

AMPHETAMINE AND APOMORPHINE RESPONSES IN THE RAT FOLLOWING 6-OHDA LESIONS OF THE NUCLEUS ACCUMBENS SEPTI AND CORPUS STRIATUM

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SUMMARY

Eight μg of 6-hydroxydopamine (6-OHDA) injected bilaterally into the nucleus accumbens septi (NAS) or the caudate nucleus of the rat resulted in 79% and 50% depletion of endogenous dopamine (DA) at these respective sites. Fourteen days after the injection a low dose of amphetamine failed to induce the characteristic locomotor response in the NAS-lesioned rats but did so in the caudate-lesioned animals. By contrast the caudate lesion, but not the NAS lesion, abolished intense forms of stereotyped behaviour induced by higher doses of amphetamine. Both lesioned groups exhibited supersensitivity to the dopamine agonist, apomorphine; the NAS group showed enhanced locomotor activity and the caudate group enhanced stereotyped behaviour. The block of amphetamine locomotion and the enhanced response to apomorphine were maximal around 14 days after the operation and gradually attenuated up to 90 days. There is evidence that remaining DA levels in the NAS are greater at 90 than at 14 days postoperatively. Thus recovery of behavioural effects correlated with an increase in the remaining levels of DA in the NAS.

INTRODUCTION

In the rat, the behavioural effects of amphetamine are dose-dependent. While low doses (1.5 mg/kg) enhance locomotor activity, higher doses (5 mg/kg) produce repetitive stereotyped behaviour in which components of the normal behavioural repertoire such as sniffing, head movements, rearing, licking and gnawing occur with abnormal frequency. The purpose of this investigation was to discover whether the locomotor activity and stereotypy produced by amphetamine might be mediated by separate neural systems.

Amphetamine is known to influence the uptake and release of both noradre-

naline (NA) and dopamine (DA) in the brain^{3,13,16,20,34}. However, it would seem from studies employing specific catecholamine depleting agents that locomotor activity and stereotyped behaviour induced by amphetamine are more dependent upon brain dopamine levels than those of noradrenaline. For example, the tyrosine hydroxylase inhibitor, α -methyl-*p*-tyrosine, depletes the brain of both noradrenaline and dopamine and abolishes the amphetamine responses^{24,27,35}. Depletion of noradrenaline alone, however, by means of dopamine- β -hydroxylase inhibitors does not abolish amphetamine-induced locomotor activity or stereotypy^{25,28}.

Dopamine has been localised to specific anatomical systems which can be differentiated on the basis of the location of the neurone cell bodies which give rise to the pathways, and terminal plexi. The striatal innervation has its cells of origin in the substantia nigra, designated A8 and A9 in the histofluorescence mapping studies²⁹. By contrast, the dopaminergic mesolimbic pathway has its origin in the A10 group of neurones which lie medial to the A9 group in the midbrain, and has terminals in the nucleus accumbens septi (NAS) and tuberculum olfactorium²⁹.

Dopaminergic and noradrenergic nerve fibres can be selectively destroyed by local intracerebral injection of 6-hydroxydopamine (6-OHDA), which is a chemical analogue of the catecholamine transmitters^{32,33}. The behavioural results of various 6-OHDA treatments have been studied and can be correlated with the relative depletions of NA and DA induced by this neurotoxin. Rats which received 6-OHDA intraventricularly as neonates, were found, as adults, to have virtually total depletion of forebrain DA and substantially reduced forebrain NA levels⁶. The locomotor activity and stereotypy responses to D-amphetamine were blocked in these rats⁶. Apomorphine, which is a direct central dopamine receptor stimulating agent, produced locomotion and stereotyped behaviour in these animals at dose levels which were ineffective in normal rats. This is thought to be a result of the dopaminergic receptors being 'supersensitive' following denervation by 6-OHDA. Hollister *et al.*¹⁵ report that repeated small doses of 6-OHDA which result in NA but not DA depletion do not alter the amphetamine responses.

There is now strong evidence that a dopamine mechanism in the corpus striatum mediates amphetamine stereotypy. 6-OHDA lesions to the substantia nigra⁵, 6-OHDA lesions to the caudate nucleus^{2,7}, and electrolytic lesions of the caudate nucleus^{10,12}, convincingly block amphetamine stereotypy. These treatments have the common feature of inducing substantial depletion of striatal dopamine. Further, stereotyped behaviour can be produced by local application of DA or DA agonists such as apomorphine to the caudate nucleus^{11,12}.

However, it has proved more difficult to establish a definite relationship between striatal DA depletion and blockade of the locomotor activity induced by amphetamine. The intracerebral 6-OHDA neonate preparation⁶, intraventricular 6-OHDA treatment after MAO inhibition in the adult rat¹⁵, and 6-OHDA substantia nigra lesions which result in virtually total loss of striatal DA⁸, have been reported to abolish amphetamine-induced locomotor activity. However, in addition to DA depletion these manipulations also result in substantial forebrain NA loss, which made it impossible to attribute the blockade of amphetamine-induced locomotor

activity solely to the severe DA loss from the striatum. In addition the manipulations which successfully damage the substantia nigra and its terminal projection in the striatum may also damage the origins of the closely related mesolimbic DA system originating in the A10 neurone group. It has not been possible to evaluate the contribution of such damage as DA levels in these regions were not measured in earlier studies. The possibility that the mesolimbic DA system mediates the locomotor activity induced by amphetamine recently gained support from the observation of Pijnenburg and van Rossum²³, that in the rat bilateral injection of dopamine directly into the nucleus accumbens, produced a strong enhancement of locomotor activity. In the present study we have investigated the motor responses to amphetamine and the dopamine agonist, apomorphine, in rats with bilateral 6-OHDA lesions to the nucleus accumbens septi (NAS) or the caudate nucleus.

METHODS

Subjects

Thirty six male albino Sprague–Dawley rats, mean operative weight 310 g were housed in groups of 6 with free access to food and water. They were maintained in controlled conditions with a 12 h light–dark cycle.

Surgical procedures

The rats were anaesthetised with Equithesin (3.75 ml/kg) and positioned in a stereotaxic apparatus. Twelve rats received bilateral injections of 6-OHDA into the nucleus accumbens septi (NAS) using stereotaxic coordinates A 3.4, L 1.7, V 7.2, according to the atlas of Pellegrino and Cushman²², and 12 rats received the sham procedure, with the cannula being lowered into the NAS but no solution injected. Six rats received bilateral injections of 6-OHDA into the caudate nucleus of the corpus striatum using stereotaxic coordinates A 2.0, L 3.0, V 4.7, and 6 rats received the sham procedure. Stereotaxic injections were made from a 10 μ l microsyringe through a 30-gauge stainless steel cannula. The total injection volume was 2 μ l on each side, injected at a rate of 1 μ l/min. The injection volume and rate of infusion were selected to minimise non-specific damage at the site of injection.

Drugs

6-Hydroxydopamine (6-OHDA) hydrobromide (Sigma Ltd.) was dissolved in ice-cold, 0.9% saline containing ascorbic acid (1 mg/ml) as an antioxidant. The dose used was 8 μ g of 6-OHDA in 2 μ l of solution. D-Amphetamine sulphate (Smith, Kline and French Ltd.) and apomorphine hydrochloride (McFarlan Smith) were freshly dissolved in 0.9% saline and injected intraperitoneally.

Behavioural testing

Locomotor activity was measured in a bank of 12 wire cages (25 cm $\times$ 40 cm $\times$ 20 cm), each with two horizontal photocell beams along the long axis. The cages

were contained in a room which was masked with white noise and maintained at 22 °C. Non-cumulative recordings of photocell beam interruptions were taken every 10 min in each experimental session. Prior to any drug manipulations the animals were habituated to the photocell cages for 30 min and spontaneous locomotor activity was recorded. Following amphetamine administration locomotor activity was recorded for 2 h, and following apomorphine it was recorded for 90 min. Beam interruptions by locomotor activity are reliably recorded. However, stereotypy is inconsistently recorded and may yield high or low counts depending on the physical location of the rat in relation to the photocell and the amount of concurrent locomotor activity. It is therefore important to combine photocell recording of amphetamine responses with direct observation. Accordingly the nature of the response being repeated and the degree of repetition was assessed by means of a rating scale, previously developed from observations of the behaviour of normal rats exposed to increasing doses of amphetamine. The ratings used were a slightly modified form of the scale described by Creese and Iversen⁶

- 0 — asleep or stationary
- 1 — active
- 2 — predominantly active with *bursts* of stereotyped sniffing or rearing.
- 3 — stereotyped activity predominantly sniffing and rearing *over a large area of the cage*.
- 4 — stereotyped behaviour maintained in *one location*.
- 5 — stereotyped behaviour in one location *with bursts of gnawing or licking*.
- 6 — *continual* gnawing or licking of the cage bars.

Stereotypy ratings for each animal were made immediately after the number of photocell beam interruptions for the preceding 10 min had been recorded.

In 6 NAS- and 6 caudate-lesioned rats with their appropriate controls the behavioural responses to a low dose of amphetamine (1.5 mg/kg) were measured 3, 14, 22, and 90 days postoperatively and the responses to apomorphine (1 mg/kg) were measured 2, 7, 10, 18, 28, 40 and 78 days following surgery. The behavioural response to a high dose of amphetamine (5 mg/kg) was measured 20 days postoperatively in the caudate-lesioned animals and 31 days postoperatively in the nucleus accumbens-lesioned animals. The brains of these animals were assayed 90–110 days postoperatively. In the remaining 6 NAS lesioned and control rats the response to 1.0 mg/kg apomorphine was tested on postoperative days 2 and 8, and to 1.5 mg/kg D-amphetamine on days 3 and 13. Assays were performed on these animals 18 days postoperatively.

Biochemical methods

Regional levels of noradrenaline and dopamine in the brain were determined by a sensitive radioenzymatic assay⁹. The rats were stunned and killed by decapitation. Their brains were rapidly removed and chilled on an ice-cold glass plate. The olfactory tubercles, nuclei accumbens septi and striatum were dissected as previously described^{14,16}. A fourth region, referred to here as 'forebrain', consisted of that tissue, anterior to a frontal cut through the optic chiasm, not included in any of the

other regions. Dopamine was assayed in 3 regions and noradrenaline in the forebrain segment.

Statistical analysis

The mean number of photocell beam interruptions per unit time was subjected to analysis of variance, as were variations in body weight between the groups. Differences between two groups were analysed with the Students *t*-test. As the stereotypy ratings constituted neither an interval nor an ordinal scale, traditional statistical methods were inapplicable. The frequency distribution of ratings could, however, be analysed with the information statistic¹⁹. Catecholamine levels were expressed as percentages of the appropriate sham values and compared using the Mann-Whitney *U*-test.

RESULTS

Postoperative body weight

The animals which received lesions in the nucleus accumbens lost approximately 8.5% of their preoperative weight (mean value 298 g) in the two days following surgery, but by the third day had recovered to over 99% of their preoperative weight. The caudate-lesioned group lost 5% of their preoperative weight (mean value: 323 g) in the two days after the lesion and by the third day their average weight was still only 95% of its preoperative value. The NAS sham operates lost only 1% of their preoperative weight (mean value: 308 g) during the first two postoperative days and the caudate sham operates (mean preoperative weight: 311 g) actually made a small gain in weight during this period (1%). The differences in the mean weights for the 4 groups of animals two days postoperatively were not significant ($F = 2.2$, $df = 3,20$). At 10 days postlesion the NAS lesion groups mean weight was 116% preoperative level, the caudate lesion group 110% and the NAS sham operates and caudate sham operates 118% and 112% respectively. These differences were not significant ($F = 0.20$, $df = 3,20$).

At no time in this experiment did any of the animals have to be given highly palatable food or be force fed in order for them to gain weight.

Motor behaviours

The stereotypy ratings were averaged over the sessions but were also tabulated in terms of frequency of a given rating per session per lesion group. These values for 1.5 mg/kg amphetamine at 14 days, 5.0 mg/kg amphetamine at 20 days in the caudate-lesioned group and 31 days in the NAS group and 1.0 mg/kg apomorphine in both lesioned groups at 10 days are presented in Table I. The information statistic was used to analyse differences in the distributions of scores over the 7 rating categories.

Once an overall significant difference in distributions for a given dose of a drug had been demonstrated the contributions of the various lesions and sham lesions to this difference were calculated by making the following comparisons: caudate lesion group *versus* NAS lesion + NAS sham + caudate sham; NAS lesion *versus*

TABLE I

MEAN FREQUENCY PER SESSION OF STEREOTYPY RATINGS 0-4 IN CAUDATE AND NUCLEUS ACCUMBENS 6-OHDA LESIONED RATS AND THEIR SHAM OPERATED CONTROLS

<i>Frequency of ratings</i>						
<i>1.5 mg/kg amphetamine at 14 days (2 h)</i>						
	0	1	2	3	4	Total
NAS lesion	24	22	18	0	8	72
NAS sham	4	25	21	13	9	72
Caud. lesion	3	37	32	0	0	72
Caud. sham	0	7	29	36	0	72
<i>5.0 mg/kg amphetamine at 20-31 days (2 h)</i>						
	0	1	2	3	4	Total
NAS lesion	1	0	2	2	67	72
NAS sham	0	0	0	4	68	72
Caud. lesion	0	0	18	29	25	72
Caud. sham	0	0	0	5	67	72
<i>1.0 mg/kg apomorphine at 10 days (1.5 h)</i>						
	0	1	2	3	4	Total
NAS lesion	12	8	9	25	0	54
NAS sham	41	1	3	4	5	54
Caud. lesion	29	3	1	5	16	54
Caud. sham	28	5	3	13	5	54

NAS sham + caudate sham; NAS sham *versus* caudate sham. These breakdowns of the information statistic are presented in Table II.

Amphetamine-induced locomotor activity

When tested on the third postoperative day there was no significant difference between the mean locomotor activity to 1.5 mg/kg D-amphetamine of the NAS-lesioned and caudate-lesioned animals ($F = 0.93$, $df = 1,10$). In addition, there were no significant differences between the two sham operated groups ($F = 0.002$, $df = 1,10$) or between the accumbens-lesioned and accumbens sham rats ($F = 0.06$, $df = 1,10$). The caudate-lesioned rats were significantly less active, however, than the caudate shams ($F = 5.97$, $df = 1,10$, $P < 0.05$). This effect was seen only at 3 days.

When retested 14 days postoperatively with the same dose of amphetamine (1.5 mg/kg) it was found that the mean locomotor activity of the accumbens-lesioned group was significantly less than that of the caudate lesioned group ($F = 20.57$, $df = 1,10$, $P < 0.005$) following the drug injection. Whereas the former recorded a mean of only 50 beam interruptions per 10 min throughout the 2 h session, the latter recorded a mean of 263 beam interruptions per 10 min (Fig. 1, left). There was no significant difference between the mean activity to 1.5 mg/kg D-amphetamine of the caudate-lesioned group and two sham operated groups ($F = 0.92$, $df = 2,15$).

Almost identical results were seen at 22 days postoperatively (Fig. 1, right). The accumbens lesioned rats were again significantly less active than the caudate-lesioned

TABLE II

INFORMATION STATISTIC VALUES INDICATING THE CONTRIBUTION OF THE VARIOUS LESIONS AND THEIR SHAM PROCEDURES TO THE OVERALL SIGNIFICANT INFORMATION STATISTIC VALUE

Source	$2\hat{f}$	df	Significance
<i>1.5 mg/kg amphetamine at 14 days</i>			
1. Caudate lesion vs. NAS lesion NAS sham C sham	+ 55.864	4	$P < 0.001$
2. NAS lesion vs. NAS sham C sham	+ 76.900	4	$P < 0.001$
3. NAS sham vs. C sham	41.282	4	$P < 0.001$
Overall	174.046	12	$P < 0.001$
<i>5 mg/kg amphetamine</i>			
1. Caudate lesion vs. NAS lesion NAS sham C sham	+ 105.428	4	$P < 0.001$
2. NAS lesion vs. NAS sham C sham	+ 8.830	4	n.s.
3. NAS sham vs. C sham	0.122	4	n.s.
Overall	114.378	12	$P < 0.001$
<i>1.0 mg/kg apomorphine at 10 days</i>			
1. Caudate lesion vs. NAS lesion NAS sham C sham	+ 26.198	4	$P < 0.001$
2. NAS lesion vs. NAS sham C sham	+ 42.274	4	$P < 0.001$
3. NAS sham vs. C sham	10.394	4	$P < 0.05$
Overall	78.866	12	$P < 0.001$

rats ($F = 57.05$, $df = 1,10$, $P < 0.001$) in response to 1.5 mg/kg D-amphetamine. However, there was no significant difference between the caudate and the two sham operated groups, ($F = 0.3$, $df = 2,15$). The mean beam interruptions per 10 min were 82 for the accumbens-lesioned rats, 306 for the caudate-lesioned rats, 284 for the accumbens shams and 258 for the caudate shams.

At 90 days postoperatively it was found that there was no longer a significant difference in the mean locomotor activity of the accumbens and accumbens sham rats following injection of 1.5 mg/kg D-amphetamine ($F = 2.12$, $df = 1,10$), (Fig. 3, lower).

The marked attenuation of locomotor stimulation at 14 days was verified

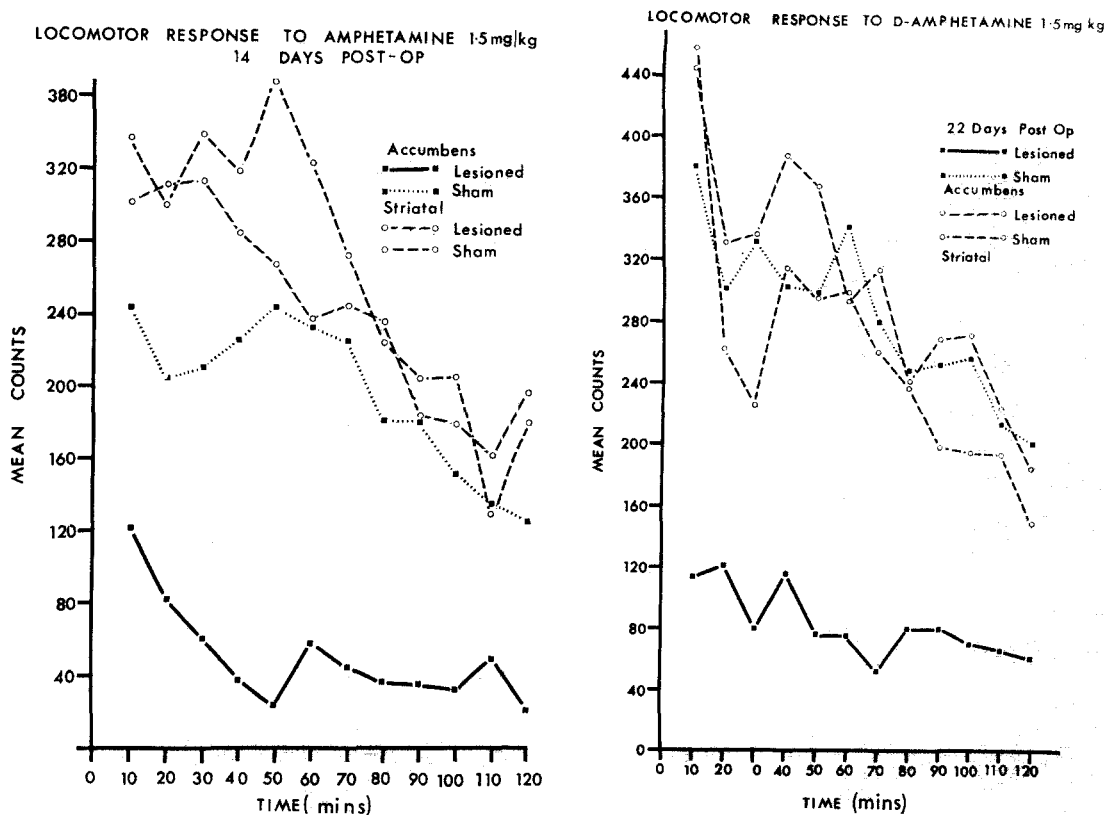


Fig. 1. Locomotor activity response to 1.5 mg/kg D-amphetamine on post-operative days 14 (left) and 22 (right). Mean photocell beam interruptions per 10 min for caudate and nucleus accumbens 6-OHDA lesioned rats and their sham operated controls.

in the parallel groups of NAS-lesioned and sham operated animals. They were tested with 1.5 mg/kg D-amphetamine 13 days postoperatively and then killed for brain amine assays at 18 days.

Apomorphine-induced locomotor activity

The dopamine agonist, apomorphine, induced significantly more locomotor activity, as measured by photocell counts, in the NAS-lesioned rats than in caudate-lesioned or sham operated controls. This was true at 2, 7, 10, 18, 28, 40 and 78 days postoperatively. The result at 10 days is illustrated in Fig. 2. However, the magnitude of the enhanced locomotor response varied over the postoperative period, reaching a peak between 10 and 18 days after the operation (Fig. 3, upper). The enhanced apomorphine locomotor response in the NAS-lesioned animals showed a significant drop between 18 and 40 days postoperatively (Students *t*-test, $t = 3.3$, $df = 5$, $P < 0.05$). The time course of the gradually increasing and decreasing apomorphine response mirrored the degree of blockade of the response to 1.5 mg/kg D-amphetamine (Fig. 3).

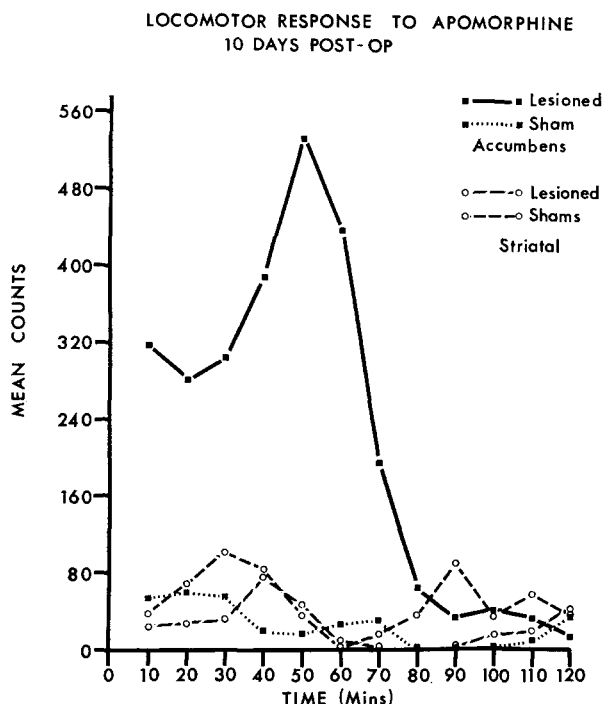


Fig. 2. Locomotor activity response to 1.0 mg/kg apomorphine on postoperative day 10: mean photocell beam interruptions per 10 min for caudate and nucleus accumbens 6-OHDA lesioned rats and their sham operated controls.

Amphetamine-induced stereotyped behaviour

At 1.5 mg/kg D-amphetamine both lesion groups showed an abnormal distribution of ratings ($P < 0.001$; Table I). The NAS-lesioned animals showed the highest frequency of total inactivity (rating 0) which is consistent with the recorded block of locomotor activity. Furthermore, when active they were less likely than their controls to show continuous sniffing while moving around the cage (*i.e.* rating 3). When stereotypy was seen it occurred dissociated from locomotor activity (*i.e.* rating 4). The NAS lesion therefore appears to attenuate locomotor activity and sniffing. By contrast, the caudate lesion group was not inactive, showed stimulation of locomotion together with sniffing but did not show intense stereotypy of rating 4 or higher.

These results are borne out by inspection of the mean stereotypy ratings to 1.5 mg/kg D-amphetamine shown in Table III. At 14 days postoperatively both the caudate- and NAS-lesioned rats showed reduced stereotypy relative to their respective controls. The mean stereotypy rating in the NAS group gradually increased during the postoperative period and between day 22 and 90 the rating increased significantly (Students *t*-test, $t = 3.94$, $df = P < 0.02$).

At 1.5 mg/kg amphetamine produces only weak stereotypy in the second hour after administration and is not by itself a valid dose to study if one is interested in

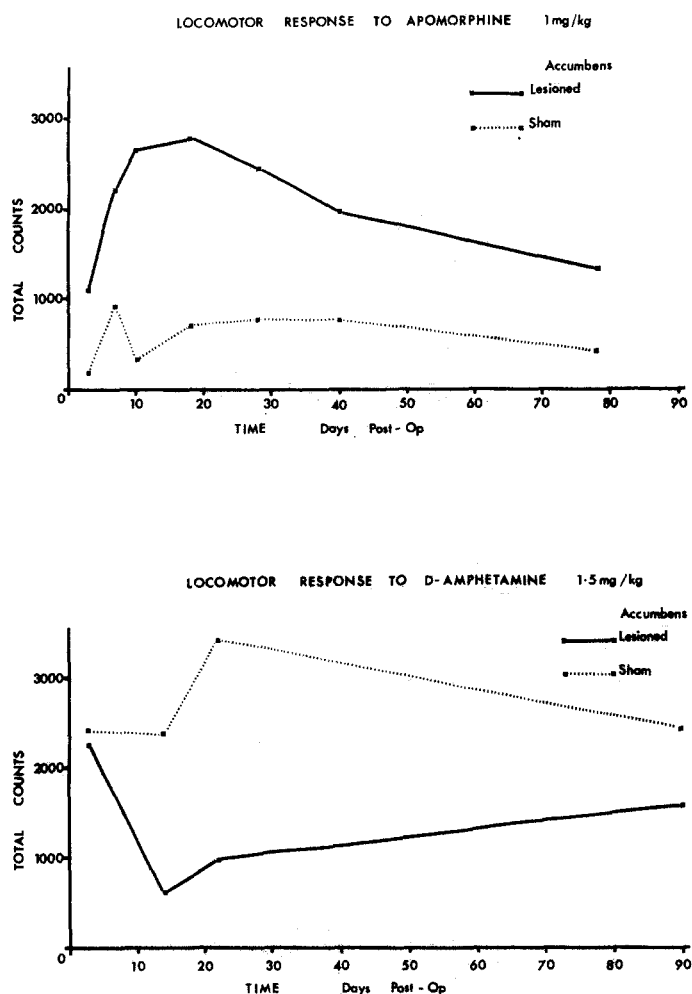


Fig. 3. Locomotor activity in total counts per session of nucleus accumbens lesioned animals and their sham operated controls. Lower: to 1.5 mg/kg D-amphetamine administered on postoperative days 3, 14, 22, and 90. Upper: to 1.0 mg/kg apomorphine tested on postoperative days 2, 7, 10, 18, 28, 40 and 78.

manipulations which block stereotypy. The results with 5 mg/kg are of far more relevance in this respect. Turning to this dose the results are very clear. Neither the NAS lesion nor the sham procedures significantly modify the rating frequency distribution (Table I) or the mean rating over the session, (Table III). All 3 groups showed intense stereotypy in one location (*i.e.* rating 4) (Fig. 4, left) and, hence, low recorded locomotor activity (Fig. 4, right). By contrast, the caudate lesion group showed a reduced frequency of rating 4 (Table I), a reduced mean rating over the session (Fig. 4, left and Table III) and maintained high levels of locomotor activity to this stereotypic dose of amphetamine (Fig. 4, right).

TABLE III

MEAN STEREOTYPY RATING PER 10 MIN IN CAUDATE AND NUCLEUS ACCUMBENS 6-OHDA LESIONED RATS AND THEIR SHAM OPERATED CONTROLS

Observations were made for 2 h after amphetamine injections and for 1.5 h after the apomorphine injection.

	<i>NAS lesion</i>	<i>NAS sham</i>	<i>Caudate lesion</i>	<i>Caudate sham</i>
14 days				
1.5 mg/kg D-amphetamine	1.3	2.0	1.4	2.4
22 days				
1.5 mg/kg D-amphetamine	1.9	2.6	1.5	2.0
90 days				
1.5 mg/kg D-amphetamine	2.5	2.7	—	—
5.0 mg/kg D-amphetamine	3.9	4.0	3.1	3.9
10 days				
1.0 mg/kg apomorphine	3.0	1.5	3.8	2.8

Apomorphine-induced stereotypy

At 1.0 mg/kg of apomorphine both the caudate lesion and the NAS lesion show significantly different distributions of stereotypy ratings. The contribution of the NAS lesion to this difference is the greater and is due to a marked increase in rating 3, persistent sniffing associated with locomotor activity. This may reflect a supersensitive response to apomorphine of the neural substrate mediating these forms of exploratory behaviour. The caudate-lesioned animals also show an abnormal distribution but in this case the occurrence of intense stereotypy maintained at one location in the cage (rating 4) is enhanced. No increase in locomotor activity with sniffing was observed. The frequency ratings for 1.0 mg/kg apomorphine were analysed in detail only on day 10. However, if the number of 3 and 4 ratings for the NAS- and caudate-lesioned rats are summed over the 7 sessions on which 1.0 mg/kg apomorphine was administered, the NAS group achieve 120 rating 3 and 0 rating 4, whereas the caudate-lesioned animals achieve 21 rating 3 and 54 rating 4.

With 1.0 mg/kg apomorphine both lesioned groups showed enhancement of stereotypy but of different elements. The mean ratings Table III reflect this difference with a value of 3 for the NAS group and a value close to 4 for the caudate group.

Biochemical results

The regional assay data for DA and NA content are presented in Table IV. Values obtained for the lesioned groups are expressed as percentages of the values from the sham operated animals. In the animals killed at 90–110 days postoperatively, the nucleus accumbens lesion produced a 79% depletion of dopamine in the nucleus accumbens itself ($U = 2$; $N_1 = 6$; $N_2 = 5$; $P < 0.01$) and in the tuberculum olfactorium ($U = 2$; $N_1 = 6$; $N_2 = 5$; $P < 0.01$). It also produced large depletions of forebrain noradrenaline (89%, $U = 0$; $N_1 = 6$; $N_2 = 5$; $P < 0.005$). The caudate

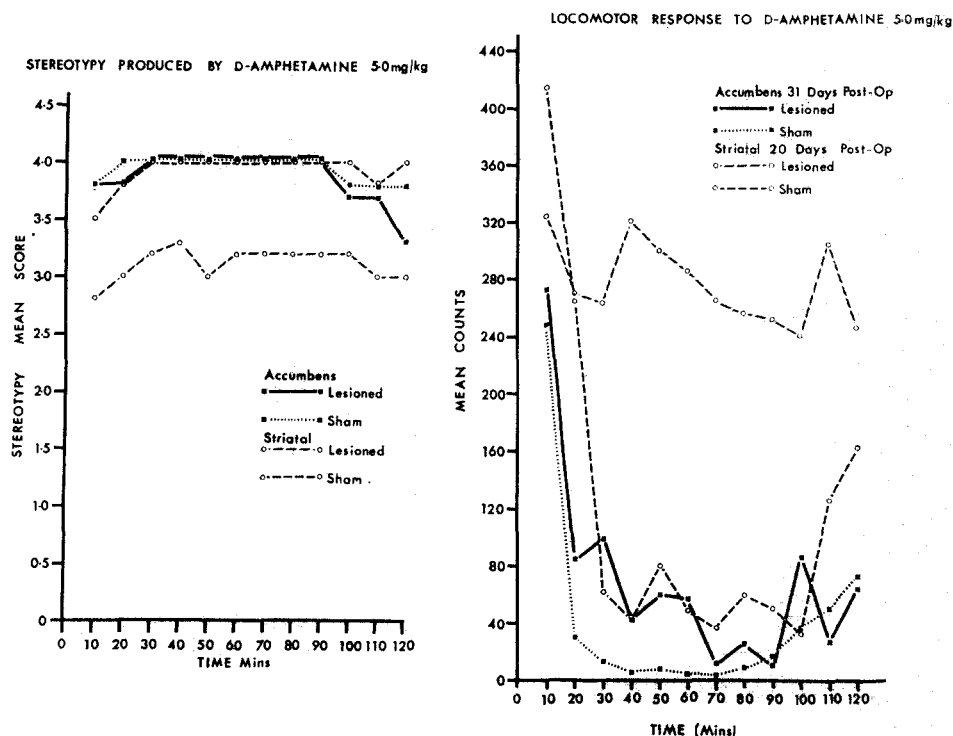


Fig. 4. Left: stereotypy response to 5.0 mg/kg D-amphetamine in caudate and nucleus accumbens 6-OHDA lesioned rats and their sham operated controls. Right: activity response to 5.0 mg/kg. D-amphetamine in caudate and nucleus accumbens 6-OHDA lesioned rats and their sham controls.

lesion resulted in only a 51% depletion of striatal dopamine ($U = 0$; $N_1 = N_2 = 6$; $P < 0.001$).

In NAS-lesioned rats assayed at 18 days after the operation a significantly greater depletion of DA from the nucleus accumbens was found ($U = 4$; $N_1 = N_2 = 6$; $P < 0.02$). However, there was not a significantly greater depletion of DA from the tuberculum olfactorium or striatum, or of forebrain NA, in NAS animals assayed at 18 days compared with those assayed at 90–110 days.

TABLE IV

REGIONAL BRAIN ASSAY FOR DOPAMINE AND NORADRENALINE EXPRESSED AS % OF VALUES IN APPROPRIATE SHAM OPERATED CONTROLS

Lesion	Dopamine levels as % of control			Noradrenaline as % of control
	Olfactory tubercle	Nucleus accumbens	Striatum	Forebrain
Caudate 90 days	90 ± 12	102 ± 13	49 ± 6	76 ± 4
Nucleus accumbens 90 days	21 ± 8	21 ± 6	90 ± 13	11 ± 2
Nucleus accumbens 18 days	22 ± 4	7 ± 2	83 ± 6	9 ± 2
Sham DA. NA. values µg/g	5.5 ± 0.64	5.28 ± 0.78	6.95 ± 0.96	0.23 ± 0.02

DISCUSSION

Low and high doses of amphetamine and 1 mg/kg of the dopamine agonist, apomorphine, have been used to probe the responsiveness of the mesolimbic and striatal dopamine systems after their selective damage with 6-hydroxydopamine. Lesions to the mesolimbic system reduced the locomotor activity response to low doses of amphetamine while resulting in an enhanced locomotor activity response to apomorphine. Stereotypy induced by high doses of amphetamine was not reduced.

By contrast, caudate lesions attenuated the stereotyped biting, licking and gnawing induced by 5 mg/kg amphetamine but did not reduce the locomotor activity induced by low doses of amphetamine.

These results verify that dopamine in the caudate is essential for amphetamine to induce intense stereotypy^{2,5,7}. The block of amphetamine stereotypy was seen most clearly when 5 mg/kg was administered at 20 days postoperatively. When apomorphine was given to the caudate-lesioned rats they showed more intense stereotypy than their sham operated controls. The block of amphetamine stereotypy observed in this study is less dramatic than that reported by Creese and Iversen⁷, probably on account of the less complete striatal DA depletion obtained in the present study. It has previously been shown that the size of the caudate lesion (and therefore presumably the degree of dopamine depletion) is positively correlated with the degree of blockage of amphetamine-induced stereotypy.

The nucleus accumbens lesions in the present study resulted in substantial depletion of dopamine from both the tuberculum olfactorium and the nucleus accumbens, but did not reduce intense amphetamine-induced stereotypy or enhance such responses to apomorphine. The fact that these animals do not show a reduction in the licking, biting and gnawing elements of intense stereotypy serves to confirm Creese and Iversen's⁷ conclusion that the tuberculum olfactorium is not concerned in the mediation of intense stereotypy. Indeed this conclusion is strengthened as the nucleus accumbens lesion in the present study resulted in more than 75% of dopamine depletion from the tuberculum olfactorium, whereas Creese and Iversen⁷ achieved only a 50% depletion.

By contrast, 14 days after the operation, the nucleus accumbens-lesioned animals showed a clear block of the locomotor activity induced by low doses of amphetamine. These lesions resulted in equally severe dopamine loss from both the tuberculum olfactorium and the nucleus accumbens and it is therefore premature to ascribe this result purely to damage of the dopamine system of the nucleus accumbens. Creese and Iversen⁷ claim that tuberculum olfactorium lesions do not block amphetamine locomotor stimulation but, only 50% of tuberculum olfactorium dopamine was depleted in that study. We cannot exclude at present the possibility that more complete and selective depletion of dopamine from the tuberculum olfactorium would block locomotor activity and sniffing produced by amphetamine. At present we favour the nucleus accumbens as the site for locomotor control for two reasons. Firstly, the assay data shows that over the postoperative period, changes in DA levels in the nucleus accumbens accompany the changing locomotor response to amphetamine,

whereas the DA levels in the tuberculum olfactorium do not change over the same period. Secondly, locomotor activity can be induced by local application of DA to the nucleus accumbens²³. However, it may be that both the tuberculum olfactorium and the nucleus accumbens contribute to the spectrum of behavioural responses to low doses of amphetamine. The tuberculum olfactorium may be important in the sniffing behaviour.

Inspection of the pattern of forebrain amine depletion in the nucleus accumbens preparation suggests a different interpretation of the result; namely that the substantial *forebrain* noradrenaline depletion in these animals underlies their changed amphetamine response. This is not considered likely as selective damage to the dorsal noradrenaline pathway does not block amphetamine-induced locomotor activity⁸. However, this interpretation can also be excluded by the use of a preparation in which DMI pretreatment is used to protect the NA systems before 6-hydroxydopamine is locally applied to the nucleus accumbens. Preliminary biochemical results on such a preparation indicate that pretreatment with pargyline and DMI result in a similar DA depletion from the nucleus accumbens and tuberculum olfactorium together with a markedly smaller reduction in forebrain NA. This preparation has now been tested with 1.5 mg/kg D-amphetamine and shows a block of locomotor activity 14 days post-operatively. This result strongly suggests that NA loss is not responsible for the block of locomotion. The conclusion is therefore that DA in the nucleus accumbens septi is essential for amphetamine to produce its characteristic increase in locomotor activity.

In several preparations in which dopamine-containing terminals were destroyed with 6-OHDA we have found that if the depletion of dopamine is sufficient to block amphetamine-induced behaviours, direct stimulation of the DA receptor will produce abnormally high levels of these behaviours. The postsynaptic sites are thought to have become 'supersensitive'. As the nucleus accumbens is seen as the site mediating locomotor activity, it was not surprising to find an enhanced locomotor response to apomorphine after the lesion. By contrast, the nucleus accumbens lesion would not be expected to show enhanced stereotypy to apomorphine. However, inspection of the frequency of occurrence of the different classes of stereotypy in the two lesion groups revealed that the nucleus accumbens group showed an enhancement of stereotypy rating 3 whereas the caudate-lesioned animals showed a peak in rating 4. Rating 3 scores continuous stereotypy over a wide area of the cage. By observation this is an active, sniffing rat. Sniffing is usually described as an element of the stereotypy response to amphetamine. However, it is suggested that sniffing is an exploratory act of behaviour which is closely coupled to locomotor behaviour in the environment. The mesolimbic dopamine system may therefore be considered as a neural substrate for certain aspects of investigative behaviour⁴. If this is so, the earlier results of Simpson and Iversen²⁶ that electrolytic lesions to the substantia nigra, which resulted in more than 50% depletion of striatal dopamine, did not abolish stereotyped neck movements could be explained. In retrospect the finding was surprising, as similar damage to the caudate has been found to block stereotypy^{10,12}. However, it seems that in

selecting neck movements as a measure of stereotypy, they may have been studying a function of the mesolimbic rather than the striatal dopamine system.

Finally, there is evidence in these results that the behavioural responses to amphetamine and apomorphine in the NAS animals change over the postoperative period. The block of amphetamine-induced locomotor activity and the enhanced locomotor response to apomorphine, were maximal on test day 14. By 80 days considerable attenuation of both of these effects was observed. The recovery of these effects appears to correlate with recovery of DA levels in the nucleus accumbens since we observed that the DA level was less at 18 days than at 90–110 days. DA levels in the tuberculum olfactorium did not show this difference, suggesting that the damage to DA systems in the NAS rather than in the tuberculum olfactorium correlated more closely with the block and subsequent recovery of drug-induced locomotor activity. It appears that between 18 and 90 days some functionally significant recovery takes place in the mesolimbic dopamine system. The anatomical and biochemical nature of this recovery remains to be explored.

It has been suggested that dysfunction of forebrain DA systems may be related to psychotic disorders^{1,21}. The drug-induced rotational behaviour of rats with unilateral lesions of the nigro-striatal pathways has proved a useful *in vivo* model for assessing the effects of drugs which interact with striatal DA receptors^{17,30,31}. The supersensitive mesolimbic-lesioned preparation may prove to be an equally useful model system for assessing the effects of neuroleptics and DA agonists on mesolimbic DA receptors¹⁸.

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