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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

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Abstract. Intra-accumbens *d*-amphetamine enhances responding for reward-related stimuli (conditioned reinforcers, CRs), whereas intra-caudate *d*-amphetamine has only weak and variable effects (Taylor and Robbins 1984). The present experiment further examined the involvement of the nucleus accumbens and the role of dopamine (DA) in this effect. Thirsty rats were trained to associate a flash of a light and movement of a dipper (CR) with water. After implantation of permanent guide cannulae aimed at the nucleus accumbens, they were assigned to one of four groups, receiving either bilateral 6-OHDA (4 mg/ml free base in 2 µl 0.1% ascorbic acid/0.9% saline) or sham (vehicle) infusions into the nucleus accumbens or the caudate nucleus. In the test phase, two novel levers were available. Responding on one lever (CR lever) produced the light and dipper stimuli without water presentation, whereas responding on the other (NCR lever) had no effect. All four groups received four counterbalanced intra-accumbens infusions of *d*-amphetamine (3, 10, 20 µg/2 µl) or vehicle. On the 5th test day, subjects were pretreated subcutaneously with apomorphine (0.1 mg/kg). Intra-accumbens *d*-amphetamine in both sham-lesioned groups produced a dose-dependent increase in responding on the CR lever, but no significant change on the NCR lever. No selective increases in responding on either lever were found in animals with 6-OHDA-induced depletion of DA (>80%) in the nucleus accumbens following intra-accumbens *d*-amphetamine; however, in subjects with DA depletion of the posterior caudate nucleus (>80%), increases in responding on the CR lever were observed to be similar in magnitude to those of both the sham-lesioned groups. Following systemic administration of apomorphine, only rats in the nucleus-accumbens-lesioned group continued to respond, preferring the CR lever, thus suggesting the involvement of DA receptors in these effects. These results indicate that enhanced responding for CR following administration of psychomotor stimulant drugs is critically dependent on dopaminergic activation of the nucleus accumbens, rather than the caudate nucleus.

Key words: Conditioned reinforcement – *d*-Amphetamine – Nucleus accumbens – Caudate nucleus – 6-Hydroxydopamine – Rat

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Activation of mesolimbic regions rich in dopamine (DA) innervation following the administration of psychomotor stimulant drugs may be critically involved in mediating reinforcement (Wise 1981). For example, self-administration of *d*-amphetamine directly into the nucleus accumbens has been reported (Lenard et al. 1980; Hoebel et al. 1983), and self-administration of intravenous stimulant drugs is attenuated following 6-hydroxydopamine (6-OHDA)-induced depletion of catecholamines in the nucleus accumbens (Roberts et al. 1977, 1980; Lyness et al. 1979; Pettit et al. 1984). Place-preference conditioning with *d*-amphetamine is also blocked by 6-OHDA-lesions of the nucleus accumbens (Spyraki et al. 1982). The dopaminergic substrate involved in place-preference conditioning has been further indicated by the demonstration that infusions of *d*-amphetamine into mesolimbic regions (e.g. the nucleus accumbens) result in a place-preference, whereas infusions into neostriatal regions (e.g. the caudate nucleus) do not have this effect (Carr and White 1983).

Although place-preference conditioning and self-administration studies show that stimulant drugs and stimuli associated with them can act as reinforcers, the behavioural mechanism by which they acquire this capacity has not been identified. Thus, it is unclear if the reinforcing effects of stimulants result from their interoceptive effects or whether they enhance the reinforcing impact of salient (exteroceptive) events. In addition, the possible role of drug-induced increases in activity in reinforcement or appetitive effects of stimulants cannot easily be controlled using self-administration or place-preference procedures (c.f. Swerdlow and Koob 1984) and the reduction of stimulant self-administration following dopaminergic lesions is difficult to interpret unambiguously as an attenuation of drug reinforcement (Roberts et al. 1977, 1980).

One way to examine whether a drug affects the processing of stimuli associated with reward, and also to control for non-specific increases in activity induced by a drug, is the use of a two-lever, new-response procedure with conditioned reinforcement (see Robbins 1978). This procedure specifies a relationship between the presentation of an environmental stimulus and reward, thereby establishing the stimulus as a conditioned reinforcer (CR) when presented in extinction (Mackintosh 1974). Psychomotor stimulants, notably pipradrol, can potentiate the effects of conditioned reinforcers (Hill 1967; Robbins 1978; Beninger et al. 1980). Dose-dependent increases in responding for CR on a previously inactive or absent lever have been found following

systemic injections of pipradrol and other stimulant drugs, including *d*-amphetamine (Robbins 1978; Beninger et al. 1980; Robbins et al. 1983a). These results cannot be explained by non-specific increases in activity produced by stimulant drugs, because responding on a second lever that produces no CR (NCR) is either not increased (Beninger et al. 1980) or is even decreased (Robbins 1978), compared with responding on the CR lever following administration of stimulant drugs. The selective stimulation also depends on the positive contingency between the stimulus and reward because the drug does not increase responding reinforced by the same stimulus that was only randomly paired with reward in training (Taylor and Robbins 1984), thereby controlling for unconditioned influences of the CR stimulus on responding.

The behaviourally selective potentiation of responding for CR following the central administration of *d*-amphetamine into the nucleus accumbens (Taylor and Robbins 1984) provides indirect support for a catecholaminergic involvement in this behavioural effect. In this study, selective increases in responding on the CR lever following nucleus accumbens activation were consistent across subjects, whereas intra-caudate *d*-amphetamine infusions produced variable effects. Some of the positive effects seen with intra-caudate infusions may have resulted from spread of the drug to proximal areas of the nucleus accumbens because of the relatively large infusion volumes used (2 µl), or both areas may be implicated.

The purpose of the experiment reported here was to examine further the involvement of the nucleus accumbens, and the role of catecholamines, in the potentiation of responding for CR using infusions of *d*-amphetamine into the nucleus accumbens in rats with 6-OHDA lesions of the nucleus accumbens or the caudate nucleus and in sham operated controls. The direct DA agonist apomorphine was also systemically administered to investigate the specific importance of DA rather than noradrenaline (NA).

Materials and methods

The following behavioural procedures and general surgical techniques have been previously described (Taylor and Robbins 1984) with the exception of those involving 6-OHDA.

Subjects and apparatus. Subjects were male Sprague Dawley rats (OLAC Bicester, England), which weighed approximately 225 g at the start of the experiment. They were housed in pairs under natural daylight. Access to water was limited to 30 min each day, except during surgery and recovery. Food was continuously available.

Four double-lever operant chambers were used (26.5 cm long, 22 cm wide, 20 cm high, Campden Instruments). Each chamber contained a model 441 water dipper (cup capacity 0.06 ml) and two levers, both 3.8 cm wide (removed during training), which were positioned 1.6 cm from the side walls and 5.5 cm from the grid floor. The force required to produce switch closure on each of the levers was 12 g. Access to water was obtained by pressing a hinged Plexiglas panel, located between the two levers, when the water dipper was in an elevated position. The panel tray could be illuminated by a 2.5-W, 24-V light situated 10 cm behind the panel. The operant chamber could be lit by means of a similar light located on the ceiling.

The operant chambers were housed in sound-attenuating boxes and external noise was masked by ventilating fans mounted on the side of each box. The apparatus was controlled and the data collected by an ACORN microcomputer located in an adjacent room.

Surgical procedures. At the time of surgery the rats weighed approximately 325 g. Sodium pentobarbitone/chloral hydrate anaesthesia was used during surgery. Ten rats were randomly assigned to one of four groups, designated variously to receive bilateral nucleus-accumbens lesions, nucleus-accumbens sham surgery, caudate nucleus lesions or caudate-nucleus sham surgery. During surgery all 40 subjects also received bilateral implantation of stainless-steel guide cannulae (23 gauge) aimed to give access to the nucleus accumbens (AP +3.4 from bregma, Lat +/−1.7, Vert −6.0 from dura). All stereotaxic coordinates were determined from Pellegrino and Cushman (1967).

Lesions were made using 6-hydroxydopamine hydrobromide (6-OHDA; Sigma, Poole) dissolved in a vehicle of ice-cold 0.9% saline containing 0.1 mg/ml ascorbic acid at a concentration of 4 mg/ml 6-OHDA (calculated in terms of the free base). The volume of 6-OHDA or vehicle solution was 2 µl, infused for 4 min in each side. The coordinates used for the nucleus accumbens or caudate nucleus (lesion or sham lesion) were AP +3.4, Lat +/−1.7, Vert −7.2 and AP +2.0, Lat +/−3.5, Vert −5.5, respectively.

For subjects in the nucleus accumbens lesion or sham-lesion groups, guide cannulae aimed at the nucleus accumbens were implanted before the 6-OHDA or vehicle was infused. Following this bilateral cannulae implantation, a lesion or sham lesion was made by manually inserting a 30-gauge infusion cannula filled with 6-OHDA or vehicle solution down the cannula shaft and the appropriate 2-µl solution infused for 4 min into each side. Both caudate nucleus lesion subjects and caudate nucleus sham-lesion subjects received infusions of 6-OHDA or vehicle directly into the caudate nucleus prior to the permanent implantation of guide cannulae aimed at the nucleus accumbens. After surgery the subjects were given free access to sweetened baby cereal moistened with water and their weights monitored daily. Eight subjects became ill with gastrointestinal difficulties before being tested and were excluded from the experiment.

Intracerebral infusions were made bilaterally using a Harvard Apparatus compact infusion pump. Rats were hand-held (except during the 6-OHDA or vehicle infusion) while 30-gauge injection needles were placed into the surgically implanted guide cannulae. The injection needles were attached to syringes (S.G.E. 10 µl) by tubing (PE 10) filled with the drug or the vehicle solution. The infused volume was 2 µl over a 4-min period and an additional 2 min allowed to elapse prior to the removal of the injection needle. Animals were returned to their home cages for 10 min before the test session commenced.

Drug doses of 3, 10 and 30 µg *d*-amphetamine sulphate (Smith Kline and French, Welwyn Garden City, England) were given. All doses were calculated in terms of the salt and were dissolved in 0.9% saline, which was used as a vehicle (control) solution. Apomorphine hydrochloride (MacFarlan Smith Ltd., Edinburgh, Scotland), in a dose of 0.1 mg/kg, dissolved in 0.9% saline containing 0.2% ascorbic acid was administered subcutaneously in the back of the neck.

Total responses on each of the two levers, total number and time spent panel-pushing, and frequency of the stimulus presentations were recorded at 3-min intervals during each test session. Lever responses were analysed by parametric analysis of variance with the data subjected to a square-root transformation to achieve homogeneity of variance, as recommended by Winer (1971). Subsequent comparisons were made following determination of simple interactions and simple main effects (Winer 1971).

At the completion of the experiment all the animals were decapitated and their brains quickly removed. Brains were dissected over ice according to the procedures previously described by Koob et al. (1978). The frontal cortex, nucleus accumbens, and anterior and posterior caudate nucleus (left and right sides separately) were dissected from coronal slices. Tissue was then stored in liquid nitrogen until it could be assayed for the presence of DA (and for NA in the frontal cortex) using high-pressure liquid chromatography with electrochemical detection (modified according to Mefford 1981). During the dissection the nucleus accumbens was examined closely to determine whether there was an area of gliosis or damage indicating the location of the infusion tip, as detailed histological analysis could not be performed in the same brains that were analysed neurochemically. No obvious signs of gliosis or nonspecific damage to the nucleus accumbens by the cannulae implantation were observed. Analyses of the biochemical data were made using two-tailed *t*-tests to compare each of the two lesioned groups with the appropriate sham-lesioned group.

Behavioural procedures. For the experiment there were two phases: training and testing. The training phase consisted of classical conditioning to establish an association between a previously neutral stimulus and water. The test phase examined the efficacy of the stimulus (CR) to act as a reinforcer for the acquisition and maintenance of a new response (lever pressing) in the absence of actual water reward. The test was conducted following the administration of various doses of *d*-amphetamine or control (vehicle) into the nucleus accumbens in nucleus accumbens lesioned, caudate nucleus lesioned and sham-operated controls. At the completion of the experiment, an additional test was given in which apomorphine was injected (0.1 mg/kg) immediately before the session.

Training was identical for all of the 40 rats. After initial dipper training there were two sessions (15 min each) in which the water dipper was elevated every 9 s preceded by the 1-s illumination of the tray-light and house-light offset. When operated, the dipper produced a characteristic sound and it remained in an elevated position for 5 s. During the first of these two preliminary training sessions the hinged panel was permanently taped open. In subsequent sessions subjects were required to make a panel-push response in order to gain access to the water. Two sessions of preliminary training were usually sufficient to establish panel-pushing and drinking from the dipper cup.

During training a reinforcement schedule (random-time 30-s schedule of presentations) was used in which there were 30 presentations of the 1-s tray-light stimulus and house-light offset followed by elevation of the water dipper for 5 s. The subjects were trained not to panel-push when the dipper was not elevated by delaying for 3 s the next possible random presentation of water reward if they responded during this time. The training sessions were variable, lasting usually between 30 and 40 min and becoming shorter as training progressed. After being trained for 14 days, the subjects were divided into four groups (designated to be, nucleus-accumbens lesioned, nucleus-accumbens sham lesioned, caudate nucleus lesioned and caudate-nucleus sham lesioned) and surgery was performed, as described. During the recovery period (1 week) the animals were allowed free access to food and water. Two additional training sessions were then given to confirm that the number of panel-pushes and the duration of the session were comparable to pre-surgical levels. Each rat was then assigned to receive a series of counterbalanced single *d*-amphetamine infusions into the nucleus accumbens (3, 10, 20 μg) and a control (vehicle) infusion determined by a 4×4 Latin Square. This design enabled each rat to serve as its own control during the test. The four test sessions for each subject were separated by 60 h to ensure recovery from the effects of the drug. Seventy-two hours after the final test session an additional session was conducted in which no *d*-amphetamine was infused; instead, a single subcutaneous injection of apomorphine (0.1 mg/kg) was given before the test in the neck region.

During the test sessions, water was never presented and panel-pushing had no programmed consequences apart from being recorded. The two levers were present in the box for the first time. Responding on the CR lever, one of the two novel levers, resulted in the presentation of the compound stimulus that served as the CR (tray-light on, house-light off, and characteristic sound of the dipper elevating), while responding on the other lever (NCR lever) did not result in any stimulus presentation. The probability of the presentation of the CR contingent on a CR lever response was 0.5 (RR-2) for the duration of the test sessions. The lever designated to produce CR (left or right) was counterbalanced over subjects in each of the lesion or sham-lesioned groups; however, this assignment remained the same for each rat during the test sessions. Sessions began with the illumination of the house light. Subjects were allowed 30 min to make a response on the CR lever. If no CR response was made the session was terminated; if a CR response was emitted the initial response automatically produced the CR stimulus and began the 30-min test session. There were 32 subjects tested in the four groups: nucleus-accumbens sham lesion ($N=7$), caudate-nucleus sham lesion ($N=8$), nucleus accumbens lesion ($N=8$), and caudate nucleus lesion ($N=9$).

Results

Biochemical analysis. Assay of DA and NA concentrations in the nucleus accumbens, caudate nucleus, and frontal cortex revealed the pattern of catecholamine (CA) depletion by 6-OHDA shown in Table 1. In general, contrasting patterns of depletion were observed in the nucleus accumbens and caudate nucleus in the two groups.

Infusions of 6-OHDA into the nucleus accumbens resulted in a statistically significant reduction in DA levels to 17.35% of control (nucleus accumbens sham lesions) values. Small non-significant depletions of DA were found in both the left and right anterior caudate nucleus (this area of caudate nucleus was dissected from the same coronal slice that contained the nucleus accumbens), as well as even smaller depletions in the posterior caudate. There

Table 1. Mean regional DA levels (μg/g tissue, wet weight) for the four groups: nucleus-accumbens lesioned ($N=7$), nucleus-accumbens sham lesion ($N=8$), caudate-nucleus lesioned ($N=9$), caudate-nucleus sham lesioned ($N=8$). The mean and standard errors (SEM) are shown for the five sampled areas; nucleus accumbens (N.Acc.), anterior caudate nucleus (ACN, left and right), posterior caudate nucleus (PCN, left and right). NA levels in the frontal cortex (FCx) are also depicted. CA levels for each region of the 6-OHDA-lesioned groups were compared with the corresponding sham lesioned group using two-tailed *t*-tests

Surgery			Lesioned	Sham	Depletion	*t* value
	DA Assayed					(*df* 1,13)
N.Acc.	N.Acc.	Mean	0.98	5.97	84%	6.34***
		SEM	1.02	1.55		
	ACN left	Mean	9.81	10.90	10%	0.69
		SEM	1.90	3.46		
	right	Mean	10.03	11.31	11%	0.76
		SEM	3.44	2.61		
	PCN left	Mean	9.99	10.59	6%	0.69
		SEM	1.79	1.40		
	right	Mean	8.85	9.26	4%	0.32
		SEM	2.69	1.90		
	NA Assayed					
	FCx	Mean	0.045	0.199	77%	3.64**
		SEM	0.021	0.102		
	DA Assayed					(*df* 1,15)
CN	N.Acc.	Mean	5.29	6.69	20%	2.46*
		SEM	1.61	1.49		
	ACN left	Mean	3.45	9.48	66%	3.46**
		SEM	2.82	3.76		
	right	Mean	3.07	11.49	65%	6.28***
		SEM	3.28	1.78		
	PCN left	Mean	1.97	10.12	84%	10.16***
		SEM	1.82	1.29		
	right	Mean	1.39	9.25	85%	14.55***
		SEM	0.96	1.74		
	NA Assayed					
	FCx	Mean	0.151	0.214	30%	2.27**
		SEM	0.063	0.044		

*$P<0.05$, **$P<0.01$, ***$P<0.001$

were depletions of NA in the frontal cortex, although there were also significant depletions of NA in the frontal cortex of caudate-lesioned subjects.

6-OHDA infusions into the caudate nucleus resulted in reductions of more than 80% of DA in the posterior caudate nucleus (the area of the intended depletion). There was also a significant depletion of DA (>60%) in the anterior caudate nucleus and a small, but significant, depletion of DA (20.97%) in the nucleus accumbens. The data for the left and right sides of the caudate nucleus revealed no substantial asymmetries in the amount of DA depletion that might have influenced the behavioural results.

Behavioural analysis. Figure 1 shows the effects of sham or 6-OHDA lesions of the nucleus accumbens or caudate nucleus on responding for stimuli formerly correlated with water reward (CR) following a range of doses of intra-accumbens *d*-amphetamine. In three of the groups the drug produced a selective increase in responding on the CR lever as opposed to on an inactive lever (NCR lever), which produced no stimuli. However, this selective increase in responding was blocked by 6-OHDA lesions of the nucleus accumbens. This difference in responding on the CR and NCR lever following intra-accumbens *d*-amphetamine in the four groups was substantiated by a statistically significant interaction among Group, Dose, and Lever factors ($F=2.04$, *df* 9,84, $P<0.05$), which is further analysed as follows.

In both of the sham-lesioned groups (Fig. 1, panels 1 and 2) infusions of *d*-amphetamine into the nucleus accumbens produced a dose-dependent increase in responding on the CR lever, with a mean of 63.0 and 45.0 responses (for the nucleus accumbens and caudate nucleus sham-lesioned groups, respectively) during the session under control conditions and a maximum of 301.0 and 246.0 responses (respectively) in 30 min under the highest dose of the drug administered (20 μg). No increases were observed on the NCR lever; there were means of 14.0 and 12.0 responses from the two groups under control conditions and 22.0 and 26.0 following the administration of 20 μg *d*-amphetamine. These dose-dependent, selective increases in responding following intra-accumbens infusions of *d*-amphetamine in the nucleus accumbens and caudate nucleus sham-lesioned groups were confirmed by significant interactions between dose and lever in both groups ($F=6.44$ and $F=3.64$, *df* 3,84, $P<0.05$). The absence of increases in responding on the NCR lever compared with those on the CR lever following the administration of the drug was indicated by a non-significant effect of dose on the NCR lever ($F=0.75$ and $F=0.54$, *df* 3,84) and a significant effect of dose on the CR lever ($F=9.66$ and $F=8.22$, *df* 3,84, $P<0.001$) for the nucleus accumbens and caudate-nucleus sham-lesioned groups, respectively.

No selective dose-dependent increase in responding on either the CR or NCR lever was found following intra-accumbens infusions in animals in the group with 6-OHDA-induced DA depletion of the nucleus accumbens (Fig. 1, panel 3). This was confirmed by a non-significant interaction between dose and lever ($F=0.53$, *df* 3,84) and no main effect of dose ($F=0.91$, *df* 3,84) in the nucleus accumbens lesioned group. However, irrespective of the dose (including, vehicle control) of *d*-amphetamine, there were significantly more CR than NCR responses ($F=14.92$, *df* 1,28, $P<0.01$), confirming that, as in the other groups, there was still a significant effect of CR in establishing a preference for lever responding, in the nucleus accumbens 6-OHDA-lesioned group.

In the caudate-nucleus 6-OHDA-lesioned group there was a dose-related increase in responding on the CR lever that was similar to that of both the sham-lesioned groups, except at the highest dose of the drug (Fig. 1, panel 4). Detailed analysis, however, revealed that the interaction between dose and lever just failed to reach statistical significance at the $P<0.05$ level ($F=2.50$, *df* 3,84) using the conservative pooled error term employed in all the analyses. Nevertheless, a separate analysis of responding on CR and NCR levers in the caudate nucleus 6-OHDA-lesioned group confirmed that responding was equivalent to the sham-lesioned groups: there was a significant increase in responding on the CR lever ($F=8.58$, *df* 3,84, $P<0.001$) and a non-significant effect of dose on the NCR lever ($F=1.28$, *df* 3,84). Although responding on the NCR lever increased

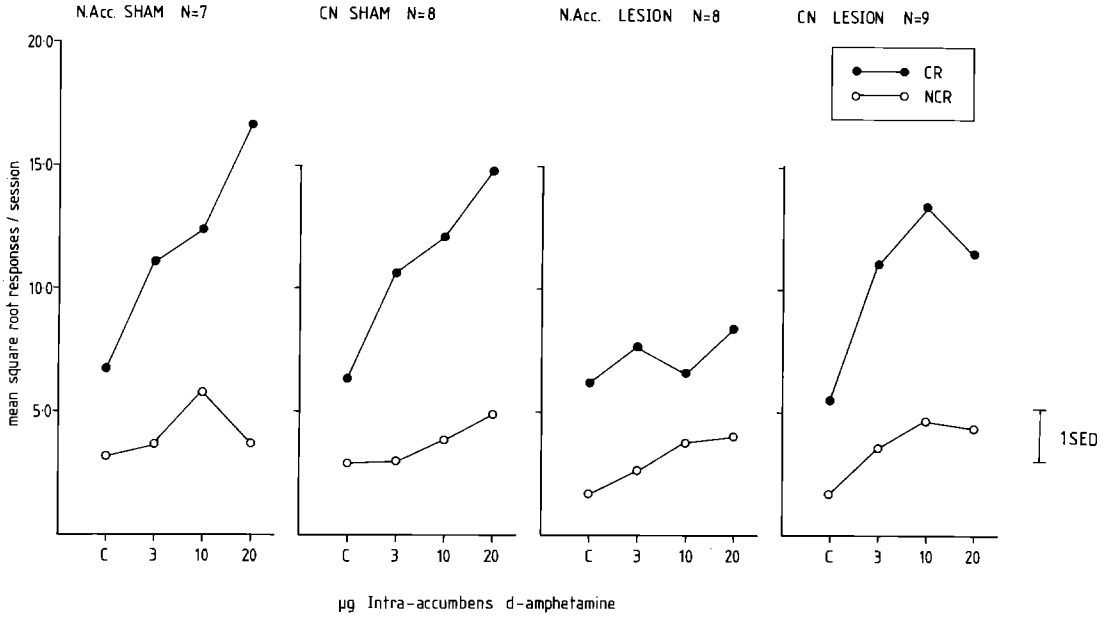

Fig. 1. Effects of bilateral microinfusions of *d*-amphetamine and control (vehicle) into the nucleus accumbens on responding on a lever providing conditioned reinforcement (*CR*) and a lever providing no conditioned reinforcement (*NCR*) in four groups of subjects: nucleus-accumbens sham lesioned (*panel 1*), caudate-nucleus sham lesioned (*panel 2*), nucleus-accumbens lesioned (*panel 3*), caudate-nucleus lesioned (*panel 4*). *Ordinate:* mean number of responses on the CR and NCR lever, subjected to a square-root transformation, during the session. The *bar* represents 1 standard error of the difference between the means (SED) for the 3-way interaction between Group × Dose × Lever (i.e., computed by the square-root of the mean square of the residual error term). *Abscissa:* infusion of control (saline) and three doses of *d*-amphetamine (3, 10, 20 µg)

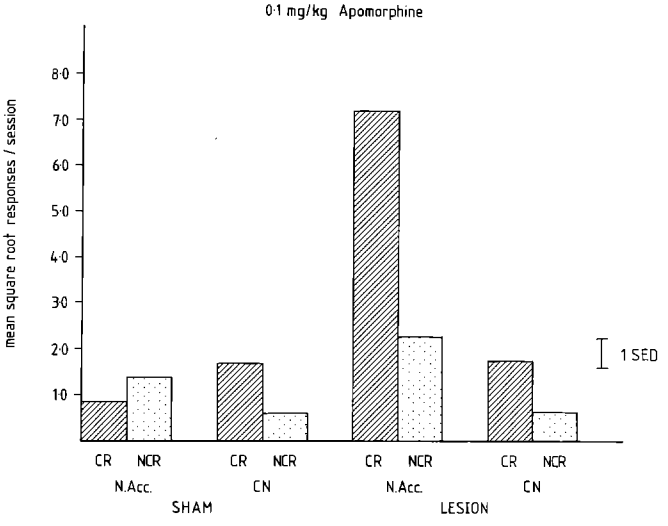

Fig. 2. Pharmacological assessment of the 6-OHDA-induced lesion using 0.01 mg/kg apomorphine administered subcutaneously prior to the test. *Ordinate:* Mean square-root number of responses on the CR (diagonal-striped histograms) and NCR (dotted histograms). The *bar* represents 1 standard error of the difference between the means (SED) for the 3-way interaction between Group × Dose × Lever. *Abscissa:* nucleus-accumbens (*N. Acc.*) sham lesion, $N=8$; caudate nucleus (*CN*) sham lesion, $N=8$; nucleus-accumbens lesion, $N=7$; caudate-nucleus lesion, $N=9$

at the highest dose of *d*-amphetamine, this result was not statistically significant.

All subjects, except for those in the nucleus accumbens lesioned group, were observed to be hypoactive in response to systemic apomorphine and hardly responded on either lever during the test, as shown in Fig. 2. For the nucleus accumbens and caudate nucleus sham-lesioned groups and the caudate-lesioned group the mean number of responses on the CR lever was 1.71, 5.25 and 6.33, respectively. The mean number of responses on the NCR lever was 4.43, 0.74 and 1.0 for these three groups, respectively. However, subjects that had received injections of 6-OHDA into the nucleus accumbens continued to respond selectively on the CR lever following administration of apomorphine, responding with a mean of 58.63 on the CR lever and 12.37 on the NCR lever during the test session. This difference between the four groups was confirmed by a significant interaction between group and lever ($F=10.04$, *df* 3,28, $P<0.001$).

Discussion

Use of combined central microinfusion and selective neurotoxic lesion techniques led to the finding that dopaminergic activation of the nucleus accumbens with *d*-amphetamine produces enhanced responding for CR. The dose-dependent increases in responding for stimuli associated with reward following intra-accumbens infusions of *d*-amphetamine in sham-operated controls confirms and extends previous results to include lower (3 µg) doses (Taylor and Robbins 1984). The main new finding in this study is that the enhanced responding with CR could be blocked selectively by 6-OHDA-induced catecholamine depletion of the nucleus accumbens, but not of the caudate nucleus. This result will be now discussed in terms of its behavioural, neuroanatomical and neurochemical specificity.

Behavioural specificity. Intra-accumbens saline (control) response rates on the CR lever in subjects with DA-depletion

of the nucleus accumbens were similar to both the sham lesioned and the caudate nucleus lesioned groups, showing that the nucleus accumbens lesioned rats had no impairment in learning the new contingency with conditioned reinforcement under saline. The nucleus accumbens lesion therefore specifically blocked the selective increases in responding produced by *d*-amphetamine. It probably cannot be argued that the lesion blocked *d*-amphetamine's effect with CR either by antagonizing its locomotor action (Kelly et al. 1975) or by impairing the capacity of the rats to press levers at high rates. First, the use of a choice procedure makes it difficult to argue that locomotor hyperactivity produced by *d*-amphetamine contributes to the results, unless it is allowed that this hyperactivity is actually an expression of a more specific behavioural process (c.f. Taylor and Robbins 1984). Second, rats with 6-OHDA lesions of the nucleus accumbens have been shown not to be deficient in responding under schedules engendering low or intermediate rates of responding (Roberts et al. 1977; Robbins et al. 1983b) comparable to those produced here by *d*-amphetamine; although high rates may be transiently reduced (Robbins et al. 1983b).

Neuroanatomical specificity. In contrast to the attenuation of *d*-amphetamine-induced increases in CR responding resulting from lesions of the nucleus accumbens, there was little effect of 6-OHDA-induced reductions of DA levels in the anterior (65%) and posterior (>80%) caudate. Intra-accumbens *d*-amphetamine administered to caudate-nucleus lesioned subjects produced dose-dependent selective increases in responding for the presentation of stimuli associated with reward which were similar to both groups of sham-lesioned controls, though not as robust at the highest dose. This (not significant) difference between the sham-lesioned groups and the caudate-nucleus lesioned group after the highest dose of *d*-amphetamine (20 µg) might have resulted from the small (20%), but significant, depletion of DA in the nucleus accumbens of caudate-nucleus lesioned subjects – given that 80% DA depletion almost blocks the *d*-amphetamine-induced potentiation of responding for CR. The complementary effect of DA depletion in the caudate nucleus of the nucleus accumbens lesioned group was probably not a relevant factor, as there was no significant (approximately only 5% in posterior caudate) depletion of DA in the caudate nucleus for this group. Intra-caudate *d*-amphetamine was previously reported to produce variable and weak increases in responding for CR (Taylor and Robbins 1984), which was suggested to result from either spread of the drug from the caudate nucleus to the nucleus accumbens or from the caudate being partly involved in enhanced responding for CR. The demonstration here that 6-OHDA lesions of the caudate nucleus do not block enhanced responding for CR following intra-accumbens *d*-amphetamine suggests that this spread of the drug may indeed have occurred. Therefore, DA activation of the nucleus accumbens, but not in the caudate nucleus, is suggested to be critical for enhanced responding for CR.

This conclusion has to be reconciled with the findings reported by Robbins and Everitt (1982) – that 6-OHDA-induced depletion of DA from the caudate nucleus led to a more effective blockade of enhanced responding produced by the systemic administration of pipradrol than did DA depletion from the nucleus accumbens. This apparent discrepancy is clearly important and cannot easily be resolved at present. However, it must be pointed out that although the degree of caudate nucleus and nucleus accumbens DA depletion was similar in the two experiments, the actual sites of 6-OHDA administration within the caudate varied slightly, with the present placement being made more lateral. As Sabol et al. (1985) have recently shown, the effects of DA depletion from the caudate nucleus depend critically upon its precise pattern. Thus, DA depletion from ventrolateral caudate nucleus impaired paw-use in a skilled motor task, whereas depletion from more anteromedial regions among other effects also apparently influenced motivational variables. It is then possible that in the experiment reported here the pattern of DA depletion within the caudate nucleus differed to that of Robbins and Everitt (1982) – their results depending upon more medial DA depletion, including of course the nucleus accumbens itself (which was approximately 30% depleted in that experiment compared with 20% in the present one). Since profound DA depletion specifically from the nucleus accumbens did not produce a larger effect than the caudate nucleus depletion in Robbins and Everitt (1982), this would imply a possible contribution of caudate nucleus DA also for their effect. There is also a precedent for such a contribution – Fink and Smith (1980) and Winn and Robbins (1985) reported that the locomotor hyperactivity produced by *d*-amphetamine was more effectively blocked by DA depletion from the anteromedial caudate in addition to the nucleus accumbens itself. Another, possibly related, reason for the discrepancy is that in the Robbins and Everitt (1982) study the rats treated with repeated systemic pipradrol were exhibiting more perseverative (stereotyped) forms of lever pressing, which were more susceptible to DA depletion than in the present study. In this sense, nucleus accumbens and caudate nucleus DA would be contributing to somewhat different facets of the potentiation of responding by stimulants. Some support for this view is gained from the fact that the caudate-nucleus 6-OHDA group exhibited a strong, though not significant, trend towards an attenuation of responding at the highest dose of *d*-amphetamine (20 µg). Finally, it should be mentioned that whereas *d*-amphetamine was administered directly into the nucleus accumbens, systemic pipradrol would be expected to affect catecholamines simultaneously in both the nucleus accumbens and caudate nucleus. This fact may explain why the rats treated with pipradrol in the nucleus accumbens group of the Robbins and Everitt (1982) study perseverated in responding in the vicinity of the water dipper, whereas no such effect was observed in this study.

Whatever the explanation of the differences in results of this study and that described by Robbins and Everitt (1982), it is evident that profound DA depletion in the caudate nucleus need not attenuate the enhanced responding for CR seen with *d*-amphetamine – even though such depletion can produce significant behavioural impairments (Sabol et al. 1985). Our emphasis solely on the nucleus accumbens should, however, be tempered with the possibility that other structures in the vicinity of the nucleus accumbens might be responsible for some of the effects, such as parts of the medial caudate-nucleus and perhaps olfactory tubercle, which is also depleted by 6-OHDA lesions of the nucleus accumbens (e.g. Robbins et al. 1983b) and has recently been found to be more sensitive than the nucleus accumbens to the locomotor effects of intracerebral DA (Cools 1985). Notably, there is neuroanatomical evidence

suggesting that the medial region of the ventral caudate nucleus should be considered part of the mesolimbic system since this area, like the nucleus accumbens, receives projections from the ventral tegmental area (Nauta and Domesick 1984).

Neurochemical specificity. Although we should like to argue that DA in the nucleus accumbens is mainly responsible for the mediation of these effects, we have to consider the obvious possibility that incidental damage to NA neurons is a contributing factor; especially in view of the large (~77%) loss of cortical NA in the nucleus-accumbens 6-OHDA group, by comparison with the much smaller (~30%), but significant, depletion of cortical NA in the caudate-nucleus 6-OHDA group. There is evidence that the nucleus accumbens contains a quantity of NA (Fonnum and Waalas 1979, 1981) and it is therefore possible that some of the effects of *d*-amphetamine in the nucleus accumbens are mediated by NA release, although there is also NA in the caudate nucleus (Hörtnagel et al. 1983). However, the concentration of NA in the nucleus accumbens is small compared with DA (Brownstein et al. 1974) and *d*-amphetamine is thought to be a poor releaser of NA compared with DA (Kuczenski 1983) which makes this possibility remote. The results of pharmacological challenge with apomorphine, which is known to selectively stimulate DA receptors (Andén et al. 1967), strongly indicates that the effects of intra-accumbens *d*-amphetamine are dependent specifically upon DA, and not NA, neurotransmission. Intracerebral injection of 6-OHDA into the nucleus accumbens destroys the presynaptic terminals containing DA and leaves DA receptors intact, becoming "supersensitive" to direct DA agonists such as apomorphine (Ungerstedt 1971; Kelly et al. 1975). Low doses of apomorphine result in hypoactivity in normal subjects (Montanaro et al. 1983), whereas they produce hyperactivity in subjects with 6-OHDA-induced depletion of DA in the nucleus accumbens because of the supersensitive DA receptors (Kelly et al. 1975; Kelly and Roberts 1983). Low doses of systemic apomorphine were found only to maintain responding for CR in the nucleus-accumbens lesioned group. Conversely, animals with sham lesions of the nucleus accumbens or caudate nucleus and caudate nucleus-lesioned animals were hypoactive and hardly responded on the levers. Notably, the effect of apomorphine on supersensitive DA receptors in the nucleus accumbens did not result in non-specific increases in lever pressing as often occurs with normal rats (c.f. Robbins et al. 1983a) because the preference for increased responding for the stimulus associated with reward was maintained. Assessment of the role for DA with this pharmacological tool provides some evidence that NA is not crucial in this effect. Therefore, the enhanced responding for CR found after infusions of *d*-amphetamine in the nucleus accumbens and attenuation of responding for CR after 6-OHDA lesions of the nucleus accumbens are probably not due to release of NA by *d*-amphetamine or to depletion of NA in the nucleus accumbens, respectively. However, a possible role for NA in mediating some of these results cannot definitively be excluded at present.

Additional support for a dopaminergic substrate within the nucleus accumbens mediating enhanced responding comes from the finding that infusions of DA itself directly into the nucleus accumbens, but not into the caudate nucleus, also enhances responding for CR (Taylor 1985). However, these effects were weaker and were less selectively influenced by the associative significance of the stimuli compared with intra-accumbens *d*-amphetamine. Enhanced responding for CR following intra-accumbens DA was also found to be blocked by pretreatment with the DA receptor antagonist alpha-flupenthixol (Taylor 1985).

In conclusion, psychomotor stimulant drugs can come to control and exaggerate behaviour determined by salient environmental stimuli or events, such as conditioned reinforcers. The mechanism by which this behavioural effect occurs may underlie the ability of stimulant drugs to be "reinforcing" and thus help to explain the addictive nature of these drugs. The potentiation of conditioned reinforcers resulting from psychomotor stimulant drugs, such as *d*-amphetamine or cocaine, may result in an affective state perceived as "pleasant" or "euphoric" in humans. There is now considerable evidence that the neural mechanism by which *d*-amphetamine evokes enhanced responding for stimuli associated with reward is through increased dopaminergic activation of the nucleus accumbens.

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References

Andén N, Rubenson A, Fuxe K, Hökfelt T (1967) Evidence for dopamine receptor stimulation by apomorphine. J Pharm Pharmacol 19:627–629

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

Brownstein M, Saavedra JM, Palkovitis M (1974) Norepinephrine and dopamine in the limbic system of the rat. Brain Res 79:431–436

Carr G, White N (1983) Conditioned place preference from intra-accumbens but not intra-caudate amphetamine injections. Life Sci 33:2551–2557

Cools AS (1985) Mesolimbic dopamine and its control of locomotor activity in rats: differences in pharmacology and light/dark periodicity between the olfactory tubercle and nucleus accumbens. Psychopharmacology (in press)

Fink JS, Smith GP (1980) Relationships between selective denervation of dopamine terminal fields in the anterior forebrain and behavioural responses to amphetamine and apomorphine. Brain Res 201:107–127

Fonnum F, Waalas I (1979) Localization of neurotransmitters in the neostriatum. In: Divac I, Oberg RGE (eds) The Neostriatum. Pergamon, Oxford

Fonnum F, Waalas I (1981) Localization of neurotransmitters in the nucleus accumbens. In: Chronister RB, De France JF (eds) The neurobiology of the nucleus accumbens. Haer Institute for Electrophysiological Research, pp 259–272

Hill RT (1967) A behavioral analysis of the psychomotor stimulant effects of a drug: The interaction of pipradrol with conditioned reinforcement. Ph.D. dissertation, Columbia University, New York, USA

Hoebel BG, Monaco AP, Hernandez L, Aulisi EF, Stanley BG, Lenard L (1983) Self-injection of amphetamine directly into the brain. Psychopharmacology 81:158–163

Hörtnagel H, Schlögl H, Sperk G, Hornykiewicz O (1983) The topographical distribution of the monoaminergic innervation in the basal ganglia of the human brain. Prog Brain Res 58:269–274

Kelly PH, Roberts DCS (1983) Effects of amphetamine and apomorphine on locomotor activity after 6-OHDA and electrolytic lesions of the nucleus accumbens septi. Pharmacol Biochem Behav 19:137–143

Kelly PH, Seviour PW, Iversen SD (1975) Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Res 94:507–522

Koob GF, Riley SJ, Smith SC, Robbins TW (1978) Effects of 6-hydroxydopamine lesions of the nucleus accumbens septi and olfactory tubercle on feeding, locomotor activity and amphetamine anorexia in the rat. J Comp Physiol Psychol 92:917–927

Kuczenski R (1983) Biochemical actions of amphetamine and other stimulants. In: Creese I (ed) Stimulants: neurochemical, behavioural and clinical perspectives. Raven, New York, pp. 31–61

Lenard L, Hernandez L, Hoebel BG (1980) Self-injection of amphetamine directly into the nucleus accumbens. Proceedings of the International Union of Physiological Science 14:2217

Lyness WH, Friedle NM, Moore KE (1979) Destruction of dopaminergic nerve terminals in nucleus accumbens: effects on *d*-amphetamine self-stimulation. Pharmacol Biochem Behav 11:553–556

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

Mefford IN (1981) Application of high performance liquid chromatography with electrochemical detection to neurochemical analysis: Measurement of catecholamines, serotonin and metabolites in rat brain. J Neurosci Meth 3(3):202–224

Montanaro N, Vaccheri A, Dall'Olio R, Gandolfi O (1983) Time course of rat motility response to apomorphine: A simple model for studying preferential blockade of brain dopamine receptors mediating sedation. Psychopharmacology 81:214–219

Nauta WJH, Domesick VB (1984) Afferent and efferent relationships of the basal ganglia. In: Evered D, O'Connor M (eds) Functions of the basal ganglia. Ciba Foundation Symposium 107. Pitman, London, pp. 3–23

Pettit HO, Ettenberg A, Bloom FE, Koob GF (1984) Destruction of dopamine in the nucleus accumbens attenuates cocaine but not heroin self-administration in rat. Psychopharmacology 84:167–173

Pellegrino LJ, Cushman AJ (1967) A stereotaxic atlas of the rat brain. Century Crofts, New York

Robbins TW (1978) The acquisition of responding with conditioned reinforcement: Effects of pipradrol, methylphenidate, *d*-amphetamine and nomifensine. Psychopharmacology 58:78–87

Robbins TW, Everitt BJ (1982) Functional studies of the central catecholamines. Int Rev Neurobiol 23:303–365

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

Robbins TW, Roberts DCS, Koob GF (1983b) Effects of *d*-amphetamine and apomorphine upon operant behaviour and schedule-induced licking in rats with 6-hydroxydopamine-induced lesions of the nucleus accumbens. J Pharmacol Exp Ther 222:662–673

Roberts DCS, Corcoran ME, Fibiger HC (1977) On the role of the ascending catecholaminergic systems in intravenous self-administration of cocaine. Pharmacol Biochem Behav 6:615–620

Roberts DCS, Koob GF, Klonoff P, Fibiger HC (1980) Extinction and recovery of cocaine self-administration following 6-OHDA lesions of the nucleus accumbens. Physiol Biochem Behav 12:781–787

Sabol KE, Neill DB, Wages SA, Church WH, Justice JB (1985) Dopamine depletion in a striatal subregion disrupts performance of a skilled motor task in the rat. Brain Res 335:33–43

Spyraki C, Fibiger HC, Phillips AG (1982) Dopaminergic substrates of amphetamine-induced place-preference conditioning. Brain Res 253:185–193

Swerdlow NR, Koob GF (1984) Restrained rats learn amphetamine-conditioned locomotion, but not place preference. Psychopharmacology 84:163–166

Taylor JR (1985) Neural mechanisms of the potentiation of conditioned reinforcement by psychomotor stimulant drugs. Unpublished Ph.D. dissertation, University of Cambridge, England

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

Ungerstedt U (1971) Post-synaptic supersensitivity after 6-hydroxydopamine-induced degeneration of the nigrostriatal dopamine system. Acta Physiol Scand Suppl 367:69–93

Winer BJ (1971) Statistical principles in experimental design. Tokyo, McGraw-Hill, Kogakusha

Winn P, Robbins TW (1985) Comparative effects of infusions of 6-hydroxydopamine into nucleus accumbens and anterolateral hypothalamus on the response to dopamine agonists, body weight, locomotor activity and measures of exploration in the rat. Neuropharmacology 24(1):25–31

Wise RA (1981) Brain dopamine and reward. In: Cooper SJ (ed) Theory and psychopharmacology Vol. 1. Academic Press, London pp. 102–122

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