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THE PHARMACOLOGICAL AND ANATOMICAL SUBSTRATES OF THE AMPHETAMINE RESPONSE IN THE RAT

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SUMMARY

Bilateral 6-hydroxydopamine microinjections into the substantia nigra abolished both the locomotor *and* stereotypy responses to D-amphetamine in adult rats. The lesions resulted in a depletion of over 99% of striatal tyrosine hydroxylase activity (indicating a near total lesion of the nigro-striatal dopamine pathway) as well as severe noradrenaline depletions. However, lesion of the dorsal or ventral noradrenergic pathways resulted in similar noradrenaline depletions but with no effect on striatal tyrosine hydroxylase levels and without the concomitant blockage of the amphetamine response. The substantia nigra lesioned rats were behaviourally supersensitive to apomorphine and L-DOPA and did not show a locomotor response to cocaine. The substantia nigra lesioned rats were not aphagic or adipsic. It was concluded that both the locomotor and stereotyped responses induced by amphetamine are dependent on the functional integrity of the nigro-striatal dopamine pathway.

INTRODUCTION

Amphetamine influences the uptake and release of the catecholamines, noradrenaline (NA) and dopamine (DA), in the brain[4,5,16,25,42]. It has been shown that the inhibition of catecholamine synthesis abolishes the behavioural effects of amphetamine suggesting that a release of catecholamines may mediate these effects[33,43].

Since amphetamine interacts with both NA and DA, interest has attached to the relative roles of these two amines in the production of the drug-induced behaviour-

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al changes. Low doses of amphetamine increase running behaviour in the rat, while at higher doses stereotyped motor responses such as gnawing or repetitive sniffing predominate.

Over the past few years many experimenters have endeavoured to find out if one or other or both of the catecholamine transmitters mediates these two highly characteristic and dose-dependent behavioural effects of amphetamine. There is strong evidence that DA rather than NA is centrally involved in the elicitation of stereotyped behaviours[14,28]. However, the evidence that NA is more important than DA in mediating the stimulation of locomotor activity[2,15,24,36,44] is less compelling, much evidence favouring their joint involvement in this behaviour[6,23,31,34] or the primacy of dopaminergic mechanisms[7,8,29,32,37].

We have attempted to investigate this question with both pharmacological and anatomical techniques. The amine transmitters are localized in different anatomical systems in the brain: NA in a dorsal pathway innervating mainly the cortex and limbic structures and in a ventral pathway innervating the hypothalamus; and DA principally in the nigro-striatal (NS) pathway[38]. It is possible to selectively lesion these pathways by stereotaxic injections of 6-hydroxydopamine (6-OHDA)[39,41]. The functional integrity of the remaining amine systems can then be examined by assessing the behavioural responses of lesioned animals to pre- and postsynaptic pharmacological manipulations of the catecholamine transmitters.

In this report evidence is presented which suggests that the integrity of the nigro-striatal DA pathway is necessary for amphetamine to induce stereotyped behaviour *and* stimulate locomotor activity in the rat.

METHODS

Subjects. Male albino Wistar rats were housed in groups of three with free access to food and water. The rats were maintained in controlled conditions with a 12 h light–dark cycle (8 a.m.–8 p.m.).

Surgery. The rats were anaesthetized with Equithesin (3.75 ml/kg) i.p. Nine rats (mean preoperative weight 260 g) received bilateral injections of 6-OHDA into the substantia nigra (SN) stereotaxic coordinates AP2.8, L2.0, V8.0[27], in order to lesion the nigro-striatal DA pathway. Nine rats received the full sham procedure of vehicle injection into the SN. Six rats (mean preoperative weight 150 g) received bilateral injections of 6-OHDA into the dorsal NA pathway in the mesencephalon, stereotaxic coordinates AP2.2, L0.8, V5.3[21] and 6 rats received the full sham procedure. Six rats (mean preoperative weight 150 g) received bilateral injections of 6-OHDA into the ventral NA pathway in the mesencephalon, stereotaxic coordinates AP1.0, L1.5, V6.0[21] and 6 rats received the full sham procedure.

Stereotaxic injections were made from a microsyringe through a 30-gauge cannula. The total injection volume was 2 μl on each side injected over a 2 min period.

Drugs. 6-Hydroxydopamine hydrochloride (AB Biotec) was dissolved in a vehicle solution of ice-cold, 0.9% saline (pH 4.5) with 1 mg/ml ascorbic acid as an antioxidant. 6-Hydroxydopamine (8 μg, free base) in 2 μl, or an equal volume of vehicle

solution was injected in each side. D-Amphetamine sulfate (Smith, Kline and French), apomorphine hydrochloride (MacFarlan Smith), L-DOPA methylester (AB Biotec), RO4-4602 (Roche) and cocaine (May and Baker Ltd.) were dissolved in 0.9% saline and injected via the intraperitoneal route.

Behavioural testing. The animals' response to drug manipulations was measured in a bank of 12 wire cages (25 cm × 40 cm × 20 cm), each with two horizontal photocell beams along the long axis, in a room which was masked with white noise and maintained at 22 °C. Non-cumulative recordings of photocell beam interruptions, for individual animals, were taken every 10 min in each experimental session. All testing commenced at 10 a.m. The rats were habituated to the photocell cages for 1 h and their spontaneous activity was measured during these habituation periods. A drug injection was then made.

Low dose levels of amphetamine stimulate locomotor activity in the rat and the beam interruptions recorded in the photocell cages are a reliable measure of the degree of stimulated activity. However, high doses of amphetamine or the administration of apomorphine produce stereotyped responses such as continuous sniffing in one location. This stereotyped behaviour could not be quantified reliably by photocell beam interruptions since intense stereotypy with little locomotor activity yielded low counts, while less intense stereotypy associated with a greater degree of locomotor activity might yield high or low counts. A stereotypy rating scale, previously developed from observations of the behaviour of normal rats exposed to increasing doses of amphetamine was used: 0, asleep or stationary; 1, active; 2, predominantly active but with bursts of stereotyped sniffing or rearing; 3, stereotyped activity such as sniffing along a fixed path in the cage; 4, stereotyped sniffing or rearing maintained in one location; 5, stereotyped behaviour in one location with bursts of licking; 6, continual gnawing or licking of the cage bars.

During all of the drug studies observations of each animal's behaviour were made at 10 min intervals and their stereotypy response rated.

The response of the SN lesioned rats to apomorphine (1 mg/kg) was measured on days 1, 2, 7, 10, 18, 35 and 48 post-operation; to D-amphetamine (1.5 mg/kg) on days 3, 14, 22 and 33; to cocaine (20 mg/kg) on day 39; and to L-DOPA (5 mg/kg, + RO4-4602 50 mg/kg 30 min previously) 50 days post-operation. The response to D-amphetamine (1.5 mg/kg) was also measured on day 52 after 48 h of food and water deprivation.

Biochemical methods. At the end of behavioural testing the animals were killed by decapitation and the brains rapidly removed, chilled and dissected into regions according to the method of Glowinski and Iversen[17]. NA was isolated from the hypothalamus and cortex and assayed fluorimetrically according to the method of Evetts *et al.*[13]. The striatum was assayed for tyrosine hydroxylase (TOH) activity by a radiochemical technique[18]. This enzyme is highly localized in aminergic neurones, and can be used as an index of the extent of destruction of the nigro-striatal DA neurones induced by the 6-OHDA treatment[41].

Analysis of results. Regional assay data were analyzed utilizing the Student's *t*-test, distribution of scores among the stereotypy rating categories using the Mann–

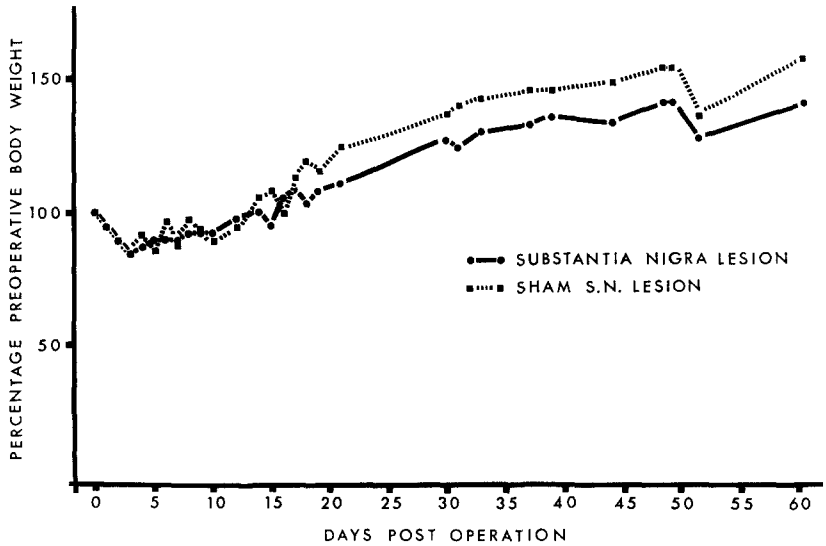

Fig. 1. Weight gain expressed as percent preoperative body weight of the substantia nigra lesioned rats and sham operated controls over the period of drug testing following lesion. The rats were completely food and water deprived for 3 days immediately after operation. The anorexic SN lesioned rats were fed on a palatable diet and the controls maintained on a restricted diet to match body weights. All rats were deprived at day 50 for 48 h.

Whitney U-test, and the distribution of photocell beam interruptions over time was subjected to Analysis of Variance.

RESULTS

Substantia nigra lesion

Body weight, food and water intake. Substantia nigra (SN) lesioned rats are usually severely aphagic and adipsic. In order to demonstrate that any initial changes in the drug induced behaviours were not simply due to the greater decrement in weight of the SN lesioned rats compared to their controls, both the SN lesioned rats and their sham lesioned controls were completely deprived of food and water for 3 days after operation. After this period the SN lesioned rats were fed on a diet of wet mash, chocolate chip cookies and normal lab chow and water *ad lib.* At no time was it necessary to tube feed the SN lesioned rats. It can be seen from Fig. 1 that the SN lesioned rats were able to increase their body weight in a regular fashion on this diet. They were anorexic but not aphagic and adipsic. The control rats were maintained on a restricted diet of lab chow and water in order that their weight gain would be of the same order as that of the SN lesioned rats.

Spontaneous motor activity. Throughout the study the SN lesioned rats were significantly less active than the controls during the habituation periods ($F = 12.33$; $df\ 1,12$; $P < 0.01$) (Fig. 2b). Within a session the SN lesioned rats did exhibit a normal pattern of habituation from their lower baseline of activity (Fig. 2a). At day 52 post-

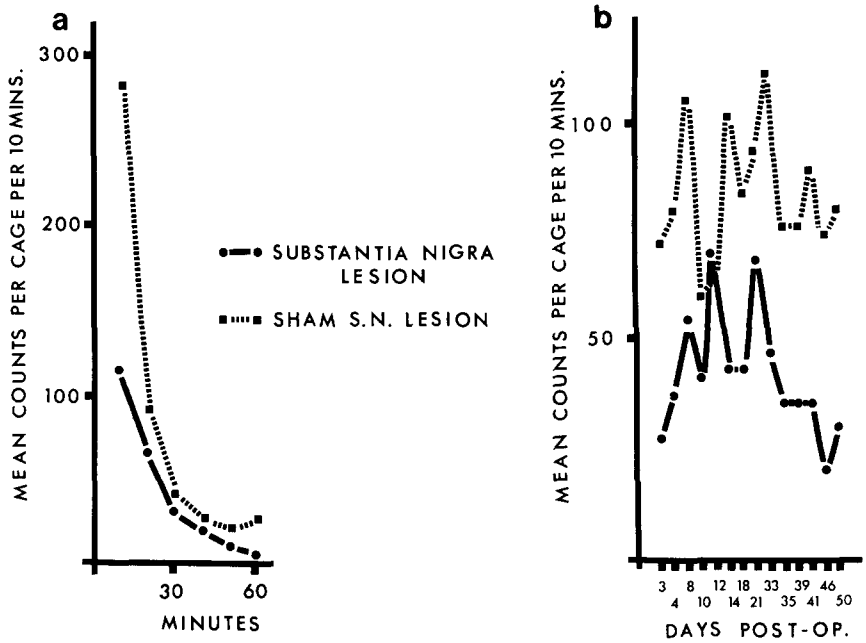

Fig. 2. a: habituation of spontaneous locomotor activity of SN lesioned and sham operated rats averaged over all habituation sessions. b: mean total activity recorded in each habituation session (1 h) on the post-operative days listed for SN lesioned and sham operated rats. The SN lesioned rats were significantly less active than the controls.

operation after 48 h food and water deprivation the control rats showed a significant increase in their spontaneous activity above their activity measured 2 days before deprivation ($F = 11.86$; df 1,72; $P < 0.01$) recording a mean of 124 beam interruptions/10 min compared to their non-deprived mean of 77. The SN lesioned rats showed no change in their mean spontaneous activity ($F = 0.293$; df 1,72) recording means of 27 and 23 beam interruptions respectively.

Amphetamine stimulated behaviour. The lesioned rats and controls were tested with 1.5 mg/kg D-amphetamine 3, 14, 22 and 33 days post-operation. This dose of amphetamine stimulates locomotor activity in normal rats and does not induce stereotypy.

The SN lesioned rats could be classified into 2 distinct groups as regards their amphetamine stimulated behaviour, a grouping which corresponded to the degree of DA terminal degeneration as measured by the TOH depletion in the striatum. The group with severest DA terminal degeneration (less than 0.5% remaining TOH activity) exhibited a completely modified response to 1.5 mg/kg D-amphetamine at day 3 post-operation. Whereas the control rats exhibited only a stimulation of locomotor activity these 7 SN lesioned rats showed vigorous stereotypy within 10 min of amphetamine injection. This stereotyped sniffing and gnawing was interspersed with violent bursts of locomotor activity. The SN lesioned rats also exhibited a behaviour not seen before of clinging to the top of the wire cages with their teeth and

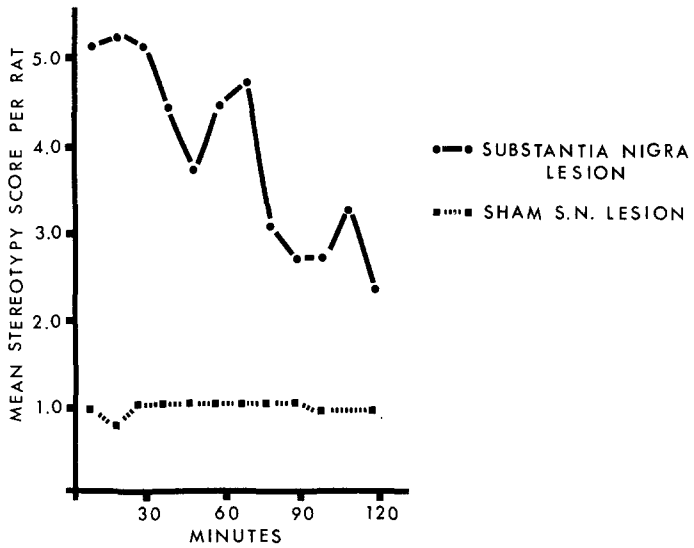

Fig. 3. Stereotypy response to 1.5 mg/kg D-amphetamine at 3 days post-operation recorded for the 7 complete SN lesioned rats and sham operated controls. The enhanced stereotypy response to amphetamine was not seen in the SN lesioned rats in any of the later amphetamine test sessions.

swinging along from the top of the cages, holding on with their forepaws and teeth. The behaviour was similar in some ways to 'obstinate progression' for, if placed in an open space, the rats would run in a straight line and 'jump up' the wall when it was reached, or if wire netting was placed in front of them, they would climb the netting even to the extent of continuing upside-down if the netting was bent over at the top. The stereotyped behaviour was maximal in the first hour and then progressively declined over the second hour (Fig. 3).

On subsequent test days these same rats showed no response to 1.5 mg/kg D-amphetamine. Little locomotor activity was seen and none of the modified stereotypy seen at 3 days post-operation was observed. The photocell beam interruptions with time over the 4 test sessions for these SN lesioned rats and controls are shown in Fig. 4. The SN lesioned rats were overall significantly less active than the controls ($F = 57.73$; df 1,12; $P < 0.001$). The control rats showed normal stimulated activtiy over the 4 test sessions and exhibited no stereotypy.

The responses of the remaining SN lesioned rats with the less complete lesion (20% remaining TOH activity in the striatum) were different (Fig. 5). These rats exhibited vigorous locomotor activity on all 4 drug sessions, recording a mean activity that was twice that of the control rats. They showed none of the bizarre stereotypy seen in the SN lesioned rats with the near complete degeneration of the nigro-striatal pathway at day 3 post-operation but they did show stereotyped locomotor activity (score 3 on the rating scale) at this time.

At day 52 post-operation, after 48 h food and water deprivation, the control rats significantly increased their locomotor activity response to amphetamine com-

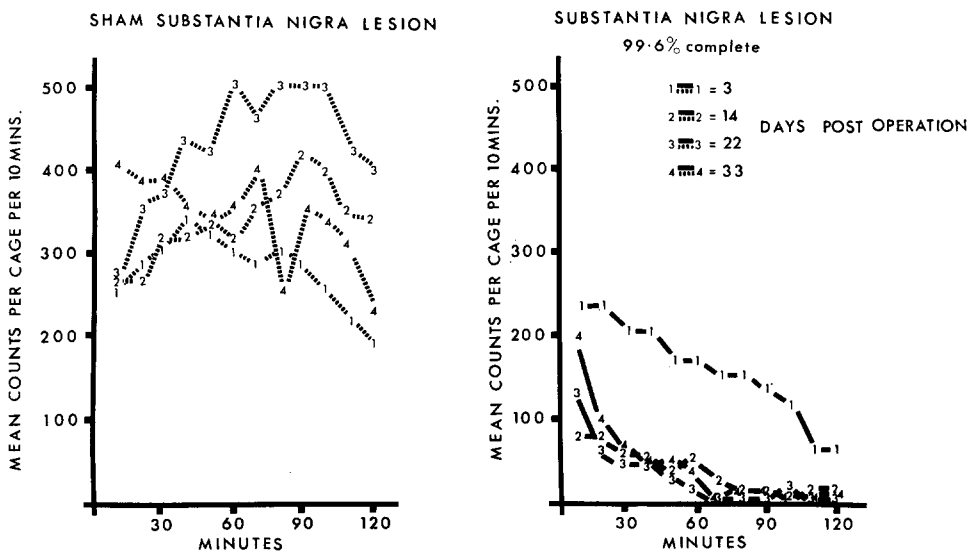

Fig. 4. Mean photocell beam interruptions/10 min for the sham operated and the 7 complete SN lesioned rats to 1.5 mg/kg D-amphetamine recorded on days 3, 14, 22 and 33 post-operation. The characteristic locomotor activity response to amphetamine was abolished in the SN lesioned rats. The response on day 3 in these animals records the presence of intense stereotypy.

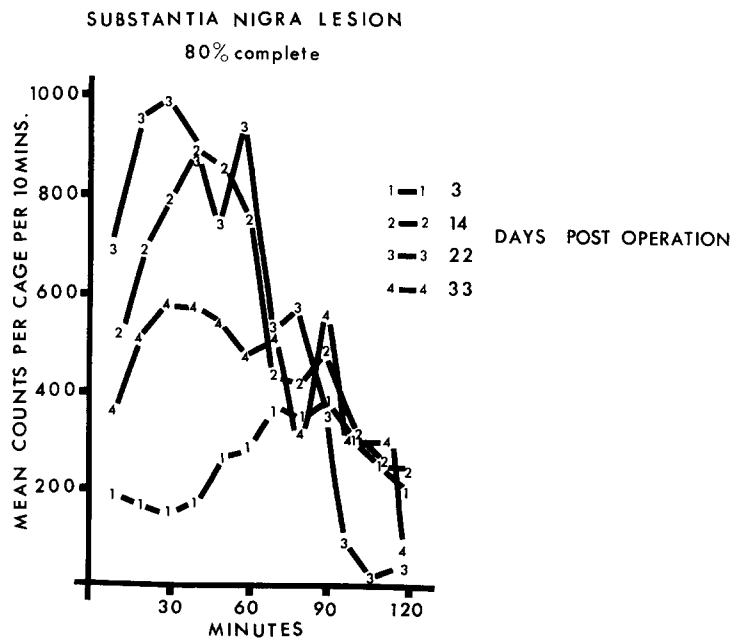

Fig. 5. Mean photocell beam interruptions/10 min for the 2 incomplete SN lesioned rats to 1.5 mg/kg D-amphetamine recorded on days 3, 14, 22 and 33 post-operation. These animals showed an enhanced locomotor activity response to amphetamine.

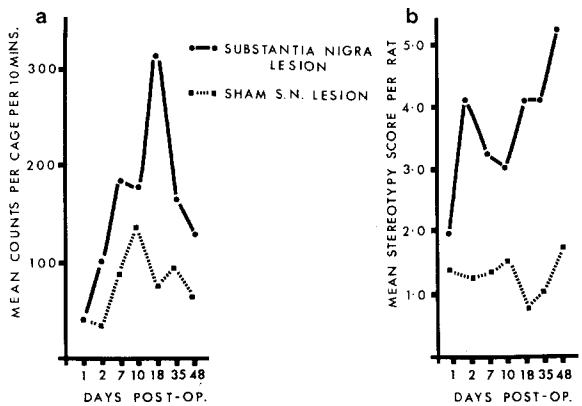

Fig. 6. a: mean total photocell beam interruptions/10 min for the 7 complete SN lesioned rats and sham operated controls to 1 mg/kg apomorphine on the post-operative days listed. b: mean total stereotypy response for the 7 complete SN lesioned rats and sham operated controls to 1 mg/kg apomorphine on the post-operative days listed. The SN lesioned rats showed a significantly enhanced response on both measures compared to their sham operated controls.

pared to their response at 33 days post-operation ($F = 4.53$; df 1,144; $P < 0.05$) but there was no significant change in the activity of the SN lesioned rats.

Apomorphine stimulated behaviour

The response of the SN lesioned and control rats to 1 mg/kg apomorphine was examined on days 1, 2, 7, 10, 18, 35, and 48 post-operation and to 0.5 mg/kg apomorphine on day 12.

On post-operative day 1, 1 mg/kg apomorphine elicited the same number of beam interruptions/10 min in the SN lesioned rats and controls with means of 42 and 41 respectively. However, on each of the subsequent tests with this dose of apomorphine, the response of the SN lesioned rats was significantly higher than that of the controls ($F = 16.05$; df 1,12; $P < 0.01$) (Fig. 6a). The magnitude of the response in the lesioned rats increased greatly up to day 18 and then declined, while the response of the controls also increased but to a much lesser extent. However, the time course of this change in activity over sessions was significantly different between the two groups ($F = 4.78$; df 6,72; $P < 0.001$).

The stereotypy response of the SN lesioned rats was significantly greater than that of the controls except on day 1 (Fig. 6b). The stereotypy of the SN lesioned rats was different in character from that of the controls. The same 'climbing' behaviour seen with 1.5 mg/kg D-amphetamine 3 days post-operation was also elicited by apomorphine on all days except day 1. A number of rats also showed bursts of very fast and intense locomotor activity interspersed with jumps onto the side walls of the cage. The data presented are for the 7 complete SN lesioned rats. However, the response of the incomplete SN lesioned rats was not qualitatively different although they did show somewhat greater increases in locomotor activity.

The response of the SN lesioned rats to 0.5 mg/kg apomorphine was also signif-

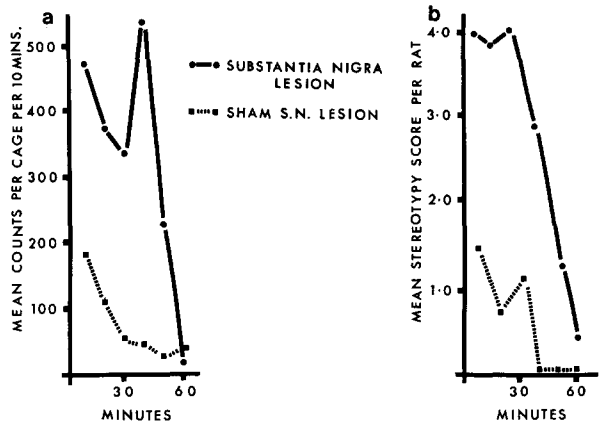

Fig. 7. a: mean photocell beam interruptions/10 min for the 7 complete SN lesioned rats and sham operated controls to 0.5 mg/kg apomorphine. b: mean stereotypy response for the 7 complete SN lesioned rats and sham operated controls to 0.5 mg/kg apomorphine. The SN lesioned rats showed a significantly enhanced response on both measures.

icantly greater than that of the controls both in terms of beam interruptions ($F = 41.65$; df 1,12; $P < 0.001$) (Fig. 7a), and stereotypy rating ($P < 0.001$) (Fig. 7b).

Cocaine stimulated behaviour. Thirty-nine days post-operation the SN lesioned rats and controls were tested with cocaine (20 mg/kg). The control rats' activity was significantly stimulated (226 counts/10 min) above their saline baseline (29 counts/10 min) ($F = 107.17$; df 1,144; $P < 0.001$). Their activity was significantly greater than that of the SN lesioned rats who recorded a mean of 35 counts/10 min ($F = 19.40$; df 1,12; $P < 0.001$). The control rats exhibited no stereotyped behaviour but showed increased activity similar to that seen with a low dose of amphetamine (Fig. 8).

L-DOPA stimulated behaviour. Fifty days post-operation the SN lesioned rats and controls were pretreated with RO4-4602 (50 mg/kg), a peripheral decarboxylase

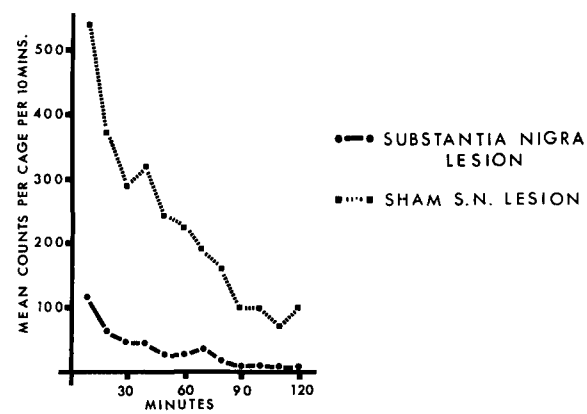

Fig. 8. Mean photocell beam interruptions/10 min for the 7 complete SN lesioned rats and sham operated controls to 20 mg/kg cocaine. The control rats showed a significantly greater response than the SN lesioned rats.

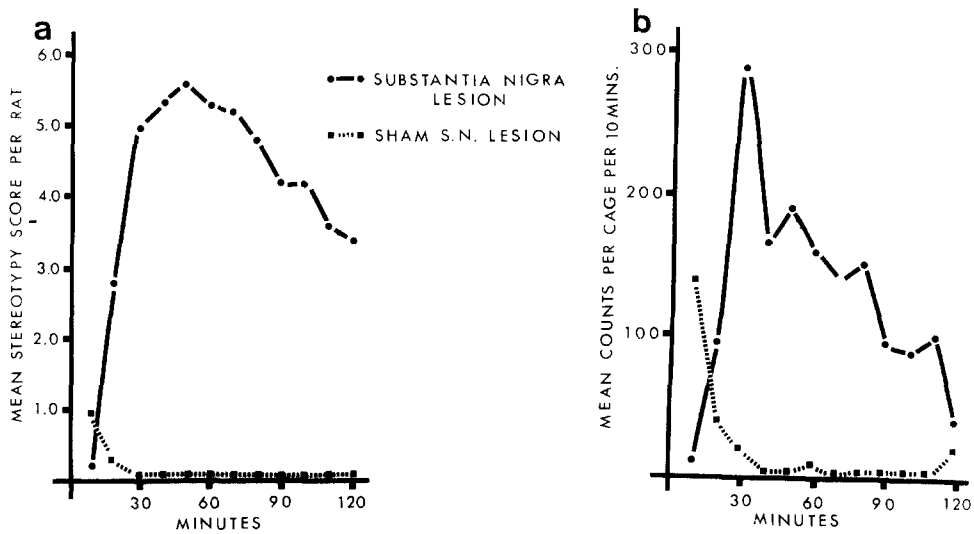

Fig. 9. a: mean stereotypy response to 5 mg/kg L-DOPA (+ RO4-4602 50 mg/kg) for the 7 complete SN lesioned rats and sham operated controls. b: mean photocell beam interruptions/10 min to 5 mg/kg L-DOPA (+ RO4-4602 50 mg/kg) for the 7 complete SN lesioned rats and sham operated controls. The SN lesioned rats showed a significantly enhanced response on both measures.

inhibitor, 30 min before being injected with L-DOPA (5 mg/kg). Within the first 30 min after the L-DOPA injection the SN lesioned rats were showing vigorous stereotypy with gnawing of their own paws and the modified climbing behaviour seen with apomorphine treatment and amphetamine on day 3 (Fig. 9a). The control rats showed no stimulation of either locomotor activity or stereotypy. The SN lesioned rats recorded significantly more beam interruptions (136/10 min) than the controls (21/10 min) ($F = 11.133$; df 1,12; $P < 0.01$) (Fig. 9b), the SN lesioned rats being significantly stimulated above their baseline activity to saline injection ($F = 104.13$; df 1,114; $P < 0.001$), while the controls were not significantly different from their baseline activity ($F = 2.12$; df 1,144).

Dorsal and ventral noradrenaline bundle lesions

Body weight. All animals gained weight in a normal fashion after operation and there was no indication of any gross disturbance in food and water intake. There were no significant differences in body weight between the lesion and control groups after operation.

Spontaneous motor activity. Fifteen days after operation, the dorsal and ventral bundle lesioned rats and their sham operated controls were habituated to the photocell cages for two 2-h periods on alternate days. There was no significant difference in mean activity over these two habituation periods between either the dorsal and sham dorsal lesioned groups ($F = 3.46$; df 1,10) or the ventral and sham ventral lesioned groups ($F = 2.18$; df 1,10) — the respective mean activity scores being 70, 95, 65 and 89 beam interruptions/10 min. All groups showed habituation of their spontaneous activity throughout the test periods, with a small but significant difference in the overall

TABLE I

REGIONAL ASSAY DATA FROM 6-OHDA SUBSTANTIA NIGRA LESIONED, DORSAL AND VENTRAL NORADRENALINE PATHWAY LESIONED RATS AND SHAM OPERATED CONTROLS

The control absolute enzyme activities and noradrenaline levels were: for the sham substantia nigra lesion–striatum TOH: 15.6 nmoles DOPA formed/h/g (subsaturating concentration of substrate); hypothalamus NA 1.4 μg/g; cortex NA 0.10 μg/g. For the sham dorsal NA pathway lesion–striatum TOH: 12.6 nmoles DOPA formed/h/g; hypothalamus NA 1.5 μg/g; cortex NA 0.14 μg/g; and for the sham ventral NA pathway lesion–striatum TOH: 16.8 nmoles DOPA formed/h/g; hypothalamus NA 1.8 μg/g; cortex NA 0.20 μg/g.

	Striatum *Tyrosine hydroxylase activity* *Expressed as % control values*	*Hypothalamus* *Noradrenaline*	*Cortex* *Noradrenaline*
Substantia nigra (N = 7) (Blocked amphetamine response)	0.4 ± 0.2%**	14.6 ± 1.5%**	36.4 ± 8.2%**
Substantia nigra (N = 2) (Enhanced amphetamine response)	19.2 ± 5.7%**	7.6 ± 0.1%**	46.9 ± 27.5%*
Sham substantia nigra (N = 7)	100 ± 7.6%	100 ± 5.2%	100 ± 25.5%
Dorsal NA pathway (N = 6)	129.7 ± 7.6%*	37.6 ± 9.1%**	23.3 ± 3.5%**
Sham dorsal NA pathway (N = 5)	100 ± 6.8%	100 ± 7.6%	100 ± 6.3%
Ventral NA pathway (N = 6)	94.4 ± 8.6%	18.1 ± 2.4%**	12.8 ± 1.5%**
Sham ventral NA pathway (N = 6)	100 ± 3.8%	100 ± 7.8%	100 ± 7.8%

* $P < 0.05$; ** $P < 0.001$; Student *t*-test.

time course of habituation between the dorsal and sham dorsal operated groups ($F = 2.14$; *df* 11,110; $P < 0.05$), the dorsal lesioned group showing a slightly faster habituation. The rats were then injected with saline (1 ml/kg) on the next alternate day. There were no significant differences in mean activity to saline between the groups ($F = 1.22$ and $F = 1.82$; *df* 1,10). However, both groups showed significant differences in the time course of their activity compared to their sham operates, for the dorsal *vs.* sham dorsal group ($F = 1.90$; *df* 11,110; $P < 0.05$) and for the ventral *vs.* sham ventral group ($F = 2.88$; *df* 11,110; $P < 0.01$), the lesioned groups showing a slightly faster return of activity to baseline levels. The rats were then tested with amphetamine (1.5 mg/kg and 5 mg/kg) and apomorphine (1 mg/kg).

Amphetamine stimulated behaviour. There was no significant difference in the mean activity stimulated by 1.5 mg/kg D-amphetamine in either the dorsal and sham dorsal lesioned groups with respective mean activity scores of 317 and 315 beam interruptions/10 min ($F = 0.00$; *df* 1,10) or the ventral and sham ventral lesioned groups with mean scores of 269 and 294 ($F = 0.26$; *df* 1,10). Neither were there any significant differences in the time course of the stimulated activity.

There were no significant differences in the mean activity between either lesioned group and its sham operated controls to 5 mg/kg D-amphetamine, either in the mean activity over the 2-h test period (for the dorsal lesion $F = 0.96$ and the ventral lesion $F = 0.97$; both df 1,10) or the time course of activity (for the dorsal lesion $F = 0.89$ and ventral lesion $F = 1.27$; df 11,110). All groups showed the characteristic decrement in beam interruptions over the first hour of the session as vigorous stereotypy occurred with the rats maintaining one location in the cage as they engaged in stereotyped sniffing.

Apomorphine stimulated behaviour. There was no significant difference in either the mean activity score, time course of activity or intensity and time course of stereotyped behaviour stimulated by 1 mg/kg apomorphine in the ventral and sham ventral lesioned rats. The dorsal lesioned group was significantly less active than their sham operated controls recording a mean of 38 beam interruptions/10 min compared to 89 ($F = 49.6$; df 1,10; $P < 0.001$). However there was no difference in the time course of the activity and there was no significant difference in the stimulated stereotyped behaviour.

Biochemical results. The results of the regional assays are presented in Table I. It is noteworthy that the substantia nigra lesioned rats which showed a blocked amphetamine response had only 0.4% remaining TOH activity in the striatum, indicating an almost complete lesion of the nigro-striatal DA pathway. The selectivity of the NA lesions was good inasmuch as they did not encroach upon the DA pathway to the striatum. The elevated TOH levels in the dorsal NA pathway lesioned group as expressed as % sham operated control probably indicated that the sham operated group's TOH levels were themselves lower than normal as can be seen from the absolute enzyme activities. In fact both the dorsal and the ventral NA pathway lesioned rats had similar absolute values of TOH activity. These results demonstrate the difficulty of making selective lesions within the NA systems at the axonal level, where spread of 6-OHDA can occur to the other proximal NA pathway.

DISCUSSION

These results, in agreement with earlier studies[10], indicate that a 90% depletion of striatal DA is not sufficient to abolish the locomotor response to amphetamine. By contrast, virtually total DA depletion, whether achieved by neonatal 6-OHDA treatment[11] or, as in the present study, by local injections of 6-OHDA into the SN of the adult rat, does block this response.

That the behavioural responses elicited by amphetamine are blocked by lesion to the nigro-striatal DA pathway, while the DA receptors are still functional (as indicated by presence of response to the DA agonist apomorphine) demonstrates that amphetamine is an indirect sympathomimetic agent acting presynaptically by releasing DA or blocking its reuptake, both actions serving to increase functional levels of DA at the receptor.

Cocaine also failed to stimulate locomotor activity in the SN lesioned rats, while it did so in controls. Cocaine has been shown to be an indirectly acting sympa-

thomimetic agent, acting by inhibiting the reuptake of DA and NA[30]. This is supported by these results where, without functional DA terminals in the striatum of the SN lesioned rats, cocaine did not stimulate locomotor activity. The low levels of activity of the SN lesioned rats to both cocaine and amphetamine correlated significantly (rho = 0.983) indicating that both drugs were having their minimal effects through the same mechanism or that the striatum was the final common output for the locomotor activity initiated by these two drugs.

The adult 6-OHDA SN preparations described in this study have provided further evidence on the pharmacological and behavioural responses of rats with substantial damage to terminal DA systems in the striatum. The responses in these preparations were variable during the immediate post-operative period but stabilized into two forms (i) those with some intact presynaptic terminals and supersensitive postsynaptic mechanisms and, (ii) those in which it was impossible to demonstrate behavioural effects with presynaptically acting drugs, such as amphetamine or cocaine, but which showed supersensitivity to postsynaptically acting agents.

Immediate postoperative responses to drugs. At day 3 post-operation, the nigrostriatal DA system was still in a state of flux. The DA receptors in the striatum were functionally denervated at day 1, since it has been shown that injection of 6-OHDA into the SN stops impulse flow in the NS neurones[39]. However, the DA terminals in the striatum do not degenerate immediately and maintain a store of DA[39]. At day 3 post-operation it is apparent from the enhanced locomotor and stereotyped responses observed in the SN lesioned rats that amphetamine was able to release this stored DA in the terminals of the degenerating NS neurones and that the DA receptors in the striatum were already supersensitive. Continuous observations and photocell beam interruptions were recorded for the SN lesioned rats during the first 3 days post-operation. The SN lesioned rats did not provide evidence of any higher activity than controls throughout this period which would have indicated the spontaneous release of DA from the degenerating neurones.

Partial SN lesions. We have previously shown that a 6-OHDA lesion to the substantia nigra is capable of blocking the stereotyped behaviours elicited by amphetamine[10]. These animals also showed a modified locomotor response to a low dose of amphetamine, with an enhanced response over the first hour after injection. TOH assay of the striata of these SN lesioned rats indicated that about 10–15% of the DA terminals in the striatum were still intact. These rats are thus comparable both pharmacologically and behaviourally to the two SN lesioned rats in this study which did not show a blocked locomotor response to amphetamine. It would thus appear that stereotyped behaviour requires a high degree of DA receptor activity in the striatum whereas locomotor activity can be elicited with a lower DA receptor activation.

The enhanced locomotor response seen in the SN lesioned rats with between 10–20% remaining DA terminals in the striatum could be due to the release of DA from these intact terminals stimulating both their own receptors and the receptors of terminals destroyed by the SN lesion. The apomorphine results indicated that these denervated DA receptors were supersensitive — thus leading to the enhanced locomotor response. However, the lesion must have been of a sufficient size such that the

maximal DA release achieved by high dose levels of amphetamine was insufficient to give rise to stereotyped behaviour.

Supersensitivity after total SN lesions. The total SN lesioned rats still responded to apomorphine with both locomotor and stereotyped activity indicating that the efferent components of the motor system were still intact. The enhanced response would appear to be the result of supersensitive DA receptors in the striatum. The response increased with time, although by day 2 post-operation there were indications that the change in sensitivity was already occurring. If the enhanced response to apomorphine were simply due to the removal of uptake sites for apomorphine as the DA terminals degenerated, thus increasing the functional concentration of apomorphine at the DA receptors, it would be expected that the behavioural response would have maximized at an earlier time as soon as degeneration was complete. In fact apomorphine has not been shown to be a competitive inhibitor of DA uptake in the striatum[35] which again argues against this hypothesis. The SN lesioned rats were shown to be behaviourally supersensitive to apomorphine but pharmacological supersensitivity of the receptors was not demonstrated directly. It is possible that the enhanced response was produced by a disruption of the balance of neuronal systems in the CNS but at present the most parsimonious explanation is that of denervation supersensitivity of the DA receptors in the striatum.

The increased sensitivity of the SN lesioned rats to DA receptor activity was also illustrated by their response to L-DOPA which is converted to DA in the CNS[1]. This result directly demonstrates the possible therapeutic value of L-DOPA in severe Parkinsonism where almost complete degeneration of the NS pathway has occurred. In view of the paucity of remaining DA terminals, L-DOPA is not likely to be acting by simply increasing intraneuronal DA levels in the few remaining intact DA terminals. More probably it is converted to DA in either 5-HT neurones[26] or in the walls of cerebral blood vessels[3] and 'spilling over' onto the supersensitive DA receptors then occurs.

Finally, these results also have bearing on two controversial issues: (i) have we now demonstrated that DA is central to the amphetamine locomotor response as well as stereotypy? (ii) why were these animals with total DA depletion not severely aphagic or adipsic?

(i) DA depletion and locomotor activity. Our only previous preparation to show a block of both stereotypy and locomotor activity was the 6-OHDA neonate preparation[11] which resulted in substantial forebrain NA depletion as well as total striatal DA depletion. In interpreting these earlier results we therefore felt obliged to consider the possibility of a central involvement of NA mechanisms in the locomotor response to amphetamine.

It can be seen from the present biochemical data that the amphetamine-blocked SN lesioned rats, besides being severely DA depleted, also had low levels of NA in the hypothalamus. However, the SN lesioned rats which did not show a blocked amphetamine response had an even lower level of hypothalamic NA. We have previously reported that neonatal treatment with intraventricular 6-OHDA resulted in adult rats with a blocked amphetamine response[11]. These rats also showed DA

degeneration in the striatum (mean TOH activity 2% control) but showed hypothalamic levels of NA which were twice those of the blocked SN lesioned rats in this study. The dorsal and ventral NA pathway lesioned rats showed no change in their amphetamine response although both groups had lower cortical levels of NA than the SN lesioned rats and hypothalamic NA levels which were the same as either the blocked SN lesioned rats or the blocked neonatally 6-OHDA treated rats of the previous study. The dorsal and ventral NA pathway lesioned rats did show slight but significant modifications of their spontaneous and drug induced locomotor behaviour, suggesting that NA exerts a modulatory influence on motor behaviour, but that its role is secondary to that of DA.

These results appear to rule out any obvious correlation between NA levels and the blockage of amphetamine stimulated behaviour observed in our various studies of the nigro-striatal pathway.

However, it remains true that a preparation in which amphetamine induced locomotor activity is blocked and in which there is a *selective and total* DA depletion has yet to be described.

The hypothesis that the DA nigro-striatal pathway is critically involved in both locomotor and stereotyped behaviour stimulated by amphetamine is supported by many biochemical studies[7,8,29,32,37]. However, the recent studies of Costall *et al.*[9] reached a diametrically opposite conclusion. They found that electrolytic lesion of the SN abolished the apomorphine response but did not affect the amphetamine response of these animals. We have also utilized electrolytic lesion of the SN and found that the amphetamine response was not blocked[19] but we pointed out how unsuccessful these types of lesion are with respect to striatal DA depletion. It appeared that even though the lesions were histologically large and centred on the SN only about a 50% depletion of striatal DA was found. Unless fluorescence histochemistry or pharmacological assay is used to substantiate the effect of various lesions it is impossible to draw conclusions about the extent of the denervation caused by the lesions and draw conclusions about the mechanism of action of various drugs. Such data was not supplied in the above mentioned paper.

(ii) DA depletion and aphagia and adipsia. It is of great interest that the SN lesioned rats were not aphagic or adipsic. Ungerstedt has made massive 6-OHDA lesions of the SN leading to depletions as great as those reported here. He found that the amphetamine response was lacking in these animals (personal communication) but the physical state of these animals made the interpretation of the results inconclusive. All of his SN lesioned rats were so severely aphagic and adipsic that they needed to be tube fed continuously after lesion and never recovered spontaneous food and water intake[40]. The rats in our study were anorexic but not aphagic or adipsic. They were able to maintain themselves on a palatable diet. This allows a more straightforward interpretation of their blocked locomotor response to amphetamine and also questions the role of the SN in aphagia and adipsia.

In our many studies with 6-OHDA SN lesions[20] we have also reported aphagia and adipsia as severe as that found by Ungerstedt but aphagia and adipsia occurred only if the lesion was sufficient to deplete the striatum of at least 90% DA and when

this occurred there was always about a 70% hypothalamic NA depletion as well (caused by the spread of 6-OHDA to the proximal ventral NA pathway). In this experiment a greater DA depletion was found with an even more severe hypothalamic NA depletion — yet the rats were not aphagic and adipsic.

The present result could be added to two others in the literature which together indicate that after damage to the nigro-striatal pathway, measuring absolute DA levels in the striatum may not be a relevant correlate of the severity of the aphagia and adipsia.

	Striatal DA levels (as % of	*Hypothalamic NA levels control level)*	*Aphagia and adipsia*
Present results	0.4	14.6	No
Zigmond and Stricker[45] intraventricular 6-OHDA after DMI + pargyline	2.5	75.0	Yes
Iversen[19] electrolytic SN lesion	64.0	73.0	Yes

It would thus appear that there might well be some strict localization of the critical pathway within the NS system, lesion of which is responsible for aphagia and adipsia, and which was spared in our study. An intensive study of localization of function within the striatal area is indicated by these results and those of Divac[12] and others[22], as the possibility now exists that the NS pathway cannot be considered as a homogeneous entity.

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