Pharmacology Biochemistry and Behavior, Vol. 45, pp. 343–350, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Microinjections of Flupenthixol Into the Caudate Putamen of Rats Produce Intrasession Declines in Food-Rewarded Operant Responding

RICHARD J. BENINGER,[1] CATHERINE M. D'AMICO AND ROBERT RANALDI

Department of Psychology, Queen's University, Kingston, Ontario K7L 3N6, Canada

Received 15 June 1992

BENINGER, R. J., C. M. D'AMICO AND R. RANALDI. *Microinjections of flupenthixol into the caudate putamen of rats produce intrasession declines in food-rewarded operant responding.* PHARMACOL BIOCHEM BEHAV 45(2) 343–350, 1993.—Results of recent studies suggest that dopamine (DA) transmission in the caudate putamen may be involved in food reward-related learning. The purpose of the present study was to evaluate the contribution of DA terminals in the dorsal caudate putamen to food-rewarded operant responding. Experiment 1, a study measuring circling behaviour in 18 rats receiving systemic amphetamine (1.5 mg/kg) and unilateral intracaudate putamen injections of *cis*-flupenthixol (0.0, 1.0, 10.0, and 25.0 μg in 0.5 μl), a DA receptor antagonist, or its pharmacologically inactive isomer *trans*-flupenthixol (25.0 μg in 0.5 μl), determined a behaviourally effective dose of *cis*-flupenthixol. Results showed that *cis*-flupenthixol dose dependently increased ipsiversive turning and *trans*-flupenthixol did not. In Experiment 2, an operant study, 36 rats were trained to press a lever for food on a variable interval 30-s schedule. Rats were then randomly assigned to four groups, three of which received one of the following bilateral intracaudate injections prior to three subsequent test sessions: saline (n = 6; 0.5 μl), *cis*-flupenthixol (n = 10; 25.0 μg/0.5 μl), and *trans*-flupenthixol (n = 10; 25.0 μg/0.5 μl). Rats in the home cage control group (n = 10) received two bilateral intracaudate putamen injections of *cis*-flupenthixol (25.0 μg/0.5 μl) in their home cages and a final injection of *cis*-flupenthixol prior to a test session. The results showed that *cis*-flupenthixol, but not *trans*-flupenthixol or saline, produced a time-dependent intrasession decline in operant responding. This pattern resembled that seen in extinction. The intrasession pattern of responding in the home cage control group did not differ significantly from that of the first test day but did differ significantly from that of the third test day of the *cis*-flupenthixol group, suggesting that the extinction-like pattern of responding in the *cis*-flupenthixol group was not a result of repeated central injections per se. These results provide support for the hypothesis that DA transmission may be involved in incentive learning and, further, that dopaminergic projections to the dorsal caudate putamen may play a role in food reward-related incentive learning.

Caudate putamen Circling *cis*-Flupenthixol Food Operant responding Reward *trans*-Flupenthixol

THERE now exists good evidence that dopaminergic neurons form a critical link in the neuronal circuitry mediating the effects of reward on behaviour [for reviews, see (3,29)]. For example, Wise et al. (30) showed that blockade of dopamine (DA) receptors with pimozide decreased the response rates of animals pressing a lever for food reward. It is noteworthy that the pattern of responding resembled that typically seen in extinction, that is, response rates did not decrease immediately following pimozide but rather declined progressively across test sessions. This extinction-like pattern of responding strongly suggested that the effect of pimozide was to attenuate the rewarding impact of food. It also ruled out explanations based upon simple motor deficits or decreases in motivation to eat that might have been expected to have uniform effects throughout the session.

Studies have been undertaken to investigate the role of the dopaminergic projections to the nucleus accumbens and to the caudate putamen in reward. There are now many data that show that the rewarding properties of psychomotor stimulants and opiates are mediated by dopaminergic projections to the nucleus accumbens (6,26,29). The nucleus accumbens and caudate putamen have both been implicated in brain-stimulation reward (22,23,25). However, the role of dopaminergic projections to the nucleus accumbens and caudate putamen in food reward is less clear.

The results of neurochemical studies of postmortem levels

[1] To whom requests for reprints should be addressed.

of DA or its metabolites have revealed evidence of increased activity in mesolimbic DA neurons projecting to the nucleus accumbens (5,12,13) and/or nigrostriatal DA neurons (5,7) following feeding or lever pressing for food. These observations have been corroborated by in vivo electrochemical studies similarly showing that feeding or lever pressing for food increased accumbens and/or caudate putamen DA release (14,15). These results implicate dopaminergic projections to both the accumbens and caudate putamen target regions in food reward.

There are a number of observations that might suggest that dopaminergic projections to the caudate putamen are critical for food reward. Thus, it has been reported that neurotoxic destruction of dopaminergic projections to the caudate putamen but not the accumbens impaired lever pressing for food (2). Evenden (11) similarly found that DA-depleting lesions of the caudate putamen but not the accumbens produced an extinction-like effect in his analysis of win-stay patterns of lever pressing for food. Other findings provide indirect support for the hypothesis that dopaminergic projections to the caudate putamen are critical for food reward. Roberts et al. (26) and Wise and Rompré (29) reported that 6-hydroxydopamine lesions of the nucleus accumbens impaired stimulant self-administration but not lever pressing for food. It also has been found that kainic acid lesions of the nucleus accumbens impaired lever pressing for morphine but not food (10). Thus, there is evidence that impaired DA neurotransmission in the caudate putamen reduces the effects of food reward on behaviour and that lesions of the nucleus accumbens do not.

On the basis of these results, it is hypothesized that disruption of dopaminergic neurotransmission in the caudate putamen using local injections of the DA receptor antagonist *cis*-flupenthixol will lead to a reduction in the ability of food reward to maintain lever pressing. It is expected that such an effect will involve a gradual within- and/or between-session decline in responding suggestive of the gradual loss of responding seen in animals experiencing nonreward. Extinction curves for animals trained in the present paradigm have been published previously (4).

An initial experiment using the circling paradigm was carried out to establish a behaviourally effective dose of *cis*-flupenthixol for use in the operant experiments. It was expected that animals pretreated systemically with amphetamine would show dose-dependent ipsiversive circling following unilateral injections of *cis*-flupenthixol into the dorsal caudate putamen.

METHOD

Treatment of rats in the present study was in accordance with the Animals for Research Act, the Guidelines of the Canadian Council on Animal Care, and relevant university policy and was approved by the Queen's University Animal Care Committee.

Subjects

Male Wistar rats (obtained from Charles River Canada) weighing between 200 and 250 g were individually housed in hanging wire cages and maintained in a climactically controlled environment (21 °C) on a 12 L : 12 D cycle (lights on a 0600 h). Rats used in the operant experiments were maintained at 85% of their free-feeding weights through daily measured food rations (Purina Rat Chow); the average daily ration was from 12–15 g. Rats used in the circling studies had access to food ad lib. Water was continuously available in the home cage for all rats.

Apparatus

Operant chambers. Operant conditioning occurred in four identically constructed experimental chambers. Each operant chamber (23.0 × 20.4 × 19.5 cm) consisted of aluminum plate sides and a transparent Plexiglas top and door. The grid floor was composed of aluminum rods spaced 1.5 cm apart. A feeder cup was positioned to the right of the lever; the lever was 5.0 cm wide at a height of 5.5 cm. The chamber was illuminated by a light (7 W) and enclosed in an outer plywood box insulated with sound-attenuating styrofoam and ventilated by a small fan. Data collection and schedules of food reward were controlled by a Digital Equipment Corporation LSI 11/2 computer.

Circling arenas. Circling was measured in three polyurethane-sealed circular wooden bases (30.0 cm in diam.) enclosed within a cylinder of wire mesh (36.0 cm high) and fitted with a Plexiglas cover.

Surgery

Animals were anaesthetized with an IP injection of sodium pentobarbital (60.0 mg/kg, MTC Pharmaceuticals) and secured in a Kopf stereotaxic instrument (Kopf, Topanga, CA) with the incisor bar set at 5.0 mm above the horizontal plane passing through the interaural line. The 18 rats in the circling experiment received unilateral guide cannulae implanted into the left dorsal caudate putamen. The 36 rats in the operant experiment received bilateral guide cannulae implants in the dorsal caudate putamen. The stainless steel guide cannulae (0.64 mm diam.) were implanted at the following coordinates (21): 1.4 mm anterior to bregma, 3.0 mm lateral to the midline, and 4.0 mm ventral to the dura mater. Guide cannulae were anchored to the skull with four stainless steel jeweler's screws and acrylic cement. They were occluded between injections with stainless steel pins (0.31 mm diam.).

Central Injections

Stainless steel injection cannulae (0.31 mm diam.) were cut to extend 1.0 mm beyond the tip of the guide cannulae. They were attached to a 10-μl Hamilton microsyringe (Hamilton Co., Reno, NV), mounted in an infusion pump (Sage Instruments), by a length of polyethylene tubing. Injections were delivered over 45 s and the injection cannula was left in place for 60 s. The doses used in the circling study were 1.0, 10.0, and 25.0 μg *cis*-flupenthixol dihydrochloride (Lundbeck, Copenhagen, Denmark) dissolved in 0.5 μl distilled water. The pH level of the *cis*-flupenthixol solution was approximately 3.0. *trans*-Flupenthixol dihydrochloride (Lundbeck), the pharmacologically inactive geometric isomer of *cis*-flupenthixol (20), in a dose of 25.0 μg in 0.5 μl distilled water served as the control for the nonspecific effects of pH and osmolality. In the operant experiment, either 25.0 μg *cis*-flupenthixol in 0.5 μl distilled water, 0.5 μl 0.9% saline, or 25.0 μg *trans*-flupenthixol in 0.5 μl distilled water were infused bilaterally.

Behavioural Testing

Experiment 1. Testing of the 18 animals involved in the circling study began 6 days following surgery. Nine animals were tested each day, thereby allowing 48 h between test days. Animals were injected with (+)-amphetamine sulphate (1.5 mg/kg, IP, Smith, Kline & French, Philadelphia, PA) 15 min prior to each session. Each test session (except no-injection;

see below) began with a central injection and placement into the circular arena. Complete turns (360°) ipsi- or contralateral to the side of the cannula were counted for each rat during seven separate sessions: a) no central injection; b) central injection of *trans*-flupenthixol (25.0 μg in 0.5 μl); c), d), and e) injection of each of three doses of *cis*-flupenthixol (1.0, 10.0, and 25.0 μg in 0.5 μl) with the order of administration counterbalanced across rats over three sessions; f) replication of *trans*-flupenthixol; g) replication of no central injection.

Three animals were observed over 60 min with central injections given at staggered intervals of 5 min. Each animal was scored at 0–5, 15–20, 30–35, and 45–50 min. The clock was stopped for the time taken (maximum 2 min) to administer a central injection. Thus, each animal was scored for a total of 20 min in four 5-min blocks at approximately equal intervals throughout the 60-min session. Circling behaviour was expressed as the ratio of ipsiversive turns to total turns (ipsiversive + contraversive). Ratio values greater than 0.5 indicate a tendency toward ipsiversive turning and values less than 0.5 toward contraversive turning. Total turns per session constituted a second dependent variable.

Experiment 2. The effect of bilateral intracaudate putamen injections of *cis*-flupenthixol (n = 10), *trans*-flupenthixol (n = 10), or saline (n = 6) on three separate occasions was compared to baseline (i.e., nondrug) responding of the same animals over three sessions. An additional group, the home cage control group (n = 10), also was included. Thirty-six animals were trained to press the lever for food reward consisting of one 45-mg food pellet (Bioserv) delivered on a variable interval (VI) 30-s schedule, that is, response-contingent reward became available every 30 s on average (range: 5–90 s). Predrug sessions lasted for 30 min per day for 10 days. Individual animals were tested in the same box, in the same order, and at the same time of the day. Animals were tested in the experimental chamber every day unless they received an injection, in which case a 72-h waiting period was imposed before the next session. This was included to minimize any possible carryover effects from one injection to the next. Animals remained in their home cages for the intervening 2 days. *cis*-Flupenthixol was expected to produce a day-to-day and/or within-session decline in responding. The saline group was included to control for possible nonspecific effects of repeated injections and the *trans*-flupenthixol group was included to control for possible nonspecific effects resulting from repeated microinjections of an acidic solution.

The home cage group served as a control for cumulative pharmacological effects, that is, if the *cis*-flupenthixol group showed a day-to-day decline one possibility is that the effective dose increased from injection to injection [cf. (30)]. If this was the case, animals receiving home cage injections of *cis*-flupenthixol prior to their first test under the drug would be expected to respond less than animals receiving the drug for the first time. Thus, home cage control animals received three bilateral central injections of *cis*-flupenthixol (25.0 μg/0.5 μl). They were returned to their home cages following each of the initial two injections but were tested in the operant chambers on the third day. On the test day, these animals had pharmacological histories identical to the *cis*-flupenthixol group on test days. They differed in their experience with the lever-press situation while drugged. In this latter respect, they were similar to the *cis*-flupenthixol group on their first drug day.

Histology

At the conclusion of behavioural testing, rats were killed with a lethal dose of sodium pentobarbital, exsanguinated with 0.9% saline, and perfused intracardially with 10% formalin. Brains were removed and stored in 4% formalin for 1 week. Frozen coronal sections (50 μm) were mounted and stained with formal-thionin (9) to verify cannulae placements.

RESULTS

Histology

Cannulae for 17 of the 18 rats in Experiment 1 and all rats in Experiment 2 were in the dorsal region of the caudate putamen as indicated by the representative section from Experiment 2 shown in Fig. 1. The rejected rat from Experiment 1 had its cannula positioned too medially, invading the ventricle.

Experiment 1

Some animals exhibited a marked turning bias during the first no-injection test. Six animals with turning ratios less than 0.15 or greater than 0.85 during the first session were excluded. Student's t-tests for correlated measures indicated that the mean (±SEM) turning ratios, although increasing from session to session, were not significantly different for the initial and final no central injection [0.50 (±0.08) vs. 0.68 (±0.09)], $t(10) = 1.50$, $p > 0.10$, or *trans*-flupenthixol [0.58 (±0.08) vs. 0.73 (±0.08)], $t(10) = 1.94$, $p > 0.10$, conditions. Therefore, the ratios for these initial and final sessions were averaged for each rat for subsequent analyses.

The effects of no-injection and *cis*- and *trans*-flupenthixol on mean turning ratios are shown in Fig. 2. The data showed increasing ipsiversive turning with increasing doses of *cis*-flupenthixol. The reliability of this effect was confirmed by a repeated-measures analysis of variance (ANOVA) revealing a main effect of dose, $F(4, 40) = 4.42$, $p < 0.005$. Note that the Greenhouse–Geisser adjusted degrees of freedom were used to reduce type I errors associated with repeated measures [see (18)]. Post-hoc comparisons using the Dunnett method showed that the *trans*-flupenthixol condition did not differ significantly from the no central injection condition; consequently, the *trans*-flupenthixol condition was used as the control for the remaining comparisons. The Dunnett tests revealed that the 25.0-μg dose of *cis*-flupenthixol produced a significantly greater amount of ipsiversive turning than *trans*-flupenthixol.

A second dependent measure from Experiment 1 was the total number of turns (ipsiversive + contraversive). The mean (±SEM) total for no-injection 1, *trans*-flupenthixol 1, *cis*-flupenthixol 1.0, 10.0, and 25.0 μg, *trans*-flupenthixol 2, and no-injection 2, respectively, were: 24.5 (±3.6), 29.0 (±4.2), 36.0 (±8.3), 35.9 (±3.9), 34.6 (±7.3), 53.9 (±8.2), and 48.0 (±9.1). It appeared that total number of turns increased from session to session in these amphetamine-pretreated animals and ANOVA confirmed a significant treatment effect, $F(4, 40) = 3.71$, $p < 0.05$. The values given above for the three *cis*-flupenthixol doses are not representative of the order in which the doses were given as that order varied from rat to rat. When mean (±SEM) total turns were computed for the first, second, and third *cis*-flupenthixol sessions ignoring dose, the corresponding values were: 29.0 (±3.7), 39.1 (±7.2), and 39.2 (±7.9). These means and those presented above for no-injection and *trans*-flupenthixol sessions confirm that total turns increased from session to session.

Turning ratios showed that intracaudate putamen injections of a 25.0-μg dose of the DA antagonist *cis*-flupenthixol affected behaviour significantly. Therefore, the 25.0-μg dose

FIG. 1. Representative photomicrograph showing dorsal caudate putamen site of bilateral cannulae for an animal from Experiment 2. Rats from Experiment 1 had unilateral cannulae located in the same target site.

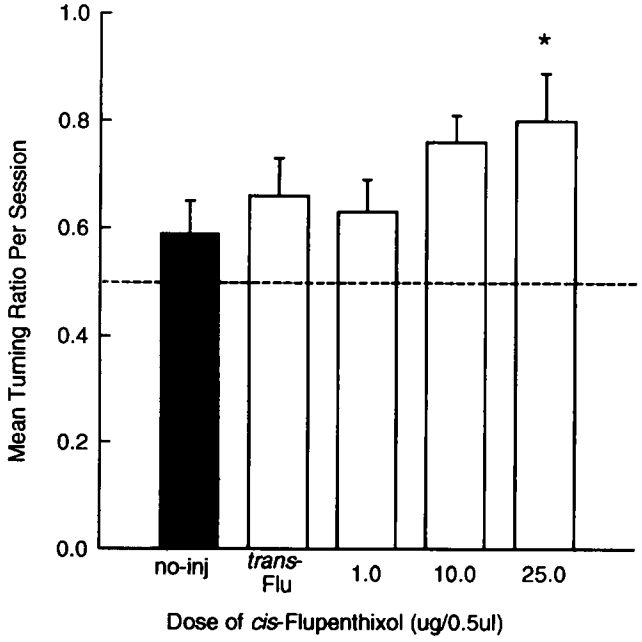

FIG. 2. Mean turning ratios [ipsiversive/(ipsiversive + contraversive)] for no-injection (no-inj), *trans*-flupenthixol (*trans*-Flu), and *cis*-flupenthixol treatments for Experiment 1. No-inj ratios for each rat (n = 11) were averaged over sessions 1 and 7 and *trans*-Flu ratios for sessions 2 and 6. Ratios greater than and less than 0.5 (broken line) indicate a tendency toward ipsiversive and contraversive turning, respectively. Vertical bars represent SEMs. *Significantly more ipsiversive turning compared to *trans*-Flu condition.

was chosen for the evaluation of responding for food following intracaudate putamen injections of *cis*-flupenthixol.

Experiment 2

Mean (±SEM) lever press responses per min for each 5-min segment of the last three 30-min baseline sessions and the three (or one) test sessions for the saline, *trans*-flupenthixol, *cis*-flupenthixol, and home cage control groups are shown in Fig. 3. For all groups, intrasession response rates during the three baseline sessions tended to increase over 5-min blocks. For the saline and *trans*-flupenthixol groups, the pattern was similar for the three test sessions following intracaudate injections. This was confirmed by statistical analyses. For each group, a three-variable repeated-measures ANOVA with time (six 5-min blocks), day (3 days), and phase (baseline and test) as the variables analysed was carried out. The Greenhouse-Geisser corrected degrees of freedom was used for all repeated-measures factors. For the saline group, only the time effect was significant, $F(5, 25) = 13.16, p < 0.005$. This result confirmed that central administration of saline had no significant effect on food-rewarded operant responding.

Results from the ANOVA for the *trans*-flupenthixol group revealed a significant effect of time, $F(5, 45) = 11.09, p < 0.005$, and an interaction of phase and time, $F(5, 45) = 3.20, p < 0.01$; this suggested that the time effect differed from the baseline to the test phase. However, tests of simple main effects revealed significant time effects in each phase, $F(5, 45) = 7.86, p < 0.005$, and $F(5, 45) = 10.82, p < 0.005$. Tests of simple main effects of phase at each time revealed no significant phase effects. Thus, the source of the significant interaction was not revealed by these tests. Inspection of Fig. 3 (also see Table 1) suggested that *trans*-flupenthixol produced a small decrease in responding during the first 5-min block of test sessions 2 and 3, possibly producing the interaction. Apart from this initial slowing of responding, *trans*-flupenthixol ap-

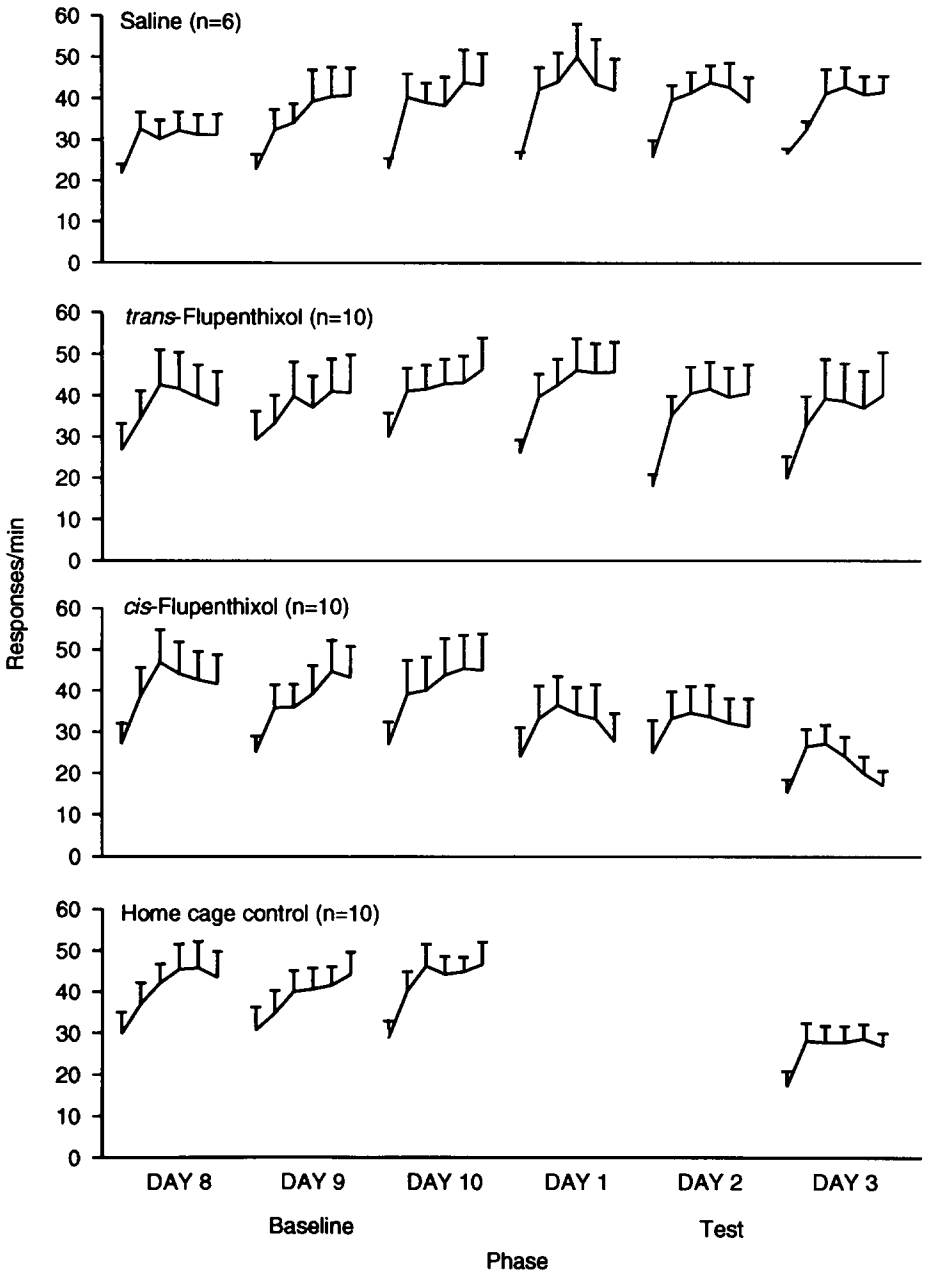

FIG. 3. Mean number of bar presses per minute for each of the six 5-min blocks in each session for the last three baseline sessions (days 8, 9, and 10) and the three test sessions for all groups from Experiment 2. Vertical bars represent SEMs.

peared to have little effect on the intrasession pattern of lever pressing for food reward.

For the *cis*-flupenthixol group, following drug injections response rates were generally lower than in baseline. Response rates showed an initial baseline-like increase over the first three 5-min blocks but then began to decline over the last three 5-min blocks. Statistical analyses confirmed that these effects were reliable. The ANOVA revealed significant time, $F(5, 45) = 16.69$, $p < 0.005$, phase, $F(1, 9) = 13.10$, $p < 0.01$, and time × phase interaction effects, $F(5, 45) = 7.14$, $p < 0.005$. Tests of simple main effects revealed a time effect in each phase, $F(5, 45) = 14.68$, $p < 0.005$, and $F(5, 45) = 10.12$, $p < 0.005$, and a phase effect at each time, $F(1, 9) = 12.07$, 6.50, 6.67, 8.68, 16.53, and 14.19, at times 1–6, respectively, $p < 0.05$. Thus, *cis*-flupenthixol significantly changed the intrasession pattern of responding but tests of simple main effects failed to reveal any differential effects at different time blocks.

In comparison with baseline, it appeared that the magnitude in the decrease in responding produced by *cis*-flupenthixol was greater in the last 5-min blocks of test sessions than in the first 5-min blocks. The magnitudes of these

TABLE 1

MEAN DIFFERENCES IN RESPONSES/min (PHASES 1 MINUS 2) DURING EACH 5-min BLOCK FOR EACH GROUP (SEM IN PARENTHESES)

	Time (5-min Blocks)					
Group	T1	T2	T3	T4	T5	T6
Saline	−3.43	−2.90	−7.63	−8.87	−3.73	−2.33
	(2.05)	(2.46)	(2.90)	(3.54)	(4.96)	(5.57)
trans	7.37	0.47	0.53	−1.57	0.50	−0.57
	(4.68)	(3.14)	(3.49)	(3.69)	(3.04)	(3.99)
cis	4.97	6.90	8.20	11.63	15.77	17.90
	(1.43)	(2.72)	(3.19)	(3.95)	(3.88)	(4.75)
Home	12.54	9.20	14.93	15.55	15.37	17.76
	(4.32)	(5.32)	(3.55)	(2.98)	(4.78)	(4.98)

cis, *cis*-flupenthixol group (n = 10); home, home cage control group (n = 10); saline, saline group (n = 6); *trans*, *trans*-flupenthixol group (n = 10).

differences were computed by subtracting the response rates for each 5-min block of the combined test days from the corresponding 5-min block of the combined baseline days. These values for all groups are shown in Table 1. It can be seen that the magnitude of the difference score increased from block to block for the *cis*-flupenthixol group. A one-way ANOVA confirmed that these values changed significantly over time, $F(5, 45) = 7.14, p < 0.01$. Thus, *cis*-flupenthixol produced a significant intrasession decline in food-rewarded operant responding.

The home cage control group was included to evaluate the possibility that repeated central administrations of *cis*-flupenthixol would lead to a decrease in responding unrelated to experience in the test environment. When this group was tested following their third injection of *cis*-flupenthixol, their response rates were generally lower than in baseline and response rates in the final four 5-min blocks failed to show progressive increases like those seen in baseline. For data analyses, response rates for each 5-min block of the three baseline sessions were averaged and compared to the response rates in the test using a two-variable ANOVA with repeated measures on 5-min block and phase. Significant 5-min block, $F(5, 45) = 29.31, p < 0.005$, phase, $F(1, 9) = 11.64, p < 0.01$, and interaction effects were seen, $F(5, 45) = 3.72, p < 0.01$. Tests of simple main effects revealed significant time effects in each phase, $F(5, 45) = 52.18, p < 0.005$, and $F(5, 45) = 8.80, p < 0.005$. Tests of simple main effects of phase for each 5-min block revealed significantly less responding in the test phase for each 5-min block except the second, $F(1, 9) = 8.41$, 17.66, 27.29, 10.36, and 12.69 for the first, third, fourth, fifth, and sixth 5-min blocks, respectively, $p < 0.05$. These tests might suggest that the interaction occurred because *cis*-flupenthixol reduced responding in all but the second 5-min block.

As was the case for the *cis*-flupenthixol group, difference scores were computed by subtracting response rates in each 5-min block in the test from the corresponding block in baseline (Table 1). The magnitude of these differences tended to increase within the session. This was confirmed to be reliable by the results of a one-way repeated-measures ANOVA revealing a significant block effect, $F(5, 45) = 3.72, p < 0.05$. Thus, when given for the first time in the test environment *cis*-flupenthixol produced a significant intrasession decline in food-rewarded operant responding, replicating the results seen in the *cis*-flupenthixol group.

The *cis*-flupenthixol group was expected to show an increasing effect of the drug over days. Although there appeared to be less responding and a greater intrasession decline on the third injection day for the *cis*-flupenthixol group, neither of these effects achieved significance in the ANOVA reported above. The home cage control group was included to evaluate the possibility that the expected trends were due to the effects of repeated injections per se rather than an effect of the combination of drug injection and behavioural testing. It was planned to compare the test results of the home cage control group to test days 1 and 3 of the *cis*-flupenthixol group. If possible increasing effects of the drug in the *cis*-flupenthixol group were related to experience with the test environment while drugged, the home cage control group, although receiving the drug for the third time, should have performed like the *cis*-flupenthixol group on the first test day. The ANOVA comparing the results of the home cage control group to test day 1 of the *cis*-flupenthixol group revealed no significant main effect of group or interaction, suggesting that performance was similar. On the other hand, the ANOVA comparing the home cage control group to test day 3 of the *cis*-flupenthixol group revealed a significant time × group interaction, $F(5, 90) = 2.45, p < 0.05$. Tests of simple main effects of group at each 5-min block revealed that the *cis*-flupenthixol group responded significantly less than the home cage control group during the sixth 5-min block, $F(1, 18) = 4.52, p < 0.05$. These results suggest that the *cis*-flupenthixol group tended to respond less with repeated injections of the drug and that this tendency was not simply related to the effects of repeated drug injections.

DISCUSSION

Results of Experiment 1 revealed that unilateral intracaudate putamen microinjections of *cis*-flupenthixol dose dependently (25.0 but not 10.0 or 1.0 μg in 0.5 μl) increased ipsilateral turning in amphetamine-pretreated rats. In Experiment 2, when the effective dose (25 μg), but not saline or the geometric isomer *trans*-flupenthixol, was injected bilaterally into the dorsal caudate putamen of animals responding for food on a variable interval schedule a time-dependent decrease in re-

sponding was seen. This effect was similar to the gradual loss of responding seen in animals no longer receiving reward [cf. (4)]. Results from the home cage control group suggested that the pattern of responding seen over days in the *cis*-flupenthixol group did not result simply from having received *cis*-flupenthixol previously. Overall, results may indicate a role for dopaminergic projections to the dorsal caudate putamen in mediating the ability of food reward to maintain operant responding.

The finding that intracaudate putamen injections of *cis*-flupenthixol dose dependently produced ipsilateral turning in amphetamine-treated animals is in excellent agreement with previous studies showing ipsilateral asymmetry following intracaudate putamen *cis*-flupenthixol plus systemic apomorphine (8). It is noteworthy that when *cis*-flupenthixol was injected into the caudate putamen of animals not pretreated with a stimulant drug no turning was seen (8,16). The series of five central injection sessions in Experiment 1 was preceded and followed by no-injection sessions. Although ipsilateral turning scores appeared to be higher in the second no-injection session, the difference was not significant, suggesting that neither chronic cannulation of the dorsal caudate putamen nor the series of five central injections had significant enduring effects on directional bias, as reported previously in studies from this laboratory (16,27). The related finding that *trans*-flupenthixol injections, which preceded and followed the series of three *cis*-flupenthixol sessions, did not produce a significant directional bias further shows that *cis*-flupenthixol-produced turning did not result from mechanical stimulation of striatal tissue with fluid or from other properties of the drug not related to its action as a DA receptor antagonist. Finally, the observation of significant dose-dependent turning following *cis*-flupenthixol, even though the doses were given in a counterbalanced order across rats, provides strong support for the conclusion that the effects of the drug were related to its action as an antagonist of DA receptors.

In Experiment 1, directionality and total turns were evaluated. However, it appeared that only the former dependent variable was influenced in a consistent manner by the treatment condition; total turns were observed to show a significant increase across treatments regardless of treatment condition. As turning ratios were dose dependent despite this trend, this finding underscores the utility of directional bias or relative turning as a sensitive measure reflecting bilateral differences in striatal function. The general increase in total turns across sessions may reflect a sensitization to the effects of systemic amphetamine, which was injected prior to each session. Thus, it has been reported frequently that the stimulant effects of amphetamine increase with repeated injections, especially if those injections are paired with a particular test environment (17,19).

An issue that pertains to both Experiments 1 and 2 concerns the possibility of diffusion of *cis*-flupenthixol [cf. (28)]. If *cis*-flupenthixol diffused away from the site of injection, to ventral regions of the striatum, for example, it would be incorrect to attribute its behavioural effects to an action in the dorsal caudate putamen. Ahlenius et al. (1) investigated this question by taking advantage of the effects of DA receptor antagonists on levels of DA metabolites. They injected *cis*-flupenthixol (40 μg in 1.0 μl) into either the caudate putamen or nucleus accumbens and measured the concentrations of the DA metabolites 3,4-dihydroxyphenylacetic acid and homovanillic acid at the site of injection and in neighboring areas. They found that the metabolites were affected only at the site of injection and concluded that *cis*-flupenthixol has a low rate of diffusion from the site of injection. Especially when it is emphasized that the dose and volume of *cis*-flupenthixol used in the present experiments were lower than those used in the studies of Ahlenius et al. (1), it seems possible to attribute the behavioural effects of *cis*-flupenthixol observed here to its action in the dorsal caudate putamen.

In Experiment 2, *cis*-flupenthixol, but not its inactive geometric isomer *trans*-flupenthixol or saline, produced a time-dependent intrasession decline in operant responding for food. Although there was a tendency for response rates to decline over *cis*-flupenthixol test sessions, this effect was not statistically significant. However, when the home cage control group, which had received two home cage injections of *cis*-flupenthixol several days prior to its first test session with the drug, was compared to the *cis*-flupenthixol group, evidence supporting an increasing effect of *cis*-flupenthixol over days of testing was found. Thus, the intrasession pattern of responding for food of the home cage control group did not differ significantly from the day 1 pattern of the *cis*-flupenthixol group but did differ from the day 3 pattern; the home cage control group responded significantly more than the *cis*-flupenthixol group in the latter 5-min blocks of the session. The observation of an intrasession decline in responding for food of rats having received *cis*-flupenthixol into the dorsal caudate putamen and the finding of some evidence for an increasing effect of the drug with repeated testing resemble the extinction patterns of responding seen in animals no longer receiving food reward [cf. (4,30)]. These results support the hypothesis that dopaminergic projections to the dorsal caudate putamen may mediate the usual effects of food reward on operant responding.

Some recent results are congruent with the present findings. G. Phillips et al. (24) reported that the dopamine D_2 receptor antagonist sulpiride, when injected into the dorsal caudate putamen, produced a gradual intrasession decline in operant responding. Further, they found that intraaccumbens sulpiride, although decreasing responding, did not produce a gradual extinction-like pattern. The finding from the dorsal caudate putamen is in excellent agreement with the present results. The additional finding that accumbens injections of a DA antagonist failed to produce a gradual effect provides further indirect support for the present hypothesis that dopaminergic projections to the dorsal striatum play a critical role in mediating the usual effects of food reward on operant behaviour.

A number of other findings are consistent with this hypothesis. Thus, neurotoxic destruction of dopaminergic projections to the caudate putamen but not the accumbens impaired lever pressing for food (2); further, in that study the effect of the lesion was observed to increase from test session to test session, suggesting a gradual loss of the effects of food reward on operant behaviour. Evenden (11) similarly found that DA-depleting lesions of the caudate putamen but not the accumbens produced an extinction-like effect in his analysis of win-stay patterns of lever pressing for food. Indirect support also comes from the observation that 6-hydroxydopamine lesions of the nucleus accumbens that impaired psychomotor stimulant self-administration failed to affect operant responding for food (26,29). Similarly, excitotoxic destruction of the nucleus accumbens impaired lever pressing for morphine but not food (10). Together with the present findings, these results suggest that dopaminergic projections from the substantia nigra to the caudate putamen may mediate the ability of food reward to maintain operant responding for food.

There is evidence from postmortem neurochemical studies

that levels of DA and its metabolites in the caudate putamen may increase following feeding or lever pressing for food (5, 7). In vivo electrochemical studies similarly have shown that eating or lever-pressing for food increased accumbens and/or caudate putamen DA release (14,15). These results further implicate caudate putamen DA in food reward.

In conclusion, the present results, showing that intracaudate putamen injections of the DA receptor antagonist *cis*-flupenthixol produced intrasession decreases in food-rewarded operant responding, support the hypothesis that dopaminergic projections to the caudate putamen play a critical role in mediating the effects of food reward on operant responding. It has been suggested that food reward, and therefore DA, produces incentive learning involving a change in the ability of stimuli associated with reward to elicit approach and other responses in the future (3). Present and related results would suggest that incentive learning involving food reward may be mediated by dopaminergic projections to the dorsal caudate putamen.

ACKNOWLEDGEMENTS

trans- and *cis*-Flupenthixol were the generous gifts of H. Lundbeck and amphetamine was the gift of Smith, Kline, and French, Canada, Ltd. This research was supported by a grant from the Natural Sciences and Engineering Research Council of Canada to R.J.B.

REFERENCES

1. Alhenius, S.; Hillegaart, V.; Thorell, G.; Magnusson, O.; Fowler, C. J. Suppression of exploratory locomotor activity and increase in dopamine turnover following the local application of *cis*-flupenthixol into limbic projection areas of the rat striatum. Brain Res. 402:131–138; 1987.
2. Amalric, M.; Koob, G. F. Depletion of dopamine in the caudate nucleus but not in nucleus accumbens impairs reaction-time performance in rats. J. Neurosci. 7:2129–2134; 1987.
3. Beninger, R. J. The role of dopamine in locomotor activity and learning. Brain Res. Rev. 6:173–196; 1983.
4. Beninger, R. J.; Cheng, M.; Hahn, B. L.; Hoffman, D. C.; Mazurski, E. J.; Morency, M. A.; Ramm, P.; Stewart, R. J. Effects of extinction, pimozide, SCH 23390, and metoclopramide on food-rewarded operant responding of rats. Psychopharmacology (Berl.) 92:343–349; 1987.
5. Blackburn, J. R.; Phillips, A. G.; Jakubovic, A.; Fibiger,H. C. Increased dopamine metabolism in the nucleus accumbens and striatum following consumption of a nutritive meal but not a palatable nonnutritive saccharin solution. Pharmacol. Biochem. Behav. 25:1095–1100; 1986.
6. Bozarth, M. A.; Wise, R. A. Involvement of the ventral tegmental dopamine system in opioid and psychomotor stimulant reinforcement. In: Harris, L. S., ed. Problems of drug dependence. NIDA Research Monograph 67. Washington, DC: U.S. Government Printing Office; 1986:190–196.
7. Church, W. H.; Sabol, K. E.; Justice, J. B., Jr.; Neill, D. B. Striatal dopamine activity and unilateral bar pressing in rats. Pharmacol. Biochem. Behav. 25:865–871; 1986.
8. Costall, B.; Kelly, M. E.; Naylor, R. J. The production of asymmetry and circling behaviour following unilateral, intrastriatal administration of neuroleptic agents: A comparison of abilities to antagonise striatal function. Eur. J. Pharmacol. 96:79–86; 1983.
9. Donovik, P. J. A metachromatic stain for neural tissue. Stain Tech. 49:49–51; 1974.
10. Dworkin, S. I.; Guerin, G. F.; Goeders, N. E.; Smith, J. E. Kainic acid lesions of the nucleus accumbens selectively attenuate morphine self-administration. Pharmacol. Biochem. Behav. 29: 175–181; 1988.
11. Evenden, J. L. Reinforcers and sequential choice: "Win-stay" and the role of dopamine in reinforcement. In: Commons, M. L.; Church, R. M.; Stellar, J. R.; Wagner, A. R., eds. Quantitative analyses of behaviour. Hillsdale, NJ:Lawrence Erlbaum; 1988: 183–206.
12. Heffner, T. G.; Hartman, J. A.; Seiden, L. S. Feeding increases dopamine metabolism in the rat brain. Science 208:1168–1170; 1980.
13. Holmes, L. J.; Smythe, G. A.; Storlien, L. H. Monoaminergic activity at the level of the hypothalamus and striatum: Relationship to anticipated feeding and pancreatic insulin responses. Brain Res. 496:204–210; 1989.
14. Joseph, M. H.; Hodges, H. Lever pressing for food reward and changes in dopamine turnover and uric acid in rat caudate and nucleus accumbens studied chronically by in vivo voltammetry. J. Neurosci. Meth. 34:143–149; 1990.
15. Joseph, M. H.; Hodges, H.; Gray, J. A. Lever pressing for food reward and in vivo voltammetry: Evidence for increases in extracellular homovanillic acid, the dopamine metabolite, and uric acid in the rat caudate nucleus. Neuroscience 32:195–201; 1989.
16. Josselyn, S. A.; Beninger, R. J. Behavioural effects of intrastriatal caffeine mediated by adenosinergic modulation of dopamine. Pharmacol. Biochem. Behav. 39:97–103; 1991.
17. Kalivas, P. W.; Stewart, J. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res. Rev. 16:223–244; 1991.
18. Keppel, G. Design and analysis: A researcher's handbook. Englewood CliffF, NJ: Prentice Hall; 1982.
19. Mazurski, E. J.; Beninger, R. J. Environment-specific conditioning and sensitization with (+)-amphetamine. Pharmacol. Biochem. Behav. 27:61–65; 1987.
20. Moller-Neilsen, I.; Pedersen, V.; Nymark, M.; Franck, K. F.; Boeck, V.; Fjalland, B.; Christensen, A. V. The comparative pharmacology of flupenthixol and some reference neuroleptics. Acta Pharmacol. Toxicol. 33:353–362; 1973.
21. Pellegrino, L. J.; Pellegrino, A. S.; Cushman, J. A. A stereotaxic atlas of the rat brain. New York: Plenum; 1979.
22. Phillips, A. G.; Brooke, S. M.; Fibiger, H. C. Effects of amphetamine isomers and neuroleptics on self-stimulation from the nucleus accumbens and dorsal noradrenergic bundle. Brain Res. 85: 13–32; 1975.
23. Phillips, A. G.; Carter, D. A.; Fibiger, H. C. Dopaminergic substrates of intracranial self-stimulation in the caudate putamen. Brain Res. 104:221–232; 1976.
24. Phillips, G.; Willner, P.; Muscat, R. Anatomical substrates for neuroleptic-induced reward attenuation and neuroleptic-induced response decrement. Behav. Pharmacol. 2:129–141; 1991.
25. Prado-Alcala, R.; Wise, R. A. Brain stimulation reward and dopamine terminal fields. I. caudate putamen, nucleus accumbens and amygdala. Brain Res. 297:265–273; 1984.
26. Roberts, D. C. S.; Corcoran, M. E.; Fibiger, H. C. On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. Pharmacol. Biochem. Behav. 6:615–620; 1977.
27. Thanos, P. K.; Jhamandas, K.; Beninger, R. J. *N*-methyl-D-aspartate unilaterally injected into the dorsal striatum of rats produces contralateral circling: Antagonism by 2-amino-7-phosphonoheptanoic acid and *cis*-flupenthixol. Brain Res. 589: 55–61; 1992.
28. Wise, R. A.; Hoffman, D. C. Localization of drug reward mechanisms by intracranial injections. Synapse 10:247–263;1992.
29. Wise, R. A.; Rompre, P.-P. Brain dopamine and reward. Annu. Rev. Psychol. 40:191–225; 1989.
30. Wise, R. A.; Spindler, J.; deWit, H.; Gerber, G. J. Neuroleptic-induced "anhedonia" in rats: Pimozide blocks reward quality of food. Science 201:262–264; 1978.