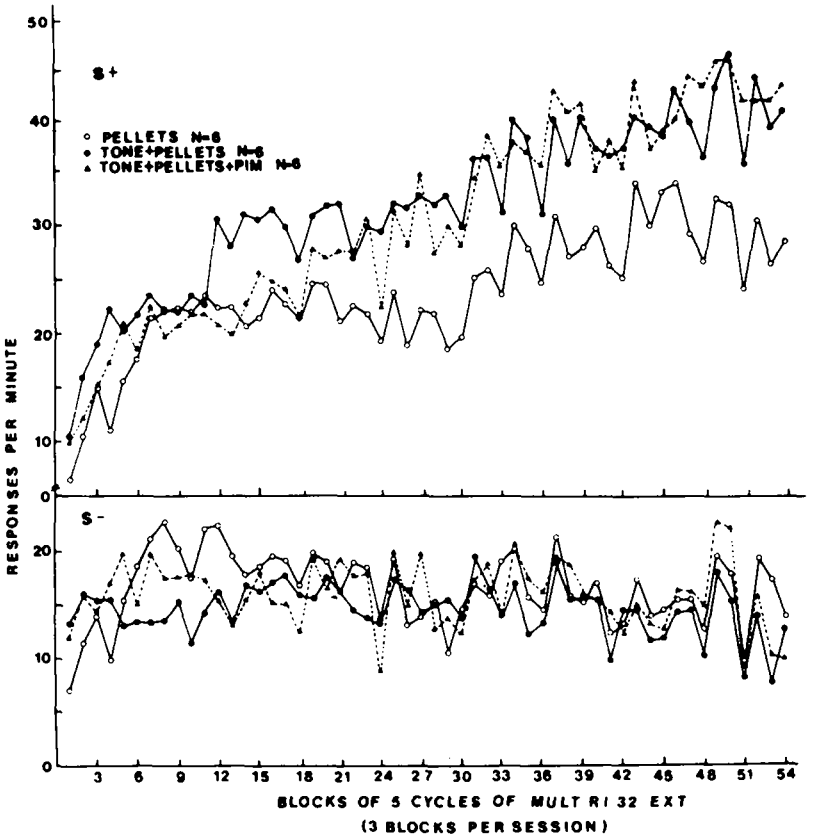

FIG. 2. Mean response rate (resp/min) for each group for each block (3 blocks per session) in the RI (S+) and EXT (S−) components of the multiple schedule for the 18 training sessions.

injected rats received pairings of a prod with electric shock. When subsequently tested for prod burying [23,24] while undrugged, the groups did not differ [4]. Ahlenius *et al.* [1] showed that injections of the neuroleptic, haloperidol during pre-exposure to a maze had no effect on latent learning when tested later in a drug-free state. These observations support the contention that neuroleptics do not affect S-S associative learning. Contrary evidence was provided by Hunt [13] who reported that chlorpromazine-treated rats given clicker-shock pairings showed weaker conditioned emotional responses to the clicker when later tested in a drug-free state. These effects of the less specific dopamine antagonist, chlorpromazine (see [2]) notwithstanding, the data from experiments using haloperidol and pimozide support the conclusion that neuroleptics do not weaken S-S associative learning. That pimozide fails to affect the accuracy of a well-learned discrimination [27] and that short term memory appears to be unaffected by haloperidol [3] further support this conclusion.

According to the state dependent learning hypothesis, drug-produced stimuli may form part of the CS that becomes associated with the US during associative learning [19]. Thus, the speed at which the Tones-Pellets-Pimozide group acquired the discrimination could be influenced by the absence of those drug-related stimuli during testing. This possibility was not supported by the results of Beninger and Phillips [7]. The failure of neuroleptics to weaken associative learning in the studies discussed above also provides data contrary to this hypothesis.

The possibility remains that the Tones-Pellets-Pimozide group acquired the discrimination at a slower rate than the Tones-Pellets group because pimozide interfered with the mechanism by which classical conditioning influences instrumental behavior. Accordingly, during tone-pellet pairings in undrugged animals, *two* learning processes are involved. The first, associating the auditory stimulus and food pellet, appears unaffected by pimozide. The second learning process involves a mechanism by which (learned) associations acquire the ability to affect instrumental behavior. The nature of this learning mechanism is unknown although some theorists have considered the role of Pavlovian conditioned responses. Rescorla and Solomon [26], for example, suggested that Pavlovian CRs do not act as specific mediators of instrumental behavior but rather serve as an index of a neural process which mediates the behavior. Regardless of its specific nature, the present data from the Tones-Pellets-Pimozide group suggest that this type of learning is disrupted by pimozide. Pharmacological evidence for a specific blockade of dopamine receptors by pimozide [22] would suggest further that dopaminergic activity is involved in this learning.

Further conjecture regarding the learning mechanism by which classical conditioning may influence instrumental responding in an appetitive situation arises in the context of dopaminergic substrates of brain-stimulation reward [15, 20, 21, 31] and the blockade of conditioned reinforcement by dopamine antagonists [7, 10, 11]. It has been postulated that dopaminergic neurons mediate the acquisition of incentive

motivational properties by neutral stimuli paired with biologically significant (reinforcing) stimuli [17]. By definition, incentive stimuli facilitate operant behavior [8]. When pimozide is administered during tone-food pairings, learning of the S-S association is not blocked but the tone fails to acquire incentive motivation and consequently can not be used as a conditioned reinforcer to maintain instrumental responding in extinction tests [7]. In the present study, animals treated with pimozide during classical conditioning clearly learned the association between tone and pellet as indicated by their superior performance, relative to controls, in the discrimination task. Slower acquisition of the discrimination by the Tones-Pellets-Pimozide group, as compared to the group not treated with pimozide during classical conditioning, may reflect decreased incentive value of the tone. Comparable performance by these two groups after seven sessions of discriminated responding for food may indicate that the tone acquired additional incentive value through tone-pellet pairings that occurred during responding on the multiple schedule while in the undrugged state. This interpretation of the data is supported by the observation that group differences in performance on the discrimination were attributable largely to S+ rates.

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