Psychopharmacology (1993) 113:110–118

Psychopharmacology

Dopamine D_1 and D_2 antagonists attenuate amphetamine-produced enhancement of responding for conditioned reward in rats

Robert Ranaldi and Richard J. Beninger

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

Received November 20, 1992 / Accepted April 19, 1993

Abstract. It has been suggested that the dopamine D_1 receptor may play an important role in reward. The present study was undertaken to investigate the roles of dopamine D_1 and D_2 receptor subtypes in responding for conditioned reward. This was done by examining the effects of the D_1 antagonist SCH 23390 and the D_2 antagonists pimozide and metoclopramide on amphetamine-produced enhancement of responding for conditioned reward. The procedure consisted of three distinct phases. During the pre-exposure phase the rats were exposed to an operant chamber containing two levers. One lever produced a lights-off stimulus (3 s) and the other a tone stimulus (3 s). This was followed by four conditioning sessions during which the levers were removed and the rats were exposed to pairings of the lights-off stimulus with food. This phase was followed by two test sessions during which the levers were present and the number of responses made on each was calculated as a ratio of the number of responses made during the pre-exposure phase. A group receiving the vehicle during the test sessions showed a greater ratio of responding for the lights-off stimulus than the tone stimulus, indicating that the lights-off stimulus had become a conditioned reward. Amphetamine (0.1, 1.0, 2.0 and 5.0 mg/kg, IP, 5 min prior to test) specifically enhanced responding on the lever producing conditioned reward. SCH 23390 (5.0 and 10.0 μg/kg, SC, 2 h before test) and pimozide (0.1 and 0.2 mg/kg, IP, 4 h before test) dose-dependently shifted the peak in the amphetamine dose-response function to the right, indicating an attenuation of conditioned reward. Metoclopramide (1.0, 5.0 and 7.5 mg/kg, IP, 1 h before test) reduced the amphetamine-produced enhancement of responding for conditioned reward but failed to shift the amphetamine dose-response function. These results provide evidence that both D_1 and D_2 receptor subtypes are essential in responding for conditioned reward.

Correspondence to: R.J. Beninger

Key words: D_1 receptors – D_2 receptors – Conditioned reward – Reward – Dopamine – Amphetamine – SCH 23390 – Pimozide – Metoclopramide

A neutral stimulus can be made to acquire incentive motivational properties by pairing it with an unconditioned rewarding stimulus such as food after which it elicits approach and related responses similar to those elicited by the unconditioned reward (Bindra 1974). The previously neutral stimulus also can be shown to act as a conditioned reward (Beninger 1989b, 1991). For example, animals pressed a lever that produced a stimulus (e.g., light) previously paired with reward (e.g., food) more than another lever that produced a stimulus (e.g., tone) not paired with reward (Mackintosh 1974).

Animals treated with indirect dopamine (DA) agonists show an enhancement of responding for conditioned reward (Hill 1970; Robbins 1975, 1976; Robbins and Koob 1978; Beninger et al. 1980, 1981; Robbins et al. 1983; Taylor and Robbins 1984, 1986; Mazurski and Beninger 1986; Cador et al. 1991; Kelley and Delfs 1991a,b; Beninger and Ranaldi 1992). Furthermore, DA antagonists given during pairing sessions resulted in an attenuation or blockade of the conditioned reward effect (Beninger and Phillips 1980; Hoffman and Beninger 1985). These studies strongly suggest that DA transmission is involved in responding for conditioned reward and corroborate an extensive literature implicating DA in reward-related learning (for reviews see Beninger 1983; Wise and Rompré 1989).

The role of DA receptor subtypes in reward has been examined. D_1 and D_2 antagonists produced intra-session declines in responding for brain stimulation reward (Fenton and Liebman 1982; Nakajima and McKenzie 1986; Nakajima and O'Regan 1991) and food (Nakajima 1986; Beninger et al. 1987) and reduced the rewarding efficacy of self-administered cocaine or amphetamine (Roberts and Vickers 1984; Koob et al. 1987; Kleven and

Woolverton 1990; Corrigall and Coen 1991). Thus, both D_1 and D_2 receptor antagonists attenuate reward.

The effects of agonists also have been investigated. The D_2 agonists, bromocriptine and quinpirole, enhanced responding for conditioned reward whereas the D_1 agonist, SKF 38393, did not and at higher doses appeared to block the conditioned reward effect (Beninger and Ranaldi 1992). For brain stimulation reward quinpirole decreased thresholds whereas SKF 38393 had no significant effect at moderate doses and completely suppressed responding at higher ones (Nakajima and O'Regan 1991). Both D_1 and D_2 agonists were self-administered (Woolverton et al. 1984; Self and Stein 1992) and both, when injected directly into the nucleus accumbens of rats, produced a place preference (White et al. 1991).

It appears that D_2 agonists facilitate the rewarding effects of stimuli in conditioned reward and brain stimulation reward studies. D_1 agonists impair, or do not increase, responding in these paradigms although place conditioning and self-administration were produced by D_1 agonists. It may be that tonic stimulation of D_1 receptors by D_1 agonists impairs the ability of discrete environmental stimuli (e.g., a lever) to control responding for reward by increasing the ability of other stimuli in the environment to elicit responses. By this same mechanism, D_1 agonists may produce place conditioning where the measured response is simply approach to the drug-paired environment (Beninger 1992). Thus, the role of the D_1 receptor in reward is best understood by considering the nature of the measured response. In this way, the D_1 receptor may be seen as crucial in reward processes.

The present study examined the hypothesis that stimulation of D_1 receptors is critical for reward-related learning. Accordingly, the dose-dependent enhancement of responding for conditioned reward seen with amphetamine was expected to be shifted to the right following injection of the selective D_1 antagonist, SCH 23390 (Hyttel 1983; Iorio 1983). For comparison the effects of the D_2 antagonists, pimozide (Seeman 1981) and metoclopramide (Jenner et al. 1978; Rotrosen et al. 1981; Harrington et al. 1983), also were assessed.

Materials and methods

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.

Subjects. Male Wistar rats ($n=249$), obtained from Charles River Canada, weighing between 225 and 275 g (free-feeding) were individually housed in a temperature-controlled environment (21°C) on a 12-h light-dark cycle (lights on at 0600 hours). Rats were habituated to the housing environment for approximately 1 week and their weights increased by 25–40 g. Weights were then reduced to 80% of these values for the 11-day duration of the experiment through daily feedings with measured rations.

Apparatus. The experimental environments consisted of four similarly constructed operant chambers. The dimensions were 29 cm in length, 23 cm in width and 19 cm in height. The chambers were constructed of aluminum sides and plexiglass tops and doors. The floors consisted of aluminum grids. Each chamber was placed in a ventilated sound-attenuating box. Each 29 cm wall of each chamber contained a 7.5-cm by 3.5-cm removable lever. A force of approximately 0.09 N was required to depress each lever. At the center of the 23-cm wall was stationed a 2.0- by 4.0-cm feeder cup at a height of 2.5 cm from the floor. An illuminated 2-W light bulb was positioned on each side (8.5 cm apart) of the feeder cup at a height of 10 cm from the floor. Each chamber also contained a 4.9-Khz tone generator positioned at 14 cm from the floor between the two light bulbs and at the center of the 23-cm wall. The tone generator was adjusted to deliver a tone 10 dB above the background noise level.

Procedure. Each group was exposed to an experimental design that consisted of three distinct phases referred to as the pre-exposure, conditioning and test phase. Following is a description of the procedure employed for the vehicle group. All groups followed exactly the same procedure except that drugs were injected prior to each test session, as described below.

The pre-exposure phase consisted of five 40-min sessions held one per day on 5 consecutive days at approximately the same time each day. Two levers were present. One lever produced the tone stimulus and the other the lights-off stimulus. Both stimuli lasted 3 s. Two of the chambers had the tone-producing lever on the right wall and the lights-off-producing lever on the left wall while the relationship between lever side and stimulus was reversed for the other two chambers. The number of responses on each lever was measured for each pre-exposure session.

The conditioning phase consisted of four 60-min sessions held one per day for the 4 consecutive days following the last day of the pre-exposure phase. During conditioning both levers were removed from the operant chamber and the rats were exposed to 80 presentations of the 3-s lights-off stimulus according to a random time 45-s schedule. That is, the average time between lights-off stimulus presentations was 45 s (range was 5–90 s). During the first conditioning session each lights-off stimulus presentation was terminated with the delivery of one 45-mg food pellet (Bioserv). During the remaining three conditioning sessions food delivery occurred following a random 33% of the lights-off stimulus presentations. This procedure was employed as Knott and Clayton (1966) observed that partial pairing resulted in a greater magnitude of conditioned reward than continuous pairing.

The test phase consisted of two 40-min sessions held on the 2 consecutive days following the last day of conditioning. The levers were again present in the operant chambers and the number of responses on each lever was measured. Conditioned reward was observed as a relative increase in the number of responses on the lights-off stimulus lever in the test phase compared to the pre-exposure phase.

A total of 26 groups was tested. One group ($n=12$) received 0.9% saline, IP, 5 min prior to and Tween 80, SC, 2 h prior to each test session. Four groups ($n=11$–13) received amphetamine in doses of 0.1, 1.0, 2.0 and 5.0 mg/kg, IP, 5 min prior to each test session. All other groups received one of three doses of amphetamine (1.0, 2.0 or 5.0 mg/kg, always IP, 5 min prior to each test session) and a D_1 or D_2 antagonist. Thus, three groups ($n=10$) received the three doses of amphetamine and 5.0 μg/kg SCH 23390, SC, 2 h prior to each test session and three other groups ($n=10$) received the amphetamine doses and 10.0 μg/kg SCH 23390 2 h prior to each test session. Nine groups ($n=9$–10) received the three amphetamine doses and 1.0, 5.0 or 7.5 mg/kg metoclopramide, IP, 1 h prior to each test session. Six groups ($n=7$–9) were administered the three amphetamine doses and 0.1 or 0.2 mg/kg pimozide, IP, 4 h prior to each test session.

The experiments were conducted with 16–32 rats at a time. Each experiment consisted of randomly assigning these rats to groups of four and assigning each group to one of the drug conditions. This ensured that different groups were tested at any one time, randomizing any possible error associated with the testing of different squads. Each drug condition was replicated at least once, bringing the *ns* for each group to eight or more.

Drug preparation. (+)-Amphetamine sulphate (Smith, Kline and French Canada Inc.) was dissolved in saline and injected in a concentration of 1 ml/kg body weight. SCH 23390 was suspended in a small quantity of the polymer, polyoxyethylene sorbitan monooleate (Tween 80) and added to distilled water at an appropriate concentration to yield an injection volume of 1 ml/kg. Metoclopramide was dissolved in distilled water and injected in a concentration of 1 ml/kg. Finally, pimozide was dissolved in boiling tartaric acid (at a concentration of 6 mg/ml distilled water) and injected in a concentration of 1 ml/kg. All drugs were prepared daily immediately prior to injection.

Data analyses. The data within the last 30 min provided the most stable estimate of pre-conditioning rates. In previous studies (Hoffman and Beninger 1985) the number of responses in each 10-min segment of pre-exposure sessions was analyzed and it was found that rates were higher in the first 10 min but did not differ significantly for the remaining 10-min periods. Therefore, only data from the last 30 min were used for pre-exposure sessions in the analyses of the present results. The number of responses made on each lever during the last 30 min of the five sessions in the pre-exposure phase was averaged for each rat. The number of responses made on each lever during the last 30 min of each session in the test phase was averaged for each rat. Finally, the number of responses on each lever in the test phase was divided by the number of responses on that lever in the pre-exposure phase [adding 1.0 to each value entering into the ratio to reduce the influence of numerically small numbers (see Winer 1971)]. These ratios were square root transformed to normalize their distribution for the purposes of analyses (Keppel 1982). Thus, the data consisted of two numbers for each rat.

To evaluate the conditioned reward effect in the vehicle group, a one-way analysis of variance (ANOVA) compared the ratios for each lever. A significantly higher ratio of responding on the lights-off lever than on the tone lever was taken as evidence that conditioned reward had occurred. The results for groups receiving each drug were subjected to two-way ANOVAs with repeated measures on the lever factor. When only a dose effect was seen, multiple comparisons, using the Newmann Keuls procedure, determined the source of this effect. When a lever effect or a lever by dose interaction was seen, the ANOVA was conducted again, this time including the vehicle group. The error term for the interaction from this ANOVA was then used to make interaction comparisons between the vehicle group and each drug dose (Keppel 1982). When the ratio for the lights-off lever was greater than the ratio for the tone lever for a drug dose and there was a significant interaction of lever and dose in the comparison with vehicle, it was concluded that the dose enhanced responding for conditioned reward.

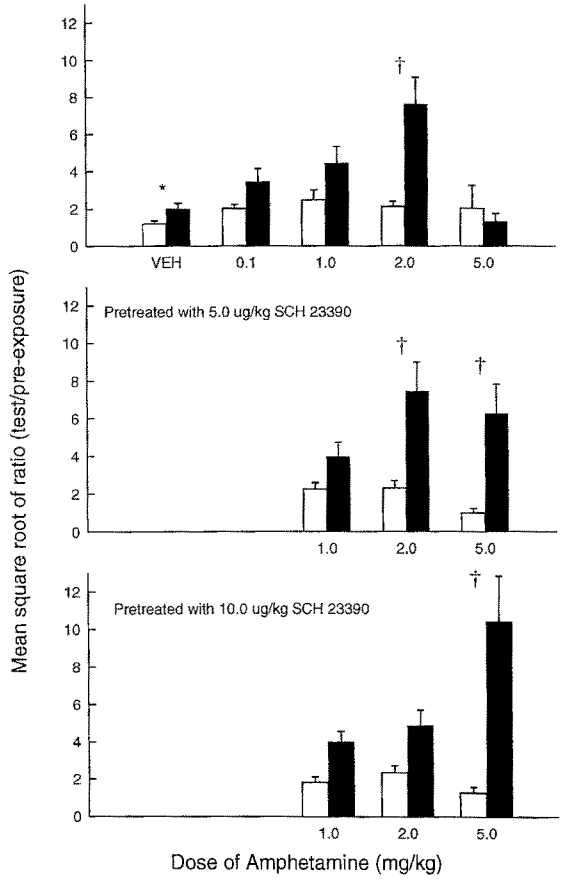

Fig. 1. Mean square roots of ratios of test phase responding relative to pre-exposure responding on each lever for groups receiving vehicle, amphetamine or combined amphetamine and SCH 23390 treatments. *Vertical bars* represent the standard errors of the mean. Lights-off (■) was the conditioned stimulus; tone (□) was the neutral stimulus. Amphetamine was administered IP, 5 min prior to testing. SCH 23390 was administered SC, 2 h prior to testing. *Significant ($P<0.01$) conditioned reward effect in the vehicle group. †Significant enhancement of the conditioned reward effect when compared to vehicle

Results

Figure 1 shows the mean (±SEM) square roots of ratios of responding on each lever in the test phase for the vehicle, amphetamine and SCH 23390-pretreated groups. The untransformed baseline mean (±SEM) rates (responses per 30 min) of pressing on both levers during both phases for the vehicle group were as follows: 8.11 (±1.33) and 10.91 (±2.66) on the tone and lights-off levers, respectively, during the pre-exposure phase and 13.71 (±3.98) and 38.92 (±9.15) on the tone and lights-off levers, respectively, during the test phase. The vehicle group showed greater ratios of responding on the lever that produced the lights-off stimulus than on the lever that produced the tone stimulus, suggesting a conditioned reward effect. The amphetamine groups appeared to show a dose-related inverted U function for responding on the conditioned reward lever; the effect appeared to peak in the 2.0 mg/kg group. The groups pretreated with 5.0 μg/kg SCH 23390 (middle panel) also showed a dose-related increase in responding with more responding on the lights-off lever. However, a specific increase in responding on the lights-off lever in the group that received 5.0 mg/kg amphetamine was observed, an effect that was not seen in the 5.0 mg/kg amphetamine-alone group. Groups pretreated with 10.0 μg/kg SCH 23390 tended to show a dose-related increase in responding with more presses on the lights-off lever. Interestingly, the amphetamine-produced enhancement of responding in the 2.0 mg/kg amphetamine group was reduced by 10.0 μg/kg SCH 23390 while responding for the lights-off stimulus in the group receiving the higher dose of amphetamine was greater. In other words, both doses of SCH 23390 appeared to shift the amphetamine effect to the right. The statistical analyses supported these observations.

An ANOVA on the vehicle data revealed a significant lever effect, [$F(1,11)=10.98$, $P<0.01$], confirming that the

lights-off stimulus acted as a conditioned reward. Groups treated with amphetamine showed a significant lever by dose interaction, [$F=(3,41)=6.27$, $P<0.005$]. This interaction indicated that amphetamine produced a greater increase in responding on the lights-off lever than on the tone lever and that this conditioned reward effect differed depending on the dose. To determine if the conditioned reward effect in the amphetamine groups was different from that in the vehicle group the ANOVA was repeated, this time including the vehicle group. The analyses revealed a significant lever by dose interaction, [$F(4,52)=6.37$, $P<0.005$]. Interaction comparisons revealed that the conditioned reward effect in the 2.0 mg/kg amphetamine group was significantly greater than that in the vehicle group, [$F=(1,52)=19.75$, $P<0.001$].

A two-way ANOVA on the data of the groups pretreated with 5.0 μg/kg SCH 23390 revealed a significant lever effect, [$F(1,27)=25.38$, $P<0.005$], indicating a conditioned reward effect in these groups. To determine if this conditioned reward effect was different from that in the vehicle group the ANOVA was repeated, this time with the data from the vehicle group included. The analysis revealed a significant lever by dose interaction, [$F(3,38)=4.10$, $P<0.01$]. Interaction comparisons revealed that the 2.0 and 5.0 mg/kg amphetamine groups showed significant enhancements of the conditioned reward effect.

A two-way ANOVA on the data from the groups pretreated with 10.0 μg/kg SCH 23390 revealed a significant lever by dose interaction, [$F(2,27)=7.02$, $P<0.01$]. This interaction indicated that the magnitude of the conditioned reward effect differed depending on the dose of amphetamine. The ANOVA was repeated, this time including the vehicle group, in order to determine if the conditioned reward effect in these groups was different from that in the vehicle group. The analysis revealed a significant lever by dose interaction, [$F(3,38)=9.10$, $P<0.005$]. Interaction comparisons revealed that the group receiving 5.0 mg/kg amphetamine showed a significant enhancement of the conditioned reward effect.

Figure 2 shows the mean (±SEM) square roots of ratios of responding on each lever in the test phase for the groups pretreated with pimozide. Groups receiving 0.1 mg/kg pimozide showed a dose-related increase in responding on the lever that produced the lights-off stimulus. Similar to the groups pretreated with 5.0 μg/kg SCH 23390, this dose of pimozide led to increased responding for the conditioned reward in the group receiving 5.0 mg/kg amphetamine, an effect that was not seen in the amphetamine-alone groups. Groups pretreated with 0.2 mg/kg pimozide showed greater increases in responding for the lights-off lever than for the tone but none of these groups appeared to show response patterns different from that in the vehicle group.

The statistical analyses supported the above observations. A two-way ANOVA on the 0.1 mg/kg pimozide data alone revealed a significant lever effect, [$F(1,19)=25.59$, $P<0.005$], confirming a conditioned reward effect in these groups. To investigate if this effect was different from that of the vehicle group the two-way ANOVA was repeated, this time including the data from

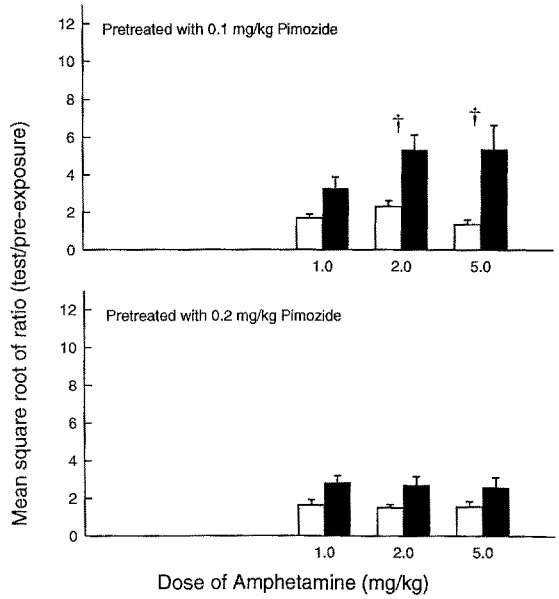

Fig. 2. Mean square roots of ratios of test phase responding relative to pre-exposure responding on each lever for groups receiving combined amphetamine and pimozide treatments. *Vertical bars* represent the standard errors of the mean. Lights-off (■) was the conditioned stimulus; tone (□) was the neutral stimulus. Pimozide was administered IP, 4 h before testing. † Significant ($P<0.01$) enhancement of the conditioned reward effect when compared to the same vehicle group as in Fig. 1

the vehicle group. This analysis revealed a significant lever by dose interaction, [$F(3,30)=3.78$, $P<0.05$], suggesting that there occurred an enhancement of the conditioned reward effect in these groups. Interaction comparisons located the enhanced conditioned reward effect in the groups administered 2.0 and 5.0 mg/kg amphetamine.

A 2-way ANOVA on the data from the groups pretreated with 0.2 mg/kg pimozide revealed a significant lever effect, [$F(1,22)=24.01$, $P<0.005$]. To determine if this conditioned reward effect was different from the effect in the vehicle group the ANOVA was repeated with the inclusion of the vehicle data. The analyses revealed a significant lever effect, [$F(1,33)=35.38$, $P<0.005$], but no significant lever by dose interaction. This ANOVA suggests that the conditioned reward effect in the groups pretreated with 0.2 mg/kg pimozide did not differ significantly from the vehicle group.

Figure 3 shows the mean (±SEM) square roots of ratios of responding on each lever in the test phase for the groups pretreated with increasing doses of metoclopramide. Groups pretreated with 1.0 mg/kg metoclopramide showed a dose-related increase in responding with more presses on the lever producing the lights-off stimulus. Although the greatest enhancement in responding occurred in the 2.0 mg/kg amphetamine group, it was slightly lower than in the amphetamine-alone group. Groups pretreated with 5.0 mg/kg metoclopramide (panel 2) responded less relative to the amphetamine-alone and 1.0 mg/kg metoclopramide groups. Only the 2.0 mg/kg amphetamine group appeared to show a conditioned reward effect. Finally, groups pretreated with 7.5 mg/kg

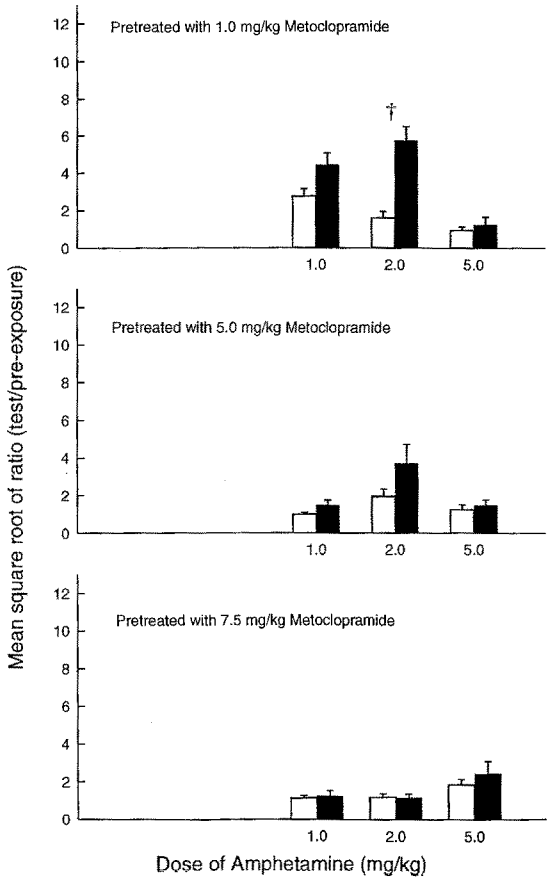

Fig. 3. Mean square roots of ratios of test phase responding relative to pre-exposure responding on each lever for groups receiving combined amphetamine and metoclopramide treatments. *Vertical bars* represent the standard errors of the mean. Lights-off (■) was the conditioned stimulus; tone (□) was the neutral stimulus. Metoclopramide was administered IP, 1 h before testing. †Significant ($P<0.01$) enhancement of the conditioned reward effect when compared to the same vehicle group as in Fig. 1

metoclopramide also showed decreased responding. This effect appeared to be much greater than with the smaller metoclopramide doses and none of these groups appeared to show responding that was greater than in the vehicle group. In general groups pretreated with increasing doses of metoclopramide showed a dose-related decrease in responding without shifting the peak amphetamine effect. The statistical analyses were in accordance with these observations.

A two-way ANOVA conducted on the data from the groups treated with 1.0 mg/kg metoclopramide revealed a significant lever by dose interaction, [$F(2,25)=9.73$, $P<0.005$], indicating a conditioned reward effect that depended on the dose of amphetamine. To determine if this conditioned reward effect differed from that in the vehicle group the two-way ANOVA was repeated with the inclusion of the vehicle group. This analysis revealed a significant lever by group interaction, [$F(3,36)=10.27$, $P<0.005$]. Interaction comparisons revealed that the group treated with 2.0 mg/kg amphetamine showed a significant enhancement of the conditioned reward effect.

A two-way ANOVA on the data from the groups receiving 5.0 mg/kg metoclopramide revealed a significant lever effect, [$F(1,25)=7.19$, $P<0.01$], indicating a conditioned reward effect in these groups. To determine if the conditioned reward effect was different from that in the vehicle group the two-way ANOVA was repeated, this time including the vehicle group. The analysis revealed a significant lever effect, [$F(1,36)=12.98$, $P<0.005$]. The failure to observe a significant lever by dose interaction indicates that the conditioned reward effect in the 5.0 mg/kg metoclopramide groups was not significantly different from that in the vehicle group.

A two-way ANOVA on the data from the groups treated with 7.5 mg/kg metoclopramide revealed a dose effect, [$F(2,25)=3.53$, $P<0.05$]. Inspection of Fig. 2 suggests that the significant dose effect occurred because of slightly less responding in the two lower amphetamine doses compared to the higher one. These groups failed to show a conditioned reward effect.

Discussion

Pairing of the lights-off stimulus with food pellets resulted in the lights-off stimulus becoming a conditioned reward. In previous experiments the conditioned stimulus was negatively correlated with food and conditioned reward was not seen (Beninger and Phillips 1980; Hoffman and Beninger 1985), demonstrating the importance of the positive contingency between food and the conditioned stimulus. The present observation of conditioned reward is consistent with many previous studies (Skinner 1938; Stein 1958; Hill 1970; Robbins 1978; Robbins and Koob 1978; Hoffman and Beninger 1985; Mazurski and Beninger 1986; Files et al. 1989).

Amphetamine dose-dependently enhanced responding for conditioned reward, an effect found previously in this (Mazurski and Beninger 1986; Beninger and Ranaldi 1992) and other laboratories (Robbins et al. 1983; Cohen and Branch 1991). Alternative explanations include the possibilities that amphetamine increased responding for the lights-off stimulus through (1) its ability to induce hyperactivity and stereotypy (Ungerstedt 1979) and/or by (2) altering the rats' sensitivity to light (Isaac 1971; Goetsch and Isaac 1983). In the first case, increases in responding on both levers might be expected. In fact, apomorphine, a direct acting DA agonist that produces hyperactivity and stereotypy (Ungerstedt 1979), did increase responding on both levers (Robbins et al. 1983; Mazurski and Beninger 1986; Beninger and Ranaldi 1992). If enhanced DA activity simply increases bar pressing behaviour then both apomorphine and amphetamine should have produced similar patterns of responding. That they did not strongly suggests that a general motor stimulant effect of amphetamine cannot explain the present results. With regard to altered sensitivity to light, we have shown that amphetamine, in rats exposed to no food, tones or lights-off stimuli during the conditioning phase, did not produce a response pattern significantly different from vehicle in the test. In control groups conditioned to the tone amphetamine produced

an enhancement of responding in the test (Beninger and Ranaldi 1992). These results strongly support the conclusion that amphetamine enhances responding for conditioned reward.

6-hydroxydopamine (6-OHDA) lesions of the meso-accumbens pathway eliminated the enhancement of responding for conditioned reward produced by intra-accumbens amphetamine (Taylor and Robbins 1986). However, lesions that depleted norepinephrine in the nucleus accumbens had no effect (Cador et al. 1991). These results suggest that in the nucleus accumbens it is amphetamine's action on dopaminergic, not noradrenergic, neurotransmission that is important in conditioned reward. Thus, amphetamine may have enhanced responding for conditioned reward in the present study by acting on dopaminergic neurotransmission.

Our finding that the D_1 antagonist, SCH 23390, decreased the enhancement of responding for conditioned reward produced by amphetamine is in agreement with the recent report by Chu and Kelley (1992) that SCH 23390 similarly decreased the effects of intra-accumbens amphetamine. Our results show further that SCH 23390 produced a dose-dependent shift to the right in the amphetamine dose-response function without reducing asymptotic responding. The failure to observe a complete reduction in the amphetamine-produced enhancement of responding with SCH 23390 may have been related to the relatively low doses used here. Higher doses of SCH 23390 might have reduced the asymptote in the amphetamine dose-response function. The present results show that SCH 23390 attenuated the reward-enhancing effects of amphetamine.

Our finding that the D_2 antagonist, pimozide, decreased the enhancement of responding for conditioned reward produced by amphetamine also is in agreement with the report by Chu and Kelley (1992) that raclopride, another D_2 antagonist, reduced the effects of intra-accumbens amphetamine. Our results show further that a low dose of pimozide shifted the peak amphetamine-produced enhancement of responding for conditioned reward to the right, suggesting decreased reward. The higher dose of pimozide eliminated the amphetamine-produced enhancement but not the conditioned reward effect itself. A higher dose of pimozide might have eliminated the conditioned reward effect. The shifts in dose-response function make it difficult to attribute the effects of pimozide and SCH 23390 to a simple motor impairment. These results suggest that pimozide and SCH 23390 acted to reduce the reward-enhancing effects of amphetamine and are in accordance with many studies showing that D_1 and D_2 antagonists impair reward (Fenton and Liebman 1982; Roberts and Vickers 1984; Nakajima 1986; Nakajima and McKenzie 1986; Beninger et al. 1987; Koob et al. 1987; Kleven and Woolverton 1990; Nakajima and O'Regan 1991).

Besides antagonizing D_1 and D_2 receptors, respectively, SCH 23390 and pimozide have actions on 5-HT_2 receptors (Hicks et al. 1984; Bischoff et al. 1986). Although there is no evidence that responding for conditioned reward is dependent on serotonergic mechanisms, at present this possibility cannot be ruled out.

Metoclopramide reduced responding without shifting the peak effect in the amphetamine dose-response function. The middle dose appeared to eliminate the amphetamine effect whereas the highest dose seemed to eliminate the conditioned reward effect itself. The failure of increasing doses of amphetamine to reinstate responding suggests that metoclopramide produced its reward-attenuating effects differently than SCH 23390 or pimozide.

The reward-enhancing effects of amphetamine may be understood through its mechanism of action. Dopamine is released when an animal is presented with rewarding stimuli (Blackburn et al. 1986; Hernandez and Hoebel 1988; Radhakishun et al. 1988; Nakahara et al. 1989; Phillips et al. 1991), suggesting that there may be a dopamine signal involved in reward. Amphetamine, which enhances the neurogenic release and inhibits the re-uptake of dopamine (Scheel-Kruger 1971; Westerink 1979), enhanced responding for conditioned reward possibly by enhancing this putative DA signal. Apomorphine, which directly stimulates DA receptors, impaired responding for conditioned reward (Robbins et al. 1983; Mazurski and Beninger 1986; Beninger and Ranaldi 1992), suggesting that tonic stimulation of DA receptors may have masked the DA signal.

Previous studies have shown that blockade of either D_1 or D_2 receptors attenuated responding for food, chemical or brain stimulation reward (Beninger et al. 1987; Kurumiya and Nakajima 1988; Winocour et al. 1988; Nakajima and Baker 1989; Corrigall and Coen 1991; Hiroi and White 1991; Nakajima and O'Regan 1991). The present findings suggest that intact functioning of both receptor subtypes is important in conditioned reward as well. The manner in which each receptor subtype contributes to reward may be different. Recently it was suggested that the D_1 receptor is important in mediating reward (Beninger et al. 1989; Miller et al. 1990; Beninger 1991). Beninger and Ranaldi (1992) demonstrated that D_1, but not D_2, agonists eliminated responding for conditioned reward, suggesting that tonic stimulation of D_1 receptors may mask the reward signal leading to a loss of control over behaviour by the conditioned stimulus. The present results showing that SCH 23390 attenuated the reward-enhancing effects of amphetamine can be explained as direct antagonism of the putative D_1 reward signal. Increasing the dose of amphetamine led to reinstatement of responding possibly through increased DA release and a reinstatement of the DA reward signal at the D_1 receptor.

Some evidence may appear to contradict the D_1 signal hypothesis. Everitt and Robbins (1992) reported enhanced responding for conditioned reward with intra-accumbens SKF 38393. Tonic stimulation of D_1 receptors apparently failed to mask the putative DA signal associated with reward. However, Chu and Kelley (1992) failed to observe enhanced responding for conditioned reward when CY 208–243, another D_1-selective agonist, was infused into the nucleus accumbens. It has been reported that SKF 38393 may be neurotoxic (Kelley et al. 1990). Perhaps the results of Everitt and Robbins (1992) were related to this action of the drug.

Enhanced conditioned reward seen with intra-accumbens DA (Cador et al. 1991) or a combination of CY 208–243 plus quinpirole and the failure of CY 208–243 to disrupt responding for conditioned reward (Chu and Kelley 1992) does not exclude the possibility that a DA signal at the D_1 receptor in some other DA terminal region, e.g. the caudate-putamen, may be sufficient for reward. In support, a small increase in responding for conditioned reward was seen with intra-caudate-putamen injections of amphetamine (Taylor and Robbins 1984; Kelley and Delfs 1991b). In studies examining enhanced responding for conditioned reward produced by accumbens amphetamine, 6-OHDA lesions of the caudate-putamen had little effect except possibly at the highest dose (Taylor and Robbins 1986) whereas nucleus accumbens lesions eliminated the amphetamine effect but, surprisingly, not the conditioned reward effect itself. This might suggest that the signal is in both the nucleus accumbens and the caudate-putamen and that either signal is sufficient for conditioned reward. This leads to the prediction that a disruption of the putative DA signal in both structures might be required to impair the increase in responding for conditioned reward produced by amphetamine and the conditioned reward effect itself. In support of this speculation one study, performed to evaluate the contributions of both accumbens and caudate-putamen dopamine to avoidance responding, a behaviour which can be understood in terms of reward processes (Beninger 1983, 1989a, 1991), showed that disruption occurred only when both sites were injected with 6-OHDA (Koob et al. 1984). Thus, the data of Everitt and Robbins and others do not require rejection of the hypothesis that there may be a signal at the D_1 receptor that is critical for reward-related learning.

D_2 receptors contribute to reward differently from D_1 receptors. The present results suggest that DA activity at the D_2 receptor may be necessary for reward. However, experiments showing that conditioned reward can be enhanced with D2 agonists suggests that this activity may not need to be temporally coupled to the conditioned stimulus (Beninger and Ranaldi 1992). Perhaps stimulation of D_2 receptors constitutes part of the behaviourally arousing or energizing aspects of reward. Indeed, the nucleus accumbens, which is involved in motor activation (Le Moal and Simon 1991), may also serve to link limbic areas and the striatum (Milner 1977; Mogenson et al. 1980; White and Milner 1992). Perhaps DA release in this region provides the energizing component of reward. Microinjections of D_2 agonists and antagonists in the accumbens produced facilitatory and inhibitory effects (White et al. 1991; Hiroi and White 1991), respectively, on reward-related learning. It is possible that D_2 stimulation provides behavioural arousal which is controlled by the conditioned reward through a DA signal. In this way, D_2 antagonists would lead to the present observation of reduced responding for reward by blocking its energizing component (cf. Miller et al. 1990). The recent report by Chu and Kelley (1992) that intra-accumbens quinpirole failed to increase responding for conditioned reward would appear to be inconsistent with this interpretation. Further studies with D2 agonists clearly are needed.

The different effects produced by pimozide and metoclopramide may be related to their pharmacological properties. There is evidence that metoclopramide binds in the striatum more than in the nucleus accumbens (Costall and Naylor 1976; Elliott et al. 1977; Jenner et al. 1978; Maidment and Marsden 1987) and that blockade of D_2 receptors in the striatum produces Parkinsonian symptoms. Thus, metoclopramide may have produced motor effects. SCH 23390 and pimozide, with a more even distribution, may have produced greater blockade of reward.

The present study showed that DA transmission is important in reward. In addition, the results suggest that amphetamine-produced enhancement of responding for conditioned reward may require intact functioning of both D_1 and D_2 receptors.

Acknowledgements. Pimozide was the generous gift of Janssen Pharmaceutica. Supported by a grant from the Natural Sciences and Engineering Research Council to R.J.B.

References

Beninger RJ (1983) The role of dopamine in locomotor activity and learning. Brain Res Rev 6:173–196

Beninger RJ (1989a) Methods for determining the effects of drugs on learning. In: Boulton AA, Baker GB, Greenshaw AJ (eds) Neuromethods, Volume 13: Psychopharmacology. Humana Press, Clifton, NJ, pp 623–685

Beninger RJ (1989b) Dissociating the effects of altered dopaminergic function on performance and learning. Brain Res Bull 23:365–371

Beninger RJ (1991) Receptor subtype-specific dopamine agonists and antagonists and conditioned behaviour. In: Willner P, Scheel-Kruger J (eds) The Mesolimbic Dopamine System: From Motivation to Action. Wiley, Chichester, pp 273–299

Beninger RJ (1992) D-1 receptor involvement in reward-related learning. J Psychopharmacol 6[1]: 34–42

Beninger RJ, Phillips AG (1980) The effects of pimozide on the establishment of conditioned reinforcement. Psychopharmacology 68:147–153

Beninger RJ, Ranaldi R (1992) The effects of amphetamine, apomorphine, SKF 38393, quinpirole and bromocriptine on responding for conditioned reward in rats. Behav Pharmacol 3:155–163

Beninger RJ, Hanson DR, Phillips AG (1980) The effects of pipradrol on the acquisition of responding with conditioned reinforcement: a role for sensory preconditioning. Psychopharmacology 69:235–242

Beninger RJ, Hanson DR, Phillips AG (1981) The acquisition of responding with conditioned reinforcement: effects of cocaine, (+)-amphetamine and pipradrol. Br J Pharmacol 74:149–154

Beninger RJ, Cheng M, Hahn BL, Hoffman DC, Mazurski EJ, Morency MA, Ramm P, Stewart RJ (1987) Effects of extinction, pimozide, SCH 23390, and metoclopramide on food-rewarded operant responding of rats. Psychopharmacology 92:343–349

Beninger RJ, Hoffman DC, Mazurski EJ (1989) Receptor subtype-specific dopaminergic agents and conditioned behavior. Neurosci Biobehav Rev 13:113–122

Bindra D (1974) A motivational view of learning, performance, and behavior modification. Psychol Rev 81:199–213

Bischoff S, Heinrich M, Sonntag JM, Krauss J (1986) The D-1 dopamine receptor antagonist SCH 23390 also interacts potently with brain serotonin (5-HT_2) receptors. Eur J Pharmacol 129:367–370

Blackburn JR, Phillips AG, Jakubovic A, Fibiger HC (1986) Increased dopamine metabolism in the nucleus accumbens and

striatum following consumption of a nutritive meal but not a palatable non-nutritive saccharin solution. Pharmacol Biochem Behav 25:1095–1100

Cador M, Taylor JR, Robbins TW (1991) Potentiation of the effects of reward-related stimuli by dopaminergic-dependent mechanisms in the nucleus accumbens. Psychopharmacology 104:377–385

Chu B, Kelley AE (1992) Potentiation of reward-related responding by psychostimulant infusion into nucleus accumbens: role of dopamine receptor subtypes. Psychobiology 20:153–162

Cohen SL, Branch MN (1991) Food-paired stimuli as conditioned reinforcers: effects of *d*-amphetamine. J Exp Anal Behav 56:277–288

Corrigall WA, Coen KM (1991) Cocaine self-administration is increased by both D1 and D2 dopamine antagonists. Pharmacol Biochem Behav 39:799–802

Costall B, Naylor RJ (1976) A comparison of the abilities of typical neuroleptic agents and of thioridazine, clozapine, sulpiride and metoclopramide to antagonise the hyperactivity induced by dopamine applied intracerebrally to areas of the extrapyramidal and mesolimbic systems. Eur J Pharmacol 40:9–19

Elliott PNC, Jenner P, Huizing G, Marsden CD, Miller R (1977) Substituted benzamides as cerebral dopamine antagonists in rodents. Neuropharmacology 16:333–342

Everitt BJ, Robbins TW (1992) Amygdala-ventral striatal interactions and reward-related processes. In: Aggleton JP (ed) The amygdala. Wiley-Liss, New York, pp 401–429

Fenton HM, Liebman JM (1982) Self-stimulation response decrement patterns differentiate clonidine, baclofen and dopamine antagonists from drugs causing performance deficit. Pharmacol Biochem Behav 17:1207–1212

Files FJ, Branch MN, Clody D (1989) Effects of methylphenidate on responding under extinction in the presence and absence of conditioned reinforcement. Behav Pharmacol 1:113–121

Goetsch VL, Isaac W (1983) The effects of *d*-amphetamine on visual sensitivity in the rat. Eur J Pharmacol 87:465–468

Harrington RA, Hamilton CW, Brogden RN, Linkewich JA, Romankiewicz JA, Heel RC (1983) Metoclopramide: an updated review of its pharmacological properties and clinical use. Drugs 25:451–494

Hernandez L, Hoebel BG (1988) Food reward and cocaine increase extracellular dopamine in the nucleus accumbens as measured by microdialysis. Life Sci 42:1705–1712

Hicks PE, Schoemaker H, Langer SZ (1984) 5-HT receptor antagonist properties of SCH 23390 in vascular smooth muscle and brain. Eur J Pharmacol 105:339–343

Hill RT (1970) Facilitation of conditioned reinforcement as a mechanism of psychomotor stimulation. In: Costa E, Garatinni S (eds) Amphetamines and related compounds. Raven Press, New York, pp 781–795

Hiroi N, White NM (1991) The amphetamine conditioned place preference: differential involvement of dopamine receptor subtypes and two dopaminergic terminal areas. Brain Res 552:141–152

Hoffman DC, Beninger RJ (1985) The effects of pimozide on the establishment of conditioned reinforcement as a function of the amount of conditioning. Psychopharmacology 87:454–460

Hyttel J (1983) SCH 23390 – the first selective dopamine D1 antagonist. Eur J Pharmacol 91:153–154

Iorio LC (1983) SCH 23390, a benzazepine with atypical effects on dopaminergic systems. Pharmacologist 23:136

Isaac W (1971) A study of the relationship between the visual system and the effects of *d*-amphetamine. Physiol Behav 6:157–159

Jenner P, Clow A, Reavill C, Theodorou A, Marsden CD (1978) A behavioural and biochemical comparison of dopamine receptor blockade produced by haloperidol with that produced by substituted benzamide drugs. Life Sci 23:545–550

Kelley AE, Delfs JM (1991a) Dopamine and conditioned reinforcement: II. Contrasting effects of amphetamine microinjection into the nucleus accumbens with peptide microinjection into the ventral tegmental area. Psychopharmacology 103:197–203

Kelley AE, Delfs JM (1991b) Dopamine and conditioned reinforcement: I. Differential effects of amphetamine microinjections into striatal subregions. Psychopharmacology 103:187–196

Kelley AE, Delfs JM, Chu B (1990) Neurotoxicity induced by the D-1 agonist SKF 38393 following microinjection into rat brain. Brain Res 532:342–346

Keppel G (1982) Design and Analysis: A Researcher's Handbook. 2nd edition. Prentice-Hall, Englewood Cliffs, New Jersey

Kleven MS, Woolverton WL (1990) Effects of continuous infusions of SCH 23390 on cocaine- or food-maintained behavior in rhesus monkeys. Behav Pharmacol 1:365–373

Knott PD, Clayton KN (1966) Durable secondary reinforcement using brain stimulation as the primary reinforcer. J Comp Physiol Psychol 61:151–153

Koob GF, Simon H, Herman JP, Le Moal M (1984) Neuroleptic-like disruption of the conditioned avoidance response requires destruction of both the mesolimbic and nigrostriatal dopamine systems. Brain Res 303:319–329

Koob GF, Le HT, Creese I (1987) The D1 dopamine receptor antagonist SCH 23390 increases cocaine self-administration in the rat. Neurosci Lett 79:315–320

Kurumiya S, Nakajima S (1988) Dopamine D1 receptors in the nucleus accumbens: involvement in the reinforcing effect of tegmental stimulation. Brain Res 448:1–6

Le Moal M, Simon H (1991) Mesocorticolimbic dopaminergic netwok: functional and regulatory roles. Physiol Rev 71:155–234

Mackintosh NJ (1974) The psychology of animal learning. Academic Press, London

Maidment NT, Marsden CD (1987) Acute administration of clozapine, thioridazine and metoclopramide increases extracellular DOPAC and decreases extracellular 5-HIAA, measured in the nucleus accumbens and striatum of the rat using in vivo voltammetry. Neuropharmacology 26:187–193

Mazurski EJ, Beninger RJ (1986) The effects of (+)-amphetamine and apomorphine on responding for a conditioned reinforcer. Psychopharmacology 90:239–243

Miller R, Wickens JR, Beninger RJ (1990) Dopamine D-1 and D-2 receptors in relation to reward and performance: a case for the D-1 receptor as a primary site of therapeutic action of neuroleptic drugs. Prog Neurobiol 34:143–183

Milner PM (1977) Theories of reinforcement, drive and motivation. In: Iversen LL, Iversen SD, Snyder SH (eds) Handbook of psychopharmacology. Principles of behavioral pharmacology. Plenum Press, New York, pp 181–200

Mogenson GJ, Jones DL, Yim CY (1980) From motivation to action: functional interface between the limbic system and the motor system. Prog Neurobiol 14:69–97

Nakahara D, Ozaki N, Kapoor V, Nagatsu T (1989) The effect of uptake inhibition on dopamine release from the nucleus accumbens of rats during self- or forced stimulation of the medial forebrain bundle: a microdialysis study. Neurosci Lett 104:136–40

Nakajima S (1986) Suppression of operant responding in the rat by dopamine D1 receptor blockade with SCH 23390. Physiol Psychol 14:111–114

Nakajima S, Baker JD (1989) Effects of D2 dopamine receptor blockade with raclopride on intracranial self-stimulation and food-reinforced operant behaviour. Psychopharmacology 98:330–333

Nakajima S, McKenzie GM (1986) Reduction of the rewarding effect of brain stimulation by a blockade of dopamine D1 receptor with SCH 23390. Pharmacol Biochem Behav 24:919–923

Nakajima S, O'Regan NB (1991) The effects of dopaminergic agonists and antagonists on the frequency-response function for hypothalamic self-stimulation in the rat. Pharmacol Biochem Behav 39:465–468

Phillips AG, Pfauss JG, Blaha CD (1991) Dopamine and motivated behavior: insights provided by in vivo analyses. In: Willner P, Scheel-Kruger J (eds) The mesolimbic dopamine system: from motivation to action. Wiley, Chichester, pp 199–224

Radhakishun FS, van Ree JM, Westerink BH (1988) Scheduled eating increases dopamine release in the nucleus accumbens of food-deprived rats as assessed with on-line brain dialysis. Neurosci Lett 85:351–356

Robbins TW (1975) The potentiation of conditioned reinforcement by psychomotor stimulant drugs: a test of Hill's hypothesis. Psychopharmacologia 45:103–114

Robbins TW (1976) Relationship between reward-enhancing and stereotypical effects of psychomotor stimulant drugs. Nature 264:57–59

Robbins TW (1978) The acquisition of responding with conditioned reinforcement: effects of pipradrol, methylphenidate, *d*-amphetamine, and nomifensine. Psychopharmacology 58:79–87

Robbins TW, Koob GF (1978) Pipradrol enhances reinforcing properties of stimuli paired with brain stimulation. Pharmacol Biochem Behav 8:219–222

Robbins TW, Watson BA, Gaskin M, Ennis C (1983) Contrasting interactions of pipradrol, *d*-amphetamine, cocaine, cocaine analogues, apomorphine and other drugs with conditioned reinforcement. Psychopharmacology 80:113–119

Roberts DCS, Vickers G (1984) Atypical neuroleptics increase self administration of cocaine: an evaluation of a behavioural screen for antipsychotic activity. Psychopharmacology 82:135–139

Rotrosen J, Stanley M, Lautin A, Wazer D, Gershon S (1981) Discrimination of functionally heterogeneous receptor subpopulations: antipsychotic and antidopaminergic properties of metoclopramide. Psychopharmacol Bull 17:110–113

Scheel-Kruger J (1971) Comparative studies of various amphetamine analogues demonstrating different interactions with the metabolism of the catecholamines in the brain. Eur J Pharmacol 14:47–59

Seeman P (1981) Brain dopamine receptors. Pharmacol Rev 32:229–313

Self DW, Stein L (1992) The D1 agonists SKF 82958 and SKF 77434 are self-administered by rats. Brain Res 582:349–352

Skinner, BF (1938) The behavior of organisms. Appleton Century Crofts, New York

Stein L (1958) Secondary reinforcement established with subcortical stimulation. Science 127:466–467

Taylor JR, Robbins TW (1984) Enhanced behavioural control by conditioned reinforcers following microinjections of *d*-amphetamine into the nucleus accumbens. Psychopharmacology 84:405–412

Taylor JR, Robbins TW (1986) 6-hydroxydopamine lesions of the nucleus accumbens, but not of the caudate nucleus, attenuate enhanced responding with reward-related stimuli produced by intra-accumbens *d*-amphetamine. Psychopharmacology 90:390–397

Ungerstedt U (1979) Central dopamine mechanisms and unconditioned behaviour. In: Horn AS, Korf J, Westerink BHC (eds) The neurobiology of dopamine. Academic Press, London, pp 577–596

Westerink BHC (1979) The effects of drugs on dopamine biosynthesis and metabolism in the brain. In: Horn AS, Korf J, Westerink BHC (eds) The neurobiology of dopamine. Academic Press, London, pp 255–291

White NM, Milner PM (1992) The psychobiology of reinforcers. Annu Rev Psychol 43:443–471

White NM, Packard MG, Hiroi N (1991) Place conditioning with dopamine D1 and D2 agonists injected peripherally or into nucleus accumbens. Psychopharmacology 103:271–276

Winer BJ (1971) Statistical Principles in Experimental Design. 2nd edition. McGraw Hill, New York

Winocour G, Chawla K, Sampson D, Sophokleous S, Muscat R (1988) Tests of functional equivalence between pimozide pretreatment, extinction and free feeding. Psychopharmacology 95:423–426

Wise RA, Rompre P-P (1989) Brain dopamine and reward. Annu Rev Psychol 40:191–225

Woolverton WL, Goldberg LI, Ginos JZ (1984) Intravenous self-administration of dopamine receptor agonists by rhesus monkeys. J Pharmacol Exp Ther 230:678–683