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Conditioned Stereotypy: Behavioral Specification of the UCS and Pharmacological Investigation of the Neural Change

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HIROI, N. AND N. M. WHITE. *Conditioned stereotypy: Behavioral specification of the UCS and pharmacological investigation of the neural change.* PHARMACOL BIOCHEM BEHAV 32(1) 249–258, 1989.—Previous work has shown that conditioned stereotypy can be produced by repeated treatments with d-amphetamine or apomorphine. We replicated this phenomenon and found that, as in previous reports, the amplitude of conditioned stereotypy was about one-third that of the unconditioned stereotypy. On the basis of the hypothesis that the UCS in this conditioning situation is a specific stimulation level of dopamine receptors expressed as a peak behavioral effect (UCR), rats were exposed to the experimental boxes for a brief interval during the peak behavioral effect of the drugs. This procedure produced an amplitude of conditioned stereotypy about two-thirds that of unconditioned stereotypy. The issue of the synaptic mechanism mediating conditioned stereotypy was addressed by examining the effect of pimozide on the behavior. A dose of pimozide that completely blocked apomorphine-unconditioned stereotypy also blocked apomorphine-conditioned stereotypy with no sign of motor impairment. d-Amphetamine-conditioned stereotypy was not completely blocked by a dose of pimozide that completely blocked d-amphetamine-unconditioned stereotypy. The implications of these findings for understanding the neural basis of conditioned stereotypy are discussed.

d-Amphetamine Apomorphine Stereotypy Conditioned stereotypy Pimozide Dopamine Neural change Pharmacological conditioning

DOPAMINE agonists induce motor behaviors such as hyperactivity and stereotypy. Hyperactivity has been shown to be conditioned in classical conditioning paradigms (2, 18, 22, 23, 27). An early study suggested that stereotypy could also be classically conditioned (9). In a well-controlled study, Schiff (22) showed that amphetamine- and apomorphine-induced stereotypy can be conditioned. The two types of conditioned motor behaviors are of particular interest because of evidence that they are mediated by central dopaminergic mechanisms (1, 5, 11, 20). Their study may, therefore, reveal information about the role of this neurochemical in learning. The present study addresses two critical issues concerning conditioned stereotypy.

One issue is the precise identification of the unconditioned stimulus (UCS) in this drug conditioning paradigm. The UCS has been vaguely defined as the drug (2, 15, 23), administration of the drug (18) or the pharmacological action of the drug (22). The experimental design based on these definitions has produced relatively weak conditioned stereotypy. Experiments 1 and 2 deal with the identification of the unconditioned stimulus in this conditioning situation.

The second issue is the nature of the neural change associated with the conditioning of stereotypy. There are contradictory views concerning this question. One argument is that the neural change is presynaptic; that dopamine release controlled by the presence of the conditioned stimulus mediates the expression of this learned behavior. This argument has been supported by biochemcial and pharmacological analyses (22,23). The alternative view is that conditioned stereotypy is, once established, independent of dopamine release. This view implies that the change may be postsynaptic (15). Such a change could take place during conditioning trials in the postsynaptic neuron or neural network that is activated by dopamine receptor activation. A study of amphetamine-conditioned hyperactivity provides some indirect support for this idea (2).

All of these experiments have examined the effect of dopamine blockers on the conditioned stereotypy produced by amphetamine; there have been no reported studies on the effect of dopamine receptor blockade on apomorphine-conditioned stereotypy. Apomorphine directly acts on both the presynaptic and postsynaptic receptors at high doses, the

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former causing inhibition of the release and synthesis of dopamine in the dopaminergic neurons. Because selective stimulation of the presynaptic receptors causes hypomotility but not stereotypy (26), apomorphine-unconditioned stereotypy is thought to be a postsynaptic event, and it might be assumed that the conditioned stereotypy produced by this drug is also postsynaptic. In Experiment 3, the effect of dopamine receptor blockade on amphetamine- and apomorphine-conditioned stereotypy was examined to identify the locus of the neural change responsible for conditioned stereotypy.

EXPERIMENT 1

In this experiment the conventional procedure for producing conditioned stereotypy with d-amphetamine and apomorphine was used. Three aspects of the unconditioned and conditioned stereotypy were compared: their topography, their amplitude, and their time-course within the training and test sessions.

The design of experiments investigating conditioned stereotypy incorporates both a pharmacological control and a conditioning control. On training days the animals in the experimental group (the pretrial drug group) receive a drug injection and are placed into the test box. This results in the pairing of the drug's pharmacological action and of any behaviors produced by this action with the stimuli that make up the test box (the CS). The animals in the pharmacological control group (the pretrial saline group) receive an injection of the vehicle and are placed in the test box. The animals in the conditioning control group (the posttrial drug group) are placed into the test box without receiving any injection because of the possibility that the injection itself may act as a CS (9). Some time after their removal from the boxes they receive a drug injection. Therefore, these animals experience both the CS and the effect of the drug, but in an unpaired manner, preventing the establishment of a conditioned association between them. This group controls for the possibility that the drug-induced behavior may appear on the test day as a result of a nonassociative process such as sensitization. On the test day the animals in the pretrial drug and saline groups receive saline injections and are placed into the test boxes. The animals in the posttrial drug group are simply placed into the box. The conclusion that conditioning has occurred is justified if the drug-induced behavior is exhibited by the pretrial drug group but not by the two control groups.

Two parameters of the conditioning procedure used were selected on the basis of data from preliminary experiments. The numbers of conditioning (training) trials producing the highest amplitude of conditioned responding on the test day were found to be seven for d-amphetamine and five for apomorphine. The doses producing the highest amplitude of responding were found to be 4.0 mg/kg d-amphetamine and 0.4 mg/kg apomorphine (10).

METHOD

Subjects

Sixty-five male hooded rats (290–320 g) obtained from Charles River Canada, St. Constant, Quebec were used. The rats were housed individually in their home cages, with Purina rat chow and water continuously available.

Apparatus

Stereotypy was measured in an open-field Plexiglas box (45×45×27 cm) with a wire-mesh floor. Three identical boxes were used. Each rat was assigned to a single box for the entire experiment.

Procedure

The subjects were randomly assigned to six groups, with eight subjects in each apomorphine group and fourteen subjects in each amphetamine group. One rat in the pretrial amphetamine group died during conditioning sessions.

Three groups were used to test conditioned stereotypy with each drug. The subjects in group 1 were given pretrial drug injections immediately before they were placed into the experimental boxes; those in group 2 were given pretrial saline injections immediately before they were placed into the experimental boxes. The rats in group 3 were given drug injections in their home cages 4 hours after they were removed from the experimental boxes. The pretrial drug and pretrial saline groups did not receive any injections after the training sessions.

On the basis of data from the preliminary experiments, the eighth session for the d-amphetamine groups and the sixth session for the apomorphine groups were the test sessions. For the test sessions, the pretrial drug and pretrial saline groups received saline injections prior to the test trial. The posttrial drug groups received no treatment prior to the test session.

All injections were given subcutaneously on the back. The animals in groups 1 and 2 were placed into the observation boxes for 45 min immediately following the injections. The animals in group 3 were also placed into the experimental boxes for 45 min. All animals were returned to their home cages immediately after each training session. The experimental boxes were cleaned after each animal was removed.

In all training and test sessions, behavioral measurements were taken during five 90-sec periods at 5, 15, 25, 35, and 45 min after injection. Stereotypy was measured by observation during the 90-sec periods by noting, every 3 sec, the behavior that the animal engaged in.

The following behaviors were classified as stereotypy: sniffing downward, nose poking, foot shuffling, gnawing, licking, repetitive head movement, and repetitive locomotion on the same path. The stereotypy score for each time interval was obtained by counting the number of times out of 30 observations that one of these behaviors was recorded. The nonstereotyped behaviors were standing still, lying down, walking, rearing, sniffing upward, and grooming.

RESULTS AND DISCUSSION

The results for d-amphetamine- and apomorphine-induced sterotypies are shown in Fig. 1. Data are shown for the training sessions on the odd numbered days and for the no-drug test sessions. The unconditioned stereotypy produced in the pretrial drug groups in comparison to the pretrial saline and posttrial drug groups is clearly shown.

During the test session the presence of conditioned stereotypy for both d-amphetamine and apomorphine is evident. For the amphetamine groups, the overall treatment effect in the test session, comparing the pretrial drug group, pretrial saline group and posttrial drug group, was significant, $F(2,38)=6.45$, $p<0.01$ (Fig. 1a). Planned comparisons showed that there was no significant difference between the pretrial saline and posttrial amphetamine groups, $F(1,38)=0.16$, $p>0.05$. The mean of these two groups was

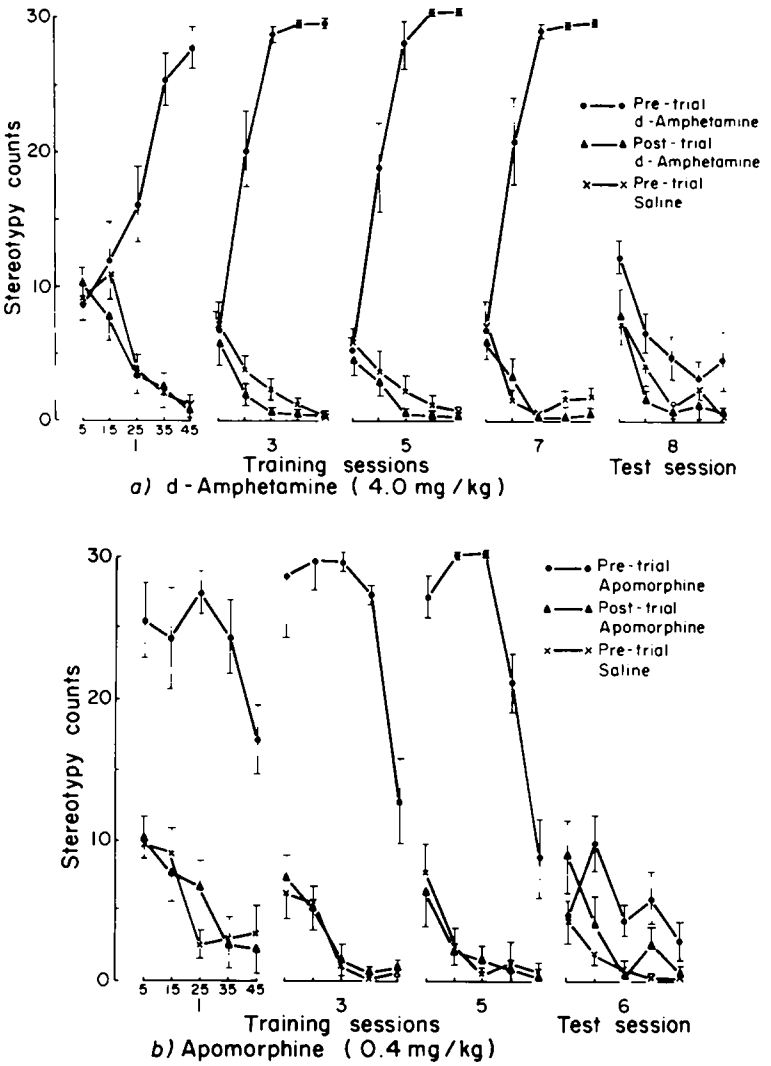

FIG. 1. Stereotypy (Training Sessions) and conditioned stereotypy (Test Session) induced by (a) d-amphetamine (4.0 mg/kg) and (b) apomorphine (0.4 mg/kg).

significantly different from that of the pretrial amphetamine group, $F(1,38)=12.77$, $p<0.01$. The interaction effect was not significant, $F(8,152)=0.67$, $p>0.05$.

The analysis of the test session for the apomorphine groups showed that the drug treatment effect was significant, $F(2,21)=3.74$, $p<0.05$ (Fig. 1b). Planned comparisons revealed that there was no significant difference between the pretrial saline and posttrial apomorphine groups, $F(1,21)=1.82$, $p>0.05$. There was a significant difference between the mean of these two groups and that of the pretrial apomorphine group, $F(1,21)=5.66$, $p<0.05$. The interaction was not significant, $F(8,84)=1.94$, $p>0.05$.

The topographical analysis of unconditioned (Session 1) and conditioned (Test Session) stereotypy is shown in Table 1. The unconditioned stereotypy produced by d-amphetamine consisted primarily of sniffing downward, $F(2,38)=28.92$, $p<0.01$. Although some repetitive head movements were observed in the pretrial amphetamine group, there were no significant differences among the three groups, $F(2,38)=2.64$, $p>0.05$. During the test session, the conditioned stereotypy consisted of sniffing downward only, $F(2,28)=12.06$, $p<0.01$. There were no group effects in gnawing, $F(2,38)=0.17$, $p>0.05$, or repetitive head movement, $F(2,38)=1.08$, $p>0.05$. The unconditioned stereotypy produced by apomorphine consisted primarily of sniffing downward, $F(2,38)=32.41$, $p<0.01$. There were no group effects for repetitive head movement or licking. During the test session apomorphine-conditioned stereotypy also consisted primarily of sniffing downward, $F(2,21)=7.35$, $p<0.01$. Some gnawing observed in the pretrial drug group was not statistically significant, $F(2,21)=1.59$, $p>0.05$.

The amplitude of the conditioned stereotypy observed in both the amphetamine and apomorphine groups was approximately one-third that of the unconditioned stereotypy, which is consistent with previous reports (22,23). This magnitude of conditioned stereotypy, consisting primarily of

TABLE 1
MEAN STEREOTYPY COUNTS

Behavior	1st Session SD	1st Session RHM	1st Session GN	Test Session SD	Test Session RHM	Test Session GN
Pretrial Amphetamine	81.5(10.1)	8.9(5.7)	0.0(—)	31.5(5.1)	0.1(0.1)	0.6(0.4)
Posttrial Amphetamine	24.4(2.6)	0.0(—)	0.7(0.5)	9.9(2.5)	0.0(—)	1.1(0.6)
Pretrial Saline	24.7(2.9)	0.0(—)	1.9(1.1)	12.6(1.7)	0.0(—)	0.9(0.7)
Significance	†	NS	NS	†	NS	NS

Behavior	1st Session SD	1st Session RHM	1st Session GN	Test Session LI	Test Session SD	Test Session GN
Pretrial Apomorphine	115.8(14.1)	0.1(0.1)	0.0(—)	2.4(2.4)	26.1(5.2)	0.4(0.3)
Posttrial Apomorphine	28.3(4.8)	0.0(—)	0.5(0.3)	0.0(—)	13.4(2.9)	3.4(2.5)
Pretrial Saline	26.3(4.5)	0.0(—)	0.9(0.7)	0.0(—)	7.3(1.8)	0.0(—)
Significance	†	NS	NS	NS	†	NS

Means (±SEM) of stereotypy counts of the first and test sessions in Experiment 1. Data were pooled from the five 90-sec observation periods. SD; sniffing downward; RHM; repetitive head movement; GN; gnawing; LI; licking. Statistical differences among the three groups are indicated as NS (nonsignificant), and †$p<0.01$.

sniffing downward, is equivalent to that observed in the no-drug control groups during the first training session (see Fig. 1 and Table 1). This issue will be addressed in Experiment 2.

Möller *et al.* (15) reported that gnawing and licking were conditioned at 0.5 and 2.0 mg/kg apomorphine and that sniffing and licking were conditioned with 0.18 mg/kg apomorphine. However, in Möller *et al.*'s study stereotypy was defined as the presence of licking or sniffing at least once during a 20-min period. In the present study, when stereotypy was defined as a repetitive behavior, only sniffing downward was conditioned, probably because of the predominance of this behavior as an unconditioned response at the doses tested. In our previous study (10), it was also found that sniffing downward, although not significant, was the only behavior observed on the test day for 0.2, 0.6, 0.8, 1.0 and 1.2 mg/kg apomorphine groups. Thus, the differences between Möller's and the present results might be due to differences in the criteria used to define stereotypy.

EXPERIMENT 2

In this experiment, an hypothesis about conditioned stereotypy based on a behavioral analysis of the conditioning procedure was tested. In the conditioning paradigm used in Experiment 1, the drug administration constituted the unconditioned stimulus (UCS), and the behavioral effect of these drugs (stereotypy) constituted the unconditioned response (UCR). The experimental boxes were the conditioned stimuli (CS), and the conditioned stereotypy observed on the test day was the conditioned response (CR).

In Experiment 1, the rats were exposed to the CS (the test boxes) for a 45-min period. However, as can be seen in Fig. 1, the peak UCR (the specific to-be conditioned behavior) did not persist during this entire period. Amphetamine stereotypy was at its peak during the 35- and 45-min periods after injection, whereas apomorphine stereotypy was at its peak during the 15- and 25-min periods. Therefore, during a large part of their time in the test boxes, the animals were exposed to the CS in the absence of the peak effect. If the peak effect is the UCR, then this procedure should reduce the amplitude of the conditioned response due to the effect called latent inhibition (14).

The hypothesis tested in this experiment was that the UCR was not the entire course of stereotypy produced by the drugs administered but rather only the peak behavioral effect observed. On the basis of this hypothesis, it can be predicted that if rats are exposed to the CS only during the peak effect of the drugs, higher amplitude conditioned stereotypy than that observed in Experiment 1 should be established.

On the basis of the data of Experiment 1 (Fig. 1) and of data from a pilot study, exposure of the animals to the test boxes in the training sessions in this experiment was limited to 10 min: 35–45 min after injections for amphetamine and 10–20 min after injections for apomorphine.

METHOD

Subjects

The subjects were forty-five male hooded rats similar to those used in Experiment 1.

Apparatus

The apparatus was identical to that used in Experiment 1.

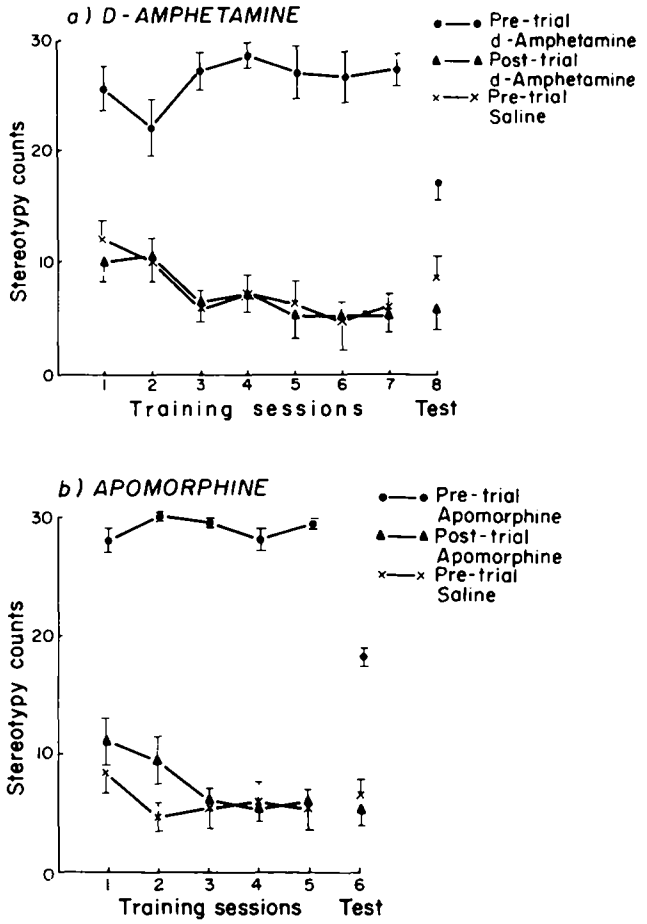

FIG. 2. Unconditioned (Training Session) and conditioned (Test Session) stereotypy induced by (a) d-amphetamine (4.0 mg/kg) and (b) apomorphine (0.4 mg/kg) with 10-min drug-environment pairings.

Procedure

There were pretrial drug, pretrial saline, and posttrial drug groups for both d-amphetamine (4.0 mg/kg) and apomorphine (0.4 mg/kg). Eight rats randomly assigned to each group were used for each d-amphetamine group and seven were used for each apomorphine group. Animals in the amphetamine groups were placed in the test boxes 35 min after injection and left there for 10 min. Animals in the apomorphine groups were placed in the test boxes 10 min after injection and left there for 10 min. All animals were kept in their home cages during the interval between injection and the start of the trials. The pretrial drug and saline groups received injections prior to training trials but no treatment after the trials. Animals in the posttrial drug groups received no treatment before the trials; after the training trials, they were returned to their home cages and received drug injections 4 hours later. In the test session, the two pretrial drug and saline groups received saline injections prior to the test trial; the posttrial group received no treatment.

In both training and test sessions, a single 90-sec behavioral observation was made for each rat starting 5 min after it had been placed in the box.

RESULTS AND DISCUSSION

The procedure used in this experiment produced an amplitude of conditioned stereotypy equal to about two-thirds of the amplitude of unconditioned stereotypy (Fig. 2a,b). This is about twice the amplitude of the conditioned stereotypy observed using the more conventional training procedure in Experiment 1.

A one-way independent-group analysis of variance was calculated on the data for the test session. For the amphetamine groups, the treatment effect was significant, $F(2,21)=13.04$, $p<0.01$. Planned comparisons showed that there was no significant difference between the posttrial amphetamine and pretrial saline groups in the test session, $F(1,21)=1.09$, $p>0.05$. The mean of these two groups was significantly different from that of the pretrial d-amphetamine group, $F(1,21)=25.00$, $p<0.01$.

The d-amphetamine unconditioned stereotypy in the first session consisted of sniffing downward, $F(2,21)=15.77$, $p<0.01$, and repetitive head movement, $F(2,21)=14.00$, $p<0.01$. On the test day, only sniffing downward was found to be significant, $F(2,21)=14.22$, $p<0.01$ (Table 2).

For the apomorphine groups, an analysis of variance showed that there was a significant treatment effect in the test session, $F(2,18)=37.80$, $p<0.01$. Planned comparisons showed that there was no significant difference between the pretrial saline group and the posttrial apomorphine group, $F(1,18)=0.38$, $p>0.05$. There was a significant difference between the mean of these two groups and the mean of the pretrial apomorphine group, $F(1,18)=75.23$, $p<0.01$.

Apomorphine-unconditioned stereotypy consisted primarily of sniffing downward, $F(2,18)=44.87$, $p<0.01$; apomorphine-conditioned stereotypy consisted solely of sniffing downward, $F(2,18)=37.45$, $p<0.01$ (Table 2).

In previous studies, fairly long conditioning sessions ranging from 25 min (22,23) to 60 min (15) were used and the conditioned stereotypy observed was relatively weak. By increasing the amplitude of conditioned stereotypy, the procedure used in the present experiment supports the hypothesis that the unconditioned response is the drugs' peak effect which is assumed to be produced by a specific stimulation level of dopamine receptors (UCS).

Operationally defined, the procedure used in the present experiment was backward conditioning because the CS (exposure to the test box) came after the UCS (drug administration). However, based upon the hypothesis described above, in which the UCS was a brief period of a specific level of dopamine receptor stimulation which then causes the behavioral peak effect, the procedure was simultaneous conditioning. This analysis supports the hypothesis that the UCS for conditioned stereotypy is the brief period of a specific level of dopamine receptor stimulation that coincides with the observed period of maximal stereotypy (UCR) produced by 4.0 mg/kg d-amphetamine and 0.4 mg/kg apomorphine.

There is evidence, however, that a mere behavioral peak effect does not constitute the UCS because it was found in our previous study (10) that 0.8, 1.0 and 1.2 mg/kg of apomorphine, which produced a peak effect during the entire 45-min training session, failed to establish conditioned stereotypy. Furthermore, it seems that the optimal UCS occurs at a specific interval after injection because it was found in pilot studies that 10-min exposures of the animals to the CS at other intervals (0 min after d-amphetamine injections and 20 min after apomorphine injections) produced weaker conditioned stereotypy than that observed at the intervals

TABLE 2
MEAN STEREOTYPY COUNTS

	1st Session			Test Session			
Behavior	SD	RHM	LI	SD	RHM	GN	NP
Pretrial Amphetamine	23.8(2.2)	1.0(0.3)	0.0(—)	17.4(1.5)	0.3(0.2)	0.0(—)	0.1(0.1)
Posttrial Amphetamine	10.0(1.8)	0.0(—)	0.0(—)	5.8(1.6)	0.0(—)	0.3(0.2)	0.1(0.1)
Pretrial Saline	11.9(1.6)	0.0(—)	0.1(0.1)	8.4(1.8)	0.0(—)	0.1(0.1)	0.1(0.1)
Significance	†	†	NS	†	NS	NS	NS

	1st Session		Test Session	
Behavior	SD	GN	SD	GN
Pretrial Apomorphine	28.0(0.9)	0.0(—)	18.3(0.8)	0.1(0.1)
Posttrial Apomorphine	11.3(2.0)	0.0(—)	5.7(1.2)	0.0(—)
Pretrial Saline	8.3(1.7)	0.1(0.1)	6.7(1.3)	0.0(—)
Significance	†	NS	†	NS

Means (±SEM) of stereotypy counts of the first and test sessions in Experiment 2. SD; sniffing downward; RHM; repetitive head movement; LI; licking; GN; gnawing; NP; nose poking. Statistical differences among the three groups are indicated as NS (nonsignificant), and †$p<0.01$.

reported here. Therefore, the optimal UCS must preferentially occur at a certain time after injection of a certain dose of dopamine agonists.

EXPERIMENT 3

The second issue addressed in the present study is the nature of the neural change associated with conditioned stereotypy. There would appear to be two possibilities with respect to the precise locus of this neural change. One possibility is presynaptic. A presynaptic change might take the form of increased dopamine release in the presence of the conditioned stimuli. The other possibility is that the neural change associated with conditioned stereotypy is a postsynaptic event. Such a change could take place in the postsynaptic neuron or neural network that is activated by dopamine receptor stimulation.

If the neural change representing conditioned stereotypy is in some postsynaptic sites, activation of these sites by dopamine might be unnecessary for the expression of conditioned stereotypy once it is acquired. If postsynaptically-mediated conditioned stereotypy can occur in the absence of dopaminergic activation, pimozide, which is a dopamine receptor blocker, would have no effect on this behavior. Alternatively, if dopaminergic activation of postsynaptic receptors mediates conditioned stereotypy, pimozide would be expected to block this behavior.

In the first part of this experiment, the effect of pimozide on d-amphetamine- and apomorphine-unconditioned stereotypy was determined. In a pilot study several doses of pimozide, ranging between 0.05 and 0.5 mg/kg, were tested for their ability to block amphetamine and apomorphine stereotypy. Doses of 0.25 and 0.2 mg/kg of pimozide were selected for use with amphetamine and apomorphine, respectively on the basis of two criteria: 1) these doses completely blocked the drug-induced unconditioned stereotypy in both cases; 2) neither dose produced significant decreases in normal levels of motor function.

In the second part of this experiment the effect of these doses of pimozide on conditioned stereotypy was examined.

METHOD

Subjects

The subjects were ninety-four male hooded rats similar to those used in Experiment 1.

Apparatus

The apparatus was identical to that used in Experiment 1.

Procedure

The procedure of the present experiment was identical to that of Experiment 2 except for pimozide injections. There were pretrial drug, posttrial drug and pretrial saline groups for both amphetamine and apomorphine.

In the first part of the experiment, six rats were used for each amphetamine and apomophine group.

Pimozide was dissolved in boiling tartaric acid (0.2 μmol/ml) and then cooled to room temperature before injections. Pimozide was injected four hours before drug or saline injections, which were given 35 min or 10 min before each trial for the amphetamine and apomorphine groups, respectively. Pimozide, 0.2 and 0.25 mg/kg, was used for the

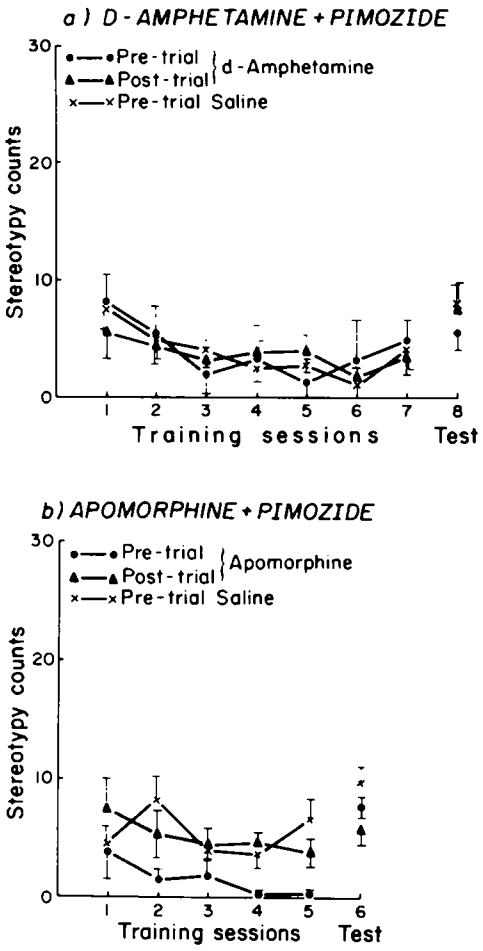

FIG. 3. The blocking effect of pimozide on unconditioned stereotypy. Mean stereotypy counts for (a) d-amphetamine (4.0 mg/kg) and (b) apomorphine (0.4 mg/kg) stereotypy challenged by pimozide 0.25 and 0.2 mg/kg, respectively.

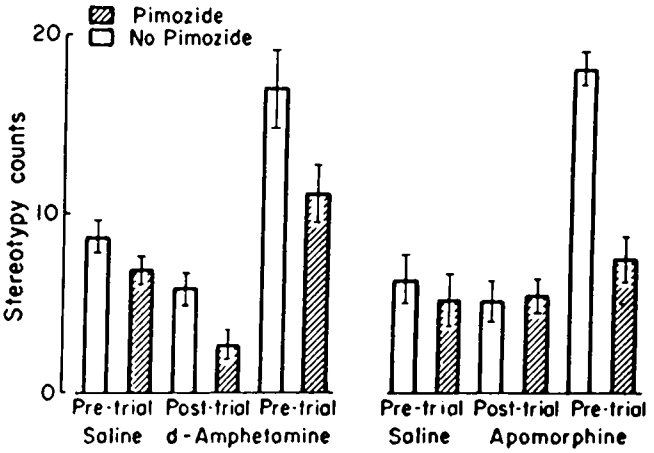

FIG. 4. Effects of pimozide on d-amphetamine- and apomorphine-conditioned stereotypy. The dopamine agonists were not administered on the test day. Pimozide, 0.25 mg/kg and 0.2 mg/kg, injected 4 hours before testing, was used for d-amphetamine- and apomorphine-conditioned stereotypy, respectively.

apomorphine and d-amphetamine groups, respectively. For the test sessions, saline injections given on the same schedule were substituted for all drug injections.

In the second part of the experiment using different animals, the effect of pimozide on both amphetamine- and apomorphine-conditioned stereotypy was investigated. Eight and twelve rats were used for each apomorphine and amphetamine group, respectively. Two rats in the amphetamine groups died in the sixth conditioning session. No pimozide injections were given on the conditioning days. Pimozide was injected four hours before saline injections on the test sessions only.

RESULTS AND DISCUSSION

Figure 3 shows the data for the first part of the experiment, in which pimozide was given on the training days. The complete blockade of unconditioned stereotypy, as well as the absence of conditioned stereotypy, are evident.

A one-way independent-group design was used to analyze the data for each drug in each session. Pimozide (0.25 mg/kg) blocked amphetamine-induced unconditioned stereotypy over the seven conditioning sessions (Fig. 3a) No treatment effects were found in the first, $F(2,15)=0.58$, $p>0.05$, second, $F(2,15)=0.09$, $p>0.05$, third, $F(2,15)=0.60$, $p>0.05$, fourth, $F(2,15)=0.39$, $p>0.05$, fifth, $F(2,15)=1.26$, $p>0.05$, sixth, $F(2,15)=1.39$, $p>0.05$, or seventh, $F(2,15)=0.09$, $p>0.05$, conditioning sessions. In the drug-free test session no conditioned stereotypy was observed, $F(2,15)=0.35$, $p>0.05$.

Pimozide (0.2 mg/kg) also blocked apomorphine-induced unconditioned stereotypy over the five conditioning sessions (Fig. 3b). There were no treatment effects in the first, $F(2,15)=0.85$, $p>0.05$, and third, $F(2,15)=1.04$, $p>0.05$, conditioning sessions. The stereotypy counts of the pretrial apomorphine group were significantly suppressed in the second, $F(2,15)=4.02$, $p<0.05$, fourth, $F(2,15)=8.17$, $p<0.01$, and fifth, $F(2,15)=7.19$, $p<0.01$, conditioning sessions. Conditioned stereotypy was not observed in the drug-free test session, $F(2,15)=3.10$, $p>0.05$.

The finding that conditioned stereotypy was not observed on the test day shows that dopamine receptor stimulation is essential for the acquisition of this behavior.

It is clear from the data for the first day of Experiment 3-1 that the doses of pimozide used blocked drug-induced unconditioned stereotypy while failing to reduce the normal activity levels. Although repeated administration of pimozide did produce some evidence of reduced activity levels in the apomorphine groups, the first day data are the relevant ones for comparison with the test day in Experiment 3-2, where pimozide was administered for the first time.

In the second part of the experiment, when the effective doses of pimozide were administered four hours before saline injections on the test day, there was a differential effect of pimozide on conditioned stereotypy established by d-amphetamine and apomorphine (Fig. 4). These data were analyzed together with those of Experiment 2 where conditioned stereotypy in the absence of pimozide was observed. A two-way independent-group analysis of the d-amphetamine groups showed that there was a significant difference between pimozide and nonpimozide groups,

$F(1,52)=15.12$, $p<0.01$, with no interaction, $F(2,52)=0.76$, $p>0.05$, indicating that the stereotypy counts of all three groups were lowered by pimozide (0.25 mg/kg). There was also a significant overall group effect, $F(2,52)=29.85$, $p<0.01$. Planned comparisons were used to investigate the nature of the significant overall group effect for the data of the pimozide groups. There was no difference between the pretrial saline and posttrial d-amphetamine groups, $F(1,31)=3.76$, $p>0.05$. There was a significant difference between the mean of these two groups and that of the pretrial d-amphetamine group, $F(1,31)=29.40$, $p<0.01$, indicating that d-amphetamine-conditioned stereotypy was not completely blocked.

A two-way independent-group analysis was performed for the apomorphine groups. Since there was a significant interaction effect, $F(2,39)=8.83$, $p<0.01$, simple main effects were examined. There were no significant differences among the three pimozide groups of Experiment 3-2, $F(2,39)=1.09$, $p>0.05$. A simple main effect within each apomorphine group revealed that there were no differences between the two pretrail saline groups tested with and without pimozide, $F(1,39)=0.69$, $p>0.05$, and between the two posttrial apomorphine groups tested with and without pimozide, $F(1,39)=0.09$, $p>0.05$, indicating that pimozide (0.2 mg/kg) did not produce a cataleptic effect. Planned comparisons showed that there was no difference between the pretrial saline group and the posttrial apomorphine group tested with pimozide, $F(1,21)=0.00$, $p>0.05$. The mean of these two groups was not significantly different from that of the pretrial apomorphine group, $F(1,21)=1.71$, $p>0.05$, indicating that pimozide completely blocked apomorphine-conditioned stereotypy.

In the present study, amphetamine-conditioned stereotypy was not completely blocked by pimozide, suggesting that the neural change representing conditioned stereotypy takes place at least partly at some postsynaptic site. However, apomorphine-conditioned stereotypy was blocked by pimozide, a finding that is inconsistent with the conclusion suggested by the amphetamine data, and supports the alternative hypothesis that conditioned stereotypy is mediated by released dopamine acting on postsynaptic receptors.

The notion of postsynaptic involvement in conditioned stereotypy is suggested by the resistance of amphetamine-conditioned stereotypy to the blocking effect of pimozide in the present study, and in previous studies reviewed in the Introduction. Because 0.25 mg/kg of pimozide was shown to block amphetamine-unconditioned stereotypy completely in Experiment 3-1, the possibility that the blocking effect was incomplete can be ruled out.

Another possible explanation, that pimozide produced the stereotypy observed on the test day through its action of promoting dopamine release by blockade of presynaptic autoreceptors, can also be ruled out for two reasons: 1) the two control groups that received pimozide showed no eivdence of stereotypy, and 2) the complete blockade of drug-induced stereotypy demonstrated in Experiment 3-1 would also have blocked any stereotypy that might have been produced by pimozide-induced dopamine release.

Yet, because amphetamine-conditioned stereotypy was reduced by pimozide and the two control groups also showed reduction in stereotypy counts, there are the possibilities that this conditioned behavior is at least partly mediated by released dopamine or that pimozide causes some motor impairment after repeated administration of d-amphetamine.

Poncelet *et al.* (19) reported that amphetamine-conditioned stereotypy and locomotor activity were blocked by clonidine, which decreases norepinephrine release. They suggested that the resistance of amphetamine-conditioned stereotypy and locomotor activity to pimozide may not necessarily indicate postsynaptic involvement of purely dopamine-activated systems, but may suggest conditioned stereotypy and locomotor activity are mediated by noradrenergic systems. However, clonidine produces sedation within the ranges of doses used in Poncelet's study (7). Thus, the blockade of d-amphetamine-conditioned stereotypy by clonidine might have been due to a secondary effect of clonidine on motor movement rather than on conditioned stereotypy itself.

The resistance of d-amphetamine-conditioned stereotypy to pimozide suggests that this learned behavior is at least partly independent of dopamine release, and that it may be mediated by some postsynaptic neural change. Such a change might be produced by dopamine release during the conditioning sessions. However, the data suggest that once this change has occurred it becomes at least partly independent of dopamine function.

The finding that apomorphine-conditioned stereotypy was completely blocked by pimozide suggests that dopamine receptor activation by released dopamine is necessary for the expression of apomorphine-conditioned stereotypy. Motor impairment by pimozide, which would have deprived rats of the capacity to show conditioned stereotypy, can be ruled out because pimozide did not decrease the stereotypy counts of the two control groups.

Another possible presynaptic mechanism of apomorphine-conditioned stereotypy could be a rebound of excitability of dopamine neurons in the test session following six sessions of suppression by daily apomorphine injections via the presynaptic receptors (autoreceptors). However, there is evidence that this does not occur. Selective stimulation of the presynaptic dopamine autoreceptors causes a decreased function of the dopamine neurons, which is expressed as hypomotility. Hypomotility was shown to be conditioned as hypomotility (15).

As discussed in the Introduction, apomorphine-unconditioned stereotypy is probably a postsynaptic phenomenon. Thus, it is highly likely that the neural change involved in apomorphine-conditioned stereotypy occurs at some postsynaptic site(s). Nevertheless, the present results suggest that activation of postsynaptic sites (which were blocked by pimozide in the present experiment) by dopamine released from presynaptic neurons is necessary for apomorphine-conditioned stereotypy. This suggests the possibility that apomorphine-conditioned stereotypy may be expressed by released dopamine mediated by a neuronal loop which influences dopaminergic terminals. This idea is discussed further in the General Discussion section.

The differential effects of pimozide on d-amphetamine and apomorphine-conditioned stereotypy were unexpected findings. It remains unclear why the two responses, both mediated by dopamine systems, seem to be mediated by different neuronal mechanisms. Further work is needed to explain these effects.

GENERAL DISCUSSION

A key question in Experiments 1 and 2 was the precise identification of the UCS and the UCR in conditioned stereotypy. The hypothesis that the UCS is a specific level of stimulation of dopamine receptors expressed as maximal stereotypy (UCR) was supported in the two experiments.

The interpretation that the UCS was a specific level of stimulation of dopamine receptors expressed as the peak effect of stereotypy suggested that an operational definition of stimuli and responses in pharmacological conditioning is not valid. Traditionally, stimuli and responses have been defined from an operational point of view. Physical events and overt responses are labelled as stimuli and responses. This view has produced confusion in understanding the nature of pharmacological conditioning. Taste aversion, for example, was reported to be formed by backward conditioning (3,6) which is generally ineffective in forming classical conditioning (21). In these studies, the UCS was defined as the drug administration which preceded the CS taste stimulus. However, the illness produced by the UCS occurs with a delay so that, in fact, the UCR occurs after the CS (8). Although taste aversion is backward conditioning from a purely operational point of view, it can be seen as forward conditioning from a pharmacological viewpoint. In support of the idea that the UCS is a pharmacological rather than a behavioral event, it has been demonstrated that conditioning of d-amphetamine-induced locomotor activity is observed on a drug-free test day even if the acutal occurrence of locomotor activity is prevented on training days (25). This also suggests that it is inappropriate to define stimuli and responses in physical terms.

In Experiment 3, the question of the locus of the neural mechanisms responsible for conditioned stereotypy was addressed. The finding that apomorphine-conditioned stereotypy was blocked by pimozide suggested that the conditioned neural event might be the release of dopamine from the terminals of presynaptic neurons. A possible explanation of this phenomenon involves postulating the existence of a neural loop.

Neuroanatomical data have shown that sniffing downward, which was the main conditioned behavior, is mediated by the mesolimbic dopamine system projecting to the nucleus accumbens (4, 12, 13). The nucleus accumbens has efferent projections to the globus pallidus, substantia innominata, preoptic region, bed nucleus of the stria terminalis, lateral septal nucleus, lateral and dorsal hypothalamic nuclei, amygdala, substantia nigra, septum, habenula, paratenialis and dorsomedial nuclei of the thalamus (16,24). Afferents to the nucleus accumbens are from the ventral tegmental area of Tsai, insular cortex, perirhinal cortex, entorhinal cortex, primary olfactory cortices, subiculum, hippocampus, intralaminar nuclei of the thalamus and amygdala (17,24). Thus, the amygdala seems to be the only brain area which has reciprocal projections with the nucleus accumbens. On the basis of these neuroanatomical facts, it can be suggested that the amygdala which gives rise to axons to the nucleus accumbens may make axo-axonal contact with the terminals of the mesolimbic dopamine pathway. Some neural change responsible for conditioned stereotypy may be formed in the amygdala which receives afferents from the nucleus accumbens. Thus, the amygdala might control the release of dopamine in the nucleus accumbens by the axo-axonal contact.

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