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ORIGINAL INVESTIGATION

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Effects of *d*-fenfluramine and metergoline on responding for conditioned reward and the response potentiating effect of nucleus accumbens *d*-amphetamine

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Abstract These studies investigated the effects of the 5-hydroxytryptamine (5-HT) releaser, and re-uptake inhibitor, *d*-fenfluramine, and the non-selective 5-HT receptor antagonist metergoline, on responding for conditioned reward (CR), and on the potentiation of responding for CR following amphetamine injected into the nucleus accumbens. Water deprived rats were trained to associate a compound stimulus with water delivery during a conditioning phase. During a test phase, water was not delivered but the compound stimulus was delivered according to a random ratio 2 schedule following a response on one of two levers; responding on the other lever was not reinforced. Overall, rats responded at a higher rate on the lever delivering the CR. *d*-Amphetamine (1, 3 and 10 μg) injected into the nucleus accumbens dose-dependently enhanced responding on the CR lever. Treatment with *d*-fenfluramine (0.5 and 1 mg/kg) reduced responding for the CR, and abolished the potentiating effect of *d*-amphetamine. Responding on the inactive lever was also reduced by 1 mg/kg but not 0.5 mg/kg *d*-fenfluramine. The reduction of *d*-amphetamine's effect on responding for CR was prevented by prior treatment with the 5-HT receptor antagonist metergoline (1 mg/kg). Control experiments showed that changes in thirst and motor performance, as well as deficits in learning ability, cannot account for the effects of *d*-fenfluramine in this paradigm. In a separate experiment, 1 mg/kg metergoline failed to enhance responding for CR, and to augment the response potentiating effect of a low dose (2 μg) of *d*-amphetamine injected into the nucleus accumbens. Thus, elevating brain 5-HT activity appears to reduce the ability of secondary reinforcing stimuli to elicit and maintain behaviour, and antagonizes the effects of enhanced dopamine activity within the nucleus accumbens. However, reduced 5-HT function induced by blockade of $5\text{-HT}_{1/2}$ receptors does not appear to influence responding for CR, or the response potentiating effect of *d*-amphetamine. These results suggest that 5-HT may play an important role in mediating incentive motivation.

Key words Conditioned reward · *d*-Fenfluramine · *d*-Amphetamine · Metergoline · Nucleus accumbens · 5-Hydroxytryptamine dopamine

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Introduction

Dopaminergic neurotransmission within the nucleus accumbens appears to mediate the locomotor activating and rewarding effects of psychomotor stimulants such as amphetamine and cocaine (for reviews see Fibiger and Phillips 1986; Wise 1987). Direct injections of amphetamine (Pijnenburg et al. 1976) and cocaine (Delfs et al. 1990) into the nucleus accumbens increase locomotor activity, and dopamine depletion in the nucleus accumbens attenuates the hyperactivity elicited by systemic amphetamine (Kelly et al. 1975). Rats self-administer amphetamine directly into the nucleus accumbens (Hoebel et al. 1983), and dopamine depletion of this area reduces intravenous self-administration of amphetamine (Lyness et al. 1979) and cocaine (Roberts et al. 1977). In addition, injections of amphetamine into the nucleus accumbens induce a conditioned place preference (Carr and White 1983).

These locomotor activating and reward-related effects of psychomotor stimulants may result from increased incentive motivation (e.g. Beninger 1983; Fibiger and Phillips 1986), which refers to the process whereby appetitive behaviours are elicited by rewarding stimuli, including those stimuli that are predictive of reward. Clear evidence for an important role for nucleus accumbens dopamine in mediating incentive motivation comes from studies in rats of the effects

of dopaminergic manipulations on responding for a conditioned reward (CR). In this paradigm, an initially neutral stimulus is paired with a primary reinforcer (e.g. food or water). After numerous pairings animals are given access to two levers, one of which delivers the CR and one of which is inactive. Animals respond at higher rates on the CR versus inactive lever, thus showing that the CR has acquired incentive motivational properties. Psychomotor stimulants, including amphetamine and pipradrol, selectively enhance responding for CR (e.g. Robbins 1978; Beninger and Ranaldi 1992). This effect does not result from an increase in general activity, since responding on the inactive lever does not increase, and since other treatments such as apomorphine (Robbins et al. 1983; Beninger and Ranaldi 1992), and opioid injection into the nucleus accumbens (Cunningham and Kelley 1992), fail to enhance responding for CR despite inducing hyperactivity. The nucleus accumbens appears to be a critical site of action mediating this behaviourally selective effect, since injections of amphetamine (Taylor and Robbins 1984, 1986; Kelly and Delfs 1991), dopamine (Cador et al. 1991), pipradrol and cocaine (Chu and Kelley 1992) into the nucleus accumbens enhance responding for CR.

Changes in 5-hydroxytryptamine (5-HT; serotonin) function modify behavioural responses to drugs interacting with dopamine function. Amphetamine-induced hyperactivity is reduced by some treatments, including *d*-fenfluramine (Bendotti et al. 1980), that elevate 5-HT activity, and enhanced by treatments that reduce 5-HT function (Gerson and Baldessarini 1980). Such manipulations also appear to alter reward-related behaviour. Drugs which enhance 5-HT activity reduce intravenous (IV) self-administration of amphetamine (Smith et al. 1986), cocaine (Carroll et al. 1990; Richardson and Roberts 1991) and heroin (Higgins et al. 1993), oral self-administration of ethanol (e.g. Higgins et al. 1992), amphetamine-induced conditioned place preference (Kruszewska et al. 1986) and responding for rewarding brain self-stimulation (McClelland et al. 1989). In contrast to these findings, chronic reductions in 5-HT function facilitate responding for IV amphetamine (Lyness et al. 1980), morphine (Smith et al. 1987) and cocaine (Loh and Roberts 1991), and responding for medial forebrain bundle self-stimulation (Poschel and Ninteman 1971). Similarly, the non-selective 5-HT receptor antagonist metergoline enhances responding for IV amphetamine (Lyness and Moore 1983). Acute reductions in 5-HT function following peripheral or intra-raphe injections of 8-OH-DPAT elicit feeding (e.g. Fletcher 1991), induce a conditioned place preference (Papp and Willner 1991; Fletcher et al. 1993), facilitate responding for lateral hypothalamic self-stimulation (Montgomery et al. 1991; Fletcher et al., In press) and increase ethanol consumption (Tomkins et al. 1994). These results suggest that reward-related behaviour is inhibited by enhanced 5-HT function, and increased by lowered 5-HT function. However, the behavioural mechanisms by which 5-HT manipulations appear to modulate rewarded behaviour are unclear.

The present studies investigated the effects of 5-HT manipulations on responding for a CR. Thus, the effects of increased 5-HT function, following injection of the 5-HT releaser and re-uptake inhibitor *d*-fenfluramine, on responding for a conditioned stimulus paired with water, and on the response potentiating effect of intra-accumbens *d*-amphetamine, were examined. An additional experiment examined whether reduced 5-HT function, following administration of the non-selective 5-HT receptor antagonist metergoline, potentiated the effect of amphetamine injected into the nucleus accumbens. A number of control experiments were also conducted to determine any possible effects of *d*-fenfluramine on thirst, response capacity or simple learning ability that might interfere with responding for CR.

Materials and methods

Subjects

Adult male Sprague-Dawley rats (Charles River, Montreal) weighing 270–310 g at the time of surgery were used. Rats were housed individually in hanging wire mesh cages with food freely available at all times. Beginning 1 week after surgery, rats were allowed access to water for 2 h each day between 3 and 5 p.m. The housing room was maintained on a 12-h light/dark cycle (lights on at 8:00 a.m.) and at a temperature of 22 ± 2°C.

Surgery and histology

For implantation of stainless steel guide cannulae, animals were anaesthetized with sodium pentobarbital (Somnotol, 45 mg/kg IP) and placed in a stereotaxic frame. Guide cannulae (22 g stainless steel tubing, 15 mm in length) were implanted so that their tips were located 2.5 mm above the intended injection site; they were anchored to the skull by three stainless steel jeweller's screws and dental acrylic. Coordinates (relative to bregma) were AP + 3.4, L ± 1.7, V − 5.2, with the incisor bar set 5 mm above the interaural line. Stylets (28 g stainless steel wire) were used to keep the cannulae patent. On completion of the experiments rats were deeply anaesthetized with Somnotol and a volume of 0.5 µl fast-green dye was injected into each brain site. The brains were removed and stored in formaldehyde for at least 7 days, and then stored in 30% sucrose solution. Brains were then frozen, cut in a cryostat in 40-µm sections and stained with cresyl violet. Animals with injection sites outside the nucleus accumbens (Paxinos and Watson 1986) were not used in the data analysis, and group sizes given below reflect this adjustment.

Apparatus

Testing was carried out in four chambers measuring 28 cm long, 21 cm wide and 21 cm high (Med Associates, Georgia, Vt.). Each chamber contained a solenoid operated water dispenser and two retractable response levers, 4.5 cm wide and 7 cm above the floor of

the chamber. The centre of the levers were located 6.5 cm either side of a central, recessed dish positioned 3 cm from the floor of the chamber. A red stimulus light was located above each lever, and a sonalert was located behind one stimulus light. Each chamber was illuminated by a house light and housed in a sound-attenuating box equipped with a ventilating fan. The apparatus was controlled, and the data collected, by a 386-SX IBM-type computer.

Experiment 1: effects of *d*-fenfluramine on responding for CR following intra-accumbens *d*-amphetamine

A total of 34 rats with nucleus accumbens cannulae were used in this study, which consisted of three phases: habituation, conditioning and testing. Seven days after beginning the water restriction period, rats were placed in the operant boxes on 2 successive days with approximately 2 ml water in the dish, and with both levers present. Rats remained in the boxes until they had completed ten presses on one lever (approximately 5–10 min). This habituation phase was designed to accustom the rats to the boxes and to receiving water there. In addition, it was thought that this procedure would reveal any side preferences. In practice, rats did not show a preference for either lever. The conditioning phase was then begun. During this phase the levers were retracted, and rats received 30 presentations of 0.05 ml water according to a random time (RT) 30-s schedule. Water delivery was also accompanied by a compound stimulus consisting of a 3-s period of house light off, both red stimulus lights on, and a tone (29000 Hz at approximately 80 dB) delivered during the last 0.5 s of this period. Water delivery also occurred during this 0.5-s period. Following water delivery, the stimulus lights were extinguished and the houselight illuminated. The duration of the stimulus and the lighting conditions were chosen to be similar to those used by Kelley and Delfs (1991). All rats were given 14 daily conditioning sessions lasting on average 15 min per day. At this point, rats were then placed in the chambers on 2 days with both response levers extended. Responses on the left lever delivered the CR but not water, on a random ratio (RR) 2 schedule, responses on the right lever had no programmed consequences. Rats remained in the boxes until they had made ten responses on the CR lever. The purpose of this procedure was to ensure that all rats sampled the CR lever prior to drug testing. Drug testing was then initiated and was conducted in 40-min sessions with both levers present, the left delivering CR on an RR2 schedule, the right having no programmed consequence. All rats were tested four times following bilateral injection of 0.9% saline, 1,3 and 10 μg *d*-amphetamine delivered into the nucleus accumbens in a volume of 1 μl. The order of injections was randomized for each animal, and testing began immediately after each injection. Thirty minutes prior to this injection one group of rats received IP injections of saline ($n = 15$), and two other groups received either 0.5 ($n = 9$) or 1 mg/kg ($n = 10$) *d*-fenfluramine.

Experiment 2: effects of metergoline on fenfluramine-induced reduction in responding for CR

Seven rats were used in this study. The general habituation, conditioning and testing phases were identical to those described above. However, during testing all rats received intra-accumbens injections of 10 μg *d*-amphetamine on four separate occasions spaced 3 days apart. Prior to this injection, rats received four drug combinations administered in a randomized order. These combinations were vehicle + saline, vehicle + 1 mg/kg *d*-fenfluramine, 1 mg/kg metergoline + saline, and 1 mg/kg metergoline + 1 mg/kg *d*-fenfluramine. *d*-Fenfluramine was injected IP 30 min before *d*-amphetamine, and metergoline was injected SC 1 h before fenfluramine.

Experiment 3: effects of *d*-fenfluramine treatment during conditioning on responding for CR

Behavioural procedures for habituation, conditioning and testing were identical to those described above. However, during the conditioning phase one group of rats ($n = 10$) was injected with saline 30 min prior to each conditioning session, and the other group ($n = 10$) received 1 mg/kg *d*-fenfluramine. Testing was carried out, during 40-min sessions, in the absence of any drug treatment after 7 conditioning days, and again after a further 7 conditioning days.

Experiment 4: effects of *d*-fenfluramine on responding for water

Eight rats habituated to water deprivation were trained to press a lever for 0.05 ml water on a continuous reinforcement schedule in daily 40-min sessions. After 5 days on the CRF schedule, water was delivered according to a RR2 schedule. Following stabilization of response rates rats were injected IP with saline, 0.5, 1 and 2 mg/kg *d*-fenfluramine 30 min before the test session. Response rates were then recorded over a 40-min session. All rats received each dose of fenfluramine and saline in a counterbalanced order with 3 days between successive injections. On non-drug days rats were run as normal in the operant boxes.

Experiment 5: effects of *d*-fenfluramine on acquisition of responding for water

Following acclimatization to the water deprivation schedule, three groups of rats were trained to lever press for 0.05 ml water during daily 40-min sessions over 7 consecutive days. On the first 2 days, water was available according to a CRF schedule, and then for a further 5 days according to a RR2 schedule. One group of rats ($n = 7$) received IP injections of saline 30 min before each test. The other groups received IP injections of 0.5 ($n = 7$) and 1 mg/kg ($n = 7$) *d*-fenfluramine 30 min before testing.

Experiment 6: effects of metergoline on responding for CR following intra-accumbens amphetamine

Behavioural procedures for habituation, conditioning and testing were identical to those described above. However, during the testing phase, each rat ($n = 7$) was administered four treatment combinations at 3-day intervals in randomized order. Each combination consisted of an IP injection of 1 mg/kg metergoline or its vehicle, followed 3 h later by an injection into the nucleus accumbens of 2 μg *d*-amphetamine or saline. Testing lasted for 40 min and began immediately after the second injection. The longer pretreatment interval for metergoline was chosen to be similar to that used by Lyness and Moore (1983), who found a potentiating effect of metergoline on amphetamine self-administration.

Drugs and drug delivery

d-Amphetamine sulphate (Bureau of Dangerous Drugs, Ottawa) was delivered into the nucleus accumbens using a fine glass microinjector (0.1–0.2 mm tip diameter), extending 2.5 mm beyond the guide cannula tip, attached to a Hamilton syringe via a length of plastic tubing. The drug was dissolved in saline, and infused in a volume of 1 μl over a 2-min period with the needle left in place for a further 1 min. *d*-Fenfluramine HCl (Servier) was dissolved in saline, and metergoline (Farmitalia) was dissolved in 1% ascorbic acid.

Statistics

Response rates for the CR studies were subjected to square-root transformations to reduce heterogeneity of variance. These transformed values were then subjected to three-way analyses of variance. Significant three-way interactions were followed by tests of simple interactions, and significant two-way interactions were followed by tests of simple main effects. Post-hoc comparisons among means were made using Tukey's test. In the other studies, one-way analyses of variance were conducted on untransformed response rates followed, where appropriate, by Dunnett's tests for comparisons against a control mean.

Results

Histology

The distribution of injection sites for rats contributing data to experiments 1, 2 and 6 is shown in Fig. 1.

Experiment 1: effects of *d*-fenfluramine on responding for CR following intra-accumbens amphetamine

Figure 2 shows that responding was higher on the CR versus NCR lever [$F(1, 31) = 241.3$, $P < 0.0001$], and that in general *d*-amphetamine increased responding [$F(1, 31) = 15.85$, $P < 0.0001$]. The significant Amphetamine × Lever interaction [$F(3, 93) = 16.32$, $P < 0.0001$] shows that responding on the two levers was differentially affected by amphetamine. Thus responding on the CR lever [simple main effect, $F(3, 93) = 85.5$, $P < 0.0001$] was enhanced to a greater degree than responding on the NCR lever [simple main effect, $F(3, 93) = 6.24$, $P < 0.01$]. Overall, *d*-fenfluramine suppressed responding [$F(2, 31) = 30.97$, $P < 0.0001$] and tests of simple main effects showed that responding was reduced on both the CR lever [$F(2, 31) = 70.6$, $P < 0.0001$] and the NCR lever [$F(2, 31) = 6.75$, $P < 0.01$]. However the Fenfluramine × Lever interaction [$F(2, 31) = 22.5$, $P < 0.0001$] was significant. The Fenfluramine × Dose × Lever interaction was significant [$F(6, 93) = 7.43$, $P < 0.001$] and so tests of simple interactions were conducted at each level of *d*-fenfluramine treatment. These analyses showed that a significant Amphetamine × Lever interaction occurred in the saline group [$F(3, 42) = 32.57$, $P < 0.001$], but not in the groups treated with 0.5 [$F(3, 24) = 1.96$, $P > 0.1$] or 1 mg/kg *d*-fenfluramine [$F(3, 27) = 1.87$, $P > 0.1$]. This illustrates that in saline-treated rats *d*-amphetamine selectively potentiated responding on the CR lever. In rats pretreated with 0.5 mg/kg *d*-fenfluramine, this potentiating effect of *d*-amphetamine was not seen. Rats treated with 1 mg/kg *d*-fenfluramine did show a small increase in responding following *d*-amphetamine [$F(3, 27) = 4.39$, $P < 0.05$], but this increase was not selective for the CR lever.

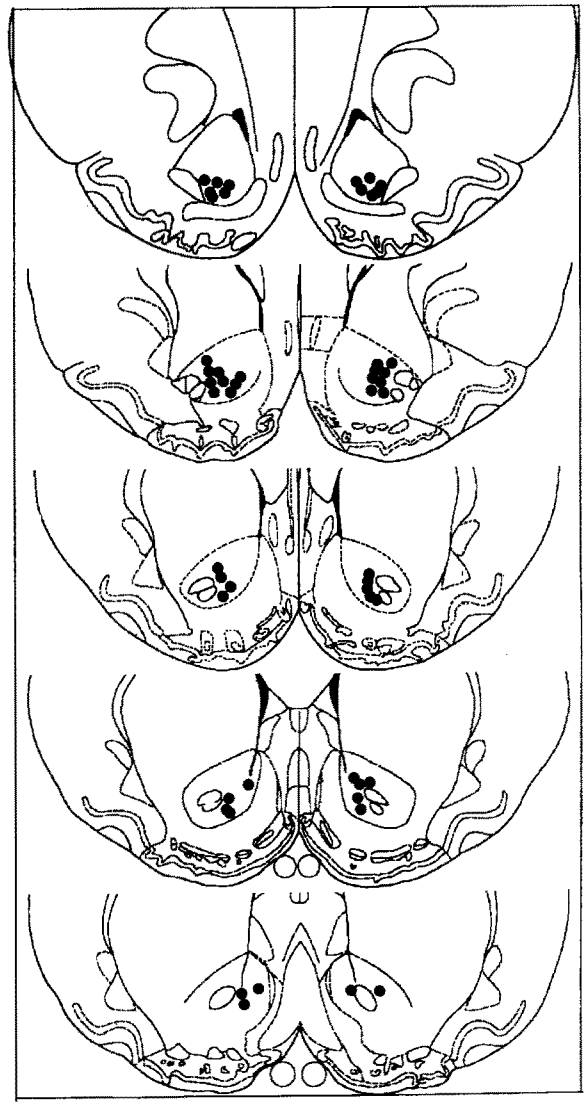

Fig. 1 Schematic diagram showing the location of injection sites within the nucleus accumbens for animals used in experiments 1, 2 and 6. The number of sites shown is fewer than the number of animals used because of overlap of injection sites. Sections are adapted from Paxinos and Watson (1986) and range from + 2.7 mm (top) to + 0.7 mm (bottom) at 0.5-mm intervals

Post-hoc comparisons among means revealed that in comparison to saline pretreatment, 0.5 mg/kg *d*-fenfluramine reduced responding on the CR lever following saline, 3 and 10 μg *d*-amphetamine injected into the nucleus accumbens. Responding on the NCR lever was not reduced, except in the rats treated with 10 μg *d*-amphetamine. However, this latter value was not significantly different from that obtained in rats receiving saline pretreatment and saline injected into the nucleus accumbens. In contrast, 1 mg/kg *d*-fenfluramine significantly reduced responding on both levers at all levels of *d*-amphetamine treatment when compared to saline pretreatment.

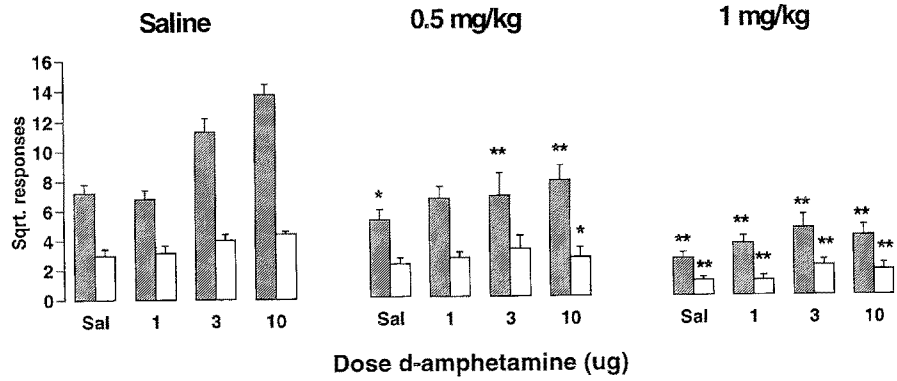

Fig. 2 The effects of pretreatment with saline ($n = 15$), 0.5 ($n = 9$) and 1 mg/kg ($n = 10$) *d*-fenfluramine on responding for CR in rats receiving bilateral injections of *d*-amphetamine (1,3 and 10 μg) or saline in the nucleus accumbens. CR (hatched columns) denotes responding on the lever delivering the conditioned reinforcer; NCR (white columns) denotes responding on the inactive lever. Values represent mean + 1SEM of square-root transformed data. $*P < 0.05$, $**P < 0.01$ compared to same lever and amphetamine dose condition in saline-pretreated rats (*left panel*). See text for statistical details showing an Amphetamine × Lever interaction for saline-pretreated but not *d*-fenfluramine-pretreated rats

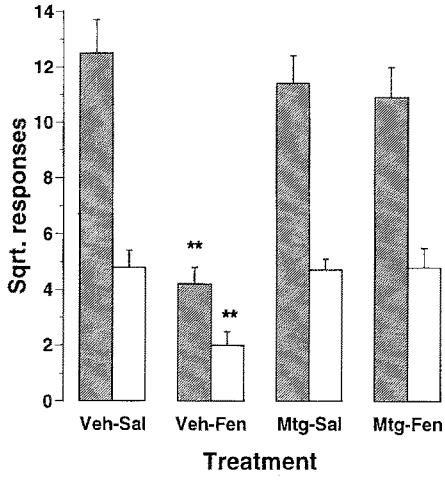

Fig. 3 Effects of combined injections of 1 mg/kg metergoline (*Mtg*) or its vehicle (*Veh*) and 1 mg/kg *d*-fenfluramine (*Fen*) or saline (*Sal*) on responding for CR in rats injected bilaterally with 10 μg *d*-amphetamine in the nucleus accumbens. Seven rats were tested under all treatment combinations. CR (*hatched columns*) denotes responding on the lever delivering the conditioned reinforcer; NCR (*white columns*) denotes responding on the inactive lever. Values represent means + 1 SEM of square-root transformed data. $**P < 0.01$ compared to Veh-Sal condition

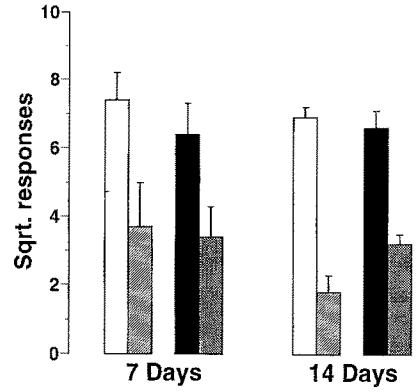

Fig. 4 The effects of treatment with 1 mg/kg *d*-fenfluramine (*Fen*; $n = 10$) or saline (*Sal*; $n = 10$) during conditioning on responding for CR after 7 and 14 conditioning sessions. CR denotes responding on the lever delivering the CR; NCR denotes responding on the inactive lever. Values represent means + 1 SEM of square-root transformed data. □ CR-Sal, ▨ NCR-Sal, ■ CR-Fen, ▨ NCR-Fen

Mean (± SEM) untransformed response rates in the saline pretreated group following saline injected into the nucleus accumbens were: CR, 57.1 ± 9.99; NCR 11.6 ± 2.89. Following 10 μg *d*-amphetamine they were: CR, 195.1 ± 20.7; NCR, 19.9 ± 1.3.

Experiment 2: effects of metergoline on fenfluramine induced reduction in responding for CR

As shown in Fig. 3, rats responded more on the CR lever versus the NCR lever [$F(1, 6) = 41.06$, $P < 0.001$]. The level of responding under the two vehicle treatments was similar to that observed for the comparable group in experiment 1. Responding was reduced by *d*-fenfluramine [$F(1, 6) = 17.36$, $P < 0.006$]. A significant Metergoline × Fenfluramine interaction [$F(1, 6) = 21.31$, $P < 0.004$] was found, which indicates that metergoline pretreatment reversed the response suppressant effects of *d*-fenfluramine.

Experiment 3: effects of *d*-fenfluramine treatment during conditioning on responding for CR

Figure 4 shows that rats responded at higher levels on the CR versus NCR lever [main effect of Lever, $F(1, 18) = 41.7$, $P < 0.001$]. Neither the main effect of Days [$F(1, 18) = 3.24$, $P > 0.05$] nor any of the interactions involving the Days factor [all $Fs < 2.0$] were significant, showing that number of days of conditioning did not affect the level of responding on either lever.

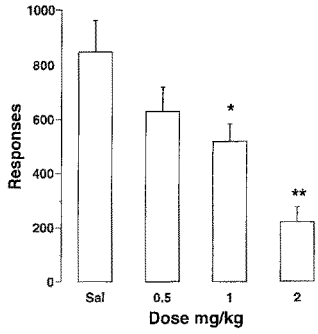

Fig. 5 Effects of *d*-fenfluramine or saline on responding for water maintained under a RR 2 schedule of reinforcement. Eight rats were tested at all dose levels. Values represent mean + 1 SEM of number of responses. $*P < 0.05$, $**P < 0.01$ compared to Sal condition

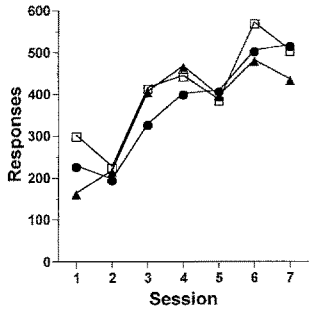

Fig. 6 Effects of saline (*Sal*), 0.5 and 1 mg/kg *d*-fenfluramine (*d*-fen) on the acquisition of responding for water reinforcement. On sessions 1 and 2 water was delivered after each bar press; on sessions 3–7 water was delivered according to a RR2 schedule. Seven rats were tested in each of the three groups. Values represent mean response rates; SEMs are omitted for clarity. □ Sal, • 0.5 *d*-fen, ▲1 *d*-fen

Treatment with *d*-fenfluramine did not significantly alter responding [$F(1, 18) = 0.21$, $P > 0.1$].

Experiment 4: effects of *d*-fenfluramine on responding for water reinforcement

Figure 5 shows that *d*-fenfluramine dose-dependently reduced responding for water on a RR2 schedule of reinforcement [$F(3, 21) = 11.96$, $P < 0.001$]. Post-hoc comparisons using Dunnett's test showed that both 1 and 2 mg/kg *d*-fenfluramine significantly reduced responding when compared to saline treatment.

Experiment 5: effects of *d*-fenfluramine on acquisition of responding for water reinforcement

Figure 6 shows the effects of daily injection of *d*-fenfluramine on the acquisition of responding for water reinforcement, where water was available according to a CRF schedule for days 1 and 2, and an RR2 schedule for days 3–7. Although response rate increased over days [$F(6, 96) = 6.22$, $P < 0.0001$], responding was not altered by *d*-fenfluramine [$F(1, 16) = 0.07$, $P > 0.8$].

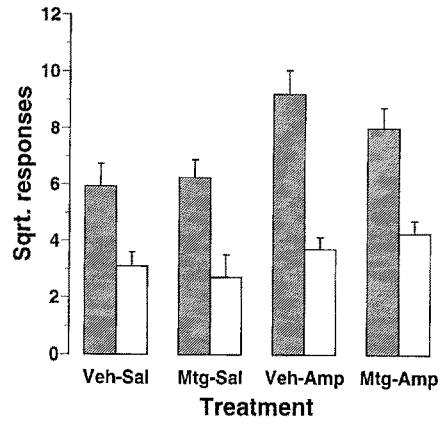

Fig. 7 The effects of pretreatment with 1 mg/kg metergoline (*Mtg*) or its vehicle (*Veh*) on responding for CR in rats receiving bilateral injections of 2 μg *d*-amphetamine (*Amp*) or saline (*Sal*) in the nucleus accumbens. Seven rats were tested under all treatment combinations. CR (*hatched columns*) denotes responding on the CR lever; NCR (*white columns*) denotes responding on the inactive lever

Experiment 6: effects of metergoline on responding for CR following intra-accumbens amphetamine

Figure 7 shows that amphetamine significantly enhanced responding [$F(1, 6) = 1\ 1.92$, $P < 0.02$], and this effect appeared greater on the CR versus NCR lever [Amphetamine × Lever interaction, $F(1, 6) = 5.87$, $P = 0.05$]. The level of responding appeared less than that observed in experiment 1 with 3 and 10 μg *d*-amphetamine. None of the factors involving metergoline was significant [largest $F(1, 6) = 3.88$, $P > 0.09$] showing that metergoline did not alter responding for CR, or the response potentiating effect of *d*-amphetamine.

Discussion

Pairing an initially neutral stimulus with water reinforcement resulted in animals selectively responding for that stimulus in a two-lever choice situation. Responding for this CR was selectively enhanced by injecting amphetamine into the nucleus accumbens. The magnitude of these effects was similar to those reported previously using a similar experimental protocol (e.g. Taylor and Robbins 1986; Kelley and Delfs 1991). Pretreating rats with 0.5 or 1 mg/kg *d*-fenfluramine generally suppressed responding. At the 0.5 mg/kg dose of *d*-fenfluramine, the reduction in responding was selective for the CR lever. At this same dose of *d*-fenfluramine, the potentiating effect of *d*-amphetamine on responding for CR was not apparent. In those rats treated with 1 mg/kg *d*-fenfluramine, responding was potentiated slightly by *d*-amphetamine, but the selectivity of *d*-amphetamine's effect on the CR lever was not apparent. *d*-Fenfluramine acts to release 5-HT and prevent its subsequent re-uptake (reviewed in Rowland

and Carlton 1986). Therefore, the acute action of *d*-fenfluramine is to increase 5-HT function. The 5-HT receptor antagonist metergoline completely reversed the reduction in responding for CR induced by *d*-fenfluramine in rats treated with the highest dose of *d*-amphetamine. This reversal provides strong evidence that the effects of *d*-fenfluramine involve enhanced 5-HT activity. Since metergoline is a non-selective antagonist at 5-HT_1 and 5-HT_2 receptor subtypes, further work involving more selective agents will be needed to determine the relative importance of the various 5-HT receptor sub-types underlying this effect. However, since the effects of *d*-fenfluramine on other motivated behaviours such as feeding (Neill and Cooper 1989) appear to involve primarily 5-HT_{1B} and 5-HT_{2C} receptors, these receptor sub-types would seem to be logical candidates.

A number of control experiments ruled out general motivational, performance and learning deficits as the cause of the effects of *d*-fenfluramine. *d*-Fenfluramine inhibits food intake, and to a lesser extent, water intake (Rowland and Carlton 1986). Thus, the reduction in responding for CR could be due to diminished thirst. The results of experiment 3 showed that in animals previously trained to respond for water, *d*-fenfluramine significantly reduced responding only at doses of 1 mg/kg and higher. However, even at 1 mg/kg, *d*-fenfluramine-treated animals still made an average of 500 responses in a 40-min session. In animals learning to bar press for water, and which exhibited lower response rates than those previously trained to respond for water, treatment with fenfluramine during training did not have any significant effect on responding. Thus, it is unlikely that *d*-fenfluramine reduced responding for CR because of lowered motivation to seek the primary reinforcer. Response rates in *d*-fenfluramine-treated rats responding for water, even when a significant reduction in total responding was observed, were equivalent to, or higher than, response rates in saline pre-treated rats responding for CR after the highest dose of *d*-amphetamine. Therefore, performance impairments can be ruled out as an explanation for the effect of *d*-fenfluramine on responding for CR. However, given that 1 mg/kg *d*-fenfluramine reduced responding on the NCR lever, it is possible that a general response suppressant action may contribute to the reduced responding for CR at this dose. Clearly though, any general response suppressant action of *d*-fenfluramine would appear to interact with the reinforcement schedule in effect.

Responding for CR depends on learning the association between the CR and the primary reward, and then learning a new motor response, lever pressing, to obtain the CR. It is possible that *d*-fenfluramine impaired these learning processes and this was expressed as a reduction in responding for CR. Although *d*-fenfluramine reduced responding on the lever delivering CR, responding was still higher than on the inactive lever, showing that the CR still retained some significance for rats injected with *d*-fenfluramine. Rats treated with *d*-fenfluramine during conditioning (experiment 3) responded appropriately for CR when tested in a drug-free state, showing that they were capable of forming stimulus-stimulus associations. Similarly, since *d*-fenfluramine treatment did not affect the acquisition of lever pressing for water, stimulus-response learning is intact under the influence of *d*-fenfluramine. Therefore, simple deficits in stimulus-stimulus and stimulus-response learning are unlikely to account for the effects of *d*-fenfluramine on responding for CR. Having ruled out these non-specific explanations, it appears that *d*-fenfluramine exerts a behaviourally selective action, such that under *d*-fenfluramine treatment the ability of CRs to elicit and maintain behaviour is reduced.

In addition to reducing responding for CR, *d*-fenfluramine also abolished the response-potentiating effects of *d*-amphetamine on CR responding following injection into the nucleus accumbens. This latter result is consistent with the findings that *d*-fenfluramine (Bendotti et al. 1980) and other treatments (Gerson and Baldessarini 1980) that elevate 5-HT function inhibit *d*-amphetamine-induced hyperactivity. The effects of 5-HT agonists on amphetamine-elicited behaviour appear to reflect an inhibitory influence of 5-HT neurons on dopaminergic function. Although the present studies do not address the central sites of action of *d*-fenfluramine, 5-HT infused into the nucleus accumbens reverses *d*-amphetamine-induced hyperactivity (Carter and Pycock 1978). Therefore, the blockade of the effect of *d*-amphetamine on responding for CR by *d*-fenfluramine may involve a local action within the nucleus accumbens.

Given that certain treatments which reduce 5-HT activity appear to enhance some types of dopamine-dependent behaviours, including hyperactivity and self-administration of psychostimulants (Lyness et al. 1980; Lyness and Moore 1983; Loh and Roberts 1991), it was predicted that 5-HT receptor blockade, using the non-selective 5-HT receptor antagonist metergoline, might enhance responding for CR, or augment the response potentiating effect of *d*-amphetamine. Neither of these effects was found, despite the fact that experiment 1 showed that 1 mg/kg metergoline is clearly an effective 5-HT receptor antagonist, since it reversed the effect of *d*-fenfluramine. The lack of effect of metergoline might indicate that the serotonergic influences over responding for CR, and dopaminergic activity in the nucleus accumbens, as inferred from the effects of *d*-fenfluramine, are not bi-directional. However, in some cases 5-HT antagonists appear ineffective in influencing rewarded behaviour, such as responding for cocaine (Lacosta and Roberts 1993), whereas chronic 5-HT depletion induces marked effects (Loh and Roberts 1991). Therefore, this conclusion should be regarded as tentative until other means of reducing

5-HT function, such as 5,7-DHT treatment, have been evaluated for their effects on responding for CR.

Drugs that increase 5-HT function, including fluoxetine (Carroll et al. 1990; Richardson and Roberts 1991) and L-tryptophan (Smith et al. 1986), reduce cocaine and amphetamine self-administration, and fenfluramine reduces heroin self-administration (Higgins et al. 1993). The behavioural mechanisms underlying these effects are unclear. It is possible that by inhibiting the effects of amphetamine and cocaine on dopaminergic neurotransmission, these treatments reduce the pharmacological efficacy of amphetamine and cocaine. However, animals compensate for reductions in amphetamine or cocaine dose, and for dopaminergic receptor blockade, by increasing rates of self-administration (Yokel and Wise 1976; de Wit and Wise 1977). Thus, the effects of 5-HT agonists on rates of responding for psychomotor stimulants are not compatible with an explanation postulating a reduction in terms of a diminished pharmacological efficacy of these drugs on dopaminergic systems. The present finding that *d*-fenfluramine inhibits responding for CR via an action involving increased 5-HT function suggests a possible alternative explanation. Often in drug self-administration paradigms discriminative stimuli such as lights are present during drug infusions (e.g. Richardson and Roberts 1991; Higgins et al. 1993) and may be operative for a short duration after infusions, during which time the drug will be exerting its effects. Stimuli paired with morphine or amphetamine serve as conditioned rewards (Davis and Smith 1975), and stimuli paired with cocaine have been shown to reinstate lever pressing in animals that have undergone extinction of this response (de Wit and Stewart 1981), illustrating that secondary reinforcing stimuli are likely to play an important role in maintaining drug self-administration. Since CRs appear to be less effective in eliciting behaviour when 5-HT function is elevated, reduced responding for drug reward following 5-HT agonist treatment may be related to a reduced capacity of stimuli associated with those drugs to elicit and maintain responding.

Another behavioural effect of indirect 5-HT agonists such as fenfluramine and fluoxetine is reduced feeding. This effect has been proposed to occur because of a more rapid onset of the process of satiation leading to an early termination of feeding (Blundell 1984). However, the onset and maintenance of feeding is likely to be under the control of secondary reinforcers, including the sight, taste, smell and texture of foods as well as stimuli previously paired with food (Weingarten 1984). Such stimuli appear to elevate nucleus accumbens dopamine activity (Blackburn et al. 1989), and the preparatory responses elicited by these stimuli are reduced by dopamine receptor blockade (Blackburn et al. 1987). It is possible, therefore, that the action of fenfluramine and other 5-HT agonists in inhibiting feeding may involve a reduced capacity of secondary reinforcing properties of foods to elicit and maintain feeding, and to inhibition of dopamine activity associated with the preparatory phase of feeding. Such effects might be particularly evident in situations involving scheduled feeding, and the presentation of highly palatable foods to satiated animals.

In summary, pretreatment with *d*-fenfluramine reduced responding for a CR previously paired with water, and abolished the potentiating effects of amphetamine injected into the nucleus accumbens on this behaviour. This effect involves an increase in 5-HT function, since it was prevented by the 5-HT receptor antagonist metergoline. In control experiments, deficits in motivation for the primary reward, learning ability or attentional mechanisms were ruled out as simple explanations for these effects of *d*-fenfluramine. The overall pattern of results suggests that for rats treated with *d*-fenfluramine conditioned rewards lose some of their effectiveness in controlling behaviour. Consequently, these findings suggest a possible role for brain 5-HT systems in controlling behaviour maintained by CRs, and in incentive motivation.

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