The amphetamine conditioned place preference: differential involvement of dopamine receptor subtypes and two dopaminergic terminal areas

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We investigated involvement of dopamine receptor subtypes and two dopaminergic terminal areas in the acquisition and the expression of the amphetamine conditioned place preference (CPP). When injected systemically before conditioning, both D1 and D2 dopamine antagonists blocked acquisition in a dose-dependent manner. When injected systemically before testing, the effects of the same D1 and D2 antagonists differed. The selective D1 antagonist SCH23390 dose-dependently blocked expression of the previously established conditioned behavior within the dose range that also blocked acquisition. In contrast, D2 antagonists failed to block expression of the amphetamine CPP at doses which blocked acquisition. Expression was, however, blocked by higher doses of D2 antagonists, which may have lost their selectivity for the D2 dopamine receptor. The expression of the CPP was also blocked by microinjections of SCH23390 or sulpiride into nucleus accumbens, but not into striatum. In a control experiment, sodium pentobarbital, which significantly reduced spontaneous locomotor activity in a manner similar to the higher doses of the dopamine antagonists, had no effect on the expression of the amphetamine CPP when given before testing. Finally, electrolytic lesions of the dorsal striatum potentiated the amphetamine CPP. These findings indicate that the dopamine released by amphetamine interacts with both D1 and D2 dopamine receptors to establish a CPP, but that the expression of the CPP may involve activation of the D1 dopamine receptor in the nucleus accumbens.

INTRODUCTION

Activation of dopamine systems causes acute behavioral changes in animals and humans and long-lasting behavioral modifications. Food, stimulants, and medial forebrain bundle stimulation unconditionally evoke dopamine release and establish incentive learning such as that measured in the conditioned place preference (CPP) paradigm.

It is most likely that activation of the mesolimbic dopamine pathway is the basis of this incentive learning. Microinjections of amphetamine into nucleus accumbens, but not other dopamine terminal areas, establish CPPs. The establishment of the amphetamine CPP is impaired by 6-OHDA lesions of the nucleus accumbens. A dopamine receptor antagonist injected into nucleus accumbens blocks acquisition of the CPP by stimulation of the medial forebrain bundle.

Another property of the mesolimbic dopamine system is that it is activated by conditioned stimuli. These are originally neutral stimuli that have been paired with events that unconditionally evoke dopamine release. When a conditioned stimulus is presented, cell bodies in VTA and medial substantia nigra fire and concentrations of dopamine metabolites increase in the nucleus accumbens. The importance of dopamine release for the expression of incentive learning was demonstrated by the finding that the expression of the amphetamine CPP was blocked by α-flupenthixol injected into the nucleus accumbens or by reserpine, a vesicle depletor.

Studies have shown that the acquisition of the amphetamine CPP is blocked by both selective D1 and D2 dopamine antagonists, and pharmacological stimulation of either dopamine receptor subtype in nucleus accumbens establishes CPPs, probably provided that the other subtype is tonically activated by endogenous dopamine release. However, little is known about how the two types of dopamine receptors are involved in the expression of the amphetamine CPP during testing. Also unknown is the involvement of the striatal dopamine system in CPP expression during testing. The findings that dopamine depletion in the nucleus accumbens attenuates the amphetamine CPP and microinjections...
of amphetamine into nucleus accumbens\textsuperscript{4,13,14}, but not into the striatum\textsuperscript{13,14}, establish CPPs, do not rule out the possibility that expression of the amphetamine CPP involves the nigrostriatal dopamine pathway. We have shown that microinjections of \( \alpha \)-flupenthixol into nucleus accumbens abolished the expression of the amphetamine CPP\textsuperscript{32}. However, it is possible that this drug spread into adjacent areas such as striatum and olfactory tubercle, although the olfactory tubercle does not seem to be involved in the expression of the amphetamine CPP since 6-OHDA lesions of this structure have no effect on this behavior\textsuperscript{15}.

The present study was designed to investigate the roles of dopamine receptor subtypes and the nigrostriatal and mesolimbic dopamine pathways in both the acquisition and the expression of the amphetamine CPP. First, we established dose–response curves for the blocking effects of D\(_1\) and D\(_2\) dopamine antagonists on acquisition and expression of the amphetamine CPP. Second, a selective D\(_1\) or D\(_2\) antagonist was injected into nucleus accumbens or striatum prior to testing to examine the relative roles of D\(_1\) and D\(_2\) dopamine receptors in these two areas in the expression of the amphetamine CPP. Third, sodium pentobarbital was used to examine the effect of performance impairment on expression, as a control for neuroleptic-induced performance impairment. Fourth, lesions were made in the dorsal striatum, the main target area of the nigrostriatal dopamine pathway, in order to examine the role of this pathway in the amphetamine CPP.

Some of the present results were previously presented in a preliminary form\textsuperscript{31}.

**EXPERIMENTS AND RESULTS**

**General methods**

**Subjects**

The subjects were 434 experimentally naive male Long-Evans rats purchased from Charles River Canada, St-Constant, Que., weighing 275–310 g at the start of the experiments. The animals were individually housed with food and water available ad libitum.

**Apparatus**

*Conditioned place preference.* The CPP apparatus was made of wood, with a Plexiglas front wall. It consisted of 3 different compartments, 2 of which were identical in size (45 \( \times \) 45 \( \times \) 30 cm). One compartment was painted white and had wood chips on a smooth floor. The other was painted black with white vertical stripes and had a floor of wire mesh. A few drops of vinegar (1 ml 2% acetic acid) were placed on the floor of this compartment below the wire mesh. These two compartments were completely separated from each other by a wooden partition. An entrance tunnel (36 \( \times \) 18 \( \times \) 20 cm) protruding to the rear of the large compartments, connected the two entrances. On conditioning days the entrances to the tunnel were blocked. The entrances were open on the pre-exposure and test days. In previous studies, we demonstrated that on a group basis, rats do not exhibit a natural preference for either compartment\textsuperscript{13,15}.

*Locomotor activity.* Locomotor activity was measured in 3 identical open-field boxes (41 \( \times \) 41 \( \times \) 28 cm) constructed of Plexiglas. The floors had nine 3 cm holes in them. Eight photocell beams, 4 in each direction, located 3 cm above the floor, partitioned the box into 25 cells. The total number of photobeam interruptions during a test was taken as the locomotor activity score.

*Cannulae.* Guide cannulae were made from 20 g (0.7 mm outer diameter) hypodermic needles. The plastic hubs were removed and the needles were cut to a length of 12.2 mm. Inner cannulae, used for microinjections, were made from 30 g (0.3 mm outer diameter) needles. They were cut to a length of approximately 30.0 mm and bent so that the tip of the inner cannulae extended beyond the tip of the guide cannula by 2.5 mm for intra-accumbens injections or 0.5 mm for intra-striatal injections. The inner cannulae were attached to a 5.0 \( \mu \)l Hamilton syringe with PE 10 tubing.

*Electrodes.* Nichrome electrodes (0.25 mm in diameter) with enamel insulation were used for electrolytic lesions. The tips (0.8 mm) of the electrodes were decoated with Strip X (GC Electronics).

**Procedures**

*Conditioned place preference.* The procedure required 6 sessions. On session 1 the rats were given a 10 min pre-exposure period: each one was allowed to move freely in the three compartments of the test apparatus. The next 4 sessions included 2 pairings with \( \alpha \)-amphetamine (2.0 mg/kg s.c.) and 2 pairings with saline. Animals in each drug and dose group were randomly assigned to the cells of a 2 \( \times \) 2 factorial design. One factor was pairing compartment (black or white) and the other was injection order. Half of the rats received \( \alpha \)-amphetamine injections before exposure to the white compartment, and the other half received amphetamine injections before exposure to the black compartment. Within each half of these subgroups, half of the rats received \( \alpha \)-amphetamine injections on even numbered sessions and saline injections on odd numbered sessions; the pattern was reversed for the remaining rats. The animals were placed into the appropriate compartment immediately after receiving s.c. injections of amphet-
amine or saline, and left there for 30 min. The entrances to the compartments were blocked so that the animals were confined to the compartments. The sixth session was the test day on which no amphetamine was injected, and the entrances to the compartments were open. The animals were placed into the tunnel and allowed to move freely in the 3 compartments for 20 min. The amount of time spend in each of the 2 large compartments was recorded by a rater who was not aware of the treatments each animal received.

**Locomotor activity.** Locomotor activity was measured for 20 min after animals were placed into the locomotor activity boxes.

**Drugs.** d-Amphetamine (Smith, Kline & French, Canada) was prepared as 2.0 mg of the salt/ml of physiological saline. SCH23390 (Schering Corp.), α-flupenthixol and metoclopramide (Nordic Laboratories Inc.) were dissolved in saline. Sulpiride (Research Biochemicals Inc.) was dissolved in 0.1 N HCl and diluted with distilled water. All the antagonists were adjusted to pH 6.5-7.0 with sodium hydroxide. Sodium pentobarbital was dissolved in a solution of 10% ethanol in 40% propylene glycol.

**Experiment 1**

This experiment examined the effects of systemically injected D1 and D2 dopamine antagonists on the acquisition and the expression of the amphetamine CPP.

**Methods**

Two sets of groups (n = 8 for each group) were used. One set of groups underwent the experimental procedure described in the CPP procedure but also received dopamine antagonist injections before each of the 4 conditioning sessions. The other set of groups was given the antagonist injections before the test session, but not before the conditioning sessions. The intervals between injections and the conditioning or test sessions were 30 min for SCH23390 (0.02–0.16 mg/kg), 150 min for α-flupenthixol (0.2–1.0 mg/kg), 45 min for metoclopramide (1.25–20 mg/kg), and 60 min for sulpiride (10–160 mg/kg).

**Results**

Fig. 1 shows the effects of dopamine antagonists on the acquisition and the expression of the amphetamine CPP. The control group showed a statistically significant CPP (P < 0.05). On the average, rats in this group spent 553 and 235 s in the amphetamine-paired and unpaired compartments, respectively, during testing. It is evident that as doses of the antagonists increased the time differences between the amphetamine-paired and saline paired compartments decreased. Two-way ANOVAs with planned comparisons were used to analyze the data using doses as one factor and compartments as the other factor (repeated factor).

SCH23390. Both the acquisition and expression of the CPP were blocked at approximately equivalent doses of this D1 receptor antagonist. As the figure reveals, the time differences approached zero as the doses increased. 0.16 mg/kg (the highest dose used) of the drug blocked both processes equipotently. For the groups given SCH23390 before the conditioning sessions, planned comparisons showed that there were significant differences between time spent in the paired and unpaired compartments at 0.04 mg/kg ($F_{1,35} = 14.17, P < 0.01$) and 0.08 mg/kg ($F_{1,35} = 4.88, P < 0.05$). These differences were not significant at 0.12 mg/kg ($F_{1,35} = 3.36, P > 0.05$) and 0.16 mg/kg ($F_{1,35} = 0.71, P > 0.05$). On the average, the 0.16 mg/kg group spent 430 and 363 s in the amphetamine-paired and unpaired compartments, respectively. For the groups given this drug before testing, there were significant time differences at 0.02
mg/kg ($F_{1,42} = 4.69, P < 0.05$), 0.04 mg/kg ($F_{1,42} = 4.54, P < 0.05$), 0.08 mg/kg ($F_{1,42} = 9.54, P < 0.01$) and 0.12 mg/kg ($F_{1,42} = 4.38, P < 0.05$), but not at 0.16 mg/kg ($F_{1,42} = 0.67, P > 0.05$). On the average, the 0.16 mg/kg group spent 421 and 326 s in the amphetamine-paired and unpaired compartments, respectively. 0.12 mg/kg blocked acquisition, but not expression. A direct comparison of the two groups, however, revealed that the time difference between the two compartments for the acquisition group was not significantly different from that for the expression group at this dose ($F_{1,28} = 1.40, P > 0.05$).

**a-Flupenthixol.** Both acquisition and expression were blocked by this antagonist; higher doses were required to block expression than acquisition. For the groups given this drug before the conditioning sessions, planned comparisons revealed that there were significant time differences at 0.2 mg/kg ($F_{1,28} = 12.30, P < 0.01$) but not at 0.1 mg/kg ($F_{1,28} = 0.74, P > 0.05$) and 0.8 mg/kg ($F_{1,28} = 2.55, P > 0.05$). On the average, rats given 0.8 mg/kg of a-flupenthixol during conditioning spent 354 and 470 s in the amphetamine-paired and unpaired compartments, respectively. For the groups given this drug before the test session, the differences were significant at 0.2 mg/kg ($F_{1,34} = 5.11, P < 0.05$) and 0.4 mg/kg ($F_{1,34} = 7.65, P < 0.01$) but not at 0.8 mg/kg ($F_{1,34} = 0.82, P > 0.05$) and 1.0 mg/kg ($F_{1,34} = 0.02, P > 0.05$). On the average, the 1.0 mg/kg group spent 454 and 475 s in the amphetamine-paired and unpaired compartments, respectively. A significant rightward shift of the antagonist curves from acquisition to expression is evidenced by the differential effects of 0.4 mg/kg (the second lowest dose) on acquisition and expression. The time difference of the acquisition group was significantly different from that of the expression group at this dose ($F_{1,28} = 44.88, P < 0.01$).

**Metoclopramide.** For the groups that received this D2 receptor antagonist before the conditioning sessions, the time differences were significant at 1.25 mg/kg ($F_{1,35} = 17.18, P < 0.01$), 2.5 mg/kg ($F_{1,35} = 12.00, P < 0.01$) and 5.0 mg/kg ($F_{1,35} = 6.64, P < 0.05$). The time difference was not significant at 10 mg/kg ($F_{1,35} = 0.52, P > 0.05$). On the average, rats in this group spent 371 and 427 s in the amphetamine-paired and unpaired compartments, respectively. For the test-day groups, the differences were significant at 1.25 mg/kg ($F_{1,42} = 7.14, P < 0.01$), 5.0 mg/kg ($F_{1,42} = 9.95, P < 0.01$) and 10.0 mg/kg ($F_{1,42} = 19.27, P < 0.01$), but not significant at 2.5 mg/kg ($F_{1,42} = 3.77, P > 0.05$) and 20 mg/kg ($F_{1,42} = 0.49, P > 0.05$). On the average, rats given 20 mg/kg of metoclopramide during testing spent 525 and 444 s in the amphetamine-paired and unpaired compartments, respectively. The highest pre-conditioning dose (10 mg/kg) of metoclopramide blocked acquisition but not expression: the time difference of the acquisition group was significantly different from that of the expression group ($F_{1,28} = 54.51, P < 0.01$).

**Sulpiride.** The acquisition of the amphetamine CPP was blocked at 40 mg/kg ($F_{1,35} = 3.47, P < 0.05$) and 80 mg/kg ($F_{1,35} = 0.19, P > 0.05$) but not at 10 mg/kg ($F_{1,35} = 6.43, P < 0.05$) and 20 mg/kg ($F_{1,35} = 6.79, P < 0.05$) when this D2 receptor antagonist was given before the conditioning sessions. On the average, rats given 80 mg/kg of sulpiride during conditioning spent 414 and 368 s in the amphetamine-paired and unpaired compartments, respectively. When given before the test session this drug blocked the expression of the amphetamine CPP at 160 mg/kg ($F_{1,49} = 0.02, P > 0.05$) but not at 10 mg/kg ($F_{1,49} = 10.39, P < 0.01$), 20 mg/kg ($F_{1,49} = 6.81, P < 0.05$), 40 mg/kg ($F_{1,49} = 9.31, P < 0.01$), 80 mg/kg ($F_{1,49} = 12.99, P < 0.01$) and 120 mg/kg ($F_{1,49} = 4.39, P < 0.05$). On the average, rats given 160 mg/kg of sulpiride during testing spent 484 and 468 s in the amphetamine-paired and unpaired compartments, respectively. 80 mg/kg of sulpiride completely blocked acquisition but not expression. The time difference of the acquisition group was significantly different from that of the expression group ($F_{1,28} = 13.94, P < 0.01$).

When given before the test session, all the antagonists potentiated expression of the amphetamine CPP at certain doses. However, none of the potentiated preferences was significantly different from the preference observed for the control group except for 10 mg/kg of metoclopramide group ($F_{1,84} = 8.94, P < 0.01$).

Visual observation revealed that all the dopamine antagonists decreased locomotion. The blockade of acquisition is, however, not due to this side effect of the antagonists, since simple inhibition of locomotion does not prevent establishment of the amphetamine CPP. However, the nature of the expression blockade remains unclear since it is unknown how reduced locomotion affects expression of the amphetamine CPP. This issue is dealt with in Experiments 2 and 3.

In summary, the data show that SCH23390 blocked acquisition and expression within similar dose ranges, but that the expression-blocking dose ranges of the other drugs were considerably higher than the acquisition blocking dose ranges.

**Experiment 2**

Since studies have shown that the mesolimbic dopamine system in the nucleus accumbens mediates acquisition of the amphetamine CPP[3,14,61], it is most likely that the site of action of dopamine antagonists on acquisition is the nucleus accumbens. The site of action
of the antagonists on expression remains unclear, however. Also the possibility exists that the expression blockade is simply due to decrease locomotion produced by the antagonists. In this experiment, we compared the effects of SCH23390 and sulpiride injected into nucleus accumbens or striatum on expression of the amphetamine CPP and spontaneous locomotor activity, in order to reveal the site of action of the antagonists on expression and to examine the effects of the antagonists on locomotion.

Methods

Using standard stereotaxic techniques, rats were bilaterally implanted with the guide cannulae under 65 mg/kg sodium pentobarbital anesthesia (A: +1.7, L: ±1.5, V: -4.5). Dummy cannulae were inserted extending the tip of the guide cannulae by 2.5 mm (A: + 1.7, L: ± 1.5, V: -7.0) for the animals which were to receive intra-accumbens microinjections. The animals which were to receive intrastriatal microinjections did not receive dummy cannulae. The guide cannulae were filled with insect pins (00) cut at the length of the guide cannulae. A week after the surgery, testing began.

Two sets of groups (n = 8 for each group) were used in the CPP paradigm. One set of groups received bilateral microinjections of vehicle, SCH23390 (0.0001, 0.001 or 0.01 μg/side) or sulpiride (0.01 or 0.1 μg/side) into nucleus accumbens before testing. The other set of groups received vehicle, SCH23390 (0.01 μg/side) or sulpiride (0.1 μg/side) bilaterally into striatum before testing. SCH23390 (0.01 μg/side) or sulpiride (0.1 μg/side) was also bilaterally injected into the nucleus accumbens to examine their effects on spontaneous locomotor activity in another set of groups (n = 6–8 for each group).

After the inner cannulae were inserted into the guide cannulae the drugs were delivered over a 30 s period. The inner cannulae were left in place for a further 60 s. After a further 90 s testing began.

After the completion of testing, animals were perfused with saline and subsequently formal saline through the heart. Brains were removed and stored in formal saline. The brains were sectioned in 20 μm slices and stained with thionin.

Results

Fig. 2 shows the locations of the tips of the inner cannulae for the intra-accumbens (A) and the intra-striatal (B) groups. Intra-accumbens injections were located in the center of the nucleus; intra-striatal injec-
Fig. 3. Effects of SCH23390 (0.0001, 0.001 and 0.01 μg/side) and sulpiride (0.01 and 0.1 μg/side) injected into the nucleus accumbens on the expression of amphetamine CPP. The ordinate represents the time spent in the two large compartments. Paired side, amphetamine-paired compartment; unpaired side, saline-paired compartment. The numbers above the columns are the differences in time (in s) spent in the two large compartments.

In both Experiments 1 and 2, injections of SCH23390 completely blocked the expression of the amphetamine CPP had no effect on this conditioned behavior when they were injected into the striatum (Fig. 4). Planned comparisons revealed that time spent in the two large compartments was significantly different for the vehicle ($F_{1,21} = 7.70, P < 0.05$), SCH23390 ($F_{1,21} = 14.89, P < 0.01$) and sulpiride ($F_{1,21} = 9.28, P < 0.01$) groups. These data indicate that the blockade of expression of the amphetamine CPP by intra-accumbens microinjections of SCH23390 or sulpiride is not due to spread of the drugs into the striatum.

Microinjections of the highest doses of SCH23390 and sulpiride into the nucleus accumbens suppressed spontaneous locomotor activity to 37 and 36% of the level of the no-treatment group, respectively (Fig. 5). A one-way ANOVA revealed that the drug effect was significant ($F_{3,22} = 14.41, P < 0.01$).

Experiment 3

In both Experiments 1 and 2, injections of SCH23390

![Figure 3](image3.png)

![Figure 4](image4.png)

![Figure 5](image5.png)

![Figure 6](image6.png)
Experiment 4

In Experiment 2, microinjections of the dopamine antagonists into striatum did not impair the expression of the amphetamine CPPs, suggesting that the nigrostriatal dopamine system is not involved in the expression of the amphetamine CPP. This experiment examined the effect of substantial damage to the dorsal striatum on the amphetamine CPP in order to confirm this conclusion.

Methods

One group of rats \((n = 12)\) received bilateral electrolytic lesions \((2.5 \text{ mA for } 20 \text{s})\) of striatum \(\text{A: } +1.0, \text{ L:} \)
Fig. 9. Effects of electrolytic lesions of the dorsal striatum on the amphetamine CPP. Sham, sham lesioned group; lesion, dorsal striatum lesioned group; paired side, amphetamine-paired compartment; unpaired side, saline-paired compartment.

±2.8, V: -5.5) and the other (n = 8) received sham lesions. After surgery, the animals' weight was monitored and they were fed with wet mash food mixed with sucrose. At the end of a 1 week recovery session, there was no significant difference between the mean weights of the group with lesions (average = 316.0 g, S.E.M. = 4.2) and the group with sham lesions (average = 322.1 g, S.E.M. = 4.1). After the recovery period, the CPP experiment began.

Results

The extent of lesions is shown in Fig. 8. They were mainly confined to the dorsal section of the striatum.

Electrolytic lesions of the dorsal striatum potentiated the amphetamine CPP (Fig. 9). A two-way ANOVA with planned comparisons showed that there were significant differences in time spent in the two compartments for the control group (Fr,rs = 11.30, P < 0.01) and for the lesioned group (Fr,rs = 31.96, P < 0.01). The time difference of the lesioned group was significantly different from that of the control group (F,,,, = 8.79, P < 0.01).

DISCUSSION

The present results demonstrate that systemically injected selective D1 and D2 dopamine antagonists have different effects on the acquisition and the expression of the amphetamine CPP. The selective D1 antagonist SCH23390 blocked acquisition and expression within similar dose ranges. The antagonists with high affinity for D2 dopamine receptors such as a-flupenthixol, metoclopramide, and sulpiride had no effect on expression at doses which completely blocked acquisition. Yet higher doses of these D2 antagonists blocked expression. Microinjections of SCH23390 or sulpiride into the nucleus accumbens, but not the dorsomedial section of the anterior striatum, also blocked expression of the amphetamine CPP. Although intra-accumbens microinjections of sulpiride reduced spontaneous locomotor activity, sodium pentobarbital (17.5 mg/kg) produced the same degree of reduction of spontaneous locomotor activity but exerted no effect on the expression of the amphetamine CPP. Finally, electrolytic lesions of the dorsal striatum potentiated the amphetamine CPP.

Acquisition of the amphetamine CPP

Although amphetamine induces dopamine release unconditionally in the nucleus accumbens and striatum11, 40,70, dopamine release in the striatum does not seem to be relevant to acquisition of this incentive learning since lesions of the dorsal striatum failed to impair the amphetamine CPP in the present study. Together with the previous demonstrations that dopamine depletion in the nucleus accumbens attenuated the amphetamine CPP61 and that microinjections of amphetamine into nucleus accumbens, but not into striatum, establish CPPs4,13,14, it seems clear that the mesolimbic dopamine system in the nucleus accumbens is a critical neural system for the establishment of this type of learning.

Since the receptor selectivity of the antagonists to both the D1 and the D2 receptors is maintained within the dose ranges used in the acquisition part of this study1,2, the present findings indicate that both D1 and D2 dopamine antagonists block acquisition. This is consistent with previous studies showing that the establishment of the amphetamine CPP was blocked by SCH2339034,39, a-flupenthixol14 and metoclopramide34.

A possible explanation of the present findings is based upon the fact that metoclopramide establishes a CPP34 and SCH23390 establishes conditioned place aversion (CPA)59,60. However, in the present studies the antagonists were given before both amphetamine-pairing and saline-pairing sessions. Even if metoclopramide established a CPP, it would have been added to both conditions so that the amphetamine-paired compartment would still have been uniquely paired with the amphetamine effect. In this hypothetical situation, animals might still be expected to choose the amphetamine-metoclopramide paired compartment over the metoclopramide paired compartment if the CPP-establishing action of amphetamine was not blocked by the antagonist. Similarly, if SCH23390 simply produced a CPA without blocking the effect of amphetamine, animals might be expected to choose the amphetamine-SCH23390 paired compartment over the SCH23390 paired compartment. The argument that CPPs or CPAs produced by the antagonists cannot explain the blockade of the amphetamine CPP is further strengthened by evidence that the dose of a-flupenthixol which completely blocked the
establishment of the amphetamine CPP in this study does not produce a CPP or CPA. Thus it seems that both D1 and D2 dopamine antagonists directly antagonize the action of amphetamine. Together with the finding that intra-accumbens injections of either D1 or a D2 agonist alone establishes CPPs, it might be suggested that co-activation of the two receptor subtypes is a necessary condition for establishing a CPP with at least one of the receptors in a state of supernormal activation.

Expression of the amphetamine CPP

Systemically injected dopamine antagonists blocked expression of previously established amphetamine CPPs when given on the test day. Microinjections of SCH23390 and sulpiride into the nucleus accumbens, but not the striatum, also blocked this behavior. It is unlikely that the antagonists blocked this conditioned behavior simply by impairing performance. Although microinjections of SCH23390 or sulpiride reduced spontaneous locomotion, the same degree of motor retardation produced by sodium pentobarbital had no effect on the expression of the amphetamine CPP. Since sodium pentobarbital produces strong aversive effects measured in the CPP paradigm, the finding that this drug injected on the test day had no effect on expression of the amphetamine CPP also indicates that the blockade of the expression of the CPP by neuroleptics is not due to any aversive effects they might have.

Together with the finding that lesions of the dorsal striatum did not impair the amphetamine CPP, it can be suggested that dopamine released from the mesolimbic, rather than the nigrostriatal, dopamine pathway has a crucial role in expressing this incentive learning.

The data also reveal different effects of systemically injected SCH23390 and the other antagonists. While the D1 antagonist was equally effective in blocking the acquisition and expression of the amphetamine CPP within the dose range which maintains selectivity for the D1 dopamine receptor, higher doses of the other antagonist were required to block expression than acquisition. The latter drugs share the property of higher affinity for D2 than D1 dopamine receptors, respectively. These ratios coincide approximately with the degree of the rightward shift of the antagonism curves from acquisition to expression (Fig. 1). Doses of 0.4 mg/kg and 0.8 mg/kg of a-flupenthixol, for example, produced complete blockade of the acquisition and expression of the amphetamine CPP, respectively. Since the high doses of the D2 antagonists seem to bind to D1 receptors in vivo, the blocking effects of the high doses of the D2 antagonists on expression might be due to blockade of D1 dopamine receptors. If the blockade of the amphetamine CPP by systemic sulpiride is due to blockade of D1 dopamine receptors, then the blockade of the expression of the amphetamine CPP by intra-accumbens injections of sulpiride might also be due to this drug’s binding to the D1 dopamine receptor in the nucleus accumbens.

Although SCH23390 also binds to 5-HT2 receptors, the blocking effect of SCH23390 on the amphetamine CPP does not seem to be due to this action. First, depletion of serotonin in the nucleus accumbens has no effect on the amphetamine CPP. Second, the doses of SCH23390 used in the present study have no effect on 5-HT2 binding sites or on 5-HT mediated behaviors.

The mesolimbic dopamine system and incentive learning

The present data are consistent with a line of evidence that conditioned stimuli paired with incentive stimuli which unconditionally cause dopamine release acquire the property of evoking firing of the mesolimbic dopamine cell bodies and to increase dopamine metabolism in the nucleus accumbens. Furthermore, when animals are engaged in amphetamine conditioned locomotor activity (CLA) in the absence of amphetamine an elevation of dopamine metabolism is observed in the terminal area and 6-OHDA lesions of the nucleus accumbens abolish the expression of amphetamine CLA.

Contrary to this line of evidence, it has been suggested that, once established, amphetamine conditioned behaviors are expressed independently of dopamine function on the grounds that a dose of pimozide which completely blocked amphetamine unconditioned locomotor activity had no effect on CLA. It should be noted, however, that studies demonstrating the non-involvement of dopamine in the expression of amphetamine conditioned behaviors used 'typical' neuroleptics such as pimozide and haloperidol. These drugs have a higher affinity for D2 than D1 receptors in vivo. The present results show that the antagonists with high affinity for D2 dopamine receptors did not block expression at the doses which blocked acquisition. Although this finding obviously pertains directly to the CPP, failure to block expression of other amphetamine conditioned behaviors may also be explained by the fact that higher doses of these drugs are required to block expression than acquisition, which in turn might be due to the weak effect of D2 antagonists on D1 dopamine receptors. Thus, the present finding calls into question the notion that conditioned behaviors can be expressed even when dopaminergic function is disrupted.

In operant paradigms, pimozide produces extinction-like response patterns in the presence of food and
amphetamine\textsuperscript{2,7,22}, which unconditionally induce dopamine release\textsuperscript{9,11,15,40,47,50,70,73}. During a genuine extinction session, responding is not followed by food or amphetamine, and the temporary persistence of responding during extinction is generally explained by the concept of conditioned reinforcement — the process whereby stimuli paired with food or stimulants acquire conditioned reinforcing properties and thereby sustain responding. If conditioned reinforcers are removed from an extinction situation, responding shows a very rapid decline and extremely low response rates are noted\textsuperscript{16,29}.

The present results suggest that the behavioral effects of stimuli paired with amphetamine are mediated by activation of the D1 dopamine receptor in the nucleus accumbens and that neuroleptics with high affinity for D2 dopamine receptors block the unconditioned effect of amphetamine in dose ranges which do not block the effect of conditioned incentive stimuli. Thus, pimozide might produce extinction-like patterns of responding in the presence of unconditioned dopamine release, because the drug does not blunt the effect of conditioned reinforcers or conditioned incentive stimuli at the doses which block that of unconditioned dopamine release, due to its higher affinity for D2 than D1 receptors. This hypothesis also explains the burst of responding that is observed under pimozide treatment when a conditioned stimulus is suddenly presented\textsuperscript{31}.

The reason why the effect of conditioned incentive stimuli involve activation of the D1, rather than D2, dopamine receptor is not clear at present. Conditioned incentive stimuli also seem to involve dopamine released from the reserpine-sensitive, \(\alpha\)-MPT-insensitive pool in the nucleus accumbens\textsuperscript{22} and interaction with the lateral nucleus of the amygdala\textsuperscript{33}. These functionally overlapping substrates might correspond to the striosome, which is characterized by weaker AChE staining than the surrounding matrix\textsuperscript{25}. Compared to the extrastriosomal matrix, the striosome has slightly higher D1 binding density\textsuperscript{1}, lower D2 binding density\textsuperscript{37,41}, and lower tyrosine hydroxylase activity\textsuperscript{20,24}. The amygdalostriatal projections mainly, but not exclusively, terminate in the striosomes\textsuperscript{51}. Although the striosomal organization in the nucleus accumbens is not as clear as it is in the striatum, the correspondence between the properties of the striosome and the functional unit that mediates the expression of the conditioned incentive learning revealed in our present and previous studies\textsuperscript{31,32,33,67} is worthy of further investigation.

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