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Relative roles of ventral striatal D_1 and D_2 dopamine receptors in responding with conditioned reinforcement

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Abstract. Several experiments investigated the involvement of D_1 and D_2 dopamine receptors in the ventral striatum in the control over behaviour by a conditioned reinforcer using an acquisition of new response procedure. Intra-accumbens infusion of either the D_1 receptor antagonist, SCH 23390, or the D_2 receptor antagonist, raclopride, completely blocked the potentiative effects of intra-accumbens *d*-amphetamine on responding with conditioned reinforcement and reduced responding to control levels. SCH 23390 was more potent than raclopride. At higher doses in the absence of *d*-amphetamine, both antagonists also blocked the preference for responding on the lever producing the conditioned reinforcer. Intra-accumbens infusions of either the D_1 receptor agonist, SKF 38393, or the $D_{2/3}$ receptor agonist, LY 171555 (quinpirole), selectively potentiated responding on the lever producing the conditioned reinforcer. Various combined infusions of the D_1 and D_2 agonists in specific low doses had additive, but not synergistic, effects on responding with conditioned reinforcement. None of the drugs affected the drinking of water in deprived subjects when infused intra-accumbens. These results suggest that both D_1 and D_2 receptors in the nucleus accumbens are involved in mediating the effects of dopamine in potentiating the control over behaviour by conditioned reinforcers.

Key words: Conditioned reinforcement – Dopamine – Reward – Nucleus accumbens – D_1 and D_2 receptors

The control of behaviour by drugs acting as reinforcers almost certainly requires a functional interaction between the drug reinforcer and environmental stimuli with which it is associated; indeed there is ample evidence to indicate that such stimuli themselves can exert control over responding, both in the Pavlovian setting of the place preference procedure and in the operant case of drug self-administration (Young and Herling 1986; Carr et al. 1989). In the former case such conditioned stimuli express properties of conditioned incentives, eliciting approach behaviour, whereas in the latter case they can act as conditioned reinforcers (CRs) which thus increase responses upon which they are contingent. (This distinction may be more procedural than real, as both functions overlap and are probably controlled by similar neural substrates). Not only can environmental stimuli gain these properties by virtue of pairing with amphetamine-like drugs (Davis and Smith 1974; Phillips and Fibiger 1990), but the power of stimuli conditioned by pairing with other, more natural reinforcers such as food (Beninger et al. 1989; Beninger 1991), water (Robbins 1976) or sex (Everitt et al. 1989), can be enhanced by treatment with psychomotor stimulant drugs.

The capacity of a CS to act as a conditioned reinforcer can be tested by evaluating the control over behaviour by conditioned stimuli in the absence of the primary reinforcer. In the first phase of the test rats are trained according to a Pavlovian conditioning procedure to associate a previously irrelevant stimulus with the presentation of a reward (e.g. food or water). In the second phase, which is carried out in the absence of the primary reinforcer, two levers are presented to the animals, a response on lever (CR lever) resulting in the presentation of the CS while a response on the other (NCR lever) has no consequence. Under these conditions rats prefer to respond on the CR lever, indicating that the CS is able to support the acquisition of a behavioural response and so act as a conditioned reinforcer (Robbins et al. 1989; Everitt and Robbins 1992). Intra-accumbens *d*-amphetamine produces a dose-dependent, selective enhancement of responding which is behaviourally, neurally and neurochemically selective. Thus, dopamine depletion from the ventral, but not the dorsal striatum prevents

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these effects of intra-accumbens *d*-amphetamine (Taylor and Robbins 1986). In addition, the effects of *d*-amphetamine on responding with CR are mimicked by intra-accumbens infusions of DA, but not noradrenaline (Cador et al. 1991). However, these findings leave open the questions of the DA receptor specificity of the effects.

Until recently, dopamine receptors were generally subdivided into two classes on the basis of their effect on adenylate cyclase. Activation of D_1 receptors had been shown to stimulate adenylate cyclase, with activation of D_2 receptors being either inhibitory, or having no effect (Clark and White 1987). Moreover, behavioural and electrophysiological studies had suggested that the D_1 and D_2 receptor systems may interact functionally having co-operative, synergistic or sometimes opposing effects (for reviews see Kelly and Nahorski 1986; Clark and White 1987; Waddington and O'Boyle 1987; Waddington 1989). However the picture has been complicated still more by the discovery of additional dopamine receptor subtypes (Kelly and Nahorski 1986; Schwartz et al. 1992). For example quinpirole, a previously characterised selective D_2 receptor agonist, has also been shown to interact with D_3 receptors and it now appears that there is a family of D_2, D_3 and D_4 receptors (Schwartz et al. 1992). In parallel, there is a separate family of DA receptors composed of the D_1 and D_5 subtypes (Schwartz et al. 1992). Furthermore, it has also recently been demonstrated that D_1 and D_2 receptors are found in different populations of striatal neurons with different projection targets in the pallidum and midbrain (Gerfen 1992a, b).

In the present study we investigated the role of the D_1 and $D_{2/3}$ receptor subtypes in mediating the control over behaviour exerted by conditioned reinforcers. It has been reported that responding with CR was dose-dependently increased by systemic administration of the D_2 agonists quinpirole and bromocriptine, whereas systemic administration of the D_1 agonist SKF 38393 had no such effect (Beninger et al. 1989; Beninger 1991). It is unclear, however, how these effects might be mediated centrally because the various DA receptor subtypes are also localised in regions not thought to mediate the effects of DA on CR. Consequently, in the present study we have investigated the effects of intra-accumbens infusions of (a) D_1 and $D_{2/3}$ agonists, both separately and combined and (b) D_1 and D_2 receptor antagonists by themselves and also in combination with intra-accumbens *d*-amphetamine.

Materials and methods

Animals

Male Lister hooded rats (Olac Bicester, UK) weighing approximately 250 g at the start of the experiments were used. The animals were housed two per cage and tested under a reversed day-night cycle (12 h: 12 h) with free access to food. Drinking was restricted to a 1 hour period at the end of the afternoon.

The animals were trained and tested in operant chambers (26.5 × 22 × 20 cm, Campden Instruments) fitted with water dippers. Each chamber contained two retractable levers (3.8 cm wide, positioned 1.6 cm from the side walls and 5.5 cm from the grid floor) which were retracted during training. The operant chamber could be illuminated by a light in the ceiling. When the water dipper was elevated, access was by way of a panel with a hinged, Plexiglas flap located equidistant between the two levers. The panel could be illuminated by a 2.5 W, 24 V light. The boxes were controlled and data logged on-line using a microcomputer (Paul Fray Computers Ltd, Cambridge).

Surgical procedure

The rats were anaesthetised with 3,3,3-tribromoethanol in tertiary amylalcohol and placed in a Kopf stereotaxic apparatus. Two stainless steel guide cannulae (12 mm long) were bilaterally implanted into the rat brain, their tips ending approximately 1.5 mm above the nucleus accumbens [coordinates: bregma +3.2 mm; lateral ±1.7 mm; vertical −5.2 m below the cortical surface; incisor bar set at +5.0 mm (Pellegrino et al. 1979)]. Anchor screws were placed into the skull, the cannulae secured with dental cement (Cranioplast, Plastics 1, USA) and, when dry, the guides were fitted with obturators cut to the same length. After surgery, the animals were allowed to recover for at least 7 days.

Infusions

Intra-cerebral infusions were carried out in hand-held animals by lowering through the guide cannulae two injection cannulae (13.2 mm long, 30 gauge) connected via PE–10 tubing to 10 µl Hamilton syringes. The infusion cannulae protruded 1.5 mm below the guide cannulae into mid-regions of the posterior-medial nucleus accumbens. Using Harvard Compact infusion pumps, drug solutions were infused bilaterally and simultaneously in a volume of 1 µl/side over a 1 min period. Cannulae were left in place for an additional 1 min before being removed and replaced by the obturators. Immediately afterwards each animal was placed into the operant chamber and testing was started.

Drugs

Dexamphetamine sulphate was obtained from Sigma Chemical Co. Ltd, (Poole, Dorset, UK). LY 171555 hydrochloride (quinpirole) was supplied by Eli Lilly (Indianapolis, IN); raclopride tartrate was supplied by Astra Arcus (Södertälje, Sweden), SKF 38393 hydrochloride was supplied by Smith, Kline and French (Hertfordshire); SCH 23390 maleate was supplied by Schering Corporation (Bloomfield, NJ). All solutions were freshly prepared in phosphate-buffered saline on the day of use.

Experimental procedure

The experiments consisted of two phases, training and testing.

Training. After initial dipper training, in the first phase the rats were required to learn that they only had access to the dipper immediately after presentation of a conditioned stimulus (CS) which consisted of a 5 s illumination of the tray light, house light offset and the characteristic sound of the dipper operation. During training, the dipper was presented for 5 s according to a Random Interval (RI) 30 s schedule. The rats were trained not to respond outside the CS/dipper presentation period by delaying the next possible random presentation by 3 s when they responded outside this period during the RI. Thus, continued responding outside the CS/dipper presentation period would result in indefinite postponement of these stimuli.

Testing. The test sessions were carried out in the absence of presentations of water. Panel pushing in this phase had no programmed consequences. Two levers were then introduced into the box; responding on one of them, predetermined for each rat (CR lever), resulted in a 0.3 s presentation of the CS (i.e. illumination of the tray light, house light offset and sound of dipper operation) according to a random ratio (RR) 2 schedule. A response on the other lever (NCR lever) had no programmed consequence. During the test session the numbers of lever presses (CR and NCR) and panel pushes were recorded.

All the experiments followed the same general procedure. After training, the rats were implanted with cannulae and, after the recovery period, retrained for a few days to the preoperative criterion of discriminated approach to the panel before they entered the test phase. The treatments were administered according to a Latin square design.

Experiment 1: Effects of the D_1 agonist SKF 38393 and the $D_{2/3}$ agonist LY 171555 on responding with conditioned reinforcement

The effects of the D_1 agonist SKF 38393 and the $D_{2/3}$ agonist LY 171555 on responding with CR were studied over several dose ranges. SKF 38393 was tested in two studies in doses of 0.1, 1.0 and 10.0 μg and in doses of 0.01, 0.03 and 0.1 μg/accumbens. The D_2 agonist LY 171555 was tested in three dose ranges; 0.01, 0.03 and 0.1 μg; 0.1, 0.3 and 1.0 μg; and 0.1, 1.0 and 3.0 μg. A few animals received LY 171555 at a dose of 10 μg/accumbens, but as this dose induced strong sedation in the animals (data not shown) the effects of this dose of LY 171555 on responding in the conditioned reinforcement paradigm were not tested.

Experiment 2: Effects of co-infusions of SKF 38393 and LY 171555 on responding with conditioned reinforcement

In order to investigate possible interactions between the D_1 and $D_{2/3}$ receptor systems in the conditioned reinforcement paradigm, the effects of simultaneous D_1 and D_2 receptor stimulation with SKF38393 and LY 171555 were studied over a wide range of dose ratios. In a first experiment, the effects of infusions of a solution containing a mixture of SKF 38393 and LY 171555 in the doses 0.01+0.02; 0.03+0.06 and 0.1+0.2 μg/μl were compared with separate infusions of each alone.

In a second experiment, the animals were infused with vehicle or a solution containing a higher range of concentrations of SKF 38393 and LY 171555 (SKF 38393+LY 171555, respectively 0.1+0.1, 0.1+1.0, 1.0+0.1 and 1.0 and 1.0 μg/μl). In a third experiment the animals were infused sequentially with SKF 38393 (0.1 μg) followed by LY 171555 (0.1 μg).

Experiment 3: Effects of the D_1 and D_2 antagonists SCH 23390 and raclopride on d*-amphetamine potentiated responding with conditioned reinforcement*

This experiment investigated the effects of D_1 and the D_2 receptor blockade on *d*-amphetamine-potentiated responding with CR. SCH 23390 was tested at the doses 0.1, 0.3 and 1.0 μg/μl/side, while raclopride was tested in doses of 0.5, 1.5 and 5.0 μg/μl/side. Each animal was first infused intra-accumbens with drug or vehicle (also infused in 1 μl volumes) according to the Latin square design and then, immediately thereafter, vehicle or *d*-amphetamine (18 μg, a reliable dose for producing potentiative effects, as determined by pilot studies) was also infused intra-accumbens. Each animal was then immediately placed into the operant chamber and the test session was begun.

Experiment 4: Effects of SCH23390 or raclopride on responding with conditioned reinforcement

Two separate studies investigated the effects of the D_1 and D_2 antagonists by themselves on the acquisition of a new response with CR (i.e. in the absence of potentiation with *d*-amphetamine). In the first study, SCH 23390 (3 μg) and raclopride (10 μg) were administered intra-accumbens. In the second study, a lower dose range of the two antagonists was investigated. SCH 23390 was administered in doses of 0.3–1.0 μg and raclopride in doses of 1.5–5.0 μg.

Experiment 5: Effects of D_1 and D_2 agonists and antagonists on drinking

Singly housed, thirsty animals received bilateral intra-accumbens infusions of either SKF 38393 (10.0 μg), LY 171555 (0.1 or 3.0 μg), SCH 23390 (3 μg) or raclopride (5 μg) exactly as described above. After 15 min, the animals were given access to drinking water in a burette. Subsequently, for each rat, the volume of water drunk was measured every 5 min for 15 min.

Histological assessment of cannula placements

At the conclusion of the experiments, animals were deeply anaesthetized (Sodium pentobarbitone IP) and perfused transcardially with 10% formal saline. Brains were blocked and sectioned at 6 μm on a freezing microtome. The sections were mounted on glass slides and stained with cresyl violet. The glial aggregation associated with placement of the infusion cannulae into the region of the nucleus accumbens was located and recorded, together any more generalized damage to the ventral striatum associated with implantation of the guide cannulae or the infusions themselves. This examination was undertaken blind to the behavioural results and occasional animals with evidence of major damage to the ventral striatum or with cannulae incorrectly located (i.e. too deep or asymmetrically placed) were excluded from the subsequent statistical analysis.

Statistical analysis

Data were analysed by ANOVA (Lane 1981) with the factors Lever and drug Dose. Data were square-root (SqRt) transformed in order to preserve the homogeneity of variance, as recommended by Winer (Winer 1971) except for data from the drinking test. Post hoc comparisons were made using the Newman-Keuls or Dunnett's *t* tests.

Results

Histological assessment of cannula placement

Histological analysis of the basal forebrain revealed that the guide cannulae were situated in the ventral part of the dorsal striatum and that a line of gliosis extending down into the nucleus accumbens indicated, in the great majority of cases, that the infusions had been made bilaterally into this region (Fig. 1). Occasionally, the infusion cannula tracks extended down into the more ventral, olfactory tubercle region of the ventral striatum. In general, cannulae were localized to the more caudal-medial parts of the nucleus accumbens, extending from its mid-region to its more posterior limit. In some instances following the 10 μg dose of SKF 38393 there was evidence of uni-

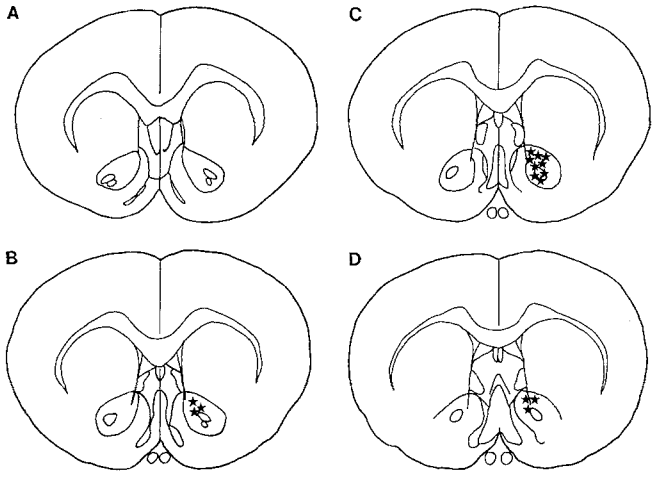

Fig. 1A–D. Schematic diagram showing the location of infusion cannulae within the posterior medial division of the nucleus accumbens region of the ventral striatum. The levels shown are from bregma +1.6 (**A**) to bregma +0.7 (**D**) according to Paxinos and Watson (1986). *Stars* denote the range of placements and relative preponderance in the posteromedial aspects of the nucleus accumbens (**C**)

or bi-lateral toxic damage to the nucleus accumbens region, as has been reported previously (Kelley et al. 1990). This problem affecting the use of SKF 38393 was not apparent at lower concentrations of the drug and when it was observed, it generally correlated with a loss of behavioural response to intra-accumbens infusions at some point in the Latin square design. In those cases where such damage was seen in the nucleus accumbens region, data from those subjects were removed from the data analysis.

Experiment 1: Effects of intra-accumbens infusions of SKF 38393 and LY 171555 on responding with conditioned reinforcement

Figure 2a shows that intra-accumbens infusions of the D_1 agonist SKF 38393 (in doses of 0.1, 1.0 and 10.0 μg/μl/accumbens) selectively potentiated responding on the CR lever. This was confirmed by ANOVA which revealed a significant Lever × Dose interaction [$F(3,54)=4.42$, $P=0.0076$]. Infusions of lower doses of SKF 38393 (0.01, 0.03 and 0.1 μg/μl/accumbens) were without significant effect on responding on either CR or NCR levers ($F<0.5$). In this low dose series, the 0.1 μg dose failed to induce a significant increase in responding on the CR lever (mean SQRT number responses on CR lever: vehicle – 5.85; SKF 38393 (0.1 μg) – 6.00), whereas it was effective in the higher dose (0.1–10 μg) series.

Figure 2b shows that intra-accumbens infusion of the $D_{2/3}$ agonist LY 171555 (0.1, 0.3 and 1.0 μg/μl/accumbens) also selectively increased responding on the CR lever. This was confirmed by ANOVA, which revealed a significant Lever × Dose interaction [$F(3,21)=4.23$, $P=0.017$]. Doses of 0.01, 0.031 μg/μl LY171555 were without effect on responding on either the CR or NCR lever ($F<0.5$).

Typical untransformed response rates at optimal doses were: SKF 38393 – CR lever = 73, NCR lever = 12; LY 171555 – CR lever = 49, NCR lever = 5 by comparison with control values CR lever = 19, NCR lever = 9. Numbers of responses were generally higher following the D_1 than the D_2 agonist and were generally lower than those typically seen after intra-accumbens infusions of *d*-amphetamine (CR lever = 120–150, NCR lever = 15–20).

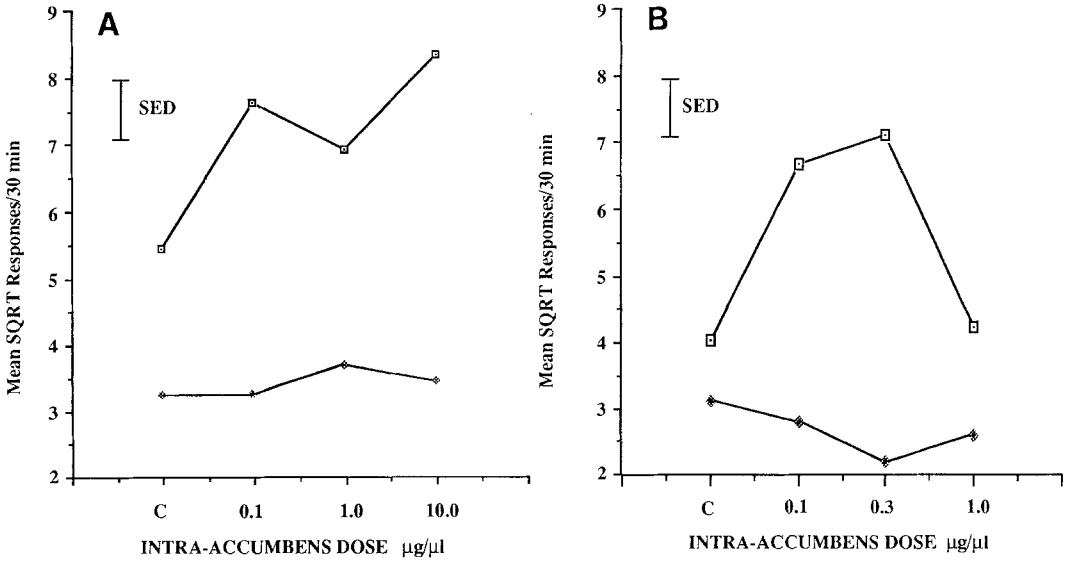

Fig. 2A, B. The effects of intra-accumbens infusions of **A** the D_1 agonist SKF 38393 and **B** the D_2 agonist LY 171555 on responding with conditioned reinforcement. CR lever = lever producing the conditioned reinforcer (—⊡—); NCR lever = control lever on which responses had no programmed effect (—◆—). SED = standard error of the difference of the means. This index of variability is derived from the appropriate error term in the ANOVA and can be used as the denominator for post hoc comparisons; *C* = control, i.e. intra-accumbens infusion of drug vehicle

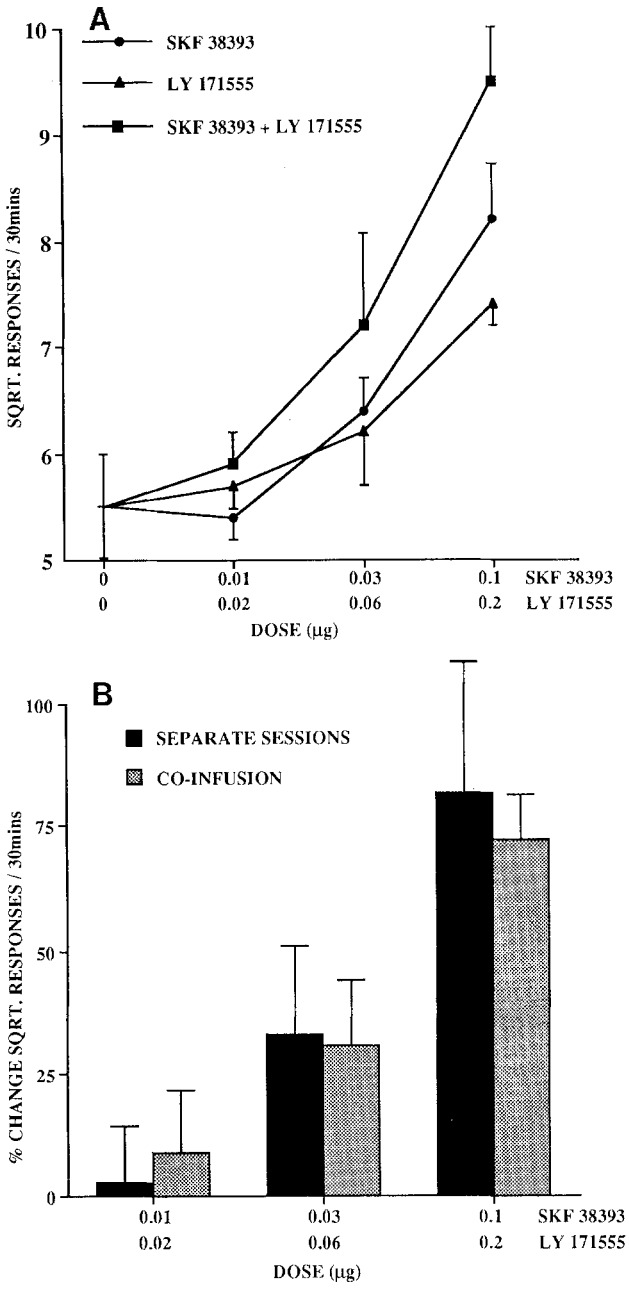

Fig. 3A, B. The effects of SKF 38393 and LY 171555 infused intra-accumbens separately or together on responding with conditioned reinforcement. In **A** the effects on responding on the CR lever of the D_1 and D_2 agonists infused separately or combined in each of three doses are shown. In **B** the results are presented as the percentage change in responding on the CR lever when the effects of the drugs infused separately are simply added together in order that they can be compared with the change in responding on the CR lever occurring after the drugs were co-infused. The simple additive effects of the co-infused D_1 and D_2 agonist can clearly be seen. Data shown are means ± SEM

Experiment 2: Effects of mixtures of SKF 38393 and LY 171555 on responding with conditioned reinforcement

In the first study, low doses of SKF 38393 (0.01, 0.03 and 0.1 μg) and LY 171555 (0.02, 0.06 and 0.2 μg) were infused, either separately or together, bilaterally into the ventral striatum. The results are shown in Fig. 3. Analysis revealed a significant Lever × Dose interaction [$F(9,45)=2.6$, $P<0.05$), indicating that drug effects on responding were confined to the CR lever [CR lever: $F(9,45)=4.0$, $P<0.001$; NCR lever: $F(9,45)=1.6$, NS]. Further analysis revealed that while responding on the CR lever was increased by separate administration of the highest doses of SKF 38393 and LY 171555 (0.1 μg and 0.2 μg respectively; Fig. 3a), co-infusion of these doses of the DA agonists enhanced responding still further [$F(1,45)=4.2$, $P<0.05$]. However, the effects of co-infusion of the DA agonists were found to be entirely comparable with the sum of the effects of their separate administration [$F(1,25)=0.01$, NS; Fig. 3b], indicating that the effects of the D_1 and D_2 agonists on responding were purely additive.

In the second study, a variety of higher dose mixtures of SKF 38393 and LY 171555 in different concentrations were infused into the ventral striatum (the dose of SKF 38393 is shown first: 0.1 + 0.1 μg; 0.1 + 1.0 μg; 1.0 + 0.1 μg; 1.0 + 1.0 μg). All combinations of the two drugs selectively increased responding on the CR lever [Lever × Dose interaction: $F(4,20)=6.373$, $P=0.002$], but there was no evidence that the mixtures resulted in enhanced or diminished effects on responding on the CR lever compared with either drug given alone.

Experiment 3: Effects of intra-accumbens SCH23390 or raclopride on the increased responding with conditioned reinforcement induced by intra-accumbens infusions of d-*amphetamine*

Intra-accumbens infusion of *d*-amphetamine (18 μg) induced a marked and selective increase in responding on the CR lever from a control level of about 40 responses/30 min to about 100 responses/30 min with very low response rates on the NCR lever in both cases (< 16/30 min). This selective increase was completely blocked to control levels by both SCH 23390 (1 μg/μl) and raclopride (5 μg/μl) infused immediately before *d*-amphetamine; responding on the NCR lever was unaffected [Drug × Lever interaction, $F(3,36)=3.74$, $P=0.019$]. Post hoc comparisons revealed that only the effect of *d*-amphetamine was significantly different from control.

Two similar experiments were performed using lower doses of SCH 23390 and raclopride. A dose of 0.3 μg/μl SCH23390, but not 1.5 μg raclopride significantly reduced the response to intra-accumbens *d*-amphetamine [Drug × Lever interaction, $F(3,30)$ 3.12, $P=0.04$; post hoc comparisons revealed that *d*-amphetamine plus SCH 23390, but not *d*-amphetamine plus raclopride were significantly different from *d*-amphetamine alone]. Neither 0.5 μg/μl raclopride, nor 0.1 μg/μl SCH 23390 significantly attenuated the effects of intra-accumbens *d*-amphetamine [Drug × Lever interaction, $F(3,21)=4.83$, $P=0.01$; post hoc comparisons revealed that *d*-amphetamine alone, or in combination with either antagonist was significantly different from vehicle controls]. The data from all three experiments, expressed as % of the

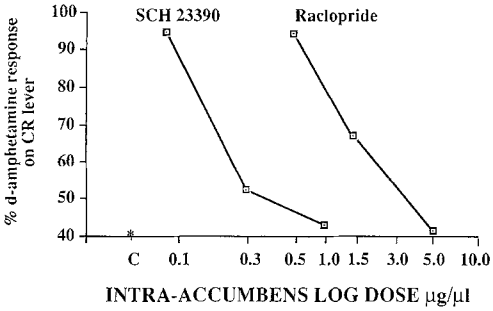

Fig. 4. Logarithmic dose-response curve for intra-accumbens SCH 23390 and raclopride antagonism of the effects of intra-accumbens *d*-amphetamine. The * at *C* = level of responding on the CR lever following vehicle infusion. Other points show the reduction of the *d*-amphetamine response (100%) following pre-infusion into the nucleus accumbens with each of three doses of each antagonist. (Note for the purposes of this comparison that 1 µmol of raclopride = 497.4 µg and 1 µmol SCH 23390 = 248.4 µg)

amphetamine response and plotted on a log-dose scale, are incorporated in Fig. 4. SCH 23390 can be seen to be approximately 10 fold more potent than raclopride in blocking the potentiative effect of *d*-amphetamine.

Experiment 4: Effects of intra-accumbens infusion of SCH23390 or raclopride alone on responding with conditioned reinforcement

Figure 5 shows that intra-accumbens infusion of either 3 µg/µl SCH23390 or 10 µg/µl raclopride resulted in a significant reduction in responding on the CR lever. This was confirmed by ANOVA, which revealed a significant Drug × Lever interaction [$F(2,19) = 5.32$, $P = 0.0183$]. In a separate experiment, lower doses of the same drugs were infused (0.3 and 1.0 µg SCH23390; 1.5 and 5.0 µg raclopride). Analysis again revealed a significant Drug × Lever interaction [$F(4,24) = 5.37$, $P = 0.003$]. Post hoc comparisons showed that neither 0.3 µg nor 1.0 µg SCH23390 affected responding on the CR lever (Fig. 5a), but that raclopride significantly reduced responding on the CR lever only at the 5 µg dose (Fig. 5b). Other comparisons confirmed significant differences between the CR and NCR levers at all other doses including control infusions for both drugs.

Experiment 5: Effects of D_1 and D_2 agonists and antagonists on drinking

Thirsty rats (23 h water deprived) were infused with appropriate drug vehicles into the nucleus accumbens and given access to water 15 min later. They drank approximately 10–11 ml over the next 15 min. The majority of the water (>80%) was drunk during the first 5 min of the 15 min period.

Bilateral, intra-accumbens infusions of the dopamine agonists SKF 38393 (10.0 µg) or LY 171555 (0.1 µg or 3.0 µg) were without effect on drinking (SKF 38393 – 10.5 ml drunk; LY 171555 – 11.0 and 12.1 ml drunk, respectively; [$F(3,18) = 0.44$, NS). Similarly, infusions of the dopamine antagonists SCH 23390 (3.0 µg) or raclopride (5 µg) were also without effect on drinking [10.3 ml and 11.0 ml drunk, respectively; $F(2,14) = 0.34$, NS]. Thus, under the conditions of these experiments, neither D_1, nor D_2, agonists and antagonists had any effect on thirst as measured in home cage drinking tests.

Discussion

The experiments reported here investigated the involvement of D_1 and D_2 receptors in the ventral striatum in the control over behaviour by conditioned reinforcement. Intra-accumbens infusion of either the D_1 receptor antagonist, SCH 23390, or the D_2 receptor antagonist, raclopride, completely blocked the potentiative effects of intra-accumbens *d*-amphetamine and reduced responding to control levels. At higher doses in the absence of *d*-amphetamine, they also blocked the preference for responding on the lever producing the conditioned reinforcer. By contrast, intra-accumbens infusions of either the D_1 receptor agonist, SKF 38393, or the $D_{2/3}$ receptor

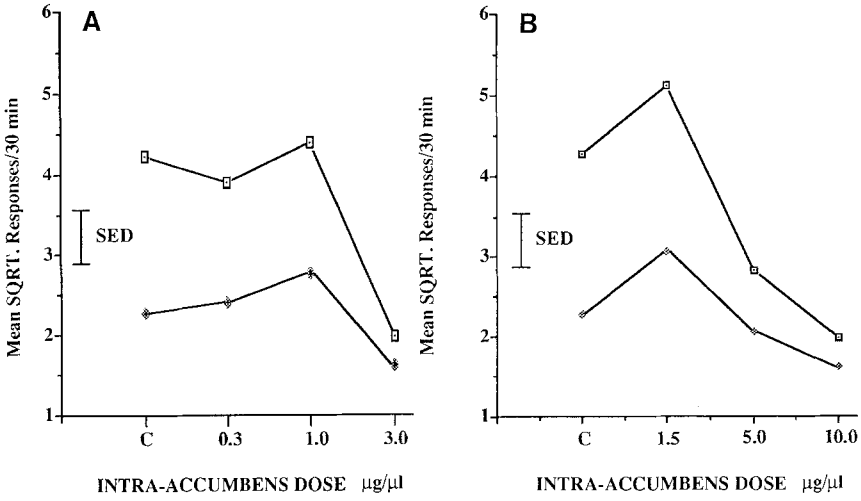

Fig. 5A, B. The effects of intra-accumbens infusions of **A** SCH 23390 or **B** raclopride on responding with conditioned reinforcement. Abbreviations as for Fig. 2. (—⊡—) CR lever; (—◆—) NCR lever

agonist, quinpirole, selectively potentiated responding on the lever producing the conditioned reinforcer. Various combined infusions of these two drugs in specific doses had additive, but not synergistic, effects on responding with CR. None of the drugs had primary motivational effects, in that they did not affect the drinking of water in deprived subjects.

The acquisition of a new response procedure utilised here provides a stringent test of conditioned reinforcement (Mackintosh 1974). Our previous work has established neurochemical, neuroanatomical and behavioural specificity of the impact of *d*-amphetamine on the control of behaviour by conditioned reinforcers. Thus, *d*-amphetamine only potentiates responses that result in presentation of the conditioned reinforcer; stimuli randomly paired with primary reward do not support the acquisition of a new response and *d*-amphetamine has no effect on responding for such stimuli (Taylor and Robbins 1984). Moreover, dopamine itself, but not noradrenaline, mimics the effects of *d*-amphetamine, so indicating that it is dopamine release that mediates the effects of *d*-amphetamine (Cador et al. 1991). This is further emphasized by the observation that 6-hydroxydopamine-induced depletion of dopamine, but not noradrenaline, from the ventral, but not the dorsal, striatum abolishes the potentiative effects of intra-accumbens infusions of *d*-amphetamine (Taylor and Robbins 1986). The present results extend this analysis of the importance of ventral striatal dopamine in reward-related processes by specifying the effects of manipulations of D_1 and $D_{2/3}$ receptors. The results indicate that *d*-amphetamine-induced potentiation of the control over behaviour by a conditioned reinforcer depends upon both D_1 and $D_{2/3}$ receptors in the ventral striatum.

Both the D_1 antagonist and the D_2 antagonist infused into the ventral striatum blocked selectively the effects of intra-accumbens *d*-amphetamine, that is neither drug altered responding on the control lever. The D_1 antagonist, SCH 23390, was about one order of magnitude more potent than raclopride in this regard. Moreover, it is important to note that doses of SCH 23390 effective in blocking the potentiating effect of *d*-amphetamine were not effective when given alone. In addition, blockade of either receptor subtype did not eliminate information about the rewarding efficacy of environmental stimuli, but merely eliminated the potentiation of their impact due to dopamine release. Higher doses of the antagonists did impair responding on the CR lever, but general motor activity was probably also depressed by such doses as indicated, for example, by the concomitant reduction in responding on the NCR lever in the experiments reported here. A similar conclusion about the relative efficacy of D_1 and D_2 antagonists has also been reached concerning the blockade of cocaine self-administration (Koob 1992), but both of these effects contrast with the greater efficacy of D_2 receptor antagonists on retarding reaction time performance (Amalric et al. 1990). Alterations in responding maintained by conditioned reinforcement cannot easily be attributed to underlying changes in primary motivation, such as have been seen following systemic administration of SCH 23390 (Clifton et al. 1991), as neither drug infused into the nucleus accumbens affected drinking when the deprived animals were given free access to water.

It should be recognized that SCH 23390 also possesses some 5–HT2 receptor-blocking activity (Ohlstein and Berkowitz 1985) and that its potent effects in the paradigm used here may be due to actions at that receptor – especially as *d*-amphetamine is known also to release 5-hydroxytryptamine (5–HT), in addition to catecholamines (Hoebel and Hernandez 1989). However, it seems unlikely that reductions in 5–HT transmission would also diminish the effects of *d*-amphetamine, indeed, the opposite result might be predicted. Thus, lesions of forebrain 5–HT neurons potentiate the effects of the dopamine reuptake inhibitor, cocaine, in a self-administration procedure (Lyness et al. 1980; Loh and Roberts 1990), while social isolation results in a marked potentiation of the effects of *d*-amphetamine in the conditioned reinforcement procedure used here and this is associated with reductions in indices of 5–HT release as measured by in vivo microdialysis (Jones et al. 1991)

Both the D_1 and $D_{2/3}$ agonists, when infused into the ventral striatum, enhanced responding with conditioned reinforcement, but to a lesser extent than was seen after infusion of *d*-amphetamine; SKF 38393 was generally more effective than LY 171555 at all doses tested. The $D_{2/3}$ agonist, LY 171555, exhibited an inverted U-shaped dose-effect relationship, with doses of 3.0 or 10.0 μg (data not shown) having a clear sedative effect on the animals. The consistent effects of SKF 38393 on responding with conditioned reinforcement are impressive in the context of its potential for inducing toxic damage in the nucleus accumbens (see Kelley et al. 1990, but note that this was only occasionally observed at the highest dose used here) and also in view of its only partial agonist activity at the D_1 receptor (Setler et al. 1978). Thus, stimulation of the D_1 receptor by a full agonist might be expected to produce greater effects. However, we have consistently found that responses to direct dopamine agonists, such as apomorphine (Taylor and Robbins 1986) and the agonists used here, whether infused singly or together, are appreciably smaller than those seen to follow intra-accumbens infusions of the indirect agonist, *d*-amphetamine. The reasons for this are not entirely clear, but may in part be due to the fact that direct agonists may activate ventral striatal outflow in a pattern that is less closely related to information processing in this structure than would be the case following more subtle modulation of pre-synaptic dopamine release. Indeed, two studies employing a conditioned reinforcement procedure similar to that used here have failed to demonstrate any effect of D_1 agonists (Beninger 1991; Chu and Kelley 1992). In the former case, this may be related to the fact that SKF 38393 was given systemically, rather than intra-accumbens, and thus the drug both may not have been delivered optimally to the ventral striatum and also may have had effects at sites other than the ventral striatum that may have obscured any potentiative effects on responding with conditioned reinforcement. These factors cannot explain the lack of effect in the study by Chu and Kelley (1992), since the drug was infused directly into the

nucleus accumbens. However, a different D_1 agonist was used so making direct comparisons difficult.

Separate infusions of SKF 38393 and LY 171555 produced effects on responding with conditioned reinforcement that were seen at doses much lower (i.e one order of magnitude or more) than those used previously in studies of the locomotor activating (Dreher and Jackson 1989; Plaznik et al. 1989), or oral stereotypy-inducing (Delfs and Kelley 1990), effects of these drugs infused into the nucleus accumbens or ventrolateral caudate-putamen, respectively. Furthermore, neither drug had any effect on drinking, indicating that alterations in primary motivation do not underlie the changes in response to conditioned reinforcement. These observations emphasize that the effects of the drugs in the present experiments are not simply dependent on their locomotor-activating actions, or on fundamental changes in consummatory behaviour. Indeed, evidence from a variety of experiments now strongly suggests that consummatory responses, such as chewing, lapping, mounting or lordosis, are associated more or less exclusively with dorsal, not ventral, striatal dopaminergic function. Moreover, a rather specific role can be defined for the ventral, but not dorsal, dopaminergic system in reward-related processes, as in the promotion of appetitive behaviour in the presence of both conditioned and unconditioned incentive stimuli, such as those reported here (Robbins et al. 1989; Everitt 1990; Robbins and Everitt 1992; Salamone 1992).

The pattern of results following the separate infusions of D_1 and D_2 agonists suggests that stimulation of both D_1 and $D_{2/3}$ receptors may be required to mimic more effectively the effects of intra-accumbens *d*-amphetamine. The experiments using combinations of SKF 38393 and LY 171555 bear this out, but only in certain dose combinations. Thus, combinations of the lower doses of the two drugs produced a larger effect on responding than did either alone, but this was significant only in the case of the 0.1 plus 0.2 μg combination of the D_1 and $D_{2/3}$ agonists, respectively. In the higher dose series, there was no such increased effect of combined infusions and this may be explained in terms of the inverted "U" dose response curve of LY 17155, in that the high dose combination pushed the response onto the downward part of the curve, i.e. behaved like a high dose of the D_2 agonist alone. Interestingly, even the 0.1 plus 0.1 μg co-infusion was without effect in this high dose series, suggesting that the experience of other drug dose combinations in the Latin square design affected the response to an otherwise effective treatment. In addition, electrophysiological studies have demonstrated that sequential infusions of SKF 38393 and LY 171555, and only in the order D_1 followed by D_2, were more effective than administration of a mixture of both compounds together, or each alone (Yang and Mogenson 1989). However, this was not observed in the present experiments.

It is therefore clear from these experiments that there is no indication whatsoever of synergy in the effects of co-infused D_1 and $D_{2/3}$ agonists, whereby the effects should be greater than the added effects of each given alone. The observed response was explainable solely in terms of a simple additive effect of the two drugs used. This observation of additive, but non-synergistic, effects of combined D_1 and $D_{2/3}$ agonists reported here contributes to a complex area of research on the effects of manipulations of striatal dopamine, since additive, potentiative and opposing actions of such combined treatments have been reported in a variety of behavioural, electrophysiological and in vitro pharmacological models (Clark and White 1987; Waddington 1989). However it seems appropriate to re-appraise some of the premises upon which such D_1/D_2 receptor interactions are based. First, not only D_1 and D_2, but D_3, D_4 and D_5 receptors recently have been identified and cloned (Schwartz et al. 1992). Apart from raising the issue that perhaps these newly identified receptors might also interact functionally with D_1 and/or D_2 receptors at those sites where they are co-localised, it has also become possible more selectively to define the receptor specificity of previously used D_1 and D_2 receptor agonists and antagonists. While detailed consideration of this subject is beyond the scope of the present paper, one point is important to emphasise. Many of the demonstrations of D_1/D_2 interactions have been based on studies involving LY 171555 (quinpirole) as a potent and selective D_2 agonist (Clark and White 1987; Dreher and Jackson 1989; Plaznik et al. 1989; Waddington 1989). However, in vitro analysis of the binding characteristics of quinpirole, along with many other dopamine agonists and antagonists, in cell lines expressing the cloned receptor DNAs has revealed that this drug is some 100 times more potent at the D_3 than D_2 receptor (Schwartz et al. 1992). Thus, part of the premise upon which D_1/D_2 synergy is based must now be questioned and it has yet to be determined whether it is interactions between D_1 and D_3, or D_1 and D_2, receptors that are revealed in the many studies using quinpirole. But this does not detract, of course, from the demonstrations of interactions between D_1 and $D_{2/3}$ receptor agonists themselves, regardless of the precise receptors through which their effects are mediated. We have used the notation $D_{2/3}$ agonist when discussing quinpirole because of the uncertainty which surrounds its actions at the present time.

In the dorsal striatum, D_1 and D_2 agonists have opposite effects on adenylate cyclase activity, for example the stimulation of cAMP efflux induced by SKF 38393 is reduced by D_2 agonists (Stoof and Kebabian 1981, 1984; Kelly and Nahorski 1986). Such opposing effects of D_1 and D_2 receptor agonists are also seen in some behavioural tests, for example the repetitive mouth and jaw movements produced by treatment with SKF 38393 are attenuated by concurrent D_2 receptor stimulation (Johansson et al. 1987; Waddington 1989). However, both behavioural and electrophysiological studies paradoxically have provided evidence of synergistic, rather than opposing, effects of D_1 and D_2 receptor stimulation (Clark and White 1987). Thus, concurrent D_1 and D_2 receptor stimulation has been shown to have synergistic effects on pallidal and substantia nigra reticulata neuronal activity (Walters et al. 1986; Carlson et al. 1987), while nucleus accumbens neurons have been shown to

respond much more to iontophoretic application of both a D_1 and a D_2 agonist, than to either given alone (Clark and White 1987). Similarly, combined systemic administration of D_1 and D_2 agonists has been demonstrated to produce either additive or synergistic effects in a number of behavioural tests, such as locomotor activity and rotation (Clark and White 1987). An explanation of the latter has been provided in terms of actions of the drugs on receptors in different locations, in particular D_1 receptors in the substantia nigra and D_2 receptors in the striatum (Robertson and Robertson 1987). Although this may be useful in explaining behavioural phenomena arising from studies in which such drugs are given systemically, it cannot explain additive or synergistic effects of D_1 and D_2 agonists infused directly into the striatum. For example, synergistic effects on locomotor activity of intra-accumbens SKF 38393 and quinpirole have been demonstrated by a number of groups (Dreher and Jackson 1989; Plaznik et al. 1989). In addition using a procedure similar to that used in the present experiments, neither a D_1 nor a D_2 agonist when infused alone into the nucleus accumbens affected responding with conditioned reinforcement, but a significant and, therefore, synergistic effect was seen when they were co-infused (Chu and Kelley 1992).

In general, the results of our experiments suggest that both D_1- and $D_{2/3}$-mediated effects of dopamine transmission in the ventral striatum are essential for the full impact of reward-related processes on behaviour. Clear evidence of additivity, but not synergy, of D_1 and $D_{2/3}$ effects was obtained and this appears to be all that is required to explain the powerful effects of dopamine-releasing psychomotor stimulants, such as *d*-amphetamine. The notion that D_1 receptor stimulation is necessary for the full expression of D_2-mediated effects (Clark and White 1987; Dreher and Jackson 1989; Waddington 1989) receives some support from the present data. Thus, the D_1 receptor antagonist completely blocked the potentiative effects of *d*-amphetamine at doses ineffective when given alone, despite the presumed occupancy of D_2 receptors by released dopamine. By contrast, the D_2 receptor antagonist only completely blocked the effects of *d*-amphetamine at a dose which had rate-reducing effects when given alone.

While more experimentation will be required to resolve these issues, recent data on the neuronal localisation of D_1 and D_2 receptors in the striatum and the intraneuronal consequences of their activation may allow putative D_1/D_2 receptor agonist interactions to be seen in a clearer light. Thus, D1 and D_2 receptors appear in general to be located on separate neuronal populations – at least in the dorsal striatum (Gerfen 1992a, b). Thus, stimulation of dorsal striatal D_2 receptors induces the expression of the pre-proenkephalin gene in a subpopulation of GABA-ergic medium spiny neurons that project to the external segment of the globus pallidus (Gerfen et al. 1990; Gerfen 1992a). However, a separate population of GABA-ergic medium spiny neurons which project to the substantia nigra and entopeduncular nucleus, express D_1 receptors and, in response to D_1 agonists, these neurons express the peptides substance P and dynorphin (Gerfen et al. 1990; Gerfen 1992a). Apart from raising an interesting question concerning the neuronal basis of opposing actions of D_1 and D_2 agonists on cAMP activity when they are acting largely upon these different populations of neurons, such anatomical data suggest that the additive effects of these agonists represent the simultaneous activation of separate output chanels from the striatum. It is not apparent how synergistic, rather than additive, effects of D_1 and D_2 co-activation might come about according to this scheme. However, Gerfen has speculated that local axon collaterals and/or striatal interneurons, such as the cholinergic or peptidergic neurons, may mediate such interactions between neurons having D_1 or D_2 receptors (Gerfen 1992a). Nor is it clear whether this scheme, which has been so precisely worked out for the dorsal striatum, also applies to the ventral striatum. Some data suggest that it does, but the additional core/shell principle of nucleus accumbens organisation (Groenewegen et al. 1989; Heimer et al. 1991) means that another layer of complexity must be taken into account when considering dopamine-dependent functions of this structure, especially since the concentrations and metabolism of dopamine, as well as the distribution of dopamine receptors, vary considerably between these two ventral striatal compartments (see Deutch and Cameron 1992).

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