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Anatomical analysis of the involvement of mesolimbocortical dopamine in the locomotor stimulant actions of *d*-amphetamine and apomorphine

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Abstract. Lesion studies employing 6-hydroxydopamine (6-OHDA) suggest that locomotor hyperactivity induced by certain stimulant drugs is dependent on dopaminergic neurotransmission in the nucleus accumbens (NACC). However, studies to date have not adequately controlled for the reported effects of 6-OHDA on baseline (non-drug) activity and on DA levels in other terminal regions. Slow bilateral infusions of 6-OHDA into the NACC, but not into olfactory tubercle (OT) or medial prefrontal cortex (mPFCx), reduced *d*-amphetamine (0.5 mg/kg SC) hyperactivity and resulted in a "supersensitive" (hyperactive) response to a low dose of apomorphine (0.1 mg/kg SC) in photocell cages. Direct observation revealed no behavioral changes in OT lesioned rats challenged with apomorphine which might correspond to a "denervation supersensitivity" syndrome. Assays of DA and 5-hydroxytryptamine (5-HT) in mPFCx, OT, NACC, and caudate-putamen revealed that 6-OHDA infusion into NACC caused substantial DA loss in NACC, OT and mPFCx, whereas infusion at mPFCx or OT sites depleted DA locally (>85% loss) with little or no remote change. Concentrations of 5-HT were little altered by 6-OHDA, except for a local depletion in mPFCx. The present results confirm the importance of nucleus accumbens DA in the expression of locomotor stimulation induced by apomorphine and *d*-amphetamine, and suggest that the mPFCx and OT do not make an important contribution.

Key words: Locomotor activity – Dopamine – Serotonin – 6-Hydroxydopamine – Nucleus accumbens – Olfactory tubercle – Medial prefrontal cortex – Amphetamine – Apomorphine

Several findings suggest that hyperactivity induced by certain psychomotor stimulant drugs (e.g., *d*-amphetamine) is mediated through dopamine (DA) release in the nucleus accumbens (NACC). Thus, bilateral injection of the neurotoxin 6-hydroxydopamine (6-OHDA) into the NACC has been reported to reduce or block *d*-amphetamine-induced locomotor activity, and this occurs even when noradrenergic terminals are pharmacologically protected (Kelly et al. 1975; Kelly and Iversen 1976; Koob et al. 1978; Joyce and Koob 1981; Koob et al. 1981; Makanjuola and Ashcroft 1982; Joyce et al. 1983; Kelly and Roberts 1983). Such lesions also produce a "supersensitive" response to low doses of the DA receptor agonist apomorphine (Kelly et al. 1975; Joyce and Koob 1981; Koob et al. 1981; Joyce et al. 1983). In addition, microinjections of dopaminergic (but not of noradrenergic) agonists into the NACC of unlesioned animals induce locomotor behavior (Pijnenburg et al. 1976; Makanjuola et al. 1980). Finally, intra-accumbens injection of a DA receptor antagonist inhibits *d*-amphetamine-induced locomotion (Pijenburg et al. 1975).

Although attention has focussed on the NACC, the above manipulations may also affect the underlying olfactory tubercle (OT) which is another major terminal area of the mesolimbic DA system. Intra-accumbens administration of 6-OHDA typically produces an extensive depletion of DA in the OT (Kelly et al. 1975; Koob et al. 1978; Joyce and Koob 1981; Makanjuola and Ashcroft 1982; Joyce et al. 1983; Robbins et al. 1983). In addition, locomotor stimulation can be elicited by microinjection of DA receptor agonists into the OT (Pijnenburg et al. 1976). Indeed, recently Cools (1986) compared the effects of microinjection of DA receptor agonists into the OT and NACC, and concluded that the OT and not the NACC mediates the stimulant effects of DA and related compounds. This conclusion is consistent with reports indicating that bilateral electrolytic or excitotoxic lesions of the NACC have little effect on amphetamine-induced hyperactivity (Makanjuola and Ashcroft 1982; Kafetzopoulos 1986).

Intra-accumbens microinfusion of 6-OHDA not only destroys DA terminals in the mesolimbic system but can also deplete DA in the prefrontal cortex (Koob et al. 1978; Taghoutzi et al. 1985). Joyce et al. (1983) injected 6-OHDA directly into the frontal cortex of rats, and despite a local depletion of DA, found no effect on spontaneous or drug-induced locomotion. However, in that study, tests of spontaneous locomotor activity were administered in the 1st week after surgery, whereas drug locomotor tests were not initiated until 12 days later. Other lesion evidence suggests that frontal cortex DA may after all play a role in the expression of locomotor activity (Carter and Pycock 1980; Robinson and Stitt 1981).

All the 6-OHDA studies cited above are somewhat compromised, because the possible effects of 6-OHDA on base-

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line (non-drug) activity were not adequately assessed. In some studies, control (i.e., non-drug) activity was not measured at all. In several others, control activity was assessed at a consistently different time interval after surgery than the drug tests. This is particularly problematic, since infusions of 6-OHDA into either NACC or mPFCx have been reported to alter levels of spontaneous activity, and these effects wane, increase, or even reverse with time after surgery (Koob et al. 1978; Carter and Pycock 1980; Robinson and Stitt 1981; Koob et al. 1981; Joyce and Koob 1981; Makanjuola and Ashcroft 1982; Joyce et al. 1983; Kubos et al. 1987). In yet other studies, "baseline" activity has been assessed only during short habituation sessions given directly before a longer drug session; this kind of experimental design is also undesirable, since intra-accumbens infusions of 6-OHDA are reported to affect exploration in a behaviorally selective way (Taghoutzi et al. 1985). Finally, some investigators have employed long pre-drug habituation sessions so that baseline activity is so low that a depressant effect of 6-OHDA alone may be difficult to detect. As a result of these methodological problems, the magnitude of drug effects is difficult to determine, and unambiguous comparisons between lesioned and control animals are therefore problematic.

The experiments described below addressed three issues:

(1) The first experiment investigated the respective roles of NACC and OT DA in spontaneous and drug-induced locomotion. Three drugs were tested: *d*-amphetamine, apomorphine and scopolamine. Apomorphine was administered in a low dose which reduces locomotion in intact rats but induces hyperactivity in 6-OHDA lesioned animals presumably by acting at "supersensitive" receptors. Scopolamine provided a test of pharmacological selectivity.

(2) The second experiment was similar to the first, but direct behavioral observation replaced automated activity monitoring. The purpose of this experiment was to detect any stereotypy or other behaviors which might have contaminated photocell locomotor scores in rats treated with *d*-amphetamine and apomorphine in experiment 1. In addition, the behavior of OT-lesioned rats treated with apomorphine was examined closely for a possible "DA supersensitivity" syndrome, by analogy with rats given 6-OHDA lesions of the nucleus accumbens or striatum (Kelly et al. 1975).

(3) The third experiment examined the possible effects of destroying DA terminals in the mPFCx on locomotor responses to *d*-amphetamine and apomorphine. In these experiments, care was taken to provide tests of baseline (saline) activity interspersed with drug tests in a counterbalanced manner.

Materials and methods

Subjects. Male Long-Evans hooded rats (Charles River) were housed two or three per cage (depending on the experiment) in a room illuminated from 8:00 A.M. to 8:00 P.M., with free access to food and water. Subjects were adapted to laboratory conditions for at least 2 weeks prior to behavioral testing.

Surgery. Rats were pretreated with pargyline HCl 50 mg/kg IP (experiments 1 and 2) or desmethylimipramine (DMI) 25 mg/2 ml dist H_2O/kg IP (experiment 3) 30 min prior to surgery, and anaesthetized with chloral hydrate 400 mg/kg IP. Subjects were placed in a stereotaxic frame (David Kopf, Tujunga, CA) with the tooth bar positioned 3.9 mm (experiments 1 and 2) or 4.2 mm (experiment 3) below the interaural line. 6-Hydroxydopamine HBr (6-OHDA – 8 µg base/2 µl/site) was made up in vehicle consisting of 0.3 mg/ml ascorbic acid in 0.9% saline solution, and kept on ice in the dark before use. Injection of 6-OHDA solution or vehicle (control subjects) was made bilaterally into the brain via 30 gauge stainless steel cannulae attached by polyethylene tubing to 5 µl Hamilton syringes driven by a syringe pump (Sage Instruments, Cambridge, MA). During injection, the 6-OHDA solution was shielded from light. For greater accuracy at ventral sites (NACC and OT), stereotaxic coordinates were derived from the mean of two systems based respectively on interaural (IA) zero and bregma. NACC coordinates were A_{IA} +10.9/A_{Bregma} +1.7, L ±1.5, V_{IA} +3.0/V_{Bregma} −7.0. OT coordinates were 2.3 mm more ventral but otherwise the same. Infusions into mPFCx were at A_{Bregma} +3.25, L ±0.5, V_{dura} −3.2. Cannulae were lowered to the final injection site through burr holes made in the skull, and after 2 min delay, 2 µl were continuously injected over 36 min (NACC or OT) or 5 min (mPFCx) at each site; after a further 5 min to allow for diffusion, the cannulae were removed and the wound closed with stainless steel clips (Autoclips, Becton, Dickinson and Co., USA).

Locomotor activity. Five circular (61 cm diameter) activity cages (BRS/LVE), each transected by two perpendicular coplanar arrays of three infrared photobeams, were used to measure locomotor activity. Photocell beam interruptions occurring more than 500 ms apart were recorded with a NOVA IV (Data General) minicomputer equipped with MANX (GC Controls) software and interface. During testing, a light-proof lid was placed over each activity cage. Prior to each test session, rats were placed in a holding cage for 50 min, in order to habituate to the test room. Each subject then received an injection of drug or saline, was replaced in the holding cage, and then introduced to the test cage 60 s after injection. The test session was initiated 60 s later and was of 60 min duration. As far as possible, equal numbers of subjects from each experimental condition were tested in each cage. All behavioral tests were carried out between 9:00 A.M. and 5:00 P.M.

Behavioral observation and stereotypy rating (experiment 2). Observation cages comprised a wire grid floor (40 × 45 cm) with plexiglass sides (45 × 45 cm). An oblique mirror permitted viewing from below the cage floor. Subjects were kept in a holding cage for 15 min prior to injection and then placed individually in observation cages immediately after injection. General observations of behavior and stereotypy ratings were made for 2 min, 20 min after injection. The observer was blind with respect to surgical treatment and drug treatment. During this time, a brief description of posture and movements was recorded, as well as the number of rears made (forepaws more than 45° above hindpaws). Stereotypy was continuously rated during the observation period, as follows (modified from Kelly et al. 1975): 0 – asleep or stationary; 1 – active; 2 – predominantly active with bursts of stereotyped sniffing and/or rearing; 3 – stereotyped activity, predominantly sniffing and rearing, over a large area of the cage; 4 – stereotyped behavior maintained in one location; 5 – stereotyped behavior in

one location with bursts of gnawing and/or licking; 6 – continual gnawing or licking of grid floor. One stereotypy score was allocated to each rat, corresponding to the behavioural category occupying the most time during the observation period.

Determination of brain amines. Rats were killed by cervical dislocation and the brain was rapidly removed. The brain was cut at the midthalamic level and coronal sections of forebrain were taken with a freezing microtome. Three tissue sections were taken; the rostral section was 1.5 mm thick, and the other two were 1.0 mm thick. The sections spanned the following anterior coordinates (from the atlas of Paxinos and Watson 1982): for medial prefrontal cortex (mPFCx), 11.2–12.7; for nucleus accumbens (NACC), 9.9–10.9; for caudate-putamen (CP), 8.9–9.9 mm. Samples of olfactory tubercle (OT) were taken from the middle and posterior sections. The brain sections were placed on ice-cold filter paper dampened with saline, and DA terminal regions were hand-dissected with the aid of binocular magnifiers. The boundary between OT and NACC was demarcated by small fiber bundles forming the olfactory radiations. Further anatomical details are given elsewhere Clarke et al. 1988. This procedure yielded mean tissue weights (left and right sides combined) of approximately: 12 mg (mPFCx), 8.5 mg (OT), 11 mg (NACC), and 21 mg (CP).

For the determination of amines and metabolites, tissue samples from left and right sides were combined, weighed, and disrupted by sonication in ice-cold 0.2 N $HClO_4$ with 0.15% $Na_2S_2O_5$ and 0.5% Na_2 EDTA (mPFCx and CT on 250 µl, NACC in 400 µl and CP in 500 µl). After centrifugation and filtration, aliquots (20–40 µl) were injected directly onto a reverse phase analytical column (µ Bondapak C18, 10 µm, 3.9 × 150 mm, Waters) with a mobile phase of 0.1 M KH_2PO_4, 0.2 mM OSA, 0.15 mM Na_2 EDTA and methanol 10.5%, pH 3.6. Quantitation of the solutes was by electrochemical detection using an LC-4B amperometric detector (Bioanalytical Systems) with a glassy carbon TL-5 working electrode. The operating potential was +0.75 V versus the Ag/AgCl reference electrode. The detection limit for all compounds under these conditions was about 0.5 pmol/20 µl tissue sample.

Drugs. Drugs and suppliers were as follows: 6-hydroxydopamine HBr, pargyline HCl, scopolamine HBr, and apomorphine HCl (Sigma Chemical Co., St Louis, MO), chloral hydrate and *d*-amphetamine sulphate (BDH, Toronto, Ont), desmethylimipramine (Merrel Dow Research Institute, Cincinnati, OH). Chloral hydrate, pargyline and desmethylimipramine were given IP; other drugs were administered SC into the flank. Unless otherwise stated, drugs were dissolved in 0.9% saline and given in a volume of 1 ml/kg. Ascorbic acid (0.1 mg/ml) was added to apomorphine to inhibit oxidation. Drug doses refer to the salt, except for apomorphine (expressed as base). The dose of apomorphine was selected so as to depress activity in intact rats but to increase activity in animals which had received intra-accumbens 6-OHDA (Joyce and Koob 1981).

Data analysis. Locomotor activity scores were assessed by univariate and multivariate analysis of variance (SPSSX software, McGraw-Hill, New York), with two possible between-subject factors: LESION (i.e., 6-OHDA versus vehicle infusion) and SITE (NACC versus OT). Each rat served as its own control, where appropriate. As in previous studies (Clarke and Kumar 1983), the drug effect was defined as the difference between drug and saline test scores. Where each subject was tested with several drugs (*d*-amphetamine, apomorphine, scopolamine), the within-subject factor DRUG refers to differences between the drugs, either with respect to the drug effects per se or with respect to the saline scores associated with each drug test. Other comparisons were made by Student's *t*-test. Probability values are 2-tailed.

Experiment 1: Effects of prior 6-OHDA infusion into NACC or OT on photocell activity changes induced by d-amphetamine, apomorphine and scopolamine. Forty rats were used, weighing 335–410 g at surgery. Subjects were randomly allocated to four surgical groups after pretreatment with pargyline (see *Surgery*) and received bilateral microinfusions of 6-OHDA or vehicle into the NACC or OT ($n=10$ per group). One rat died after surgery. Between 2 and 4 weeks after surgery, each rat received six locomotor tests spaced 2 or 3 days apart, consisting of three tests with saline and one test each with *d*-amphetamine 0.5 mg/kg, scopolamine 0.5 mg/kg, and apomorphine 0.1 mg/kg. Saline tests alternated with drug tests; half the rats in each surgery group received saline first, whilst the remainder received drug first. The order in which the three drugs were tested was counterbalanced as far as possible within each group. Subjects were sacrificed 49 days after surgery for biochemical analysis.

Experiment 2: Effects of prior 6-OHDA infusion into NACC or OT on observed behavioral changes induced by d-amphetamine and apomorphine. Forty rats (332–412 g at surgery) were given 6-OHDA or vehicle into NACC or OT, as in experiment 1. On days 12, 14 and 16 after surgery, rats were observed and rated for stereotypy (see above) following the administration of saline, *d*-amphetamine 0.5 mg/kg, or apomorphine 0.1 mg/kg, in a counterbalanced order. Subjects were sacrificed 38 days after surgery for biochemical analysis.

Experiment 3: Effects of prior infusion of 6-OHDA into medial prefrontal cortex on photocell activity changes induced by d-amphetamine and apomorphine. Twenty rats, weighing 320–360 g, were randomly allocated to two equal groups for surgery. Ten rats received bilateral infusions of 6-OHDA into the mPFCx after pretreatment with DMI (see *Surgery*). The remainder received vehicle after DMI. Twelve days after surgery, each subject received four locomotor tests spaced 2 days apart with saline (twice), *d*-amphetamine 0.5 mg/kg, and apomorphine 0.1 mg/kg. Tests were arranged in pairs and counterbalanced as in experiment 1. Subjects were sacrificed 23 days after surgery for biochemical analysis.

Results

Experiment 1: Effects of prior 6-OHDA infusion into NACC or OT on photocell activity changes induced by d-amphetamine, apomorphine and scopolamine

Preliminary analysis of saline (60 min) scores revealed an increase in baseline activity due to the administration of 6-OHDA (main effect of LESION: $F=9.23$, df 1,35, $P<$

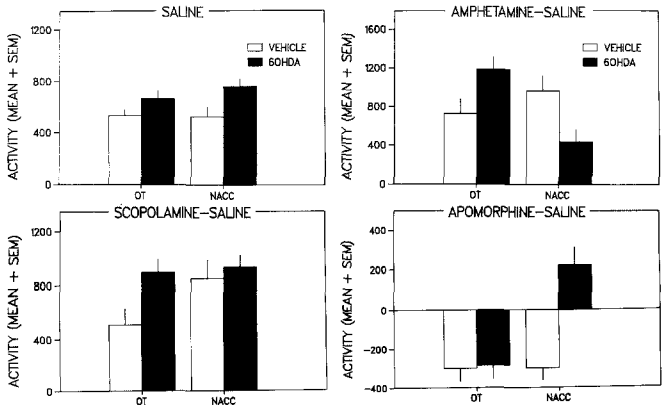

Fig. 1. Effect of 6-OHDA infusion into nucleus accumbens (NACC) or olfactory tubercle (OT) on spontaneous and drug-induced locomotor activity. Data are from experiment 1. Rats received bilateral infusions of 6-OHDA or vehicle into either structure ($n=9$ or 10 per group) and were subsequently given three locomotor tests with saline, and one each with *d*-amphetamine (0.5 mg/kg SC), apomorphine (0.1 mg/kg SC), and scopolamine (0.5 mg/kg SC). The order of testing was counterbalanced between groups. Total (0–60 min) session scores did not differ between the three saline tests and a mean saline score was taken for each rat. The panels show the group mean ± SEM total session score for saline tests (*top left*), and drug-induced changes from saline baseline (i.e., drug minus saline scores)

0.005; Fig. 1), regardless of the site of infusion (main effect of SITE: $F=0.57$, *df* 1,35; SITE × LESION: $F=0.73$, *df* 1,35). Saline activity scores, as expected, did not differ according to the drug paired with each saline test (main effect of DRUG: $F=0.05$, *df* 2,70), and interactions between DRUG, LESION and SITE were all non-significant. Therefore, the effect of a given drug was calculated for each rat by subtracting the mean score of the three saline tests from the drug test score.

The locomotor stimulant effect of *d*-amphetamine was not directly dependent upon prior treatment with 6-OHDA (main effect of LESION: $F=0.02$, *df* 1,35) or on the infusion site (main effect of SITE: $F=3.69$; *df* 1,35), but there was a significant interaction between these two factors ($F=12.6$, *df* 1,35; $P<0.001$). Comparisons between the two NACC surgical groups showed that 6-OHDA infusion reduced the stimulant effect of *d*-amphetamine (6-OHDA versus vehicle group: $t=2.63$, *df* 17, $P<0.02$); however, *d*-amphetamine continued to exert a significant stimulant action in the NACC 6-OHDA group ($t=2.95$, *df* 9, $P<0.02$). In contrast, 6-OHDA infusion into the OT increased the stimulant effect of *d*-amphetamine (6-OHDA versus vehicle group: $t=2.38$, *df* 18, $P<0.05$; Fig. 1). These effects were apparent throughout the 60-min session (Fig. 2).

Apomorphine significantly increased locomotor activity in subjects which had previously received 6-OHDA into the NACC, but significantly reduced activity in those groups which had received 6-OHDA into the OT or had received infusions of vehicle into either structure. These results are shown in Fig. 1. Analysis of variance revealed highly significant effects (main effects of LESION and SITE, respectively: $F=13.2$, *df* 1,35, $P<0.001$; $F=12.3$, *df* 1,35, $P<0.001$; LESION × SITE: $F=12.3$, *df* 1,35, $P<0.001$).

Scopolamine increased locomotor activity in all groups

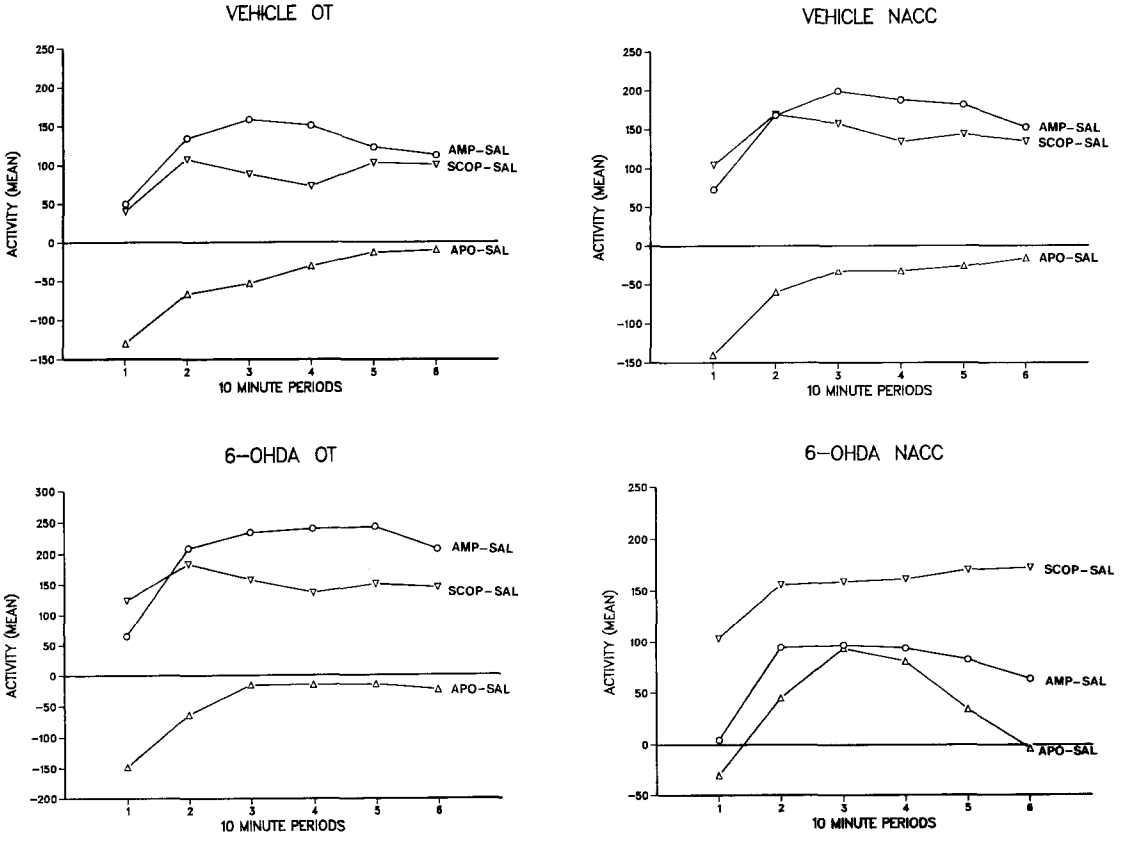

Fig. 2. Effect of 6-OHDA infusion into nucleus accumbens (*NACC*) or olfactory tubercle (*OT*) on drug-induced locomotor activity (experiment 1). Procedural details are given in the legend to Fig. 1. The panels show the group mean ± SEM drug-induced changes from saline baseline (i.e., drug minus saline scores) in successive 10-min periods of the 60-min session

Table 1. Regional concentrations[a] of DA, 5-HT and metabolite/transmiter ratios following 6-OHDA infusion into olfactory tubercle (OT) or nucleus accumbens (NACC) (experiment 1)

Group	DA	HVA/DA	DOPAC/DA	5HT	5HIAA/5HT
Medial prefrontal cortex					
OT Vehicle	0.59±0.07	0.68 ±0.14	0.41±0.03	1.95±0.14	0.76±0.06
OT 6-OHDA	0.33±0.05	0.52 ±0.20	0.38±0.09	1.94±0.11	0.76±0.04
NACC Vehicle	0.50±0.04	0.81 ±0.07	0.46±0.04	2.02±0.13	0.81±0.06
NACC 6-OHDA	ND[c]	ND[c]	ND[c]	1.70±0.11	0.95±0.08
OT % control[b]	57	77	94	99	99
NACC % control[b]	–[c]	–[c]	–[c]	84	117
Olfactory tubercle					
OT Vehicle	37.1 ±1.0	0.053±0.005	0.20±0.01	4.48±0.33	0.38±0.02
OT 6-OHDA	4.3 ±0.8	0.104±0.026	0.32±0.04	4.75±0.55	0.48±0.05
NACC Vehicle	39.5 ±1.6	0.052±0.002	0.22±0.01	4.82±0.22	0.39±0.01
NACC 6-OHDA	9.9 ±1.5	0.078±0.011	0.25±0.01	4.33±0.41	0.52±0.03
OT % control[b]	12	196	159	106	126
NACC % control[b]	25	150	114	90	133
Nucleus accumbens					
OT Vehicle	67.8 ±2.4	0.065±0.003	0.21±0.01	3.00±0.27	0.71±0.07
OT 6-OHDA	53.4 ±2.6	0.075±0.006	0.22±0.01	4.01±0.26	0.77±0.06
NACC Vehicle	69.0 ±2.4	0.074±0.004	0.22±0.01	3.01±0.17	0.68±0.02
NACC 6-OHDA	10.6 ±1.8	0.173±0.002	0.33±0.01	2.23±0.26	0.98±0.06
OT % control[b]	79	115	103	134	108
NACC % control[b]	15	233	155	74	140
Caudate-putamen					
OT Vehicle	98.1 ±2.4	0.056±0.003	0.14±0.00	1.91±0.14	0.95±0.05
OT 6-OHDA	96.9 ±2.5	0.058±0.003	0.15±0.01	2.07±0.08	0.90±0.03
NACC Vehicle	96.1 ±1.4	0.056±0.002	0.15±0.00	2.01±0.05	0.91±0.04
NACC 6-OHDA	73.5 ±3.1	0.063±0.005	0.15±0.01	1.73±0.06	0.99±0.03
OT % control[b]	99	104	107	108	95
NACC % control[b]	76	113	105	86	109

[a] Absolute concentrations are given as mean±SEM (pmol/mg wet tissue, $n=9$–10)
[b] OT and NACC % control refer to the concentration remaining in 6-OHDA lesioned subjects as a percentage of that found in rats receiving vehicle at the same site
[c] Values too low for valid determination

(Fig. 1). This effect was marginally greater in subjects lesioned with 6-OHDA (main effect of LESION: $F=4.68$, *df* 1,35, $P<0.05$), irrespective of the lesion site (main effect of SITE: $F=2.90$, *df* 1,35; LESION×SITE: $F=1.94$, *df* 1,35, $P>0.1$).

The site of 6-OHDA (but not of vehicle) microinfusion was visible as a small (approx. 0.3 mm diameter) circular area of gray discoloration. This was located with its center just medial to the anterior commissure (NACC placements) or approximately 0.3 mm above the ventral surface of the brain (OT placements), at approximately A 10.5 according to the atlas of Paxinos and Watson (1982). The location of infusion sites was highly consistent between animals.

Regional concentrations of DA, 5-HT and metabolite/transmitter ratios are shown in Table 1. Microinfusion of 6-OHDA into the NACC led to substantial depletions of DA in NACC (85%), mPFCx (>90%), and OT (75%), with a slight depletion in CP (24%). An equivalent infusion into the OT markedly depleted DA in OT (88%) but with less change in mPFCx (43%) and NACC (21%) and no detectable effect in CP. Major reductions of DA content were accompanied by local increases in metabolite/DA ratios, except in the mPFCx. Administration of 6-OHDA had little if any effect on regional concentrations of 5-HT, except for a small (26%) local loss which occurred following intra-accumbens infusion.

Experiment 2: Effects of prior 6-OHDA infusion into NACC or OT on observed behavioral changes induced by d-amphetamine and apomorphine

Stereotyped behavior was rarely observed after administration of saline, apomorphine, or amphetamine, as indicated by the virtual absence of rating scores above 1 (Table 2). These scores suggest that in saline tests, rats lesioned with 6-OHDA were generally more active than sham-lesioned subjects; this is also seen by increased rearing behavior in lesioned rats (main effect of LESION: $F=4.59$, *df* 1,35, $P<0.05$), independent of lesion site (main effect of SITE: $F=1.30$, *df* 1,35; LESION×SITE: $F=0.19$, *df* 1,35).

Drug effects on rearing were assessed as the difference scores between drug and saline tests. Amphetamine-induced rearing appeared to be reduced in NACC 6-OHDA rats (Table 3); however, all treatment effects were non-significant, possibly reflecting the fact that *d*-amphetamine only weakly increased rearing behavior in control subjects. Conversely, apomorphine tended to increase rearing in NACC

Table 2. Stereotypy scores[a] (experiment 2)

Rating	*n*	Saline				Apomorphine				*d*-Amphetamine			
		0	1	2	3	0	1	2	3	0	1	2	3
OT 6-OHDA	10	0	10	0	0	2	6	1	1	0	10	0	0
OT Vehicle	10	3	7	0	0	4	6	0	0	0	10	0	0
NACC 6-OHDA	9	0	9	0	0	0	9	0	0	1	7	0	1
NACC Vehicle	10	2	8	0	0	3	7	0	0	0	10	0	0

[a] Each rat was allocated a stereotypy score corresponding to the predominant category of behavior displayed within the observation period. The number of subjects exhibiting a given stereotypy score is shown for each group. No rat showed more than a mild degree of stereotypy, corresponding to a score of 3. The full rating scale is given in the text

Table 3. Rearing scores[a] (experiment 2)

Group	*n*	Saline	Apo–Sal[b]	Amp–Sal[b]
OT 6-OHDA	10	6.2±0.8	−0.8±1.8	3.2±1.9
OT Vehicle	10	4.5±1.3	−0.8±0.9	3.4±1.1
NACC 6-OHDA	9	7.8±0.9	4.3±1.7	1.2±1.7
NACC Vehicle	10	5.2±0.9	−0.7±1.5	3.8±1.6

[a] Mean±SEM number of rears in 2-min observation period
[b] Each subject was tested once with saline, apomorphine and *d*-amphetamine. Difference scores (drug minus saline) are shown

6-OHDA rats, whilst having no effect in any other group, but no significant lesion effects were obtained (Table 3).

Biochemical changes produced by 6-OHDA infusion were very similar to those produced by the same surgical procedure in experiment 1. Thus, 6-OHDA infusion into NACC reduced the DA concentration of OT, NACC, mPFCx, and to a lesser extent of CP, whereas infusion into OT produced a marked (83%) depletion locally with little change outside this structure (Table 4). Again, metabolite/DA ratios were increased where DA was substantially depleted. Regional concentrations of 5-HT were unaffected by 6-OHDA infusion, except for a 30% loss in mPFCx after NACC infusion, confirming a trend seen in experiment 1. In contrast to experiment 1, 6-OHDA infusions into NACC did not alter the concentration of 5-HT in this structure.

Table 4. Regional concentrations[a] of DA, 5-HT and metabolite/transmitter ratios following 6-OHDA infusion into olfactory tubercle (OT) or nucleus accumbens (NACC) (experiment 2)

Group	DA	HVA/DA	DOPAC/DA	5HT	5HIAA/5HT
Medial prefrontal cortex					
OT Vehicle	0.87±0.07	0.50 ±0.04	0.76±0.11	2.56±0.16	0.64±0.04
OT 6-OHDA	0.56±0.05	0.65 ±0.04	1.15±0.15	2.61±0.19	0.61±0.05
NACC Vehicle	0.84±0.08	0.81 ±0.17	0.74±0.10	2.89±0.13	0.68±0.04
NACC 6-OHDA	ND[c]	ND[c]	ND[c]	2.02±0.16	0.74±0.03
OT % control[b]	65	130	151	102	95
NACC % control[b]	−[c]	−[c]	−[c]	70	109
Olfactory tubercle					
OT Vehicle	43.6 ±1.1	0.050±0.003	0.23±0.01	6.19±0.28	0.36±0.01
OT 6-OHDA	7.3 ±1.1	0.117±0.025	0.39±0.02	6.08±0.13	0.44±0.01
NACC Vehicle	44.9 ±2.0	0.046±0.003	0.21±0.01	6.31±0.37	0.36±0.01
NACC 6-OHDA	9.6 ±1.5	0.070±0.004	0.30±0.02	6.16±0.67	0.47±0.04
OT % control[b]	17	234	169	98	122
NACC % control[b]	21	152	141	98	131
Nucleus accumbens					
OT Vehicle	70.1 ±2.5	0.065±0.002	0.21±0.01	3.91±0.17	0.63±0.02
OT 6-OHDA	56.1 ±3.4	0.078±0.012	0.21±0.01	3.86±0.28	0.66±0.05
NACC Vehicle	71.3 ±3.0	0.068±0.005	0.19±0.01	4.28±0.30	0.62±0.04
NACC 6-OHDA	12.9 ±1.5	0.181±0.034	0.25±0.02	4.24±0.28	0.71±0.04
OT % control[b]	80	120	96	99	105
NACC % control[b]	18	266	128	99	115
Caudate-putamen					
OT Vehicle	91.9 ±2.2	0.060±0.003	0.16±0.01	3.20±0.12	0.64±0.04
OT 6-HDA	95.3 ±2.4	0.057±0.004	0.14±0.00	3.15±0.12	0.69±0.02
NACC Vehicle	94.0 ±1.8	0.057±0.003	0.14±0.00	3.20±0.12	0.67±0.02
NACC 6-HDA	69.7 ±2.5	0.062±0.004	0.14±0.00	3.26±0.12	0.67±0.02
OT % control[b]	104	95	93	98	108
NACC % control[b]	74	109	98	102	100

[a] Absolute concentrations are given as mean±SEM (pmol/mg wet tissue, $n=9$–10)
[b] OT and NACC % control refer to the concentration remaining in 6-OHDA lesioned subjects as a percentage of that found in rats receiving vehicle at the same site
[c] Values too low for accurate determination

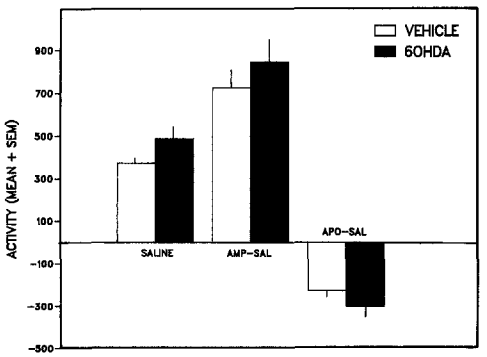

Fig. 3. Effect of 6-OHDA infusion into medial prefrontal cortex (*mPFCx*) on spontaneous and drug-induced locomotor activity. Data are from experiment 3. Rats received bilateral infusions of 6-OHDA or vehicle into either structure ($n=10$ per group) and were subsequently given locomotor tests with saline (twice), and with *d*-amphetamine (0.5 mg/kg SC) and apomorphine (0.1 mg/kg SC), in a counterbalanced order. Total (0–60 min) session scores did not differ between the two saline tests and a mean was taken for each rat. The figure shows group mean ± SEM session scores for saline tests, and drug-induced changes from saline baseline (i.e., drug minus saline scores)

Experiment 3: Effects of prior infusion of 6-OHDA into medial prefrontal cortex on photocell activity changes induced by d-amphetamine and apomorphine

Saline scores within the 60 min session did not differ significantly between 6-OHDA and sham-lesioned subjects ($t=1.85$, *df* 18; Fig. 3). Drug effects were calculated as difference scores between the drug test and the mean of the two saline tests. *d*-Amphetamine and apomorphine respectively increased and decreased locomotor activity in both groups of rats (Fig. 3), and neither drug effect differed significantly between the two groups (*d*-amphetamine: $t=0.87$, *df* 18; apomorphine: $t=1.30$, *df* 18).

Infusion of 6-OHDA into the mPFCx reduced DA concentrations in this area by at least 90% (Table 5). In the mPFCx, 5-HT was also depleted (by 57%), and there was a concomitant increase in the ratio of 5-HIAA (5-hydroxyindole acetic acid) to 5-HT. In other regions assayed, DA and 5-HT indices were unaffected.

Discussion

Anatomical selectivity within DA systems

In the present experiments, 6-OHDA was used to destroy discrete populations of dopaminergic axons and terminals. Attempts were made to improve anatomical selectivity in two ways: firstly, by combining two stereotaxic coordinate systems for more accurate cannula placements; secondly, by infusing the toxin at a lower rate than is commonly employed.

Infusion of 6-OHDA resulted in a substantial DA depletion within each target structure, ranging from 82% to over 90%. However, toxin administration into OT and NACC also produced DA loss in other areas. For example, DA content in mPFCx was reduced by 6-OHDA infusion at sites within OT (40% loss) and especially within NACC (>90% loss). Previous reports have also indicated a marked reduction of mPFCx DA (34–68%) after intra-accumbens administration (Koob et al. 1978; Robbins et al. 1983). Dopamine depletion produced remotely in mPFCx presumably reflects damage to mesocortical projection fibres in the vicinity of the 6-OHDA injection sites.

The NACC and OT were also strikingly different in their susceptibility to DA depletion following 6-OHDA infusion. Thus, 6-OHDA infusion into NACC produced almost as great a depletion in OT as in NACC itself, whereas OT injections of the toxin barely affected NACC DA whilst markedly depleting DA within the target structure. Loss of DA within the OT following 6-OHDA infusion into

Table 5. Regional concentrations[a] of DA, 5-HT and metabolite/transmitter ratios following 6-OHDA infusion into medial prefrontal cortex (experiment 3)

Group	DA	HVA/DA	DOPAC/DA	5HT	5HIAA/5HT
Medical prefrontal cortex					
Vehicle	0.80 ± 0.05	0.62 ± 0.05	0.47 ± 0.07	2.16 ± 0.14	0.79 ± 0.06
6-OHDA	0.08 ± 0.01	3.62 ± 0.42	1.05 ± 0.19	0.93 ± 0.14	1.91 ± 0.56
% control[b]	10	548	223	43	242
Olfactory tubercle					
Vehicle	42.6 ± 1.5	0.057 ± 0.005	0.25 ± 0.02	5.71 ± 0.30	0.38 ± 0.03
6-OHDA	44.4 ± 1.3	0.048 ± 0.002	0.23 ± 0.01	6.03 ± 0.25	0.34 ± 0.01
% control[b]	104	84	89	106	89
Nucleus accumbens					
Vehicle	66.9 ± 2.4	0.081 ± 0.006	0.23 ± 0.01	2.47 ± 0.07	0.90 ± 0.03
6-OHDA	68.5 ± 1.8	0.076 ± 0.074	0.22 ± 0.01	2.48 ± 0.18	0.92 ± 0.06
% control[b]	102	94	93	100	97
Caudate-Putamen					
Vehicle	91.0 ± 2.2	0.070 ± 0.005	0.16 ± 0.01	1.62 ± 0.06	1.15 ± 0.03
6-OHDA	91.0 ± 3.4	0.065 ± 0.004	0.15 ± 0.01	1.50 ± 0.08	1.26 ± 0.04
% control[b]	100	93	92	93	110

[a] Absolute concentrations are given as mean ± SEM (pmol/mg wet tissue, $n=10$)

[b] % control refer to the concentration remaining in 6-OHDA lesioned subjects as a percentage of that found in rats receiving vehicle at the same site

NACC sites was first reported by Kelly et al. (1975), and several subsequent studies have confirmed comparable depletions in OT and NACC following intra-accumbens administration (Joyce and Koob 1981; Joyce et al. 1983; Robbins et al. 1983). In contrast, Costall et al. (1977) reported that 6-OHDA infusions into NACC reduced DA by 72% within this structure whilst having little effect on the OT; comparable selectivity was associated with infusion at OT sites. Despite numerous attempts, we have been unable to obtain OT-sparing DA depletions of NACC by varying such parameters as the rate of infusion, vertical stereotaxic coordinate (V_{IA} 2.3–3.0) and injection volume.

In the experiments reported here, 6-OHDA infusion into the OT resulted in a small (20%) depletion of DA in the overlying NACC. Preliminary studies with dye injection indicate that there was little if any leakage of toxin up the cannula tract at the slow rate of infusion adopted for OT and NACC sites, in contrast to more commonly employed rates of infusion (e.g., 1 µl in 3 min). The incomplete anatomical specificity of OT lesions presumably reflects diffusion of toxin through tissue and/or the existence of collateral innervation arising from dopaminergic cell bodies in the ventral tegmentum. Comparable infusions into NACC reduced the DA content of caudate-putamen samples by 25%. Quite probably, the ventral caudate-putamen, particularly in its anterior part in the vicinity of the NACC, was considerably depleted, as others have reported (Koob et al. 1981; Joyce et al. 1983; Taghzouti et al. 1985).

Administration of 6-OHDA to the mPFCx produced no detectable alterations of dopaminergic indices in subcortical structures. For reasons presently unknown, this result is at variance with other studies which have reported elevated dihydroxyphenylacetic acid/dopamine (DOPAC/DA) ratios in the NACC and CP after 6-OHDA mPFCx lesions (Pycock et al. 1980; Martin-Iversen et al. 1986).

Where regional concentrations of DA were markedly depleted, DOPAC/DA ratios and particularly HVA/DA (homovanillic acid/dopamine) ratios were increased by up to 150%. An analogous increase in 5HIAA/5HT ratios was observed following depletion of 5HT in the mPFCx. These increased metabolite/transmitter ratios confirm that some degree of functional recovery probably occurs through increased activity among surviving neurons after neurotoxic insult (Schultz 1982).

Effects of 6-OHDA on 5-HT indices

Moderate doses of 6-OHDA are considered to have minimal effects on 5HT neurons in the rat brain (Jonsson 1983), and to some extent the present results support this notion. Thus, considerable neurochemical selectivity was shown following 6-OHDA infusion into OT or NACC; despite substantial local depletions of DA ranging from 82 to 89%, there was little if any change in local concentrations of 5HT. In contrast, administration of 6-OHDA into the mPFCx resulted in a significant (57%) depletion of 5HT as well as a virtual elimination of DA within this region. These infusions also produced a marked (142%) increase in the 5HIAA/5HT ratio. Using comparable procedures, Martin-Iversen et al. (1986) in this laboratory have reported similar but less marked changes in mPFCx 5HT and 5HIAA/5HT following infusion of 6-OHDA within this region.

Possibly, the loss of mPFCx 5HT encountered in the present study reflects a direct toxic effect of 6-OHDA on serotonergic neurons, although it is not clear why this should not also occur in either NACC or OT. Alternatively, the loss of 5HT may have been secondary to destruction of dopaminergic elements. However, this is at best a partial explanation, since intra-accumbens infusion of 6-OHDA depleted DA in the mPFCx to a similar degree as by direct intra-mPFCx infusion, but reduced 5-HT to a much smaller extent (16–30% depletion).

Spontaneous locomotor activity

In the present experiments, 6-OHDA had little effect on activity in saline tests. The only significant effect occurred in experiment 1, where subjects in the NACC-infused group were somewhat more active than sham-lesioned controls. Previous investigators have reported decreased locomotor activity after intra-accumbens administration of 6-OHDA (Koob et al. 1978, 1981; Joyce and Koob 1981; Makanjuola and Ashcroft 1982) which recovers over a few days or weeks (Joyce et al. 1983; Kubos et al. 1987). Spontaneous hyperactivity following unilateral or bilateral 6-OHDA lesions of mPFCx (Carter and Pycock 1980; Robinson and Stitt 1981) was not observed. The extent to which different locomotor testing methods and surgical procedures determine the effects of 6-OHDA on spontaneous activity remains to be determined.

Direct behavioral observation and stereotypy

High doses of *d*-amphetamine and apomorphine induce intense stereotyped behavior in rats (Kelly et al. 1975). Depending on the measuring device used, repetitive stereotyped movements may interfere with the counting of place-to-place locomotor movements. The activity meters used in the present study register whole body movements, and also, to some extent, movements confined to one location of the cage. Care was therefore taken to select low drug doses which produced approximately equal degrees of locomotor stimulation in pilot experiments without producing competing stereotypic behavior. For *d*-amphetamine and apomorphine, the absence of stereotypy was confirmed in experiment 2.

Apomorphine, a direct DA agonist, was given in a low dose which produces hypoactivity in intact animals, probably through a selective action on autoreceptors (Kelly and Roberts 1983). In rats lesioned by infusion of 6-OHDA into NACC or CP, this drug promotes locomotor activity and stereotypy, respectively, through actions on "supersensitive" postsynaptic receptors (Kelly et al. 1975; Joyce and Koob 1981; Koob et al. 1981; Joyce et al. 1983). The functional significance of DA transmission in OT being unknown, the possibility of a previously unobserved "supersensitive" response to apomorphine in OT-lesioned subjects was anticipated; however, no behavioral alteration was seen.

Anatomical substrates of responses to d-amphetamine and apomorphine

The first experiment confirmed the many previous reports indicating that bilateral 6-OHDA infusion into the NACC reduces or blocks the locomotor stimulant effect of *d*-amphetamine (Kelly et al. 1975; Kelly and Iversen 1976; Koob et al. 1978, 1981; Joyce and Koob 1981; Makanjuola and

Ashcroft 1982; Joyce et al. 1983; Kelly and Roberts 1983). With adequate measurements of baseline activity, we were able to determine the extent to which the *d*-amphetamine response was reduced. In experiment 1, a partial reduction was observed, whereas complete blockade has been observed when a higher dose of 6-OHDA is used (in preparation). Administration of 6-OHDA resulted in a slight (5–15 g) loss of body weight which recovered at least 1 week before the start of behavioral testing. Thus lesion-induced changes in drug responses probably do not reflect pharmacokinetic factors.

As discussed above, DA depletion following intra-accumbens infusion was not confined to this structure, but included the OT, mPFCx, and to some extent the CP. Infusion of 6-OHDA at OT sites produced a more selective pattern of depletion largely limited to the target structure itself; this lesion failed to reduce *d*-amphetamine-induced locomotor stimulation and actually appeared to increase it. In addition, infusion of 6-OHDA directly into mPFCx sites produced neurochemical alterations confined to this region but had no effect on responses to *d*-amphetamine.

The present results therefore suggest that the locomotor stimulant effect of *d*-amphetamine is not mediated through the DA innervation of the OT or mPFCx to any significant extent. Results with apomorphine accord well with these conclusions. In experiment 1, a hyperactive response to apomorphine was seen only in rats which had received 6-OHDA into NACC sites. Such a "supersensitive" response has been reported previously (Kelly et al. 1975; Kelly and Iversen 1976; Koob et al. 1981; Joyce et al. 1983). Infusion of toxin at OT or mPFCx sites largely spared the NACC and did not alter the hypoactive response to apomorphine which was seen in sham-lesioned rats.

As already discussed, infusions of 6-OHDA into the mPFCx reduced local concentrations of both 5-HT and DA. Since this lesion did not affect saline-test activity or locomotor responses to apomorphine and *d*-amphetamine, it seems unlikely that the loss of cortical 5-HT was behaviourally important. Nevertheless, the possibility of a behaviourally significant interaction between DA and 5-HT in this region cannot be excluded.

The present data are consistent with the hypothesis that the stimulant effects of *d*-amphetamine and direct DA agonists in rats are mediated by DA neurotransmission in the NACC (see Swerdlow et al. 1986). Several authors have found that bilateral intra-accumbens injections of *d*-amphetamine or DA receptor agonists increase locomotor activity (e.g., Pijnenburg et al. 1976; Makanjuola et al. 1980). In contrast, Cools (1986) recently reported that bilateral microinjection of DA and apomorphine into NACC sites produced only inconsistent effects, whereas similar injections into OT resulted in hyperactive responses of short latency. Unfortunately, in that study, the rate of drug injection was not stated nor was the intracerebral spread of drug assessed. It cannot be excluded that upon injection, drug solutions leaked up the cannula tract from OT sites into the NACC, so that DA receptors were activated in both structures. Nevertheless, two other recent studies, employing electrolytic and kainic acid lesions, also suggest that the locomotor stimulant effects of DA agonists are not exclusively mediated by the NACC (Makanjuola and Ashcroft 1982; Kafetzopoulos 1986).

Two other possibilities must therefore be examined: (1) that these stimulant effects are independent of DA innervation of the NACC itself, but depend on DA innervation of a neighbouring site, possibly in the overlying CP, and (2) hyperactive responses to these drugs rely for their expression on DA innervation of the NACC and of a second unidentified site. In order to test these possibilities, we have attempted to restrict the 6-OHDA lesions to the NACC, but without success (see above). Costall et al. (1977) have reported a relatively discrete 6-OHDA-induced depletion of NACC DA (72%) which left the OT largely untouched (28% depletion); this lesion was said to shorten the locomotor stimulant effect of *d*-amphetamine, but baseline activity was not sufficiently well assessed for any firm conclusion to be drawn. Fink and Smith (1979) have proposed that DA innervation of the anteromedial caudate, but not of the NACC, is necessary for the full expression of *d*-amphetamine-induced locomotion, but for the same reason, this possibility requires further validation.

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References

Carter CJ, Pycock CJ (1980) Behavioural and biochemical effects of dopamine and noradrenaline depletion within the medial prefrontal cortex of the rat. Brain Res 192:163–176

Clarke PBS, Kumar R (1983) The effects of nicotine on locomotor activity in non-tolerant and tolerant rats. Br J Pharmacol 78:329–337

Clarke PBS, Fu DW, Jakubovic A, Fibiger NC (1988) Evidence that mesolimbic dopaminergic activation underlies the locomotor stimulant action of nicotine in rats. J Pharmacol Exp Ther 246:701–708

Cools AR (1986) Mesolimbic dopamine and its control of locomotor activity in rats: differences in pharmacology and light/dark periodicity between the olfactory tubercle and the nucleus accumbens. Psychopharmacology 88:451–459

Costall B, Marsden CD, Naylor RJ, Pycock CJ (1977) Stereotyped behaviour patterns and hyperactivity induced by amphetamine and apomorphine after discrete 6-hydroxydopamine lesions of extrapyramidal and mesolimbic nuclei. Brain Res 123:89–111

Fink JS, Smith GP (1979) Abnormal pattern of amphetamine locomotion after 6-hydroxydopamine lesion of the anteromedial caudate. Pharmacol Biochem Behav 11:23–30

Jonsson G (1983) Chemical lesioning techniques: monoamine neurotoxins. In: Bjorklund A, Hokfelt T (eds) Handbook of chemical neuroanatomy. Elsevier, Amsterdam, pp 463–507

Joyce EM, Koob GF (1981) Amphetamine-, scopolamine-, and caffeine-induced locomotor activity following 6-hydroxydopamine lesions of the mesolimbic dopamine system. Psychopharmacology 73:311–313

Joyce EM, Stinus L, Iversen SD (1983) Effect of injections of 6-OHDA into either nucleus accumbens septi or frontal cortex on spontaneous and drug-induced activity. Neuropharmacology 22:1141–1145

Kafetzopoulos E (1986) Effects of amphetamine and apomorphine on locomotor activity after kainic acid lesion of the nucleus accumbens septi in the rat. Psychopharmacology 88:271–274

Kelly PH, Iversen SD (1976) Selective 6-OHDA-induced destruction of mesolimbic dopamine neurons: abolition of psychostimulant-induced locomotor activity in rats. Eur J Pharmacol 40:45–56

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

Koob GF, Stinus L, Le Moal ML (1981) Hyperactivity and hypoactivity produced by lesions to the mesolimbic dopamine system. Behav Brain Res 3:341–359

Kubos LK, Moran TH, Robinson RG (1987) Differential and asymmetrical behavioral effects of electrolytic or 6-hydroxydopamine lesions of the nucleus accumbens. Brain Res 401:147–151

Makanjuola ROA, Ashcroft GW (1982) Behavioural effects of electrolytic and 6-hydroxydopamine lesions of the accumbens and caudate-putamen nuclei. Psychopharmacology 76:333–340

Makanjuola ROA, Dow RC, Ashcroft GW (1980) Behavioural responses to stereotactically controlled injections of monoamine neurotransmitters into the accumbens and caudate-putamen nuclei. Psychopharmacology 71:227–235

Martin-Iversin MT, Szostak C, Fibiger HC (1986) 6-Hydroxydopamine lesions of the medial prefrontal cortex fail to influence intravenous self-administration of cocaine. Psychopharmacology 88:310–314

Paxinos W, Watson C (1982) The rat brain in stereotaxic coordinates. Academic Press, New York

Pijnenburg AJJ, Honig WMM, van Rossum JM (1975) Inhibition of *d*-amphetamine-induced locomotor activity by injection of haloperidol into the nucleus accumbens of the rat. Psychopharmacology 41:87–95

Pijnenburg AJJ, Honig WMM, van der Heyden JAM, van Rossum JM (1976) Effects of chemical stimulation of the mesolimbic dopamine system upon locomotor activity. Eur J Pharmacol 35:45–58

Pycock CJ, Carter CJ, Kerwin RW (1980) Effect of 6-hydroxydopamine lesions of the medial prefrontal cortex on neurotransmitter systems in subcortical sites in the rat. J Neurochem 43:91–99

Robbins TW, Roberts DCS, Koob GF (1983) Effects of *d*-amphetamine and apomorphine upon operant behavior and schedule-induced licking in rats with 6-hydroxyxdopamine-induced lesions of the nucleus accumbens. J Pharmacol Exp Ther 224:662–673

Robinson RG, Stitt TG (1981) Intracortical 6-hydroxydopamine induces an asymmetric behavioral response in the rat. Brain Res 213:387–395

Schultz W (1982) Depletion of dopamine in the striatum as an experimental model of Parkinsonism: direct effects and adaptive mechanisms. Prog Neurobiol 18:121–166

Swerdlow NR, Vaccarino FJ, Amalric M, Koob GF (1986) The neural substrates for the motor-activating properties of psychostimulants: a review of recent findings. Pharmacol Biochem Behav 25:233–248

Taghoutzi K, Simon H, Louilot A, Herman JP, Le Moal M (1985) Behavioral study after local injection of 6-hydroxydopamine into the nucleus accumbens in the rat. Brain Res 344:9–20

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