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Microinjections of flupenthixol into the caudate–putamen but not the nucleus accumbens, amygdala or frontal cortex of rats produce intra-session declines in food-rewarded operant responding

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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 this hypothesis by injecting the DA antagonist *cis*-flupenthixol (25 μg in 0.5 μl) into the dorsal caudate–putamen of rats ($n = 19$) trained to lever press for food presented according to a variable interval 30-s schedule. Additional groups received non-reward ($n = 8$), systemic *cis*-flupenthixol (0.01, 0.1 mg/kg i.p.; ns = 8), dorsal caudate–putamen injections of the inactive isomer *trans*-flupenthixol ($n = 10$), frontal cortical (dorsal to the caudate–putamen site) injections of *cis*-flupenthixol ($n = 6$), or *cis*- or *trans*-flupenthixol injected into the nucleus accumbens (*n*s = 9, 8) or amygdala (*n*s = 6, 5). Rats were tested in 30-min sessions and response rates were recorded every 5 min. As expected, non-reward produced a gradual decline in responding. A similar pattern was seen in the groups receiving systemic (0.1 mg/kg) or dorsal caudate–putamen injections of *cis*-flupenthixol. No significant effect was seen following systemic (0.01 mg/kg), cortical or amygdala *cis*-flupenthixol or dorsal caudate–putamen or amygdala *trans*-flupenthixol. Accumbens *cis*-flupenthixol reduced rates but did not produce a gradual decline in responding; however, accumbens *trans*-flupenthixol led to a time-dependent elevation in response rates making interpretation of the accumbens results difficult. It was concluded that dopaminergic projections to the dorsal caudate–putamen may play a critical role in mediating the effects of food-reward on operant responding.

INTRODUCTION

"There are converging inputs to the globus pallidus from the nucleus accumbens (ventral striatum) and the caudate nucleus (neostriatum)... The nucleus accumbens and the caudate nucleus filter signals from the limbic structures and the cerebral cortex (from the "emotive brain" and "cognitive brain")... A major challenge for future research is how the "emotive brain" and the "cognitive brain" operate together in response initiation." (Mogenson et al. 1980, ref. 18, pp. 88 and 92)

There is general agreement that dopaminergic neurons originating in the ventral mesencephalon form a critical link in the neuronal circuitry mediating the effects of reward on behaviour (for reviews see Refs. 3 and 34). A number of investigators have shown that blockade of dopamine (DA) receptors 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 to their minimum level immediately following treatment with DA antagonists but rather declined gradually within and/or across test sessions (e.g. see Refs. 4 and 35). This extinction-like pattern of responding strongly suggested that the effect of DA receptor antagonists was to attenuate the rewarding impact of food. It also ruled out explanations based on simple motor deficits produced by the drugs; such deficits would be expected to be relatively constant throughout the session.

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[6,29,34] and the nucleus accumbens and caudate–putamen both have been implicated in brain stimulation reward[22,23,28]. The role of dopaminergic projections to the nucleus accumbens

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and caudate–putamen in food reward is less clear, however.

Neurochemical and voltammetric studies implicate dopaminergic projections to both accumbens and caudate–putamen target regions in food reward. Thus, postmortem levels of DA or its metabolites suggested increased activity in mesoaccumbens[5,13,14] and/or nigrostriatal DA neurons[5,7] following feeding or lever pressing for food. In vivo electrochemical studies similarly showed that feeding or lever pressing for food increased accumbens and/or caudate–putamen DA release[15,16].

Some observations suggest that dopaminergic projections to the caudate–putamen may be critical for food reward. It has been reported that neurotoxic destruction of dopaminergic projections to the caudate-putamen but not the accumbens impaired lever pressing for food[2]. G. Phillips et al.[24] found that intra-caudate/putamen but not intra-accumbens microinjections of the DA antagonist sulpiride produced a gradual decrease in food-rewarded lever press responding. Evenden[10] 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.[29] and Wise and Rompré[34] 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[9]. 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. This might suggest that responding for food reward is more importantly influenced by signals from the cortex (that are filtered by the caudate–putamen) than by signals from limbic structures (that are filtered by the nucleus accumbens), a distinction discussed by Mogenson et al.[18].

On the basis of these results, it was 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 was 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 non-reward.

For comparison, a group receiving non-reward following saline injections and groups receiving systemic injections of *cis*-flupenthixol were included. An additional control group received intra-caudate–putamen injections of the inactive isomer, *trans*-flupenthixol. To further evaluate the specificity of the caudate–putamen in this effect, other groups receiving *cis*-flupenthixol injected into the cortex or *cis*- or *trans*-flupenthixol injected into the nucleus accumbens or amygdala were included.

MATERIALS AND METHODS

*Subjects**

Male Wistar rats ($n = 89$), obtained from Charles River Canada, weighed between 220 and 275 g upon arrival in the colony. The rats were individually housed in hanging wire cages and maintained in a climactically controlled environment (21 °C) on a 12 h light/dark cycle (lights on a 06.00 h). The rats were maintained at 85% of their free-feeding weights, adjusted for growth, through daily measured food rations (Purina Rat Chow). Water was continuously available in the home cage.

Surgery

Following preliminary lever press training (see below), 65 animals were anaesthetized with an intraperitoneal (i.p.) injection of sodium pentobarbital (60.0 mg/kg, MTC Pharmaceuticals) and secured in a Kopf stereotaxic instrument with the incisor bar set at 5.0 mm above the horizontal plane passing through the interaural line. The 65 rats in the central injection groups received bilateral stainless steel guide cannulae (0.64 mm diam) implanted at the following coordinates anterior to bregma, lateral to the midline and ventral to the dura mater[21], respectively: caudate–putamen: 1.4, 3.0 and 3.5 mm; cortex: 1.4, 3.0 and 1.5 mm; nucleus accumbens: 3.4, 2.2 and 6.5 mm; amygdala: 0.4, 4.0 and 7.5 mm. Guide cannulae were anchored to the skull with four stainless steel jeweller'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 0.5 or 1.0 mm beyond the tip of the guide cannulae. They were attached to a Hamilton microsyringe by a length of polyethylene tubing. A 10-μl Hamilton microsyringe and infusion pump (Sage Instruments) were used to deliver injections over 45 s.

* Treatment of the 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.

The injection cannula was left in place for 60 s. *cis*-Flupenthixol dihydrochloride (Lundbeck) was delivered in a dose of 25 μg in 0.5 μl of distilled water. The pH of the *cis*-flupenthixol solution was approximately 3.0. *trans*-Flupenthixol dihydrochloride (Lundbeck), the pharmacologically inactive geometric isomer of *cis*-flupenthixol[19], in a dose of 25.0 μg in 0.5 μl of distilled water served as the control for the non-specific effects of pH and osmolality. (The 25-μg dose of *cis*-flupenthixol was chosen with the use of tests of turning responses following unilateral intra-caudate–putamen injections of various doses in amphetamine-pretreated rats; the lowest dose that produced turning was chosen.)

Apparatus

Four identically constructed experimental chambers (23.0 × 20.4 × 19.5 cm) consisted of aluminum plate sides and a transparent plexiglass top and door. The grid floor was made 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 and was 5.5 cm above the floor. The chamber was illuminated by an internal light (7 W), enclosed in a plywood box insulated with sound attenuating Styrofoam, and ventilated by a small fan. Data collection and schedules of food reward were controlled by a single board computer.

Behavioural testing: central injection groups

Pre-surgery. All rats were trained to press the lever for 45 mg food pellets (Bioserv) delivered on a variable interval (VI) 30-s schedule; i.e., response-contingent reward became available every 30 s on average (range: 5–90 s). For the 65 animals in the central injection groups, presurgery 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. Following these training sessions, rats undergoing surgery were placed on free food for several days, implanted with cannulae (see above), and allowed several days to recover before again being food-deprived.

Establishment of baseline. Implanted rats were trained daily on the VI 30-s schedule, 30 min per day, until responding stabilized; the criterion for stability required that the response rate for any of 3 consecutive days did not vary by more than 10% either above of below the mean rate for the 3 days. It took from 5 to 14 days to establish criterion. Drug testing then began.

Drug testing. Each animal was tested three times. Central injections were made immediately prior to each 30-min drug testing session and sessions were separated by 48 h. Animals remained in their home cages on the intervening days. The groups were as follows: caudate *cis*-flupenthixol ($n = 19$), caudate *trans*-flupenthixol ($n = 10$), cortex *cis*-flupenthixol ($n = 6$), accumbens *cis*-flupenthixol ($n = 9$), accumbens *trans*-flupenthixol ($n = 8$), amygdala *cis*-flupenthixol ($n = 6$) and amygdala *trans*-flupenthixol ($n = 5$). The dependent variable was the number of responses during each 5-min period of the three 30-min test sessions.

Behavioral testing: systemic injection groups

The 24 animals that received non-reward or systemic *cis*-flupenthixol were trained to lever press for food and received 10 sessions of training on the VI 30-s schedule. The rats were then ranked on the basis of their average response rates during the last three sessions, selected three at a time, and randomly assigned to one of three groups: non-reward ($n = 8$), *cis*-flupenthixol 0.01 mg/kg ($n = 8$) and *cis*-flupenthixol 0.1 mg/kg ($n = 8$). Each group received three test sessions separated by 48 h. *cis*-Flupenthixol was injected i.p. 2 h before test sessions. Non-reward animals received saline 2 h before test sessions during which lever press responses no longer produced food pellets.

Statistical analyses

For each group, data consisted of the mean number of responses for each 5-min interval of each of the last three 30-min sessions of the VI 30-s schedule that preceded treatment and the corresponding means for the three treatment sessions. For each group these values were entered into a 3-variable repeated measures analysis of variance (ANOVA), the variables being: time (5-min block), day, and phase (pretreatment and treatment). For all repeated measures the more conservative Geisser–Greenhouse adjusted degrees of freedom were used[17]. Significant interactions were further analyzed with the use of tests of simple main effects followed by multiple comparisons using the method of Newman–Keuls.

Histology

At the conclusion of behavioural testing the cannulated rats were killed with a lethal dose of sodium pentobarbital, exsanguinated with 0.9% saline and perfused intracardially with 10% formalin. The brains were removed and stored in 4% formalin for a week. Frozen coronal sections (50 μm) were mounted and stained with formal-thionin[8] to verify cannulae placements.

RESULTS

Histology

Cannula placements for the 65 rats receiving central injections are indicated in Fig. 1. Caudate–putamen

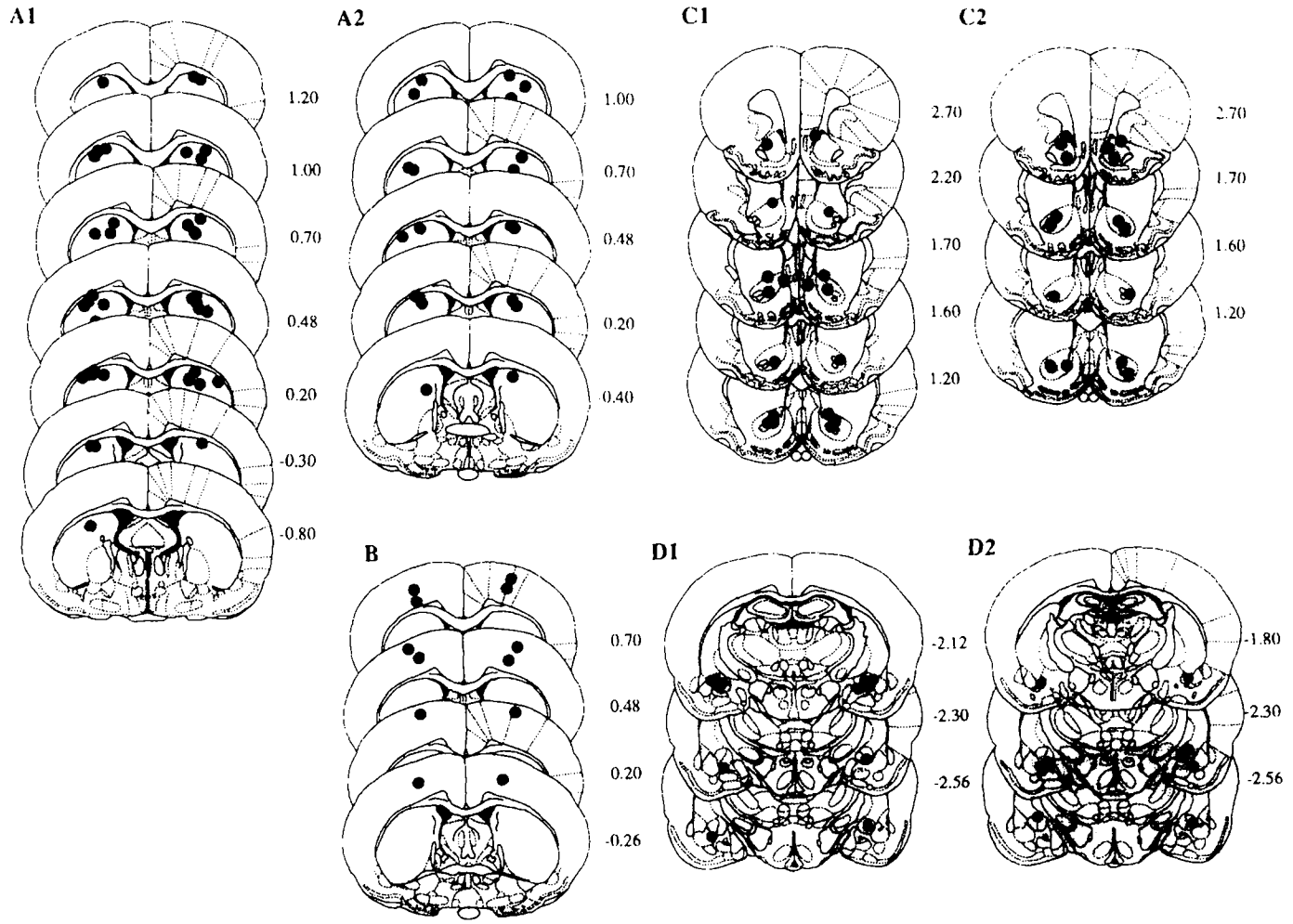

Fig. 1. Coronal sections showing the location of the cannulae tips for the rats receiving *cis*-flupenthixol (A1) or *trans*-flupenthixol (A2) into the caudate–putamen, *cis*-flupenthixol into the cortex dorsal to the caudate–putamen (B), *cis*-flupenthixol (C1) or *trans*-flupenthixol (C2) into the nucleus accumbens, or *cis*-flupenthixol (D1) or *trans*-flupenthixol (D2) into the amygdala. Drawings were adapted from Paxinos and Watson[20]; the numbers to the right of each section indicate the distance (mm) anterior to bregma.

placements were in the dorsal anterior region of the nucleus. Cortical placements were in the frontal cortex immediately dorsal to the caudate–putamen site. Accumbens placements tended to be in the anterior region of the accumbens. Cannulae tips in the amygdala group were usually in or near the central nucleus.

Behavior

Systemic injections: Fig. 2 shows the mean (+S.E.M.) responses for each 5-min segment of the three 30-min sessions of the VI 30-s schedule that preceded treatments and the same data for the three treatment sessions for groups receiving non-reward or the two doses of systemic *cis*-flupenthixol. The non-reward group showed a pattern of gradual decline in responding both within and across sessions, the classic extinction effect. The 0.01 mg/kg dose of *cis*-flupenthixol appeared to have little effect on responding. The 0.1 mg/kg dose produced an overall decrease in responding as well as a within-session decrease in responding.

This description of the data was supported by the results of statistical analyses. For the non-reward group, ANOVA revealed significant main effects of time, $F_{5,35} = 3.80$, $P < 0.05$, day, $F_{2,14} = 10.54$, $P < 0.01$, and phase, $F_{1,7} = 17.82$, $P < 0.001$, as well as significant interactions of time and phase, $F_{5,35} = 14.21$, $P < 0.001$, and day and phase, $F_{2,14} = 6.65$, $P < 0.05$. Tests of simple main effects of time within each phase revealed that the time effect was significant only in the treatment phase, $F_{5,35} = 16.24$, $P < 0.001$. Similarly, the day effect was significant only in the treatment phase, $F_{2,14} = 9.43$, $P < 0.02$. These results and an examination of Fig. 2 make it clear that the within-session and session-to-session decline produced by non-reward was highly reliable.

None of the main effects or interactions from the

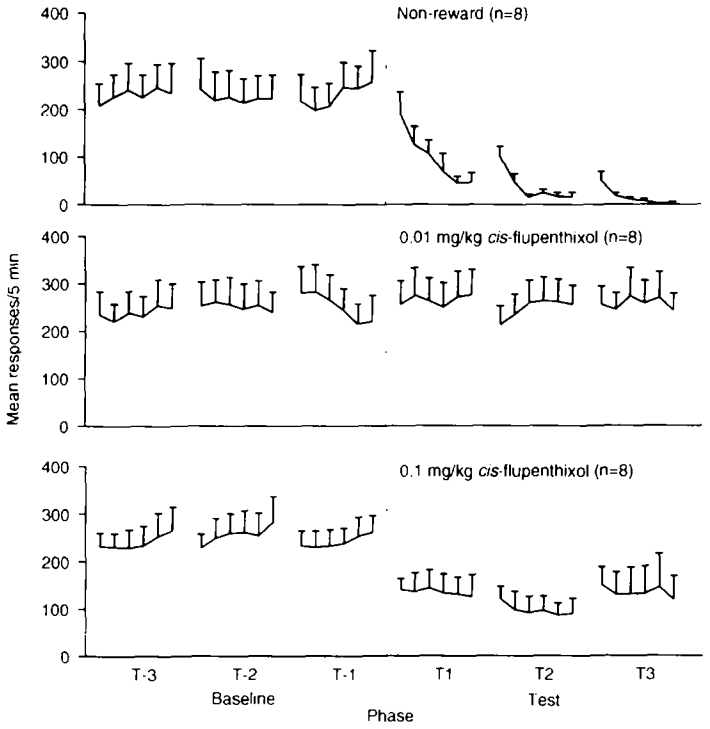

Fig. 2. Mean number of responses for each 5-min block of each of the last three 30-min sessions of the VI 30-s schedule that preceded treatment (Baseline: T-3, T-2, T-1) and the corresponding means for the three treatment sessions (Test: T1, T2, T3) for the groups receiving ip saline plus non-reward (panel 1) or an ip dose of 0.01 (panel 2) or 0.1 mg/kg (panel 3) of *cis*-flupenthixol 2 h before each treatment session. Verticle bars represent S.E.M.

ANOVA of the 0.01 mg/kg systemic dose of *cis*-flupenthixol was significant. For the 0.1 mg/kg dose, the main effect of phase was significant, $F_{1,7} = 12.34$, $P < 0.01$, showing that the overall decline in responding produced by the drug was reliable. The ANOVA also revealed a significant time × phase interaction, $F_{5,35} = 3.88$, $P < 0.05$, and tests of simple main effects revealed non-significant time effects in each phase but significant phase effects at every time, $Fs_{1,7} = 19.02$, 9.91, 12.18, 9.59, 9.66, and 15.17, $Ps < 0.02$, for 5-min blocks 1 to 6, respectively. Thus, tests of simple main effects failed to reveal the source of the interaction.

In a further effort to study the interaction of time and phase in the group receiving 0.1 mg/kg *cis*-flupenthixol, difference scores were calculated between the pretreatment phase and the treatment phase at each time, averaging over days since there was no effect of this variable. The means (+ S.E.M.) of these scores are shown in Table I and can be seen to increase in magnitude from the beginning to the end of the session. This was confirmed by a one-way repeated measures ANOVA revealing a significant time effect, $F_{5,35} = 3.88$, $P < 0.05$, and multiple comparisons revealing that the difference in the first time block was significantly smaller than the corresponding value in the sixth time block. Thus, although 0.1 mg/kg *cis*-flupenthixol produced a significant decrease in responding at every time block, the effect was greatest in the final time block, suggesting a gradual within-session decline in responding similar to that seen in the non-reward group.

TABLE I

Mean differences in responses per 5 min (phases 1 minus 2) during each 5-min block for each group (S.E.M. in parentheses)

Group	*Time (5-min blocks)*					
	T1	*T2*	*T3*	*T4*	*T5*	*T6*
Non-reward*	108.38	151.13	179.99	195.00	216.00	215.83
	(39.27)	(40.81)	(43.66)	(42.48)	(41.54)	(51.06)
cis-Flupenthixol						
Systemic						
0.01 mg/kg	14.83	3.25	-11.54	-16.92	26.21	-22.25
	(18.46)	(14.11)	(11.11)	(12.46)	(12.08)	(18.65)
0.1 mg/kg*	93.88	115.58	118.54	123.58	132.88	158.37
	(21.52)	(36.71)	(33.96)	(39.91)	(42.76)	(40.66)
Intracerebral						
Caudate–putamen*	56.74	45.68	41.26	60.09	73.82	94.21
	(11.70)	(13.80)	(11.67)	(17.68)	(15.48)	(19.34)
Cortex	-0.78	10.00	-10.06	-7.67	1.50	3.44
	(12.34)	(20.21)	(9.84)	(17.95)	(18.55)	(13.33)
Accumbens	37.63	19.78	25.41	39.44	37.19	40.22
	(17.40)	(16.02)	(16.18)	(15.42)	(8.66)	(12.26)
Amygdala	3.06	6.28	-5.11	5.56	15.61	24.61
	(13.80)	(14.36)	(15.23)	(14.10)	(14.06)	(18.77)
trans-Flupenthixol						
Caudate–putamen	61.63	14.50	-4.90	6.90	10.67	3.60
	(32.59)	(12.86)	(15.54)	(12.34)	(9.36)	(11.77)
Accumbens*	-8.96	-53.38	58.63	-65.46	-66.25	-39.13
	(15.80)	(18.60)	(20.13)	(20.19)	(24.43)	(25.61)
Amygdala	26.87	12.93	5.20	30.33	36.07	24.00
	(24.37)	(17.12)	(14.57)	(18.02)	(39.71)	(18.28)

* significant at $P < 0.05$ as determined by one-way analysis of variance.

Caudate–putamen and cortical injections: mean (+ S.E.M.) responses for each 5-min segment of three pretreatment and treatment sessions for the groups receiving caudate–putamen injections of *cis*-flupenthixol, *trans*-flupenthixol or cortical injections of *cis*-flupenthixol are shown in Fig. 3. Animals receiving caudate–putamen *cis*-flupenthixol injections showed an overall decrease in responding and an intra-session pattern consisting of an initial increase in rates from the first time block to the second or third, like that seen in baseline, followed by a decline. Intra-caudate/putamen *trans*-flupenthixol appeared to have little effect on the rate or pattern of responding except for a decrease in the first 5-min block. There was no consistent effect of cortical *cis*-flupenthixol on operant responding for food.

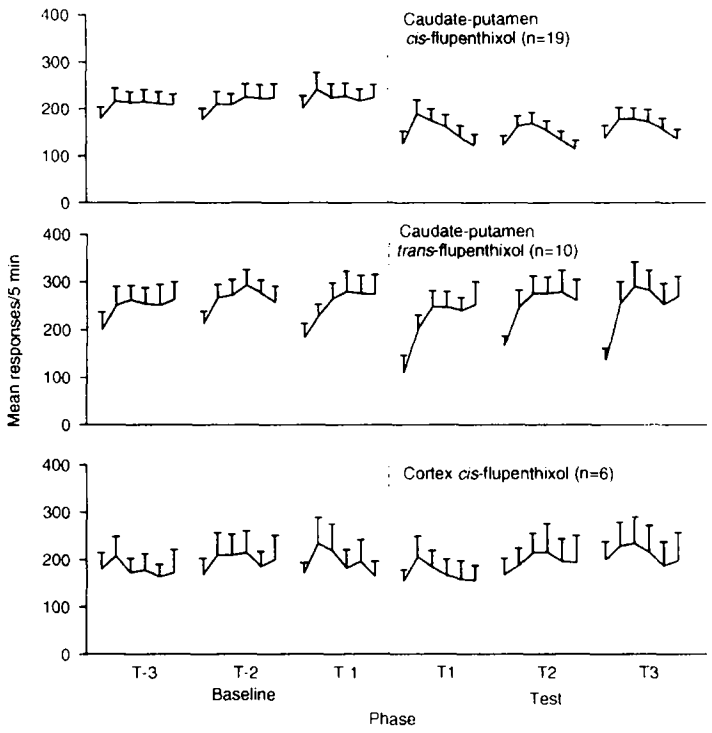

Fig. 3. Mean number of responses for each 5-min block of each of the last three 30-min sessions of the VI 30-s schedule that preceded treatment (Baseline: T-3, T-2, T-1) and the corresponding means for the three treatment sessions (Test: T1, T2, T3) for groups receiving microinjections of 25.0 μg/0.5 μl *cis*-flupenthixol into the caudate–putamen (panel 1) or the cortex just dorsal (panel 3) or 25.0 μg/0.5 μl *trans*-flupenthixol into the caudate–putamen (panel 2) immediately prior to each treatment session. Verticle bars represent S.E.M.

This description of the results was borne out by statistical tests. The analysis of the cortical *cis*-flupenthixol group revealed no significant main effects or interactions. The analysis of the caudate–putamen *trans*-flupenthixol group revealed only a significant main effect of time, $F_{5,45} = 13.84$, $P < 0.001$.

The caudate–putamen *cis*-flupenthixol group, on the other hand, was found to have significant time, $F_{5,90} = 8.13$, $P < 0.001$, phase, $F_{1,18} = 21.80$, $P < 0.001$, and time by phase interaction effects, $F_{5,90} = 5.73$, $P < 0.001$. Tests of simple main effects revealed a time effect in each phase, $Fs_{5,90} = 4.89$ and 9.00, $Ps < 0.01$, for the pretreatment and treatment phases, respectively, and a phase effect at each time, $Fs_{1,18} = 23.52$, 10.96, 12.50, 11.55, 22.74, and 23.74, $Ps < 0.005$, for times 1 to 6, respectively. Thus, as was the case for the group receiving 0.1 mg/kg *cis*-flupenthixol, tests of simple main effects failed to reveal the source of the significant phase by time interaction.

To further explore the interaction, difference scores were calculated as shown in Table I. Difference scores generally increased across the session. This was confirmed by a one-way ANOVA revealing a significant time effect, $F_{5,90} = 5.73$, $P < 0.005$. Multiple comparisons showed that times 1, 2, 3 and 4 differed from time 6, and that time 3 differed from time 5. Thus, intra-caudate/putamen injections of *cis*-flupenthixol, but not similar injections into the cortex above the caudate–putamen or intra-caudate/putamen injections of *trans*-flupenthixol, produced an intra-session decline in responding similar to that seen in the non-reward group.

Accumbens and amygdala injections: mean responses per 5 min for the last three pretreatment sessions and the three treatment sessions for the groups receiving *cis*- or *trans*-flupenthixol into the nucleus accumbens or amygdala are shown in Fig. 4. In the nucleus accumbens, *cis*-flupenthixol produced a decrease in responding especially on the first treatment day but

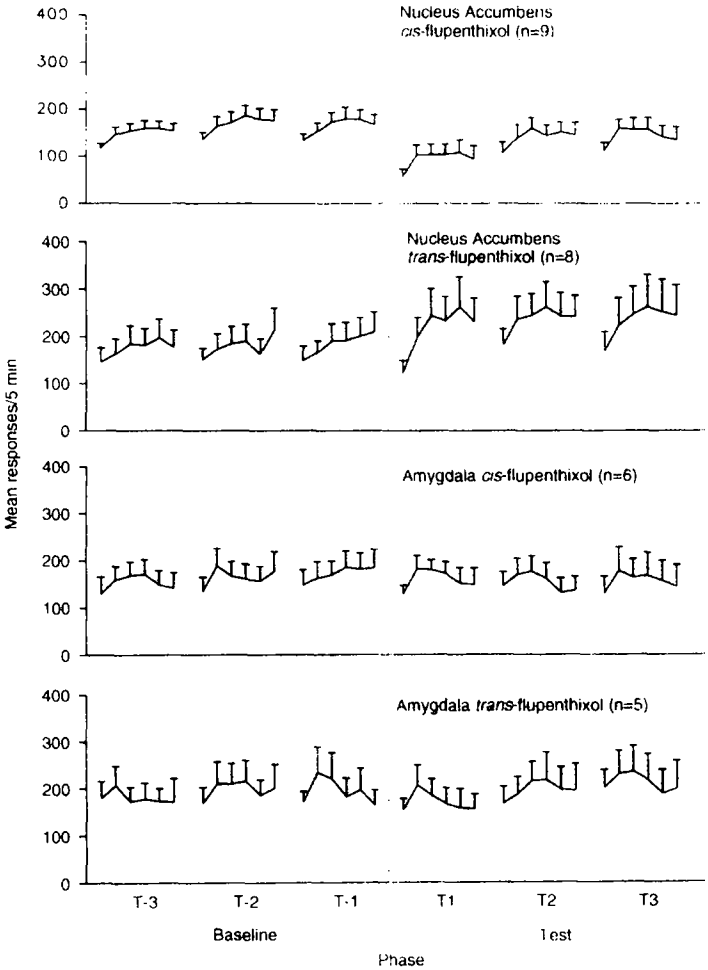

Fig. 4. Mean number of responses for each 5-min block of each of the last three 30-min sessions of the VI 30-s schedule that preceded treatment (Baseline: T-3, T-2, T-1) and the corresponding means for the three treatment sessions (Test: T1, T2, T3) for groups receiving microinjections of 25.0 μg/0.5 μl of *cis*-flupenthixol into the accumbens or amygdala (panels 1 and 3, respectively) or 25.0 μg/0.5 μl of *trans*-flupenthixol into the accumbens or amygdala (panels 2 and 4, respectively) just prior to each treatment session. Verticle bars represent S.E.M.

the intra-session pattern of responding did not appear to change; *trans*-flupenthixol produced an increase in responding. Injections of either compound into the amygdala had little effect.

These results were confirmed by ANOVAs. Thus, for the *cis*-flupenthixol nucleus accumbens group, there were significant effects of phase, $F_{1,8} = 8.97$, $P < 0.02$, day, $F_{2,6} = 10.21$, $P < 0.001$, and time, $F_{5,40} = 6.56$, $P < 0.02$. The day and time effects occurred when phases were combined; as neither of these variables interacted with phase, results suggest that treatments with *cis*-flupenthixol had little effect on the intra- or inter-session pattern of responding. The phase effect confirmed that rates decreased when animals were injected with *cis*-flupenthixol into the nucleus accumbens.

The *trans*-flupenthixol group showed significant effects of phase, $F_{1,7} = 6.86$, $P < 0.05$, time, $F_{5,35} = 6.74$, $P < 0.05$, phase × time, $F_{5,35} = 4.01$, $P < 0.05$, and day × time, $F_{10,70} = 2.08$, $P < 0.05$. Tests of simple main effects revealed a significant time effect in the treatment phase, $F_{5,35} = 7.33$, $P < 0.05$, but not the pretreatment phase. Thus, the time by phase interaction resulted from the greater increase in responding from 5-min block to 5-min block of animals treated with *trans*-flupenthixol into the nucleus accumbens compared to their own pretreatment response rates. This pattern can be seen in the difference scores shown in Table I. ANOVA of difference scores revealed a significant time effect, $F_{5,35} = 4.01$, $P < 0.05$, and multiple comparisons revealed that time 1 differed from times 2, 3, 4 and 5. It would appear that intra-accumbens *trans*-flupenthixol resulted in a significant intra-session enhancement of responding.

For the amygdala groups, only the main effect of time was significant, $F_{5,25} = 8.39$, $P < 0.01$ and $F_{5,20} = 6.32$, $P < 0.02$, for the *cis*- and *trans*-flupenthixol groups, respectively. These ANOVAs confirmed that intra-amygdala injections of *cis*- or *trans*-flupenthixol had no significant effect on operant responding for food.

DISCUSSION

Results revealed that non-reward produced a within- and between-session decline in operant responding previously rewarded with food according to a variable interval schedule. This pattern has been termed extinction and was described decades ago by Skinner[31]. Animals treated systemically (0.1 mg/kg) or with intra-caudate–putamen injections of *cis*-flupenthixol showed extinction-like intra-session declines in responding. Intra-cortical, accumbens or amygdala *cis*-flupenthixol failed to produce significant intra-session declines in responding while *trans*-flupenthixol into the caudate–putamen or amygdala had little effect. *trans*-Flupenthixol into the accumbens produced a time-dependent increase in responding. Results suggest that dopaminergic neurotransmission in the caudate–putamen plays an important role in mediating the effects of food reward on operant behavior.

It is noteworthy that non-reward and *cis*-flupenthixol did not produce identical patterns of responding (see Fig. 2). There are a number of possible explanations of this observation. For example, the dose of *cis*-flupenthixol may not have produced a complete block of reward; additionally, conditioned reward associated with food pellets, which continued to be delivered to the group receiving systemic *cis*-flupenthixol may have influenced the pattern of responding. Whatever the explanation for the differences between the drug and non-reward groups, the within-session decline observed in animals treated systemically with *cis*-flupenthixol is in good agreement with numerous previous studies (e.g. Refs. 4 and 35). The present study adds *cis*-flupenthixol to a long list of compounds producing extinction-like declines in operant responding for food including chlorpromazine, haloperidol, metoclopramide, pimozide, SCH 23390, and sulpiride. In agreement with the present results, Whishaw et al.[35] found that *cis*-flupenthixol produced a trial-to-trial decline in swimming rewarded with a platform providing safety. Some researchers raised the possibility that declines in responding seen after treatments with DA receptor blockers may be attributable to motoric effects of these compounds. Although there is little doubt that DA receptor antagonists affect motor activity, there now are many experimental findings supporting the conclusion that the extinction-like pattern produced by these compounds reflects a block of the usual effects of reward on behavior (e.g. ref. 12).

Results of the present study revealed that intra-caudate/putamen injections of *cis*-flupenthixol, like systemic treatments, produced an extinction-like intra-session decline in responding for food. Control studies showed that injections of *cis*-flupenthixol into the cortex dorsal to the caudate–putamen injection site produced no significant effect on responding. This finding, and the observation that *cis*-flupenthixol injected into the accumbens or amygdala failed to produce extinction-like effects, provides excellent anatomical control data for the caudate–putamen *cis*-flupenthixol group; Wise and Hoffman[33] have suggested that such anatomical controls are necessary before the results of local injections can be attributed to the site of delivery.

Ahlenius et al.[1] took another approach to investigating the possible spread of *cis*-flupenthixol to regions

remote from the site of injection. They injected *cis*-flupenthixol into the caudate–putamen or accumbens in different animals and then removed both structures from each group and analyzed them for the DA metabolite 3,4-dihydroxyphenylacetic acid (DOPAC). Results revealed that injections of a higher concentration (40 μg) in double the volume (1.0 μl) used here significantly reduced DOPAC levels in the target structure but had no significant effect on the other structure or on either structure in the contralateral hemisphere. These data and the anatomical controls included in the present study seem to allow the conclusion that DA in the caudate–putamen plays an important role in mediating the effects of food reward on operant responding.

Wise and Hoffman[33] further suggested that pharmacological controls, the use of inactive and active isomers, are needed to rule out possible nonspecific effects (e.g. osmolarity, pH) of the active isomer. The observation that the inactive geometric isomer of *cis*-flupenthixol, *trans*-flupenthixol was without significant effect, in the present study, when injected into the caudate–putamen provides strong support for the conclusion that DA in the caudate–putamen mediates the effects of food reward on operant responding.

Another point raised by Wise and Hoffman[33] is relevant to the present findings. They argued that drugs injected locally in the brain at their putative site of action should be effective in doses that are orders of magnitude lower than the required peripheral dose. In the present study, the effective peripheral dose of *cis*-flupenthixol was 0.1 mg/kg or 100 μg/kg; as the rats weighed approximately 250 g at the time of injection, they received 25 μg. This peripheral dose can be compared to a central dose of 25 μg per side or a total central dose of 50 μg! From these observations it might be expected that the central dose should have been as effective or more effective than the peripheral dose regardless of where it was injected. However, this clearly was not the case as delivery of the 50 μg central dose was ineffective in the cortex or amygdala and did not produce an intra-session decline in the accumbens. The timing of the injections may have affected the results as the peripheral dose was given 2 h before the session whereas the central dose was given immediately before the session. Perhaps a 2-h delay following central administration of *cis*-flupenthixol would lead to the observation of within-session declines in responding regardless of the site of injection. Further studies are needed to examine this possibility.

Injections of *cis*-flupenthixol into the nucleus accumbens resulted-in a decrease in responding but an intra-session decline was not seen. In animals treated with accumbens *trans*-flupenthixol, on the other hand, a significant intra-session increase in responding was observed. This effect of *trans*-flupenthixol was not seen in the caudate–putamen or amygdala. This result suggests that some property of flupenthixol, when injected into the accumbens, other than its action as a DA receptor antagonist, acts to produce a gradual increase in responding within the session. If this was the case, animals injected with *cis*-flupenthixol may have been influenced by both aspects of the drug, one producing an intra-session increase in responding and the other a general decrease in responding.

We attempted to remove the apparent non-specific effects of flupenthixol, those produced by the trans isomer, from the results recorded for *cis*-flupenthixol in the accumbens. To do this, the percent increase in responding over baseline produced by *trans*-flupenthixol at each time block for the three treatment sessions combined was calculated. The number of responses at each time block for animals treated with *cis*-flupenthixol was then reduced by the appropriate percent of baseline to remove the non-specific influence of the drug. This yielded response numbers ($\pm$ S.E.M.) of 85.24($\pm$ 17.71), 91.29($\pm$ 16.99), 90.97($\pm$ 17.72), 83.13($\pm$ 16.98), 79.80($\pm$ 14.93) and 92.49($\pm$ 18.02) for 5-min time blocks 1 through 6, respectively, for the three treatment sessions combined. ANOVA of these data yielded a time effect that was not significant but that approached significance, $F_{4,40} = 2.80$, $P < 0.08$. Thus, even when the possible non-specific effects of flupenthixol injected into the nucleus accumbens were removed, *cis*-flupenthixol failed to produce a significant intra-session decline in responding.

The present observation that intra-accumbens injections of *cis*-flupenthixol decreased responding for food without producing an intra-session decline is consistent with other reports. Thus, G. Phillips et al.[24] found that intra-accumbens sulpiride decreased responding for food but, unlike intra-caudate–putamen sulpiride, failed to produce an intra-session decline in responding. Salamone et al.[30] similarly found that intra-accumbens haloperidol decreased responding for food but intra-session data were not presented. These results suggest that, although DA in the nucleus accumbens plays an important role in motor control, it may not be critical for food reward. However, the present finding that *cis*-flupenthixol into the nucleus accumbens produced a non-significant intra-session decrease in responding that approached significance, when the possible non-specific influence of flupenthixol was removed, suggests that conclusions concerning the role of accumbens DA in food reward should be made with caution.

Injections of *cis*- or *trans*-flupenthixol into the central nucleus of the amygdala had no significant effect on

food-rewarded operant responding. This finding is in excellent agreement with that of G. Phillips et al.[24] who found that sulpiride injected into the basolateral amygdala failed to affect operant responding for food. These findings are consistent with a more extensive literature showing that animals undergoing excitotoxic lesions of the basolateral amygdala are not impaired in lever pressing tasks (see Ref. 11).

Our finding that injections of *cis*-flupenthixol into the dorsal anterior region of the caudate–putamen influenced lever pressing may be related to reports of regional specialization of function in this structure. Studies by Pisa and colleagues[25–27] have shown that the lateral portion of the dorsal anterior caudate–putamen controls forelimb reaching. This might suggest that injections of a DA antagonist into this region would lead to decreased lever pressing as a result of impaired forelimb motor function. However, the observation of a gradual, within-session decline in responding for food following intra-caudate–putamen injections of *cis*-flupenthixol argues against a simple motor deficit which might be expected to remain relatively consistent throughout the session. The argument that the observed effects of intra-caudate–putamen *cis*-flupenthixol may be attributable to an impairment of forelimb motor function is the same as the argument that response decrements following systemic treatment with DA antagonists may be attributable to a general impairment of motor function. As reviewed elsewhere[3,34], the *pattern* of responding seen following treatments with DA antagonists and numerous related data (e.g. Ref. 12) show that the effects of DA antagonists on responding for reward cannot be attributed solely to motor effects.

Overall, the present findings suggest that the ability of food reward to maintain operant responding may depend critically on dopaminergic projections to the caudate–putamen. Other results support this conclusion. Amalric and Koob[2] found that neurotoxic destruction of dopaminergic projections to the caudate–putamen but not the accumbens impaired lever pressing for food. Evenden[10] 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. Finally, G. Phillips et al.[24] found that intra-caudate–putamen but not intra-accumbens sulpiride produced an intra-session decline in food-rewarded operant responding. These findings support the conclusion that DA in the caudate–putamen may play a critical role in mediating the effects of food reward on operant responding.

Other findings provide indirect support for this conclusion. Roberts et al.[29] and Wise and Rompré[34] reported that 6-hydroxydopamine lesions of the nucleus accumbens impaired stimulant self-administration but not lever pressing for food. Similarly, Dworkin et al.[9] found that kainic acid lesions of the nucleus accumbens impaired lever pressing for morphine but not food. These results show that intact dopaminergic neurotransmission in the nucleus accumbens is not necessary for the control of operant responding by food reward.

Finally, neurochemical and voltammetric studies implicate dopaminergic projections to the caudate–putamen in food reward. Postmortem levels of DA or its metabolites suggested increased activity in nigrostriatal DA neurons following feeding or lever pressing for food[5,7]; accumbens DA also was affected[5,13,14]. In vivo electrochemical studies similarly showed that feeding or lever pressing for food increased accumbens and caudate–putamen DA release[15,16]. Although these findings show that lever pressing for food or simply feeding may increase both accumbens and caudate–putamen DA, when considered along with the data reviewed above, they can be seen to be consistent with the conclusion that caudate–putamen DA may play a critical role in mediating the effects of food reward on operant responding. It has been suggested that reward produces incentive motivational learning, increasing the ability of stimuli signalling reward to elicit responses in the future[3]. Mogenson et al.[18] distinguished between the limbic and cortical afferents to the ventral and dorsal striatum, respectively; from this point of view, the present results suggest that incentive motivational effects of food reward may act critically on the filtering of signals from the cortex in the caudate–putamen.

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